UNIVERSIDADE FEDERAL DE PERNAMBUCO

CENTRO DE BIOCIÊNCIAS

PROGRAMA DE PÓS-GRADUAÇÃO

EM CIÊNCIAS BIOLÓGICAS

APROVEITAMENTO DE SUBSTRATOS AGROINDUSTRIAIS NA PRODUÇÃO DE PRODIGIOSINA E BIOSSURFACTANTE POR *Serratia marcescens* UCP 1549

DAYANA MONTERO RODRÍGUEZ

Recife, 2017

DAYANA MONTERO RODRÍGUEZ

APROVEITAMENTO DE SUBSTRATOS AGROINDUSTRIAIS NA PRODUÇÃO DE PRODIGIOSINA E BIOSSURFACTANTE POR *Serratia marcescens* UCP 1549

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas da Universidade Federal de Pernambuco, como parte dos requisitos para a obtenção do título de Doutora em Ciências Biológicas.

Área de Concentração: Biotecnologia

Linha de Pesquisa: Microbiologia aplicada

Orientadora: Profª. Dra. Galba Maria de Campos Takaki

Co-orientadora: Profª. Dra. Hélvia Waleska Casullo de Araújo

Recife, 2017

Catalogação na Fonte:
Bibliotecário Bruno Márcio Gouveia, CRB-4/1788

Rodríguez, Dayana Montero
Aproveitamento de substratos agroindustriais na produção de prodigiosina e biossurfactante por *Serratia marcescens* UCP 1549 / Dayana Montero Rodríguez. – Recife, 2017.

120 f. : il.

Orientadoras: Galba Maria de Campos Takaki, Hélvia Waleska Casullo de Araújo
Tese (doutorado) – Universidade Federal de Pernambuco. Centro de Biociências. Pós-graduação em Ciências Biológicas, Recife, 2017.
Inclui referências e anexos

1. Resíduos agrícolas 2. Fermentação I. Takaki, Galba Maria de Campos (orient.) II. Araújo, Hélvia Waleska Casullo de (coorient.) III. Título.

528.746 CDD (22.ed.) UFPE/CB-2017- 546

DAYANA MONTERO RODRÍGUEZ

APROVEITAMENTO DE SUBSTRATOS AGROINDUSTRIAIS NA PRODUÇÃO DE PRODIGIOSINA E BIOSSURFACTANTE POR *Serratia marcescens* UCP 1549

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas da Universidade Federal de Pernambuco, como parte dos requisitos para a obtenção do título de Doutora em Ciências Biológicas. Área de Concentração: Biotecnologia.

Aprovada em: 23 de outubro de 2017

Banca examinadora:

Profª. Drª. Galba Maria de Campos Takaki (Orientadora)
Universidade Católica de Pernambuco- UNICAP/PE

Profª. Drª. Celuta Sales Alviano
Universidade Federal do Rio de Janeiro - UFRJ/RJ

Profª. Drª. Luciana de Oliveira Franco
Universidade Federal Rural de Pernambuco – UFRPE/PE

Profª. Drª. Maria Tereza dos Santos Correia
Universidade Federal de Pernambuco - UFPE/PE

Profª. Drª. Norma Buarque de Gusmão
Universidade Federal de Pernambuco - UFPE/PE

A Daniel Alejandro, meu filho, porque seu sorriso faz valer a pena qualquer sacrifício...

A Daniel por seu amor e seu companheirismo, por sua paciência nas horas difíceis...

Aos meus pais e meu irmão, por seu amor e seu incentivo sempre.

“() o mundo pertence a quem se atreve
e a vida é muito curta para ser insignificante"
Charles Chaplin

RESUMO

Serratia marcescens UCP 1549 foi investigada quanto à produção de prodigiosina e biossurfactante, considerando a sua habilidade de biotransformar substratos agroindustriais. A produção simultânea dessas biomoléculas por fermentação em estado sólido (FES), foi avaliada utilizando um planejamento fatorial 2^2. O máximo rendimento de pigmento vermelho (33,99 g/kg de substrato seco) e menor valor de tensão superficial (27,9 mN/m) foram evidenciados no ensaio 2 do planejamento, no meio constituído de 10 g de farelo de trigo e solução umedecedora contendo sais (KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$ e $(NH_4)_2SO_4$) e 5% de óleo de soja pós-fritura. O pigmento foi caracterizado como prodigiosina pelo pico máximo de absorbância a 536 nm e valor de R*f* de 0.9 em CCD. Um estudo subsequente utilizando este meio permitiu obter 39,81 g/kg do pigmento, que foi identificado como prodigiosina pelo resultado positivo em teste presuntivo. O pico máximo de absorbância a 537.1 nm, valor de R*f* de 0.89 em CCD e massa molecular de 323,199 *m/z* verificaram a presença da prodigiosina, e foi demonstrado seu potencial para tingimento de tecidos e velas. A produção simultânea de prodigiosina e biossurfactante foi estudada por fermentação submersa utilizando diferentes substratos agroindustriais e os melhores resultados foram obtidos no meio constituído por farelo de milho 1%. Uma fermentação adicional neste meio foi desenvolvida e o pigmento vermelho produzido foi extraído e identificado como prodigiosina pelo pico máximo de absorbância a 535 nm e valor de R*f* de 0.9 em CCD. A prodigiosina presente no meio de cultivo demonstrou maior efetividade no tingimento de tecidos e velas, quando comparado com o pigmento extraído em metanol. O biossurfactante produzido foi extraído por precipitação ácida seguida de precipitação com etanol (2,15 g/l) e caracterizado como um composto aniônico com capacidade de reduzir a tensão superficial e interfacial a 29,3 mN/m e 9,4 mN/m, respectivamente. O biossurfactante mostrou estabilidade em diferentes valores de pH, temperatura e concentração de NaCl, além de excelentes propriedades para a emulsificação, dispersão e o incremento da viscosidade de diferentes substratos hidrofóbicos, o que sugere sua potencial aplicação em diversas indústrias. Portanto, os resultados obtidos evidenciam o potencial biotecnológico de *S. marcescens* UCP 1549 na produção de prodigiosina e biosurfactante por fermentações em estado sólido e submersa utilizando substratos agroindustriais, o que minimiza os elevados custos de produção das duas biomoléculas.

Palavras chave: Fermentação em estado sólido. Fermentação submersa. Pigmento. Surfactante. Aplicações.

ABSTRACT

Serratia marcescens UCP 1549 was investigated for the production of prodigiosin and biosurfactant, considering its ability to biotransform agroindustrial substrates. The simultaneous production of both biomolecules by solid state fermentation (SSF) was evaluated using a 2^2 factorial design. The maximum yield of red pigment (33.99 g/kg of dry substrate) and lower value of surface tension (27.9 mNm) were evidenced in the assay 2 of the design, in medium constituted of 10 g of wheat bran and impregnating solution containing salts **(**KH_2PO_4, K_2HPO_4, $MgSO_4.7H_2O$ and **(**$(NH_4)_2SO_4$**)** and 5% waste soybean oil. The pigment was characterized as prodigiosin by the maximum absorbance peak at 536 nm and R*f* value of 0.9 in TLC. A subsequent study using this medium yielded 39.81 g/kg of the pigment, which was identified as prodigiosin by the positive result in presumptive test. The maximum absorbance peak at 537.1 nm, R*f* value of 0.89 in TLC and molecular mass of 323.199 *m/z* verified the presence of prodigiosin, and its potential for dyeing of textile and candles was demonstrated. Simultaneous production of prodigiosin and biosurfactant was studied by submerged fermentation using different agroindustrial substrates and the best results were obtained in a medium consisting of 1% corn bran. Further fermentation was carried out using this medium and the red pigment obtained was extracted and identified as prodigiosin by the maximum absorbance peak at 535 nm and R*f* value of 0.9 in TLC. The prodigiosin present in the culture medium showed greater effectiveness in the dyeing of textile and candles when compared to the pigment extracted in methanol. The biosurfactant produced was extracted by acid precipitation followed by precipitation with ethanol (2.15 g/l) and characterized as an anionic compound able to reduce surface and interfacial tension at 29.3 mN/m and 9.4 mN/m, respectively. The biosurfactant showed stability at different values of pH, temperature and NaCl concentration, as well as excellent properties for the emulsification, dispersion and increase of the viscosity of different hydrophobic substrates, which suggests its potential application in several industries. Therefore, the results obtained evidenced the biotechnological potential of *S. marcescens* UCP 1549 in the production of prodigiosin and biosurfactant by solid state and submerged fermentations using agroindustrial substrates, which minimizes the high production costs of the two biomolecules.

Keywords: Solid-state fermentation. Submerged fermentation. Pigment. Surfactant. Applications.

LISTA DE ILUSTRAÇÕES

CAPITULO 1

CAPITULO 2

CAPITULO 3

CAPITULO 4

CAPITULO 5

LISTA DE TABELAS

CAPITULO 1

CAPITULO 2

CAPITULO 3

CAPITULO 5

SUMÁRIO

CAPÍTULO 1

1. INTRODUÇÃO

O Brasil, em especial a região Nordeste, possui uma economia baseada na produção agrícola, o que resulta na geração de grandes volumes de resíduos líquidos e sólidos que muitas vezes não são totalmente utilizados. A grande complexidade física e química desses resíduos dificulta seu tratamento, podendo causar risco ao meio ambiente onde são descartados (FERREIRA-LEITÃO et al., 2010; BERGER et al., 2014). Contudo, o baixo custo, acessibilidade e composição química e nutricional desses rejeitos favorece seu aproveitamento para a produção de metabolitos microbianos de interesse comercial. Várias pesquisas têm sido desenvolvidas visando à utilização de resíduos agroindustriais descartados anualmente como fonte de matéria orgânica para a produção de biomoléculas de alto valor agregado, tais como enzimas, ácidos orgânicos, biossurfactantes e pigmentos (CARVALHO et al., 2014; SINGH e SAINI, 2014; RAVINDRAN e JAISWAL, 2016). Desta forma, também se agrega valor a produtos de descarte, permitindo a implementação de um ciclo sustentável e ambiental (FERREIRA-LEITÃO et al., 2010; MAKKAR et al., 2011; FERREIRA-LEITÃO et al., 2017).

A demanda mundial de produtos de origem microbiana tem aumentado nos últimos anos devido ao enorme potencial de aplicação destas biomoléculas em diversas indústrias, além das vantagems quanto comparados com os de origem sintética (VENIL et al., 2014). No entanto, a produção a grande escala destes compostos é limitada devido ao alto custo de produção, o que em parte é causado pelo custo dos meios de cultura (AHMAD et al., 2012; ARULDASS et al., 2014; SANAWER et al., 2017). Por consequência, pesquisas têm sido dirigidas para a avaliação de subproductos e resíduos agroindustriais como fontes alternativas de carbono e de nitrogênio, tornando-se uma estratégia atraente e de baixo custo.

Serratia marcescens UCP 1549, bactéria isolada do semi-árido de Pernambuco, tem sido investigada recentemente quanto a seu potencial para produzir prodigiosina, pigmento vermelho com demostradas propriedades imunossupresoras, antitumorais e antimicrobianas (ARAUJO et al., 2010; LAPENDA et al., 2014). Outros estudos realizados por Montero-Rodríguez et al. (2015) e Araújo et al. (2017) comprovaram a habilidade desta estirpe de produzir biossurfactante em meio contendo resíduos agroindustriais. Contudo, a produção simultânea de prodigiosina e biossurfactante no mesmo meio e condições de cultivo não foi ainda relatada pela literatura, sendo uma estratégia inovadora importante a ser explorada em *S. marcescens*.

Neste sentido, considerando o potencial de *S. marcescens* UCP 1549, novas investigações biotecnológico de caráter inovador foram realizadas utilizando esta linhagem selvagem, isolada do semi-árido de Pernambuco, associando ao uso de fontes nutricionais de baixo custo processo fermentativos submerso e em estado-sólido, com a finalidade de produzir biomoléculas de elevado interesse industrial como a prodigiosina e biossurfactante.

2. OBJETIVOS

2.1 Objetivo geral

Investigar o potencial biotecnológico de *Serratia marcescens* UCP 1549 na biotransformação de substratos agroindustriais para a produção de prodigiosina e biossurfactante.

2.2 Objetivos específicos

- Avaliar o potencial de *S. marcescens* para utilizar substratos agroindustriais na produção simultânea de biossurfactante e prodigiosina por fermentação em estado sólido;
- Avaliar o potencial de *S. marcescens* para utilizar substratos agroindustriais na produção simultânea de biossurfactante e prodigiosina por fermentação submersa;
- Realizar a extração, quantificação e caracterização da prodigiosina;
- Investigar o potencial de aplicação da prodigiosina produzida no tingimento de tecidos e velas;
- Realizar o isolamento e caracterização do biossurfactante produzido;
- Avaliar a estabilidade do biossurfactante produzido frente a diferentes valores de pH, salinidade e temperatura;
- Investigar o potencial de aplicação do biossurfactante produzido na emulsificação, dispersão e sua influência na viscosidade de substratos hidrofóbicos.

3. REVISÃO DA LITERATURA

3.1 Resíduos e subprodutos agroindustriais

O Brasil é um pais eminentemente agrícola e segundo a Organização para a Cooperação e Desenvolvimento da Europa (OCDE) e Organização das Nações Unidas para Agricultura e Alimentação (FAO), assumirá a liderança das exportações mundiais desse setor em 2024 (FAO, 2015). Este crescimento agroindustrial, embora favoreça o desenvolvimento, contribui para o aumento da geração de resíduos sólidos, compostos de casca, caroços, dentre outros, pois estima-se que haja perdas consideráveis deles nas diversas etapas da cadeia produtiva, desde a produção até o momento de seu consumo (FERREIRA-LEITÃO et al., 2010; DO NASCIMENTO FILHO e FRANCO, 2015).

Estes resíduos, por serem materiais ricos em macro e micronutrientes, podem proporcionar sérios problemas de poluição no solo e em águas superficiais e subterrâneas, se não são adequadamente tratados (BRAR et al., 2013; RAVINDRAN e JAISWAL, 2016). Além da contaminação direta, o maior impacto é causado pela fermentação do material, com geração de maus odores e redução de oxigênio dissolvido em águas superficiais, cuja magnitude depende da concentração de carga orgânica e da quantidade lançada. A degradação da matéria orgânica é, também, habitat para a proliferação de microrganismos e vetores de doenças (MATOS, 2005).

Existe uma enorme quantidade de resíduos e subprodutos agroindustrias produzidos no país, tais como bagaço de cana de açúcar, farelo de trigo, de milho, palha de arroz e cascas e resíduos de frutas em geral (FERREIRA-LEITÃO et al., 2010; PORTUGAL-PEREIRA et al., 2015). Estes compostos, além de possuírem baixo custo e boa disponibilidade, apresentam uma excelente composição química e nutricional que favorece o crescimento e metabolismo microbianos, despertando assim o interesse dos pesquisadores na busca de substratos alternativos para a obtenção de produtos de elevado valor comercial (KAUR et al. 2014; FERREIRA-LEITÃO et al., 2017).

3.1.1 Bagaço de cana-de-açúcar

O bagaço de cana-de-açúcar é um resíduo obtido da cana depois de esmagado para obter o suco que se utiliza na produção de açúcar e etanol (SOMERVILLE et al., 2009; CHANDEL et al., 2011, 2012).

A composição química complexa das paredes celulares do bagaço de cana limita seu uso como forragem para bovinos e ruminantes, em contraste com a palha de trigo, palha de arroz, palha de sorgo, entre outros. Geralmente, o bagaço é composto (% w/w peso seco) de hemicelulose (26,2-35,8), celulose (35-45), lignina (11,4-25,2) e outros (2,9-14,4) (ZHAO et al., 2009; CANILHA et al., 2011). A composição química desigual do bagaço depende de múltiplos fatores, incluindo a variedade de culturas, condições climáticas, localização e modo de crescimento, uso de fertilizantes e composição física e química do solo (CANILHA et al., 2011).

Diversas são as aplicações que têm sido informadas para este substrato como por exemplo etanol, xilitol, ácidos orgânicos, antibióticos, alimentação animal, bioidrógeno, alcalóides e pigmentos (PANDEY et al., 2000; CHANDEL et al., 2012) De igual forma, o bagaço de cana tem estimulado o crescimento microbiano para a produção de enzimas industriais, tais como xilanase, celulase, amilase e lacase por certas bactérias e fungos filamentosos, empregando sistemas de fermentação em estado sólido (FES) ou fermentação submersa (FS).

3.1.2 Farelo de trigo

O trigo para o consumo de alimentos humanos geralmente é processado em farinha. A taxa de extração de farinha varia de 73 a 77%, dependendo do processo de moagem, da variedade de trigo e das condições de cultivo (ELLIOTT et al., 2002). Assim, fluxos de subproduto, incluindo farelo de trigo e outros compostos, tais como germe de trigo e partes da endosperma representam cerca do 23 ao 27% da produção de moagem. A fração mais importante de todos os subprodutos é farelo, representando cerca de 25% do peso do grão (NEVES et al., 2006).

Geralmente, o farelo de trigo compreende aproximadamente 12% de água, 13-18% de proteína, 3,5% de gordura e 56% de carboidratos. Embora seja usado principalmente como ingrediente na alimentação animal, 1-3 o seu uso como fonte de fibra dietética está se tornando cada vez mais importante. Porém, considerando as grandes quantidades de biomassa de farelo acumulada e o baixo valor nutricional, outras aplicações tem sido valoradas para este subproduto (APPRICH et al., 2014).

Alguns exemplos na literatura mostram a produção de metabólitos microbianos como enzimas tem sido una das alternativas para sua utilização (KUMAR et al., 2013; DEMIR e TARI, 2014).

3.1.3 Farelo de milho

Dentre os subprodutos mais frequentes no processamento do milho o farelo, a camada externa de cereais, é muitas vezes descartado durante o processo de moagem em vez de ser usado como uma aplicação alimentar, devido às expectativas sensoriais dos consumidores e às desvantagens tecnológicas para a indústria alimentar (CODA et al., 2015). Neste processo a sua disposição econômica é a principal preocupação da indústria de alimentos em relação aos regulamentos ambientais completos (ELMEKAWY et al., 2013).

Com uma composição aproximada de 50-115 $g.kg^{-1}$ de proteínas, 40-112 $g.kg^{-1}$ de carboidrato e 13,2- 19 $g.kg^{-1}$ de lipídios entre outros componentes, os usos tradicionais para esse subprodutos do processamento do milho inclui, em grande parte, a alimentação animal. Portanto, a procura de outras aplicações para a produção de compostos com valor agregado tem estimulado seu uso como substrato para o crescimento microbiano (ROSE et al., 2010).

Ou et al. (2017) desenvolveram e otimizaram um processo para a obtenção de n-butanol por *Clostridium cellulovorans* utilizando um resíduos agrícolas de baixo valor derivados do milho. Embora o farelo favoreceu a produção do n-butanol a espiga estimulou a maior produção maior que 3 g/L. Odeniyi e Deola (2017) identificaram a produção de ácidos polihidroxialcanoicos (PHA) por *Bacillus thuringiensis* SBC4 utilizando diferentes substratos como farelo de milho, espiga de milho e farelo de trigo para produção de PHA, embora com a espiga de foi detectado o maior índice de PHA com 21,05%. Por outro lado, Lee et al. (2017) investigaram o potencial de farelo de milho como matéria-prima para a produção de lipídios pela levedura oleaginosa, *Trichosporon oleaginosus* ATCC 20509. Neste estudo o meio alternativo com farelo de milho estimulou uma acumulação de mais do 50% de lipídios. Desta forma estes autores concluíram que este subproduto pode ser uma alternativa como fonte de carbono na produção de lipídios.

3.1.4 Casca e coroa de abacaxi

O Brasil é um dos principais produtores mundiais de abacaxi com uma produção de 3,1 milhões de toneladas ao ano. O *Ananas comosus L. Merril* é o abacaxi mais importante da família Bromeliaceae, do ponto de vista econômico, e pode ser uma excelente fonte alimentar de proteínas, vitaminas e minerais (GRANADA et al., 2004; CUNHA, 2007). O abacaxi fruto é a parte comercializável da planta, entretanto, esta porção representa somente 23 % do total

da planta, enquanto que o restante formado por caule, folha, casca, coroa e talo, é considerado subproduto agrícola e não tem sido devidamente aproveitado, resultando em perdas econômicas. A composição deste resíduo pode modificar-se de acordo com a qualidade e a variedade da fruta (OLIVEIRA et al., 2008).

A composição química do abacaxi varia muito de acordo com a época em que é produzido. De modo geral, a produção ocorre no período do verão e gera frutas com maior teor de açúcares e menor acidez. O abacaxi destaca-se pelo valor energético, devido à sua alta composição de açúcares, e valor nutritivo pela presença de sais minerais (cálcio, fósforo, magnésio, potássio, sódio, cobre e iodo) e de vitaminas (C, A, B1, B2 e Niacina), no entanto, apresenta teor proteico e de gordura inferiores a 0,5% (Tabela 1). O fruto apresenta alto conteúdo em bromelina, que auxilia o processo de digestão. Trata-se de mistura de enzimas proteolíticas que em meio ácido, alcalino ou neutro, transforma as matérias proteicas em peptídeos ou aminoácidos. A bromelina pode ser isolada do suco da fruta ou do talo da planta, ocorrendo em maior concentração no cilindro central do abacaxi (OLIVEIRA et al., 2008).

Os talos, coroas e cascas do abacaxi possuem diversas propriedades nutricionais que podem ser transformados em ração para animais, farinhas para enriquecimento de produtos da panificação e para obtenção de enzimas proteolíticas. Além disso, estudos de novas perspectivas na minimização e/ou promoção do uso eficiente destes resíduos, sendo desenvolvidos vários bioprocessos capazes de utilizar estes materiais como substratos para a produção de diversas moléculas com alto valor agregado, tais como proteínas microbianas, ácidos orgânicos, etanol, enzimas e metabólitos secundários biologicamente ativos (RODA et al., 2014; de ALMEIDA et al., 2015; KHEDKAR et al., 2017).

3.1.5 Casca de tangerina

O Brasil é o maior produtor mundial de citros e o quarto na produção de tangerinas (*Citrus reticulata* Blanco) (ASSIS et al., 2010).

As frutas cítricas e seus derivados apresentam um importante efeito benéfico à saúde graças às suas propriedades nutricionais além de serem fontes de substâncias antioxidantes como ácido ascórbico, compostos fenólicos e carotenoides (NIPORNRAM et al., 2017). A Tabela 1 mostra o teor de nutrientes presentes na casca de tangerina (GONDIM et al., 2005).

Tabela 1. Teor de nutrientes da casca de abacaxi e tangerina (Fonte: GONDIM et al., 2005).

Parâmetro	100 g de amostra *in natura* da casca do fruto	
	Abacaxi	**Tangerina**
Umidade (g)	78,13	49,10
Cinzas (g)	1,03	1,75
Lipídeos (g)	0,55	0,64
Proteínas (g)	1,45	2,49
Fibras (g)	3,69	10,38
Carboidratos (g)	14,95	35,64
Calorias (Kcal)	70,55	158,30
Cálcio (mg)	76,44	478,98
Ferro (mg)	0,71	4,77
Sódio (mg)	62,63	77,76
Magnésio (mg)	26,79	159,59
Zinco (mg)	0,45	2,83
Cobre (mg)	0,11	0,58
Potássio (mg)	285,87	598,36

A casca da tangerina, além de ser rica em carotenoides, que são precursores da vitamina A e têm atividade antioxidante, é rica em fibra alimentar. Estudos sobre a ingestão adequada de fibras têm sido cada vez mais frequentes, dada sua importância na dieta devido à prevenção de doenças crônicas como diabetes mellitus, hipertensão arterial e algumas

desordens gastrintestinais (LEI et al., 2009; BERNAUD e RODRIGUES, 2013). Os resíduos sólidos da indústria de citros (cascas, sementes e polpas) são geralmente transformados em farelo para ração animal (ASSIS et al., 2010).

3.2 Processos fermentativos

Diante a problemática no país da enorme geração de resíduos como consequência do crescimento agroindustrial, os processos biotecnológicos tornam-se uma alternativa viável para o aproveitamento de quantidades significativas destes compostos desperdiçados ou subutilizados anualmente, como fonte de matéria orgânica para a obtenção de produtos de alto valor agregado, como proteínas, enzimas, ácidos orgânicos, biofertilizantes, biossurfactantes, aminoácidos, pigmentos, dentre outros metabólitos microbianos (FERREIRA-LEITÃO et al., 2010; KAUR et al., 2014; BRUMANO et al., 2016).

Existem dois tipos básicos de fermentação para a obtenção de produtos microbianos: Fermentação Submersa (FS) e Fermentação em Estado Sólido (FES).

3.2.1 Fermentação submersa

A fermentação submersa ou liquida (FS) é definida como aquela cujo substrato fica dissolvido ou suspenso em pequenas partículas no líquido, normalmente, água. Na FS a água chega a constituir cerca de 90 a 99% da massa total do material a ser fermentado (MITCHEL et al., 2000).

Esse tipo de fermentação apresenta como principais vantagens: fácil inoculação, processo contínuo, fácil acompanhamento da formação do produto e consumo do substrato e o controle dos parâmetros fermentativos como pH, temperatura, oxigenação e esterilidade. Como principais desvantagens, têm-se o grande volume de resíduos gerados e a dificuldade de separação produto/substrato, elevados consumo energético e custo tecnológico (PANDEY, 2003; SINGHANIA et al., 2010).

3.2.2 Fermentação em estado sólido

A fermentação em estado sólido (FES) é definida como aquela que ocorre na ausência de água livre entre as partículas e na qual se emprega um material natural ou sintético como substrato sólido (PANDEY et al., 2000; SINGHANIA et al., 2010; THOMAS et al., 2013).

Este processo apresenta como vantagens em relação à FS como maior concentração de produtos formados, fácil aeração, menos espaço requerido para equipamentos baixo consumo de energia, facilidade de extração do produto desejado e diminuição de problemas de contaminação microbiana. Por outro lado, as limitações técnicas ainda impedem sua ampla utilização industrial: dificuldade de remoção de calor em virtude da baixa condutividade térmica da matéria, condições estáticas e a dificuldade de se medir parâmetros como pH, oxigênio dissolvido, quantidade de substrato e concentração no estado sólido (EL-BAKRY et al., 2015; DORIYA et al., 2016; SOCCOL et al., 2017).

A FES tem-se mostrado muito promissora no desenvolvimento de vários bioprocessos, como na biorremediação e biodegradação de compostos tóxicos, desintoxificação de resíduos e subprodutos agro-industriais, biotransformação de resíduos de colheitas para enriquecimento nutricional e na obtenção de produtos de alto valor agregado, como metabólitos secundários (antibióticos, alcalóides, fatores de crescimento vegetal, etc), ácidos orgânicos, biopesticidas, biocombustíveis, compostos aromáticos e enzimas (PANDEY et al., 2000; LIZARDI-JIMÉNEZ e HERNÁNDEZ-MARTÍNEZ, 2017).

A seleção do substrato para os processos de FES depende de vários fatores, principalmente aqueles relacionados ao seu custo e eficiência. Neste contexto, a utilização de subprodutos agro-industriais torna -se um atrativo para este processo. A aplicação destesmateriais em bioprocessos tornou-se importante sob o ponto de vista ambiental, reduzindo problemas relacionados ao seu manejo inadequado e consequentes danos ambientais. Além disso, seu baixo custo e grande disponibilidade fazem dos mesmos excelentes substratos alternativos para vários processos industriais (PANDEY et al., 2000; EL-BAKRY et al., 2015).

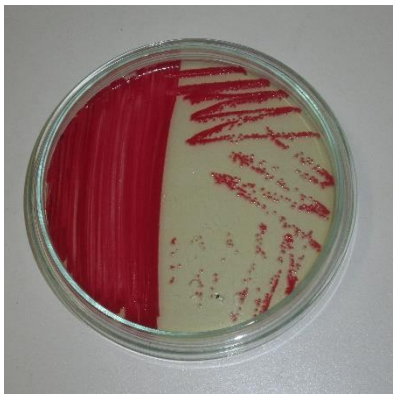
3.3 *Serratia marcescens*: produção de metabólitos de interesse industrial

O gênero *Serratia*, que pertence à família Enterobacteriaceae, é formado por bacilos Gram-negativos e distingue-se pela produção de três enzimas: DNAse, lipase e gelatinase (KHANAFARI et al., 2006; ARAÚJO, 2010). Este gênero microbiano está amplamente distribuído na natureza, encontrando-se no solo, água, plantas e no trato intestinal de seres humanos e animais homeotérmicos (KONEMAN et al., 2001; KIM et al., 2009).

S. marcescens é o membro mais importante desse género, bactéria Gram-negativa em forma de bastonete, anaeróbia facultativa, de crescimento quimioautotrófico e caraterizada

pela habilidade em produzir um pigmento vermelho denominado prodigiosina (ARAÚJO, 2010; CANTALICE, 2014) (Figura 1). Nos últimos anos do século XXI aumentou o interesse sobre esta bactéria pelo fato desta ser um patógeno oportunista, responsável por alguns casos de infecções hospitalares (SOTO-CERRATO et al., 2007; BAKKIYARAJ et al., 2012; IGUCHI et al., 2014). Contudo, entres os diferentes biótipos de *S. marcescens*, somente os não cromogênicos constituem uma ameaça real no ambiente hospitalar (CARBONELL et al., 2000; ELKENAWY et al., 2017). As espécies cromogênicas normalmente são isoladas do meio ambiente: água, solo, plantas ou insetos *(*DEORUKHKAR et al., 2007; KALIVODA, et al., 2010*).*

Figura 1- Cultivo de *Serratia marcescens* UCP/WFCC 1549 em meio Luria Bertani (LB) sólido mostrando o pigmento vermelho que a bactéria produz.



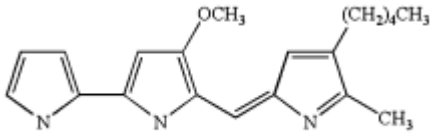
Além da prodigiosina, *S. marcescens* também é produtora de numerosas substâncias, como a serrawettina, um biossurfactante que lhe confere a propriedade de aderência no processo de colonização de superfícies (MONTANER et al., 2000; PEREZ-THOMAS et al., 2003; MATSUYAMA et al., 2011); além de enzimas como proteases, quitinases e lipases (HAMRE et al., 2015; THAKUR et al., 2016; ANDERSON et al., 2017). Dentre elas, a enzima L-asparaginase tem chamado recentemente o interesse devido à possibilidade de aplicação nas àreas terapêutica e alimentícia (BATOOL et al., 2015; CACHUMBA et al., 2016; SANAWER et al., 2017).

3.3.1 Prodigiosina

A prodigiosina é um pigmento vermelho natural pertencente à família das prodigininas. É um alcaloide de estrutura química de tripirrol linear, com peso molecular de 323,44 Dalton e de fórmula $C_{20}H_{25}N_{3O}$ (BENNETT e BENTLEY, 2000; SONG et al., 2006; KALIVODA et al., 2010) (Figura 2). Este pigmento caracteriza-se por ser bastante sensível a luz, insolúvel

em água, moderadamente em álcool e solúvel em clorofórmio, benzeno, acetona, éter e éter de etila, sofrendo variação de coloração dependendo do pH do meio em que é produzido. Segundo Bennett e Bentley (2000) e Nakashima et al. (2005), quando o pigmento é produzido em pH ácido, este se apresenta com uma pigmentação vermelha intensa com absorbância máxima em 537 nm.

Figura 2- Estrutura química da prodigiosina (Fonte: AHMAD et al., 2012).



A prodigiosina é um metabólito secundário, sendo produzido quando o metabolismo da célula na maioria das vezes alcança a fase estacionária de crescimento (VENIL; LAKSHMANAPERUMALSAMY, 2009). O pigmento é formado em condições aeróbias em torno de 30ºC; contudo, a temperatura ótima para a formação do pigmento não é necessariamente a mesma de crescimento. Assim, linhagens que crescem melhor à temperatura próxima a 37 ºC, produzem pouco ou não produzem o pigmento (KHANAFARI et al., 2006*;* ELKENAWY et al., 2017).

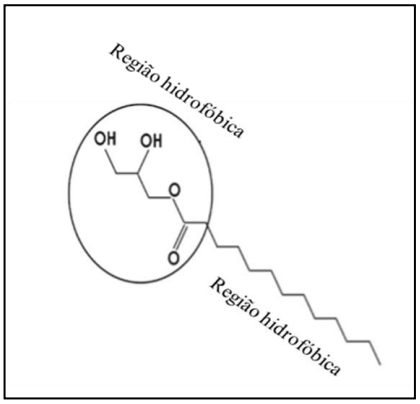
O maior interesse nas pesquisas com prodigiosina, tem sido voltado na área terapêutica, sendo considerada como uma substância promissora devido às suas características imunossupressoras, antitumorais e antiproliferativas, como mediador do efeito apoptótico em células cancerígenas humanas e sem presentar toxidez às células sadias (DEORUKHKAR et al., 2007; SUMATHI et al., 2014; SURYAWANSHI et al., 2014). Além disso, tem-se demostrado as propriedades antimicrobiana, antiviral, inseticida e antimalárica deste pigmento (CASTRO, 1967; LAPENDA et al., 2015; SURYAWANSHI et al., 2015a; ZHOU et al., 2016). Recentemente, várias estudos tem sido realizados, visando à aplicação da prodigiosina como corante natural de tecidos, papel, velas, sabões, etc. (AHMAD et al., 2012; MEHTA e SHAH, 2015; REN et al., 2017). Inclusive, sua ação protetora dos microrganismos contra a radiação ultravioleta tem sugerido sua aplicação na área dos cosméticos, como novo aditivo nos protetores solares (BORIĆ et al., 2011; SURYAWANSHI et al., 2015b).

3.3.2 Biossurfactantes

Biossurfactantes constituem um grupo bastante diverso de compostos tensoativos sintetizados por fungos filamentosos, bactérias e leveduras que possuem a típica estrutura anfifílica dos surfactantes sintetizados quimicamente (NITSCHKE; PASTORE, 2002; PEREIRA; DUVOISIN; ALBUQUERQUE, 2017).

A porção hidrofóbica dessa molécula é constituída ou de ácidos graxos de cadeia longos, ou hidroxiácidos, ou ainda α – alquil – β – hidroxi ácidos graxos, enquanto porção hidrofílica pode ser constituída ou de carboidratos, aminoácidos, peptídeo cíclico, fosfato, ácido carboxílico, ou de um álcool (Figura 3) (ABBASI et al., 2012; SATPUTE et al. 2017).

Figura 3 – Estrutura geral dos biossurfactantes.



Fonte: SATPUTE et al. 2017

3.3.2.1 Propriedades dos biossurfactantes

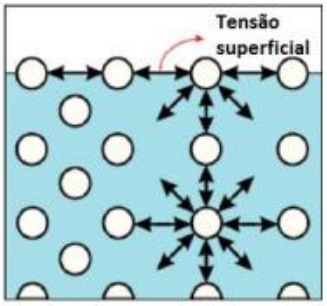
Tensão superficial e interfacial

A redução da tensão superficial e interfacial entre líquidos, sólidos e gases é responsável pelas propriedades únicas dos biossurfactantes, como: detergência, emulsificação (micro e macro), lubrificação, ação espumante e antiespumante, capacidade molhante, solubilização e dispersão de fases (XU et al., 2011b; VAZ et al., 2012; MNIF et al., 2013; LIMA et al. 2017).

Os biossurfactantes conseguem reduzir a tensão superficial e interfacial pela capacidade de distribuição dos monômeros nas interfaces entre líquidos com diferentes graus de polaridades (óleo/água ou água/óleo) formando um filme molecular ordenado, atuando no

desequilíbrio da força eletrostática das moléculas da água reduzindo a tensão interfacial, como também a tensão superficial (Figura 4).

Figura 4 - Esquema das forças intermoleculares no interior e na superfície do líquido

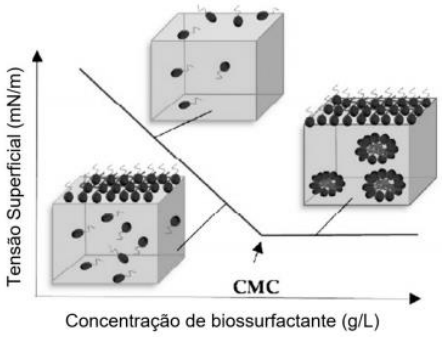


Fonte: PIRRÔLO (2006).

Concentração Micelar Crítica (CMC)

A Concentração Micelar Crítica (CMC) esta correlacionada com a tensão superficial (Figura 5). Portanto, a CMC pode ser definida como a menor quantidade de surfactante necessária para o decréscimo da tensão superficial e formação das micelas (SANTOS et al. 2016).

Figura 5- Ação do biossurfactante na formação de micelas



Santos et al. 2016

Na CMC a termodinâmica do sistema tensoativo-solvente favorece a formação de micelas. Entretanto, numa solução em que a concentração de tensoativo é baixa, as moléculas existem na forma de monômeros, quando a concentração do tensoativo vai aumentando, os monômeros vão saturando a interface. Cada vez que uma nova molécula é adicionada à solução, vai aumentando a interação desfavorável entre a fração apolar e as moléculas de água até o ponto em que os monômeros vão-se agregando formando micelas, vesículas, monocamadas e complexos polímero–surfactante (SHAVANDIA et al., 2012).

Formação de emulsões

Alguns biossurfactantes podem agir como bioemulsificantes por serem capazes de formar e estabilizar emulsões, porém nem todo bioemulsificante é capaz de possuir ação surfactante. Esta propriedade pode ser comprovada pela diferença entre densidade ótica antes e após agitação da mistura do meio de cultura e uma solução hidrofóbica (RIVARDO et al., 2009).

A emulsão é considerada como estável se seu volume, 24 h após a sua formação, ainda corresponder a 50 % do seu volume original (ANANDARA et al., 2010; SPONZA e GOK, 2011).

Estabilidade térmica, iônica e do pH

Os biossurfactantes são compostos capazes de manter sua ação surfactante mesmo após expostos as condições extremas de temperatura, pH e concentração de NaCl. Tais características permitem inúmeras aplicações em diferetentes setores industriais e em processos ambientais (CHEN; JUAN; SEM, 2015).

Estudos têm avaliado a estabilidade dos biossurfactantes de origem microbiana com o objetivo de identificar o comportamento destes compostos para viabilização industrial (BARROS et al., 2008; WEI, 2015). Alguns trabalhos têm demonstrado haver significativa estabilidade dos biossurfactantes após serem comparados aos surfactantes sintéticos (KIM et al., 1997; NITSCHKE; PASTORE, 2006)**.**

Biossurfactantes do tipo sororolipideos apresentam estabilidade em faixas extremas de pH (entre 2-10) e após adição de 20% de NaCl (DAVEREY; PAKSHIRAJAN, 2010). Por outro lado, os biossurfactantes classificados como lipopeptideos são capazes de manter estabilidade apenas em meio alcalino (8-12) e estabilidade térmica na faix de temperatura que varia de 30-100ºC (KHOPADE, et al. 2012).

3.3.2.2 Vantagens de uso dos biossurfactantes

Os biossurfactantes apresentam algumas vantagens únicas quando comparados aos surfactantes sintéticos, tais como:

- Tolerância à temperatura, pH e força iônica: muitos biossurfactantes podem ser utilizados sob condições extremas pois suas propriedades físico-químicas não são afetadas por

mudanças extremas de temperatura, pH e força iônica. Alguns deles podem suportar temperaturas de até 100°C e apresentam maior estabilidade térmica em condições extremas quando comparados aos sintéticos (MAX et al., 2012). De igual forma, possuem maior funcionalidade em valores extremos de pH, entre 5 e 12 por exemplo, e são potencialmente ativos quando submetidos a concentrações de 10% de NaCl, enquanto que 2-3% de sal são suficientes para inativar surfactantes convencionais (MAKKAR e CAMEOTRA, 2002; DAVISHI et al., 2011)

- Biodegradabilidade: são facilmente biodegradados na água e no solo, o que os torna adequados para aplicação na biorremediação e no tratamento de resíduos (COLIN et al., 2014).

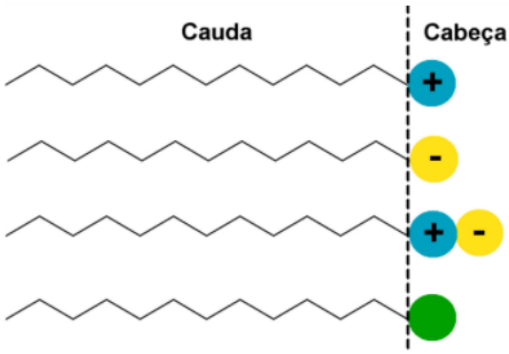
-Baixa toxicidade: apresentam baixa toxicidade quando comparados com os surfactantes sintéticos. Por isso, têm recebido maior atenção devido à crescente preocupação da população com os efeitos alérgicos dos produtos artificiais, sendo permitido em alimentos, cosméticos e produtos farmacêuticos (NITSCHKE e PASTORE, 2002; ANYANWU et al., 2011).

- Facilidade de síntese a partir de material renovável e de baixo custo: vários estudos mostram a produção de biossurfactantes produzidos através de uma grande variedade de substratos orgânicos de baixo custo, como manipueira, milhocina, melaço, óleo de sementes, resíduos de frutas, borra de café, entre outros (MAKKAR et al., 2011; ROCHA e SILVA et al., 2014).

3.3.2.3 Classificaçao dos biossurfactantes pela carga no grupo hidrofílico

Os surfactantes sintéticos e os biossurfactantes possuem sistema de classificação diferenciado. Por um lado, os surfactantes sintéticos produzidos a partir de derivados do petróleo são classificados de acordo com a carga exibida no grupo hidrofílico da molécula em: catiônicos (+), aniônicos (-), anfotéricos (carga + e -) e não-iônicos (sem carga) (Figura 6). Enquanto os biossurfactantes de origem microbiana são classificados pela carga da região hidrofílica da molécula e quanto ao tipo de micro-organismo produtor. Os biossurfactantes de origem microbiana são iônicos em sua maioria, e em raríssimos casos possuem grupamentos ricos em nitrogênio que pode dar um caráter catiônico a essas moléculas (BANAT et al., 2010; SAHARAN, et al. 2011).

Figura 6- Representação esquemática da classificação dos biossurfactantes quanto a carga da região hidrofílica da molécula.



Fonte: http://qnint.sbq.org.br/novo/index.php?hash=tema.102

3.3.2.4 Principais aplicações dos biossurfactantes

A demanda mundial dos biossurfactantes é ainda crescente devido ao enorme potencial de aplicação que eles têm em diversos setores industriais. Segundo pesquisas realizadas pelo Transparency Market Research (2012) "Biosurfactants Market - Global Scenario", o volume do mercado global de biossurfactantes é esperado para ser 476512,2 de toneladas até 2018. Desse total, 21% do consumo de volume virão de regiões como a Ásia, África e América Latina.

Na indústria de alimentos os biossurfactantes possuem uma variedade de aplicações, devido a sua habilidade de formar emulsões estáveis no processamento de matérias primas, no controle da aglomeração de glóbulos de gorduras em alimentos processados, tais como: creme de leite, manteiga, margarina, maionese, molhos para salada, entre outros (SILVA et al., 2009; SILVA, 2012). Estes bioprodutos podem ser usados também como agentes anti-adesivos durante processo de fabricação de alimentos, particularmente na redução de contaminação por patógenos ou na remoção de micro-organismos aderidos (COSTA, 2010).

Na área terapêutica os biossurfactantes também têm aplicação como agentes anti-adesivos e antimicrobianos, na formulação de produtos de limpeza e como agentes terapêuticos (COSTA, 2010; GUDIÑA et al., 2013). Além disso, tem sido demostrada a atividade antitumoral de vários biossurfactantes, o que destaca seu potencial como compostos a ser usados na terapia de diferentes tipos de câncer em humanos (BURGOS-DÍAZ et al., 2013; JANEK et al., 2013).

Alguns biossurfactantes possuem ação de detergência e propriedade espumante, o que os tornam aplicáveis na indústria de cosméticos, em sabonetes líquidos e xampus (CAROLEI e GUTZ, 2005). Dentre eles, os soforolipídeos, raminolipídeos e lipídeos manosileritirol têm apresentado excelentes propriedades e estão sendo produzidos para utilização em loções, hidratante de pele e produtos anti-rugas (LOURITH e KANLAYAVATTANAKULI, 2009).

Contudo, a área onde os biossurfactantes têm sido aplicados mais amplamente é o setor petroleiro, incluindo a limpeza de derramamento de óleos, a remoção de petroderivados de tanques de estocagem, a recuperação melhorada de petróleo e a biorremediação de ambientes terrestres e aquáticos contaminados com hidrocarbonetos (RON e ROSENBERG, 2002; SILVA et al., 2014a).

3.4 Utilização de substratos alternativos na produção de prodigiosina e biossurfactantes por micro-organismos

Apesar das propriedades apresentadas, a prodigiosina e biossurfactantes de origem microbiana não são amplamente utilizados pelas indústrias devido ao alto custo de produção, associado à baixa produtividade e ao uso de substratos caros (PANESAR et al., 2015; BRUMANO et al. 2016; DORIYA et al., 2016). Uma possível estratégia para reduzir os custos da produção seria o uso de substratos alternativos, como os resíduos agroindustriais ou da indústria alimentícia, que geralmente contêm altos níveis de carboidratos ou lipídeos necessários para a biossíntese destas biomoléculas (KAUR et al., 2014; RAVINDRAN e JAISWAL, 2016).

Várias pesquisas visam à produção de pigmentos microbianos a partir de substratos agro-industriais, o que torna o processo mais favorável em termos financeiro e ambiental (PANESAR et al., 2015; HAQUE et al., 2016). Porém, dentre delas, ainda são poucas as que se referem à produção de prodigiosina. Substratos alternativos como açúcar mascavo e sementes de sésamo trituradas têm sido utilizados para produzir prodigiosina por *S. marcescens* através de fermentação submersa, obtendo valores de rendimento do pigmento de 8,0 mg/mL e 16,68 mg/mL, respectivamente (GIRI et al., 2004; ARULDASS et al., 2014). Araújo et al. (2010) informaram a produção de prodigiosina (49,50 mg/mL) em meio constituído por 6% manipueira, suplementado com 2% manitol. Naik et al. (2012) relataram o uso de diferentes tortas de sementes, resíduos gerados no processo de produção de óleos vegetais, e obtiveram uma produção máxima de prodigiosina (39,80 mg/mL) no meio contendo torta de óleo de amendoim.

Nos últimos anos aumentou o interesse em produzir prodigiosina através de FES, pelas vantagens econômicas e operacionais que este processo tem quando comparado com fermentação submersa (XIA et al., 2016; LIZARDI-JIMÉNEZ e HERNÁNDEZ-MARTÍNEZ, 2017). Arivizhivendhan et al. (2015) obtiveram 70,40 g de prodigiosina/ kg de farelo de trigo suplementado com resíduo sólido proteico gerados pela indústria do couro em biorreator durante 96 h. Previamente, Xu et al. (2011a) tinham informado a produção 4,16 g de prodigiosina /kg de lixo de cozinha e casca de arroz, após 60 h de FES. Mais recentemente, o bagaço tem sido utilizado como matriz inerte na obtenção de prodigiosina utilizando glicerol e peptona de soja, com rendimento 40,86 g/kg de sólido seco (XIA et al., 2016). Estes resultados são promissores mas ainda insuficientes, sendo então necessário a realização de mais estudos que objetivem a produção de prodigiosina por FES.

Por outro lado, vários substratos abundantes e relativamente baratos, como subprodutos da produção de óleo vegetal, das indústrias de laticínios, carnes e açúcar, têm sido explorados para a produção de biossurfactantes (BRUMANO et al., 2016; SANTOS et al., 2016). Existem poucos trabalhos visando à produção de biossurfactantes por *S. marcescens* (ANYANWU et al., 2011; ROLDAN-CARRILLO et al., 2011; IBRAHIM et al., 2013), pertencendo a nosso grupo de pesquisa os que se referem ao aproveitamento de resíduos agro-industriais tais como manipueira e óleo de milho pós-fritura (ALVES et al., 2014; MONTERO-RODRÍGUEZ et al., 2014; 2015; ARAÚJO et al., 2017). Assim, existe uma necessidade de continuar pesquisando a habilidade de *S. marcescens* para produzir biossurfactante utilizando outros subprodutos e resíduos agroindústrias, principalmente através de FES, pois apenas Nalini e Parthasarathi (2014) reportaram o emprego deste processo para a obtenção de biossurfactante por *Serratia rubidaea*.

O alto custo da produção de biopigmentos e biossurfactantes deve-se principalmente aos custos associados aos meios de cultura, o qual é estimado em aproximadamente 80% do custo total do processo de produção (BANAT et al., 2014; CARVALHO et al., 2014; DORIYA et al., 2016). Assim, consideráveis esforços têm sido direcionados para minimizar os custos da fonte de carbono e encontrar novas fontes alternativas. Em particular, a exploração de resíduos agroindustriais e subprodutos como matérias-primas podem reduzir muito o custo, além de possibilitar um novo destino mais sustentável a esses produtos de descarte (PANESAR et al., 2015; BRUMANO et al., 2016; SANAWER et al., 2017).

REFERÊNCIAS

ABBASI, H.; HAMEDI, M. M.; LOTFABAD, T. B.; ZAHIRI, H. S.; SHARAFI, H.; MASOOMI, F.; MOOSAVI-MOVAHEDI, A. A.; ORTIZ, A.; AMANLOU, M.; NOGHABI, K. A. Biosurfactant-producing bacterium, *Pseudomonas aeruginosa* MA01 isolated from spoiled apples: Physicochemical and structural characteristics of isolated biosurfactant. **Journal of Bioscience and Bioengineering**, v. 113, p. 211 – 219, 2012.

AHMAD, Wan Azlina et al. **Applications of bacterial pigments as colorant: the Malaysian perspective.** New York: Springer Berlin Heidelberg, 84 p. 2012.

ALVES, T. S. et al. Production and Evaluation of Biosurfactant by *Serratia marcescens* UCP 1549 Using Industrial Wastes. **Br. Biotechnol. J.,** v. 4 Lingappa K, n. 6, p.708-719, 2014.

ANANDARA, J. B.; THIVAKARAN, P. Isolation and Production of Biosurfactant Producing Organism from Oil spilled Soil. **Journal Bioscience Technology**. Vol 1 (3), 120-12. 2010.

ANDERSON, M. T., MITCHELL, L. A., & MOBLEY, H. L. Cysteine biosynthesis controls *Serratia marcescens* phospholipase activity. **Journal of Bacteriology**, JB-00159. 2017.

ANYANWU, C. U., OBI S.K.C., AND OKOLO B. N. Lipopeptide biosurfactant production by *Serratia marcescens* NSK-1 Strain isolated from petroleum contaminated soil. **J. Appl. Sci. Res**.7(1):79-87. 2011.

APPRICH, S.; TIRPANALAN, Ö.; HELL, J.; REISINGER, M.; BÖHMDORFER, S.; SIEBENHANDL-EHN, S.; KNEIFEL, W. Wheat bran-based biorefinery 2: Valorization of products. **LWT-Food Science and Technology**. v. 56, n. 2, p. 222-231, 2014.

ARAUJO, H. W. C., ANDRADE, R. F., MONTERO-RODRÍGUEZ, D., SANTOS, V. P., MAIA, P. C. S., COSTA FILHO, C. F., ... & CAMPOS-TAKAKI, G. M. Biochemical and molecular identification of newly isolated pigmented bacterium and improved production of biosurfactant. **African Journal of Microbiology Research**, 11 (22), 945-954. 2010.

ARAÚJO, H. W. C. Caracterização Morfológica e Molecular de uma Nova Linhagem de *Serratia marcescens* e Potencial Biotecnológico na Produção de Biossurfactantes Prodigiosina e na Utilização de Dibenzotiofeno (DBT). Tese de Doutorado do Programa de Pós-graduação em Biotecnologia, p. 150, 2010.

ARAÚJO, H. W. C., FUKUSHIMA, K., & TAKAKI, G. M. C. Prodigiosin production by *Serratia marcescens* UCP 1549 using renewable-resources as a low cost substrate. **Molecules**, 15(10), 6931-6940. 2010.

ARIF HM, HUSSAIN Z. Important sources and medicinal applications of L-asparaginase. **Int J Pharm Sci** Rev;3:35-45. 2014.

ARIVIZHIVENDHAN K.V., MAHESH M., REGINA MARY R., SEKARAN G. Bioactive prodigiosin isolated from Serratia marcescens using solid state fermenter and its bactericidal activity compared with conventional antibiotics, **J Microb Biochem Technol**, 7, 305-312. 2015.

ARULDASS, Claira Arul et al. Brown sugar as a low-cost medium for the production of prodigiosin by locally isolated *Serratia marcescens* UTM1. **Int. Biodeterioration & Biodegrad.**, p. 1-6, 2014.

ASSIS, P., ROSA, J., OIANO-NETO, J., FURTADO, A., PACHECO, S., & GODOY, R. (2010). Composição de carotenóides das cascas e ácidos graxos da fração lipídica das sementes de tangerina murcote (Citrus reticulata Blanco). In *Embrapa Agroindústria de Alimentos-Resumo em anais de congresso (ALICE)*. In: ENCONTRO REGIONAL DA SOCIEDADE BRASILEIRA DE QUÍMICA, 24. Viçosa, MG. Integração e desafios da Química em Minas Gerais. Viçosa, MG: UFV: SBQ, 2010. 1 CD-ROM. 2010.

BAKKIYARAJ, D., SIVASANKAR, C., & PANDIAN, S. K. Inhibition of quorum sensing regulated biofilm formation in *Serratia marcescens* causing nosocomial infections. **Bioorganic & medicinal chemistry letters**, *22*(9), 3089-3094. 2012.

BANAT, I. M., SATPUTE, S. K., CAMEOTRA, S. S., PATIL, R., & NYAYANIT, N. V.. Cost effective technologies and renewable substrates for biosurfactants' production. **Frontiers in Microbiology**, 5. 2014.

BANAT, I.M.; FRANZETTI, A.; GANDOLFI, I.; BESTETTI, G.; MARTINOTTI, M.G.; FRACCHIA, L.; SMYTH, T.J.; MARCHANT, R. Microbial biosurfactants production, applications and future potential. **Appl. Microbiol. Biotechnol.**, v. 87, p. 427–444, 2010.

BARROS, F. F. C.; PONEZI, A. N.; PASTORE, G. M. Production of biosurfactant by Bacillus subtilis LB5a on a pilot scale using cassava wastewater as substrate. **Journal of Industrial Microbiology and Biotechnology,** v. 35, n. 9, 1071–1078, 2008.

BENNETT, J.; BENTLEY, Ronald. Seeing red: The story of prodigiosin. **Adv, Appl. Microbiol.,** v. 47, p.1-32, 2000.

BERGER, L. R. R. et al. Effect of Corn Steep Liquor (CSL) and Cassava Wastewater (CW) on Chitin and Chitosan Production by *Cunninghamella elegans* and Their Physicochemical Characteristics and Cytotoxicity. **Molecules,** v. 19, p.2771-2792, 2014.

BERNAUD, F. S. R; RODRIGUES, T. C. Fibra alimentar: ingestão adequada e efeitos sobre a saúde do metabolismo. (2013). Arquivo brasileiro de endocrinologia e metabolismo, 57(6), 397-405. Disponível em: http://www.scielo.br/pdf/abem/v57n6/01.pdf. Acesso em: 10/06/2016.

BORIĆ, M., DANEVČIČ, T., & STOPAR, D. Prodigiosin from *Vibrio* sp. DSM 14379; a new UV-protective pigment**. Microbial ecology**, 62(3), 528. 2011.

BRAR, S. K., DHILLON, G. S., & SOCCOL, C. R. (Eds.). Biotransformation of waste biomass into high value biochemicals. Springer Science & Business Media. 2013.

BRUMANO, L. P., SOLER, M. F., & DA SILVA, S. S. Recent advances in sustainable production and application of biosurfactants in Brazil and Latin America. **Industrial Biotechnology**, 12(1), 31-39. 2016.

BURGOS-DÍAZ, C; MARTÍN-VENEGAS, R; MARTÍNEZ, V; STORNIOLO, CE; TERUEL, JA; ARANDA, FJ; ORTIZ, A; MANRESA, Á; FERRER, R; MARQUÉS, AM. In vitro study of the cytotoxicity and antiproliferative effects of surfactants produced by *Sphingobacterium detergens*. **Int. J. Pharm.**, v. 453, p. 433–440, 2013.

CANILHA, L.; SANTOS, V.; ROCHA, G. J. M.; SILVA, J. B. M.; GIULIETTI, M. A study on the pretreatment of a sugarcane bagasse sample with dilute sulfuric acid. **J Ind Microbiol Biotechnol**. v. 38, p. 1467–1475, 2011.

CANTALICE, J. C. L. L. Avaliação da atividade antimicrobriana, citotóxica e efeitos genotóxicos da prodigiosina produzida por *Serratia marcescens* UFPEDA 398. 2014.

CARBONELL G.V., DELLA COLLETA H.H.M., YANO T., DARINI A.L.C., LEVY C.E., FONSECA B.A.L. Clinical relevance and virulence factors of pigmented *Serratia marcescens*. **FEMS Immunol. Med. Microbiol**.;28:143–149. 2000.

CAROLEI, L.; GUTZ, I.G.R. Simultaneous determination of three surfactants and water in shampoo and liquid soap by ATR-FTIR. Atlanta: [s.n.]. 2005.

CARVALHO, J C. de et al. Microbial Pigments. In: Brar, S K, Dhillon, G S, Soccol, C R (eds.) "Biotransformation of Waste Biomass into High Value Biochemicals " Springer Science, New York, p 73-97. DOI 10.1007/978-1-4614-8005-1_4. 2014.

CASTRO, A. J. Antimalarial activity of prodigiosin. Nature, 213(5079), 903-904. 1967.

CHANDEL, A. K.; DA SILVA, S. S.; CARVALHO, W.; SINGH, O. V. Sugarcane bagasse and leaves: foreseeable biomass of biofuel and bio-products. **Journal of chemical technology and biotechnology**. v. 87, n 1, p. 11-20, 2012.

CHANDEL, A. K.; SINGH, O. V, WEEDY. Lignocellulosic feedstock and microbial metabolic engineering: advancing the generation of 'biofuel'. **Appl Microb Biotechnol**. v. 89, p. 1289–1303, 2011.

CHEN, W.C.; JUANG, R.S.; WEI, Y.H. Application of a lipopeptide biosurfactant, surfactin, produced by microorganisms. **Biocemical Engineering Journal**, v. 103, p. 158-169, 2015.

CODA, R.; KATINA, K.; RIZZELLO, C.G. Bran bioprocessing for enhanced functional properties. **Curr. Opin. Food Sci**. v. 1, p. 50–55. 2015.

COLIN, V. L., CORTES, Á. J., RODRÍGUEZ, A., & AMOROSO, M. J. Surface-Active Compounds of Microbial Origin and Their Potential Application in Technologies of Environmental Remediation. In Bioremediation in Latin America (pp. 255-265). Springer International Publishing. 2014.

COSTA, S. G. V. A. O. Estudo da produção de metabólitos por *Pseudomonas aeruginosa*: ramnolipídios e polihidroxialcanoatos (PHAs). Teses de Doutorado. Rio Claro. 136 p. 2010.

CUNHA, G.A.P. Abacaxi on-line: informativo mensal da equipe Técnica de Abacaxi – ETA. Embrapa, CNPMF, v.5, n.1, 2007.

DAVEREY, A.; PAKSHIRAJAN, K. Sophorolipis from *Candida bombicola* using mixed hydrophilic substrates: Production, purification and characterization. **Colloids and Surfaces B: Biointerfaces,** v. 79, p. 246-253, 2010.

DAVISHI, P.; AYATOLLAHI, S.; MOWLA, D.; NIAZI, A. Biosurfactant production under extreme environmental conditions by an efficient microbial consortium, ERCPPI-2. **Colloids Surfaces and Biointerfaces.**, 2011.

DE ALMEIDA, F. C. G., E SILVA, T. A. D. L., GARRARD, I., ASFORA, L., SARUBBO, G. M. D. C. T., & TAMBOURGI, E. B. Optimization and evaluation of biosurfactant produced by *Pantoea* sp. using pineapple peel residue, vegetable fat and corn steep liquor. **J. Chem**, 9, 269-279. 2015.

DEMIR, H., TARI, C. Valorization of wheat bran for the production of polygalacturonase in SSF of *Aspergillus sojae*. **Industrial Crops and Products**, 54, 302-309. 2014.

DEORUKHKAR, A.A., CHANDER, R., GHOSH, S.B. & SAINIS, K.B. Identification of a red-pigmented bacterium producing a potent anti-tumor N-alkylated prodigiosin as *Serratia marcescens*. **Res Microbiol.** 158, p.399-404. 2007.

DO NASCIMENTO FILHO, W. B., & FRANCO, C. R. Avaliação do Potencial dos Resíduos Produzidos Através do Processamento Agroindustrial no Brasil. **Revista Virtual de Química**, 7(6), 1968-1987. 2015.

ELKENAWY, N. M., YASSIN, A. S., ELHIFNAWY, H. N., & AMIN, M. A. Optimization of prodigiosin production by *Serratia marcescens* using crude glycerol and enhancing production using gamma radiation**. Biotechnology Reports**, 14, 47-53. 2017.

ELLIOTT, D. C.; ORTH, R. J.; GAO, J.; WERPY, T. A.; EAKIN, D. E.; SCHMIDT, A. J. Biorefinery concept development based on wheat flour milling. In J. Crockett, & C. L. Peterson (Eds.), Bioenergy 2002. Pacific Regional Biomass Energy Program, Boise. 2002.

ELMEKAWY, A., DIELS, L., DE WEVER, H., PANT, D., Valorization of cereal based biorefinery byproducts: reality and expectations. **Environ. Sci. Technol**. v. 47, p. 9014–9027. 2013.

FERREIRA-LEITÃO, V. S., CAMMAROTA, M. C., GONÇALVES AGUIEIRAS, E. C., VASCONCELOS DE SÁ, L. R., FERNANDEZ-LAFUENTE, R., & FREIRE, D. M. G. The Protagonism of Biocatalysis in Green Chemistry and Its Environmental Benefits. **Catalysts**, 7(1), 9. 2017.

FERREIRA-LEITAO, V., GOTTSCHALK, L. M. F., FERRARA, M. A., NEPOMUCENO, A. L., MOLINARI, H. B. C., & BON, E. P. Biomass residues in Brazil: availability and potential uses. **Waste and Biomass Valorization**, 1(1), 65-76. 2010.

Food and Agriculture Organization. (2015). OCDE – Agricultura brasileira: perspectivas e desafios. Disponível em: http://apps.fao.org.br. Acesso em: 01 set. 2017.

FRIEDMAN, M. Chemistry biochemistry and safety of acrylamide. **J. Agric. Food Chem**., v. 51, p. 4504–4526, 2003.

GIRI, A. et al. A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia marcescens* isolated from soil. **BMC Microbiol.** v. 4, p.11-18, 2004.

GOKMEN, V. e PALAZOGLU, K. Acrylamide formation in foods during thermal processing with a focus on frying. **Food Bioprocess Tech**., v. 1, n. 168, p. 35–42, 2008.

GONDIM, J. A. M., MOURA, M. D. F., DANTAS, A. S., MEDEIROS, R. L. S., & SANTOS, K. M. Composição centesimal e de minerais em cascas de frutas. **Ciência e Tecnologia de Alimentos**, 25(4), 825-827. 2005.

GRANADA, G. G.; ZAMBIAZI, R.C.; MENDONÇA, C. R. B. Abacaxi: Produção, Mercado e Subprodutos. B.CEPPA, Curitiba, v. 22, n.2, p.405-422, jul/dez. 2004.

GUDIÑA, EJ; RANGARAJAN, V; SEN R, RODRIGUES LR. Potential therapeutic applications of biosurfactants. **Trends Pharmacol. Sci.**, v. 34, n. 12, p. 667-675, 2013.

HAMRE, A. G., JANA, S., HOLEN, M. M., MATHIESEN, G., VÄLJAMÄE, P., PAYNE, C. M., & SØRLIE, M. Thermodynamic relationships with processivity in *Serratia marcescens* family 18 chitinases**. The Journal of Physical Chemistry** B, 119(30), 9601-9613. 2015.

HAQUE, M. A., KACHRIMANIDOU, V., KOUTINAS, A., & LIN, C. S. K. Valorization of bakery waste for biocolorant and enzyme production by *Monascus purpureus*. **Journal of biotechnology**, 231, 55-64. 2016.

IBRAHIM, M.L., U.J.J. IJAH, S.B. MANGA, L.S. BILBIS and UMAR, S. 2013. Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. **International Biodeterioration & Biodegradation** 81, 28-34.2013.

IGUCHI, A., NAGAYA, Y., PRADEL, E., OOKA, T., OGURA, Y., KATSURA, K., ... & HAYASHI, T. (2014). Genome evolution and plasticity of *Serratia marcescens*, an important multidrug-resistant nosocomial pathogen. **Genome biology and evolution**, *6*(8), 2096-2110. 2014.

IPLIKCIOGLU CIL G and SIRELI UT. “Thermal Processing Contaminants in Foods”. **EC Pharmacology and Toxicology** 3.3 .86-89. 2017.

JANEK, T; KRASOWSKA, A; RADWAńSKA, A; ŁUKASZEWICZ, M. Lipopeptide biosurfactant pseudofactin II induced apoptosis of melanoma A375 cells by specific interaction with the plasma membrane. **PLoS ONE**, v. 8, n. 3, p. 1-9, 2013.

KALIVODA, E.J., STELLA, N.A., ASTON, M.A., FENDER, J.E., THOMPSON, P.P., KOWALSKI, R.P., SHANKS, R.M. Cyclic AMP negatively regulates prodigiosin production by *Serratia marcescens*. **Res Microbiol**. 161(2):158-67, 2010.

KAUR, S., DHILLON, G. S., SARMA, S. J., BRAR, S. K., MISRA, K., & OBEROI, H. S. Waste biomass: a prospective renewable resource for development of bio-based economy/processes. In Biotransformation of Waste Biomass into High Value Biochemicals (pp. 3-28). Springer New York. 2014.

KHANAFARI A.; ASSADI M. M.; FAKHR F. A. Review of prodigiosin, pigmentation in *Serratia marcescens.* **J Biol Sci.** v. 6, n. 1, p. 1-13, 2006.

KHEDKAR, M. A., NIMBALKAR, P. R., GAIKWAD, S. G., CHAVAN, P. V., & BANKAR, S. B. Sustainable biobutanol production from pineapple waste by using *Clostridium acetobutylicum* B 527: Drying kinetics study. **Bioresource technology**, 225, 359-366. 2017.

KHOPADE, A.; REN, B.; LIU, X.Y.; MAHADIK, K.; ZHANG, L.; KOKARE, C. Production and characterization of biosurfactant from marine *Streptomyces* species B3. **Journal of Colloids and Interface Scinces**, v. 367, p. 311-318, 2012.

KIM, Y; KIM, K.; SEO, J.; SHRESTHA, S.; KIM, H. H., NALINI, M.; YI, Y. Identification of na entomopathogenic bacterium, *Serratia* sp. ANU101, and its hemolytic activity. **Journal of Microbiology and Biotechnology,** v. 19, n. 3, p. 314-322, 2009.

KIM, H. et al. Production and properties of a lipopeptide biosurfactant from *Bacillus subtilis* C9. **Journal of Fermentation Bioengineering,** v. 84, n. 1, p. 41-46, 1997.

KONEMAN, E. W.; ALLEN, S. D; JANDA, W. M.; SCHRECKENBERGER, P. C.; Winn jr W.C. **Diagnóstico microbiológico** 5ª edição, São Paulo. 2001.

LAPENDA, J. C., SILVA, P. A., VICALVI, M. C., SENA, K. X. F. R., & NASCIMENTO, S. C. Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398. **World Journal of Microbiology and Biotechnology**, 31(2), 399-406. 2015.

LAPENDA, JC et al. Production and toxicological evaluation of Prodigiosin from *Serratia marcescens* UCP/WFCC1549 on Mannitol Solid Medium. **Int J Appl Res Nat Prod** v. 7, n. 2, p. 32-38, 2014.

LEE, J. E.; VADLANI, P. V.; FAUBION, J. Corn bran bioprocessing: Development of an integrated process for microbial lipids production. **Bioresource Technology**,v. 243, p. 196-203, 2017.

LEI, W., XUE-SU, S., CHEN-MEI, F., & BI-NING, J. . Research Progress on Bio-logical Activities and Applications of Polymethoxyflavonoids from Citrus. **Food Science,** *30* (7), 285-290. 2009.

LIMA RA, ANDRADE RFS, RODRIGUEZ DM, ARAÚJO HWC, SANTOS VP, CAMPOS-TAKAKI GM. Production and characterization of biosurfactant isolated from *Candida glabrata* using renewable substrates. **Afr. J. Microbiol. Res**. 2017; 11: 237-244. doi: 10.5897/AJMR2016.834

LINGNERT, H.; GRIVAS, S.; JAGERSTAD, M.; SKOG, K.; TORNQVIST, M.; AMAN, P. Acrylamide in food— mechanisms of formation and influencing factors during heating of foods. **Scand J Food Nutr**., v. 46: 159–172, 2002.

LIZARDI-JIMÉNEZ M.A., HERNÁNDEZ-MARTÍNEZ R.. Solid state fermentation (SSF): diversity of applications to valorize waste and biomass, 3 Biotech, 7, 44. 2017.

LOURITH. N.; KANLAYAVATTANAKUL, M. Natural surfactants used in cosmetics: glycolipids. **International Journal of Cosmetic Science**, v. 31, p. 255-261, 2009.

MAKKAR, R.; CAMEOTRA, S. An update on the use of unconventional substrates for biosurfactant production and their new applications. **Applied Microbiology and Biotechnology**, v.58, n. 4, p. 428-434, 2002.

MAKKAR, R.S.; CAMEOTRA, S.S.; BANAT, I.M. Advances in utilization of renewable substrates for biosurfactant production. **Appl. Microbiol. Biotechnol.** v. 1, p. 1–5, 2011.

MATOS, A.T. Tratamento de resíduos agroindustriais. In: CURSO SOBRE TRATAMENTO DE RESÍDUOS AGROINDUSTRIAIS, 2005, Viçosa, MG. Anais... Viçosa, MG: Fundação Estadual do Meio Ambiente. p.1-34. 2005.

MATSUYAMA, T., TANIKAWA, T., & NAKAGAWA, Y. Serrawettins and other surfactants produced by *Serratia*. In *Biosurfactants* (pp. 93-120). Springer Berlin Heidelberg. 2011.

MAX, Y.F.S.; ANDRADE, R.F.S.; SILVA, A.M.; RIBEIRO, D.L.R.; JARA, A. M. A. T.; ALENCAR, A.A.; CAMPOS-TAKAKI, G.M.; GUSMÃO, N.B. Biosurfactant production by *Rhodotorula glutinis***:** Emulsifying property and stability Microbes In **Applied Research: Current Advances and Challenges**. [S.l.]: [s.n.] 358-361 p. 2012.

MEHTA, M., & SHAH, G. Extraction of pigment from *Serratia marcescens* and its application in candle industry. **Advances in Applied Research**, 7(2), 144-146. 2015.

MITCHELL, D. A.; KRIEGER, N.; STUART, D. M.; PANDEY, A. New developments in solid state fermentation. II. Rational approaches tothe design, operation and scale-up of bireactors. **Process Biochem**, v. 35, p. 1211-1225, 2000.

MNIF, I.; ELLEUCH, M.; CHAABOUNI, S. E.; GHRIBI, D. *Bacillus subtilis* SPB1 biosurfactant: Production optimization and insecticidal activity against the carobmoth Ectomyelois ceratoniae. **Crop Protection**, v. 50, p. 66-72, 2013.

MONTANER B., et al. Prodigiosin from the supernatant of *Serratia marcescens* apoptosis in haematopoietic cancer cell lines. **Br J Pharmacol** 131, p. 585-393, 2000.

MONTERO-RODRÍGUEZ, D., ANDRADE, R. F., RIBEIRO, D. L. R., RUBIO-RIBEAUX, D., LIMA, R. A., ARAÚJO, H. W., & CAMPOS-TAKAKI, G. M. Bioremediation of petroleum derivative using biosurfactant produced by *Serratia marcescens* UCP/WFCC 1549 in low-cost medium. **Int. J. Curr. Microbiol. App. Sci**, 4(7), 550-562. 2015.

MONTERO-RODRÍGUEZ, D., ANDRADE, R. F. S., RIBEIRO, D. L. R., LIMA, R. A., ARAUJO, H. W. C., & CAMPOS-TAKAKI, G. M. Ability of *Serratia marcescens* UCP/WFCC 1549 for biosurfactant production using industrial wastes and fuels biodegradation In: Industrial, medical and environmental applications of microorganisms: Current status and trends. Madrid, 211-216. 2014.

NAIK, C.et al. Peanut oil cake: a novel substrate for enhanced cell growth and prodigiosin production from *Serratia marcescens* CF-53. **J. Res. Biol.** v. 2, n. 6, p. 549-557, 2012.

NAKASHIMA, T. et al. Characterization of bacterium isolated from the sediment at Coastal area of Omura Bay in Japan and several biological activities of pigment produced by this isolate. **Microbiol Immunol** 49, p. 407-415, 2005.

NALINI S., PARTHASARATHI R., Production and characterization of rhamnolipids produced by *Serratia rubidaea* SNAU02 under solid-state fermentation and its application as biocontrol agent, **Bioresour Technol**, 173, 231-238. 2014.

NEVES, M. A.; KIMURA, T.; SHIMIZU, N.; SHIIBA, K. Production of alcohol by simultaneous saccharification and fermentation of low-grade wheat flour. **Brazilian Archives of Biology and Technology**. v. 49, n. 3, p. 481-490, 2015.

NIPORNRAM, S., TOCHAMPA, W., RATTANATRAIWONG, P., & SINGANUSONG, R. . Optimization of low power ultrasound-assisted extraction of phenolic compounds from mandarin (Citrus Reticulata Blanco cv. Sainampueng) peel. Food Chemistry. 2017.

NITSCHKE, M.; PASTORE, G.M. Biosurfactantes: propriedades e aplicações. **Quimica Nova**, v. 25, p.772-776, 2002.

ODENIYI, O. A.; DEOLA, O. J. Production and characterization of polyhydroxyalkanoic acid from *Bacillus thuringiensis* using different carbon substrates. **International Journal of Biological Macromolecules**. 2017.

OLIVEIRA, E. C. P.; LAMEIRA, O. A.; SOUSA, F. I. B.; SILVA, R. J. F. Estrutura foliar de curauá em diferentes intensidades de radiação fotossinteticamente ativa. **Pesq. Agropec. Bras**., Brasília, v.43, n.2, p.163-169, 2008.

OU, J.; XU, N.; ERNST, P.; MA, C.; BUSH, M.; GOH, K.; LIU, X. M. Process engineering of cellulosic n-butanol production from corn-based biomass using *Clostridium cellulovorans.* Process Biochemistry. 2017.

PANDEY, A. Solid-state fermentation. **Biochemical Engineering Journal**, 13(2), 81-84. 2003.

PANDEY A, SOCCOL CR, NIGAM P AND SOCCOL VT, Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. **Bioresource Technol**. v. 74, p. 69–80, 2000.

PANESAR R, KAUR S, PANESAR PS. Production of microbial pigments utilizing agro-industrial waste: a review. **Current Opinion in Food Science**. 1: 70-76. 2015.

PEREZ-THOMAS, R. et al. The prodigiosins, proapoptotic drugs with anticancer properties. **Biochem Pharmacol.** 66, p. 1447-1452, 2003.

PEREIRA , D. D. F. ; DUVOISIN, S. JR .; ALBUQUERQUE, P.M. O estudo da produção de biossurfactantes por fungos amazônicos. **The Journal of Engineering and Exact Sciences** , v.3, n 4, p. 0688-0695, 2017.

PIRÔLLO, M.P.S. **Estudo da Produção de Biossurfactantes utilizando Hidrocarbonetos**. Rio Claro: Universidade Estadual Paulista (UNESP), 61 p. 2006.

PORTUGAL-PEREIRA, J., SORIA, R., RATHMANN, R., SCHAEFFER, R., & SZKLO, A. Agricultural and agro-industrial residues-to-energy: Techno-economic and environmental assessment in Brazil. **Biomass and Bioenergy**, 81, 521-533. 2015.

RAVINDRAN, R., & JAISWAL, A. K. Exploitation of food industry waste for high-value products. **Trends in biotechnology**, 34(1), 58-69. 2016.

REN, Y., GONG, J., FU, R., LI, Z., LI, Q., ZHANG, J. & CHENG, X. Dyeing and antibacterial properties of cotton dyed with prodigiosins nanomicelles produced by microbial fermentation**. Dyes and Pigments**, 138, 147-153. 2017.

RIVARDO, F.; TURNER, R. J.; ALLEGRONE, G.; CERI, H. MARTINOTTI, M. G. Anti-adhesion activity of two biosurfactants produced by *Bacillus* spp. prevents biofilm formation of human bacterial pathogens. **Applied Microbiology and Biotechnology**, v.3, p.541-553, 2009.

ROCHA e SILVA, N.M.P., et al. Screening of *Pseudomonas* species for biosurfactant production using low-cost substrates **Biocatal. Agric. Biotechnol.** v. 3, p. 132–139, 2014.

RODA, A., DE FAVERI, D. M., DORDONI, R., & LAMBRI, M. Vinegar production from pineapple wastes—preliminary saccharification trials. **Chem. Eng**. Trans, 37. 2014.

RODRÍGUEZ, D. M. Potencial biotecnológico de *Serratia marcescens* UCP/WFCC 1549 na degradação de combustíveis, na produção de lipídeos e de biossurfactante. Diseertação de Mestrado, Universidade Federal de Pernambuco. 2015.

ROLDAN-CARRILLO T, MARTINEZ-GARCIA X, ZAPTA-PENASO I., CASTRORENA-CORTES, G, REYES-AVILA, J., MAYOL-CASTINO M and OLGUIN-LORA P. Evaluation of the effect of nutrient ratios on biosurfactant production by *Serratia marcescens* using a boxbehuken design. Colloids and Surfaces B; **Biointerfaces**. 86(2)284-389. 2011.

RON, E. Z.; ROSENBERG, E. Biosurfactants and oil bioremediation. **Current Opinion in Biotechnology**, v. 13, p. 249-252, 2002.

ROSE, D. J.; INGLETT, G. E.; LIU, S. X. Utilisation of corn (Zea mays) bran and corn fiber in the production of food components. **Journal of the Science of Food and Agriculture**, v. 90, n. 6, p. 915-924, 2010.

SAHARAN, B.S.; SAHU, R.K. SHARMA, D. A Review on Biosurfactants: Fermentation, Current Developments and Perspectives. **Genetic Engineering and Biotechnology Journal**, GEBJ-29, 2011

SANTOS, D. K. F., RUFINO, R. D., LUNA, J. M., SANTOS, V. A., & SARUBBO, L. A. Biosurfactants: multifunctional biomolecules of the 21st century. **International journal of molecular sciences**, 17(3), 401. 2016.

SHAVANDIA, M.; MOHEBALIA, G.; HADDADIB A.; SHAKARAMIA, H.; NUHI, A. Emulsification potential of a newly isolated biosurfactant-producing bacterium, *Rhodococcus* sp. TA6 strain .**Colloids and Surfaces B: Biointerfaces**, v. 82, p.477–482, 2011.

SHAVANDIA, M.; MOHEBALIA, G.; HADDADIB, A.; SHAKARAMIA, H.; JAIN, R. M.; MODY, K.; MISHRA, A.; JHA, B. Isolation and structural characterization of biosurfactant produced by an alkaliphilic bacterium *Cronobacter sakazakii* isolated from oil contaminated wastewater **Carbohydrate Polymers** .87, 2320– 2326. 2012.

SILVA, E.J. et al. Characterization of a biosurfactant produced by *Pseudomonas cepacia* CCT6659 in the presence of industrial wastes and its application in the biodegradation of hydrophobic compounds in soil. **Colloids Surf. B Biointerfaces** 117, p. 36–41, 2014a.

SILVA, R. C. F. S. Resíduos industriais como substratos alternativos para a produção de biossurfactantes para aplicação na remoção de poluentes ambientais gerados pela indústria de petróleo. Recife. Dissertação de Mestrado, 149 p. 2012.

SILVA, T.A.L. et al. Potencial biotecnológico de uma nova linhagem de *Pseudomonas fluorescens* na produção de biossurfactante utilizando petróleo como substrato. **Exacta.** v.7, n.1, p. 31-37, 2009.

SINGH, P. B., & SAINI, H. S. Exploitation of Agro-Industrial Wastes to Produce Low-Cost Microbial Surfactants. In Biotransformation of Waste Biomass into High Value Biochemicals (pp. 445-471). Springer New York. 2014.

SINGHANIA, R. R., SUKUMARAN, R. K., PATEL, A. K., LARROCHE, C., & PANDEY, A. Advancement and comparative profiles in the production technologies using solid-state and submerged fermentation for microbial cellulases. **Enzyme and Microbial Technology**, 46(7), 541-549. 2010.

SOCCOL, C. R., DA COSTA, E. S. F., LETTI, L. A. J., KARP, S. G., WOICIECHOWSKI, A. L., & DE SOUZA VANDENBERGHE, L. P. Recent developments and innovations in solid state fermentation. **Biotechnology Research and Innovation**. 2017

SOMERVILLE, C.; YOUNGS, H.; TAYLOR, C.; DAVIS, S.C.; LONG, S.P.; Feedstocks for lignocellulosic biofuels. **Science**. v. 329, p. 790–792, 2010.

SONG M.J. et al. Purification and characterization of Prodigiosin produced by integrated bioreactor from *Serratia* sp. KH-95. **J Biosci Bioeng** v.101, p. 157-161, 2006.

SOTO-CERRATO, V.; VIÑALS, F.; LAMBERT, J. R.; PÉREZ-TOMÁS, R. The anticancer agent prodigiosin induces p21 WAF1/CIP1 expression via transforming growth factor-beta receptor pathway. **Biochemical Pharmacology**, v. 74, n. 9, p. 1340-1349, 2007.

SPONZA, D. T.; GOK, O. Effects of sludge retention time (SRT) and biosurfactant on the removal of polyaromatic compounds and toxicity **Journal of Hazardous Materials**, v. 197, p. 404– 416. 2011.

SUMATHI, C. et al. Production of Prodigiosin Using Tannery Fleshing and Evaluating Its Pharmacological Effects. **Sci. World J. v** 2014.

SURYAWANSHI, P. K. et al. Studies on Production and Biological Potential of Prodigiosin by *Serratia marcescens*. **Appl Biochem Biotechnol** v.173, p. 1209–1221, 2014.

SURYAWANSHI, R. K., PATIL, C. D., BORASE, H. P., NARKHEDE, C. P., SALUNKE, B. K., & PATIL, S. V. Mosquito larvicidal and pupaecidal potential of prodigiosin from

Serratia marcescens and understanding its mechanism of action. **Pesticide biochemistry and physiology**, 123, 49-55. 2015.

SURYAWANSHI, R. K., PATIL, C. D., BORASE, H. P., NARKHEDE, C. P., STEVENSON, A., HALLSWORTH, J. E., & PATIL, S. V. Towards an understanding of bacterial metabolites prodigiosin and violacein and their potential for use in commercial sunscreens. **International journal of cosmetic science**, 37(1), 98-107. 2015.

THAKUR, S., SHARMA, N. K., THAKUR, N., & BHALLA, T. C. Organic solvent tolerant metallo protease of novel isolate *Serratia marcescens* PPB-26: production and characterization. **3 Biotech**, 6(2), 180. 2016.

THOMAS, L., LARROCHE, C., & PANDEY, A. Current developments in solid-state fermentation. **Biochemical Engineering Journal**, 81, 146-161. 2013.

VAZ, D. A.; GUDINA, E. J.; ALAMEDA, E. J.; TEIXEIRA, J. A.; RODRIGUES, L. R. Performance of a biosurfactant produced by a Bacillus subtilis strain isolated from crude oil samples as compared to commercial chemical surfactants. **Colloids and Surfaces B: Biointerfaces**. 89, 167– 174. 2012.

VENIL, C. K. et al. Current perspective on bacterial pigments: emerging sustainable compounds with coloring and biological properties for the industry - an incisive evaluation. **RSC Adv.** 4: 39523-39529 DOI: 10.1039/C4RA06162D. 2014

VENIL, C. K.; LAKSHMANAPERUMALSAMY, P. Application of statistical design to the optimization of culture medium for prodigiosin production by *Serratia marcescens* SWML08. **Malaysi J Microbiol.** v. 5, n. 1, p. 55-61, 2009.

XIA Y., WANG G., LIN X., SONG X., AI L. Solid-state fermentation with *Serratia marcescens* Xd-1 enhanced production of prodigiosin by using bagasse as an inertia matrix, **Ann Microbiol**, 66, 1239-1247. 2016.

XU F., XIA S., YANG Q.Strategy for obtaining inexpensive prodigiosin production by Serratia marcescen. In 3rd International Conference on Chemical, Biological and Environmental Engineering. 2011.

XU, Q., NAKAJIMA, M., LIU, Z., SHIINA, T. Biosurfactants for Microbubble Preparation and Application. **International Journal of Molecular Sciences**, v. 12, p. 462-475, 2011b.

ZHENG, C.; WANG, M.; WANG, Y.; HUANG, Z. Optimization of biosurfactant-mediated oil extraction from oil sludge. **Bioresource Technology**, v. 110, p. 338–342, 2012.

ZHOU, W., ZENG, C., LIU, R., CHEN, J., LI, R., WANG, X., ... & WAN, Y. Antiviral activity and specific modes of action of bacterial prodigiosin against Bombyx mori nucleopolyhedrovirus in vitro. **Applied microbiology and biotechnology**, 100(9), 3979-3988. 2016.

ZUBAVICHUS, Y., FUCHS, O., WEINHARDT, L., HESKE, C., UMBACH, E., DENLINGER, J. D., & GRUNZE, M.. Soft X-ray-induced decomposition of amino acids: an XPS, mass spectrometry, and NEXAFS study. **Radiation research**, 161(3), 346-358. 2004.

CAPÍTULO 2

ARTIGO I

A low-cost solid fermentation medium for potential prodigiosin production by *Serratia marcescens* UCP/WFCC 1549

---

*Capitulo de livro publicado em **Microbes in the spotlight: recent progress in the understanding of beneficial and harmful microorganisms**; ISBN Print version: 978-16-2734-612-2; Pages of the chapter: 312-315 p, 2016.

A low-cost solid fermentation medium for potential prodigiosin production by *Serratia marcescens* UCP/WFCC 1549

D. Montero-Rodríguez[*,1,2], R. F. S. Andrade[2], D. Rubio-Ribeaux[1,2] , R. A. Lima[2], D. L. R. Ribeiro[3], G. K. B. Silva[2], H. W. C. Araújo[4] and G. M. Campos-Takaki[3]

[1] Center of Biological Sciences, Federal University of Pernambuco, 50670-420, Recife, Pernambuco, Brazil

[2]Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, 50050-590, Recife, Pernambuco, Brazil

[3]Chemical Engineering Department, Federal University of Pernambuco, 50740-521, Recife, Pernambuco, Brazil

[4]Chemistry Department, State University of Paraíba, 58429500, Campina Grande, Paraiba, Brazil

*Corresponding author: e-mail: dayimontero87@yahoo.es, Phone: +55 81 995012852

Nowadays, synthetic dyes are extensively used in various fields such as food and textile industries, paper production and agricultural practices. However, the interest in natural pigments is growing due to their better biodegradability and higher compatibility with the environment. Various researchers have investigated the production and application of microbial pigments as natural colorants. Due to the high cost of using synthetic medium, there is a need to develop new low-cost process for the production of pigments by microorganisms. This study was aimed for growth and prodigiosin production by *Serratia marcescens* UCP/WFCC 1549 using solid agro-industrial substrates. The results demonstrated that the bacterium was able to grow in all tested solid media, but the highest production of biomass was obtained on wheat bran (579.46 mg/g of dry substrate). However, the highest level of red pigment (126.44 mg/g of biomass) was obtained on mixture of sugarcane bagasse and wheat bran. The pigment was identified as prodigiosin by the maximum UV absorbance at 536 nm.

Keywords: prodigiosin; *Serratia marcescens*; solid-state fermentation; sugarcane bagasse; wheat bran

1. Introduction

Pigments and dyes are extensively used in various fields of everyday life such as food, cosmetic and textile industries. However, the interest for pigments obtained from natural sources has been increasing in recent years because of hazardous effects of their synthetic

counterparts [1-3]. Among natural dyes, microbial pigments are considered as good alternative to synthetic, due to their better biodegradability and higher compatibility with the environment, offering promising applications in several industrial sectors [4,5].

Prodigiosin is a red pigment produced as secondary metabolite by *Serratia marcescens* and other bacteria [6]. This biomolecule has received recent renewed attention because of its wide variety of biological properties, including antimicrobial, antimalarial, immunosuppressive and cytotoxic activities [7,8]. These properties make it one of most powerful candidate for promising use in pharmaceutical industry [9-11].

Despite of its potential commercial values, the application of prodigiosin is limited due to the high production cost, which is mainly caused by the expensive growth medium. Therefore, efforts were made to reduce the production cost of pigments and in this view, various studies have been carried out to explore the possibility of using other types of cheaper growth medium to perform this type of bioprocesses [12-14]. In recent years, various agro-industrial residues have been used as alternative substrates or additives for pigment production, which may represent an added value to the industry and also helps in solving pollution problems which their disposal may otherwise cause [5,12,14].

Another strategy that has been gaining renewed researchers attention is solid-state fermentation (SSF). This is an alternative culture method that has many advantages over submerged fermentation, including higher production yield, more effectiveness, more eco-friendly and easy recovery of by-product [15,16]. Hence, several studies have been focused to microbial production of pigments by SSF in recent years [4,17,18]. In this contexts, the aim of this work was to investigate the feasibility of two agro-industrial wastes as substrates for growth and production of prodigiosin by the bacterium *Serratia marcescens* UCP/WFCC 1549 in SSF.

2. Material and Methods

The bacterium *Serratia marcescens* UCP/WFCC 1549 was kindly supplied from the Culture Collection of the Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, Recife, state of Pernambuco, Brazil. The microorganism was maintained in Luria Bertani (LB) solid medium (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L and agar 15 g/L) at 5°C. For pre-culture, one loop of 24 h -culture of *S. marcescens* on LB medium was transferred to 50 mL of LB broth and maintained under orbital shaker at 150 rpm during 18 h at 28ºC, to obtain the seed culture.

The process of prodigiosin production was carried out in Erlenmeyer flasks through solid-state fermentation (SSF) using 10 g of dry solid substrate: sugarcane bagasse, wheat bran and

a 50:50 (m/m) mixture of these solid materials, supplemented with an impregnating solution (KH_2PO_4 3 g/L, K_2HPO_4 7 g/L, $MgSO_4.7H_2O$ 0.2 g/L, $(NH_4)_2SO_4$ 1 g/L and waste cooking oil 3%), according to Camilios-Neto et al. [19]. A 24 h-culture of *S. marcescens* on LB medium was used as inoculum. Cultivations were conducted at 28°C during 12 days. Then, the flasks were incubated for three times with 100 mL distillate water for 1 h at 200 rpm and 30°C.

After the fermentation period, the biomass was obtained by filtration and centrifuging of extracts for 20 min at 9500 rpm and was quantified by dry weight. The red pigment produced was extracted from the biomass in solvents system chloroform: methanol (2:1, 1:1 and 1:2, v/v), evaporated to dryness and quantified gravimetrically [20]. The dried pigment was dissolved in ethanol and submitted to spectrometry scan in a wavelength of 200-700 nm [11,20].

3. Results and Discussion

3.1 Effect of agro-industrial substrates on growth and pigment production

Several agro-industrial materials have been used as components of media for the production of prodigiosin by *S. marcescens*, such as cassava wastewater, peanut oil cake and brown sugar [12,20,21]. However, few researches involving production of prodigiosin through SSF are still reported [22,23]. Thus, present study was aimed to evaluate the feasibility of using solid agro-industrial substrates for the growth of *S. marcescens* UCP/WFCC 1549 and effective production of prodigiosin.

Figure 1 shows the culture media based on sugarcane bagasse, wheat bran or a mixture of both substrates, before and after cultivation with *S. marcescens*. Microbial growth was observed in all of them, as well as change in colour in solid substrates from brown to red suggesting the presence of prodigiosin in solid-state fermented media. These results were confirmed by quantification of biomass and red pigment produced after fermentation (Table 1).

The bacterium was able to grow in all tested solid media, but highest production of biomass was obtained on medium composed only by wheat bran. However, the highest level of red pigment was produced on mixture of sugarcane bagasse and wheat bran (Fig. 1 and Table 1), suggesting that the combination of both substrates may provide necessary carbon, nitrogen and essential micro nutrients for higher production of the pigment. The extracted pigment was identified as prodigiosin by the maximum peak of UV absorbance at 536 nm (Fig. 2), that is in accordance with others studies [7,20,24].

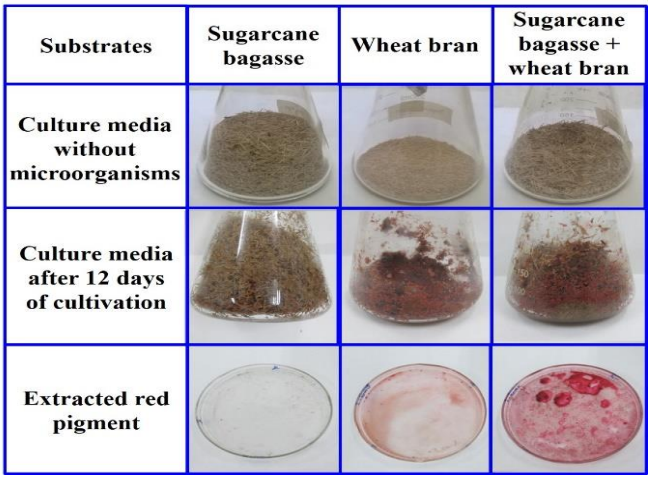


Fig. 1 Growth and red pigment production by *S. marcescens* UCP/WFCC 1549 using solid agro-industrial substrates.

Table 1 Biomass and red pigment produced by *S. marcescens* UCP/WFCC 1549 using solid agro-industrial substrates supplemented with an impregnating solution containing 3% waste cooking oil.

Medium	Biomass (mg/g of dry substrate)	Red pigment (mg/g of biomas
Sugarcane bagasse	15.60	11.65
Wheat bran	579.46	41.18
Sugarcane bagasse + wheat bra	288.54	126.44

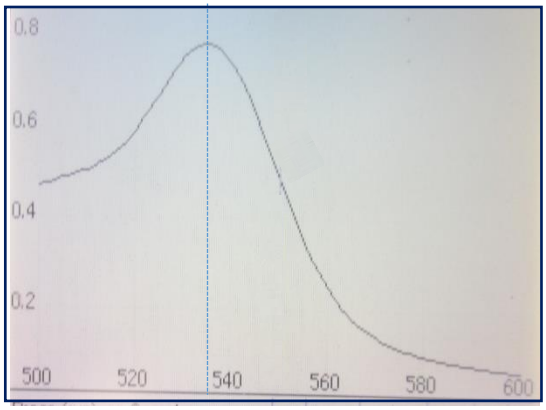


Fig. 2 Absorbance spectrum of red pigment produced by *S. marcescens* UCP/WFCC 1549 using sugarcane bagasse and wheat bran.

Several researchers have reported many conventional synthetic media such as nutrient broth, peptone glycerol broth and LB broth for prodigiosin production [11,25,26]. Although many media have been tested to replace these expensive complex to increase the production of prodigiosin, it would be desirable to design a novel nutritious and economical medium to enhance the growth of *S. marcescens* and prodigiosin production. The use of cheaper and easily available agro-industrial by-products in place of more expensive conventional complex medium should expedite large-scale production of this useful bacterial secondary metabolite [12,20,23].

Sugarcane bagasse was used previously as substrate for production of pigments by fungus [27,28] and recently, Arivizhivendhan et al. [23] employed wheat bran in combination with tannery fleshing to produce prodigiosin. However, this is the first study in that both substrates (sugarcane bagasse and wheat bran) were employed for the production of prodigiosin. Here, it was demonstrated the feasibility of using the mixture of them as a potential cheap growth medium for large-scale production of prodigiosin, which facilitate its use in the pharmaceutical industry.

Acknowledgements This work was financially supported by National Council for Scientific and Technological Development (CNPq), Coordination for the Improvement of Higher Level Education Personnel (CAPES) and Foundation for the Support of Science and Technology of the State of Pernambuco (FACEPE). The authors are grateful to CAPES for the PhD scholarship and to Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, Brazil, for use of laboratories.

References

[1] Ahmad AS, Ahmad WYW, Zakaria ZK, Yosof NZ. Applications of bacterial pigments as colorant. New York: Springer Berlin Heidelberg; 2012. pp. 57-74.

[2] Venil CK, Zakaria ZA, Ahmad WA. Bacterial pigments and their applications. Process Biochemistry. 2013; 48: 1065-79.

[3] Kumar A, Vishwakarma HS, Singh J, Dwivedi S, Kumar M. Microbial pigments: production and their applications in various industries. International Journal of Pharmaceutical, Chemical and Biological Sciences. 2015; 5: 203-212.

[4] Babitha, S. Microbial pigments. In: Biotechnology for agro-industrial residues utilisation. Netherlands: Springer; 2009. pp. 147-162.

[5] Panesar R, Kaur S, Panesar PS. Production of microbial pigments utilizing agro-industrial waste: a review. Current Opinion in Food Science. 2015; 1: 70-76.

[6] Bennett J, Bentley R. Seeing red: the story of prodigiosin. Advances in Applied Microbiology. 2000; 47: 1-32.

[7] Suryawanshi RK, Patil CD, Borase HP, Salunke BK, Patil SV. Studies on production and biological potential of prodigiosin by *Serratia marcescens*. Applied Biochemistry and Biotechnology. 2014; 173: 1209-21.

[8] Darshan N, Manonmani HK. Prodigiosin and its potential applications. Journal of Food Science and Technology. 2015; 1-15.

[9] Venil CK, Lakshmanaperumalsamy P. An insightful overview on microbial pigment: prodigiosin. Electronic Journal of Biology. 2009; 5: 49-61.
[10] Stankovic N, Senerovic L, Ilic-Tomic T, Vasiljevic B, Nikodinovic-Runic J. Properties and applications of undecylprodigiosin and other bacterial prodigiosins. Applied Microbiology and Biotechnology. 2014; 98: 3841-58.
[11] Lapenda JC, Silva PA, Vicalvi MC, Sena KXFR, Nascimento SC. Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398. World Journal of Microbiology and Biotechnology. 2015; 31: 399-406.
[12] Naik C, Srisevita JM, Shushma KN, Farah N, Shilpa AC, Muttanna CD, Darshan N, Sannadurgappa D. Peanut oil cake: a novel substrate for enhanced cell growth and prodigiosin production from *Serratia marcescens* CF-53. Journal of Research in Biology. 2012; 2: 549-557.
[13] Lopes FC, Tichota DM, Pereira JQ, Segalin J, Rios AO, Brandelli A. Pigment production by filamentous fungi on agro-industrial byproducts: as eco-friendly alternative. Applied Biochemistry and Biotechnology. 2013; 171: 616–625.
[14] Carvalho JC, Cardoso LC, Ghiggi V, Woiciechowski AL, Souza Vandenberghe LP, Soccol CR. Microbial pigments. In: Biotransformation of waste biomass into high value biochemical. Springer: New York; 2014. pp. 73-97.
[15] Barrios-González J. Solid-state fermentation: physiology of solid medium, its molecular basis and applications. Process Biochemistry. 2012; 47: 175-185.
[16] Thomas L, Larroche C, Pandey A. Current developments in solid-state fermentation. Biochemical Engineering Journal. 2013; 81: 146-161.
[17] Nimnoi P, Lumyong S. Improving solid-state fermentation of *Monascus purpureus* on agricultural products for pigment production. Food and Bioprocess Technology. 2011; 4: 1384-90.
[18] Hernández-Almanza A, Montañez-Sáenz J, Martínez-Ávila C, Rodríguez-Herrera R, Aguilar CN. Carotenoid production by *Rhodotorula glutinis* YB-252 in solid-state fermentation. Food Bioscience. 2014; 7: 31-36.
[19] Camilios-Neto D, Bugay C., Santana-Filho AP, Joslin T, Souza LM, Sassaki GL, Mitchell DA, Krieger N. Production of rhamnolipids in solid-state cultivation using a mixture of sugarcane bagasse and corn bran supplemented with glycerol and soybean oil. Applied Microbiology and Biotechnology. 2011; 89: 1395-1403.
[20] Araújo HWC, Fukushima K, Takaki GMC. Prodigiosin production by *Serratia marcescens* UCP 1549 using renewable-resources as a low cost substrate. Molecules. 2010; 15: 6931-40.
[21] Aruldass CA, Venil CK, Zakaria ZA, Ahmad WA. Brown sugar as a low-cost medium for the production of prodigiosin by locally isolated *Serratia marcescens* UTM1. International Biodeterioration and Biodegradation. 2014; 95: 19-24.
[22] Xu F, Xia S, Yang Q. Strategy for obtaining inexpensive prodigiosin production by *Serratia marcescens*. Proceedings of the 3rd International Conference on Chemical, Biological and Environmental Engineering, 2011; Singapore.
[23] Arivizhivendhan KV, Mahesh M, Regina Mary R, Sekaran G. Bioactive prodigiosin isolated from *Serratia marcescens* using solid state fermenter and its bactericidal activity compared with conventional antibiotics. Journal of Microbial and Biochemical Technology. 2015; 7: 305-312.
[24] Andreyeva IN, Ogorodnikova TI. Pigmentation of *Serratia marcescens* and spectral properties of prodigiosin. Microbiology. 2015; 84: 28-33.
[25] Samrot AV, Chandana K, Senthilkumar P, Narendrakumar G. Optimized production of prodigiosin from *Serratia marcescens* SU-10 grown as batch culture and evaluation of bioactivity of produced prodigiosin. International Journal of Medicobiological Research. 2011; 1: 145-150.
[26] Chen WC, Yu WJ, Chang CC, Chang JS, Huang SH, Chang CH, Chen SY, Chien CC, Yao CL, Chen WM, Wei YH. Enhancing production of prodigiosin from *Serratia marcescens* C3 by statistical experimental design and porous carrier addition strategy. Biochemical Engineering Journal. 2013; 78: 93-100.
[27] Carvalho JC, Oishi BO, Woiciechowski AL, Pandey A, Soccol CR. Effect of substrates on the production of *Monascus* biopigments by solid-substrate fermentation and pigment extraction using different solvents. Indian Journal of Biotechnology. 2007; 6: 194–199.
[28] Rajeswari TR, Ponnusami V, Sugumaran KR. Production of *Monascus* pigment in low cost fermentation. International Journal of Chemical and Technological Research. 2014; 6: 2929-32.

CAPÍTULO 3
ARTIGO II

Solid-state fermentation for simultaneous production of prodigiosin and biosurfactant by *Serratia marcescens* UCP/WFCC 1549

[*] Manuscrito submetido para publicação ao periódico: **Chemical Engineering Transactions**

CHEMICAL ENGINEERING TRANSACTIONS
VOL. 64, 2018
A publication of AIDIC
The Italian Association of Chemical Engineering
Online at www.aidic.it/cet
Guest Editors: Enrico Bardone, Antonio Marzocchella, Tajalli Keshavarz

ISBN 978-88-95608- 56-3; **ISSN** 2283-9216

Solid-State Fermentation for Simultaneous Production of Prodigiosin and Biosurfactant by *Serratia marcescens* UCP/WFCC 1549

Dayana Montero-Rodríguez*[ab], Rosileide F. S. Andrade[b], Daylin Rubio-Ribeaux[ab], Thayse A. L. Silva[b], Nathália S. A. A. Marques[bc], Rafael A. Kabroski[b], Hélvia W. C. Araújo[c], Galba M. Campos-Takaki[b]

[a]Center of Biological Sciences, Federal University of Pernambuco, 50670-420, Recife, Pernambuco, Brazil.
[b]Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, 50050-590, Recife, Pernambuco, Brazil.
[c]Northeast Network for Biotechnology Post-graduation Program, Federal Rural University of Pernambuco, 52171-900, Recife, Pernambuco, Brazil
[d]Chemistry Department, State University of Paraíba, 58429-500, Campina Grande, Paraíba, Brazil.
dayanamontero87@gmail.com

Solid-state fermentation (SSF) is a process that occurs in the absence or near absence of water and has gained significant attention for the development of industrial bioprocesses. It offers a cost-effective alternative that uses agricultural wastes and byproducts for the production of various high-value added microbial metabolites such as pigments and biosurfactants. In this sense, *Serratia marcescens* is a bacterium that has been investigated for its ability to produce prodigiosin, a red pigment with demonstrated antimicrobial, antimalarial, immunosuppressive and cytotoxic properties and also, biosurfactants with several industrial applications. However, the large-scale production of both biocompounds is limited due to the high cost of production medium. Then, this work aimed to use low-cost substrates for simultaneous production of prodigiosin and biosurfactant by *S. marcescens* UCP/WFCC 1549 under SSF. The experiments were carried out during 10 days at 28ºC, through a 2^2 full factorial design (FFD). The maximal yield of red pigment (34 g/kg dry solid) was achieved in condition 2 of FFD, using 10 g of wheat bran supplemented with an impregnating solution containing 5% waste soybean oil. The pigment was identified as prodigiosin by the maximum peak of UV absorbance at 535 nm and Rf of 0.9 on TLC. The best production of biosurfactant was confirmed in same condition of FFD, with reduction of surface tension of water to 27.9 mN/m. These results confirm the suitability of SSF to convert cheap and under-utilized agro-industrial byproducts into industrially relevant biocompounds.

1. Introduction

Solid-state fermentation (SSF), a process that occurs in the absence or near absence of water, has been used for the production of various high-value added microbial metabolites such as enzymes, organic acids, biosurfactants, pigments and other biotechnological products (Thomas et al., 2013). Over the last decades, SSF has gained significant attention for the development of industrial bioprocesses, particularly due to its technical and economic advantages over traditional submerged fermentation (SmF). These benefits include lower energy requirement because of the reduced aeration and agitation demands, lesser risk of contamination due to lower water activity, higher product yields and lower effluent generation (Abu Yazid et al., 2017). In addition, SSF offers a biological process to convert cheap and under-utilized agro-industrial wastes (either as carbon/energy source or as an inert carrier) into industrially relevant products (Lizardi-Jiménez and Hernández-Martínez, 2017).
Serratia marcescens is known as the major producer of prodigiosin, a red pigment with wide variety of biological properties, including demonstrated antimicrobial, antimalarial, immunosuppressive and cytotoxic activities (Granada et al., 2016). Also, this bacterium has been investigated due to its ability to produce biosurfactants, amphiphilic compounds that present surface and emulsification activity (Almansoory et al., 2017). The production

of microbial pigment and biosurfactants has received great attention during recent years due to their eco-friendly features such as low toxicity, highly efficiency and high biodegradability, contrary to the synthetic ones. They are involved in various applications including food, household, agricultural, environmental, biomedical, cosmetics and the pharmaceutical industries (Kumar et al., 2015; Varjani and Upasani, 2017). Despite of theirs potential commercial values, the large-scale production of prodigiosin and biosurfactant is limited due high production cost as well as the operational problems that occur when SmF is used. Then, SSF is being increasingly applied as a strategy to guarantee the viability of the process (Abu Yazid et al., 2017).

Brazil is one of the largest agricultural producers in the world and carries a strong potential for SSF due to its high availability of solid residues, commonly used as substrate in this kind of fermentation (Ferreira-Leitao et al., 2010). Sugarcane bagasse (SCB) and wheat bran (WB) are two of the most popular agro-industrial residues preferred by many researchers to produce value-added metabolites from various microorganisms using SSF. SCB is generated in high amounts after the crushing and extraction of the juice from sugarcane and consists of approximately 50% cellulose, 25% hemicellulose and 25% lignin. WB is a byproduct of the wheat milling industry and consists mainly of 41-60% non-starch polysaccharides, 10-20% starch and 15-20% protein (Fleuri et al., 2014; Kumari et al., 2016).
S. marcescens UCP/WFCC 1549 is a bacterium with demonstrated ability to produce biosurfactant using different agro-industrial residues and also, it is a good producer of prodigiosin (Lins et al., 2014; Araujo et al., 2017). However, there are not studies involving this strain in concomitant production of these compounds, which is a limited feature for some microorganism. Therefore, this work focused on the simultaneous production of prodigiosin and biosurfactant by *S. marcescens* UCP/WFCC 1549 through SSF, in order to develop a low-cost process to obtain both biocompounds that could replace the synthetic ones.

2. Materials and methods

2.1 Microorganism

S. marcescens UCP/WFCC 1549, originally isolated from the semi-arid soil of the state of Pernambuco, Brazil, and identified by Araujo et al. (2017), was kindly provided by the Culture Collection UCP (Universidade Católica de Pernambuco) registered in WFCC (World Federation for Culture Collection) Recife, PE, Brazil. The bacterium was maintained in Luria Bertani (LB) solid medium at 5 °C. Stored cultures were transferred first to LB medium and incubated for 18 h at 28 ºC. Then, two colonies were transferred to 50 mL of LB broth and incubated at 28 ºC and 150 rpm in an orbital shaker. Once the optical density at 600 nm reached 0.8-1.0, this culture was used as inoculum.

2.2 Agro-industrial residues

For the formulation of production media were used the solid agro-industrial residues SCB, kindly donated by Usina Japungu, Santa Rita, Paraíba, Brazil, and WB, bought at a local market in city of Recife, Pernambuco, Brazil. SCB was maintained at -4 °C until it use, then it was thawed at room temperature, oven-dried at 60 °C for 24 h and ground in a blender. WB did not receive any kind of pre-treatment. Both solid residues were sieved for separate: the fraction used was either that retained between 16- and 32- mesh sieves (opening of 1.0 and 0.5 mm, respectively). In addition, it was used waste soybean oil (WSO), kindly supplied by a local restaurant in the city of Recife, Pernambuco, Brazil.

2.3 Solid-state fermentation

SSF was carried out using a 2^2 full factorial design (FFD), to determinate the influence of percentage of WSO and the solid substrates in the production of prodigiosin and biosurfactant. Each 250 mL Erlenmeyer flasks contained 10 g of dry solid substrate: SCB, WB or a 50:50 (m/m) mixture of them, according to the FFD (Table 1). The flasks were autoclaved at 121 °C for 15 min. Then, corresponding amount of impregnating solution inoculated with seed culture at 5 % was mixed into solid substrates. The amount of impregnation solution for each dry substrate was defined as described by Camilios-Neto et al. (2011). The impregnation solution itself contained KH_2PO_4 3g/L, K_2HPO_4 7g/L, $MgSO_4.7H_2O$ 0.2 g/L, $(NH_4)_2SO_4$ 1g/L and WSO according to the FFD. The amount of WSO was quoted as a percentage (v/v; volume of WSO per total volume of impregnating solution). The inoculated flasks were incubated at 28 °C for 10 days.

Table 1: Levels of the variables studied in a 2^2 full factorial design for the production of prodigiosin and biosurfactant by S. marcescens UCP/WFCC 1549 in solid-state fermentation.

Variables	Factor levels		
	Low (-1)	Central (0)	High (+1)
Waste soybean oil (% v/v)	1	3	5
Bagasse sugarcane/wheat bran (% m/m)	0:100	50:50	100:0

2.4 Extraction of compounds from SSF

After the fermentation period, 100 mL of distilled water was added to each Erlenmeyer flask and contents were agitated for 1 h at 200 rpm and 30 °C on an orbital shaker. Then, the suspensions were filtered using

cheesecloths and the liquid excess was squeezed out manually (Nalini and Parthasarathi, 2014). This procedure was done three times. The extracts were centrifuged for 20 min at 10000 rpm and the pellet and supernatant were separated and used in the analysis described below.

2.5 Extraction and quantification of biomass and red pigment

The pellet obtained were washed twice with distilled water by centrifugation for 20 min at 10000 rpm and the biomass was quantified by dry weight. The red pigment produced was extracted from the biomass in solvents system chloroform: methanol of increasing polarity (2:1, 1:1 and 1:2, v/v), evaporated to dryness and quantified by dry weight (Araújo et al., 2010).

2.6 Characterization of red pigment

The dried pigment was dissolved in 95 % ethanol and submitted to spectrophotometry scan in a wavelength of 200-700 nm. In addition, it was subjected to thin layer chromatography (TLC) using the mixture of chloroform and methanol (9:1) (Priya et al., 2013). RF value of the extract was compared with the standard prodigiosin Rf value referred in the literature using that solvents system.

2.7 Measurement of surface tension

The surface tension was determined on metabolic cell-free liquid obtained by centrifugation and subsequent filtration of cultures, using a tensiometer model Sigma 70 (KSV Instruments Ltd., Finland) by the Du Nouy ring method at room temperature (± 28 °C). Measurements of surface tension from distilled water were used as control (Kuyukina et al., 2001).

3. Results and discussion

3.1 Solid-state fermentation for pigment production

Prodigiosin has attracted great interest for several decades for its potential clinical application because it has anti-fungal, anti-bacterial, anti-protozoal/anti-malarial, immunosuppressive and anti-cancer properties. However, high cost and low productivity of prodigiosin under SmF limits its commercial application (Lapenda et al., 2015; Xia et al., 2016). SSF offers a suitable strategy for getting inexpensive prodigiosin, but few researches involving production of this red pigment through SSF are still reported (Xu et al., 2011; Arivizhivendhan et al., 2015; Xia et al., 2016). In this study, SCB, WB and the mixture of both substrates were used through a 2^2 FFD for pigment production and the results are shown in Table 2.

Table 2: Red pigment and surface tension values obtained by Serratia marcescens UCP/WFCC 1549 using a 2^2 factorial design.

Runs	Waste soybean oil (% v/v)	Sugarcane bagasse/wheat bran (% m/m)	Red pigment (g/kg dry substrate)	Surface tension (mN/m)
1	-1	-1	12.21	33.6
2	+1	-1	33.99	27.9
3	-1	+1	9.74	30.7
4	+1	+1	14.91	31.9
5	0	0	21.01	38.9
6	0	0	25.35	38.7
7	0	0	25.53	38.8

Figure 1A illustrates the Pareto chart, with 95% of confidence level, for the estimated effects in absolute values of WSO and mixture of SCB/WB for red pigment. It is possible to observe that the only variable that had a significant and positive influence was the mixture of SCB/WB. SCB and WB were used previously in independent studies to produce prodigiosin (Arivizhivendhan et al., 2015; Xia et al., 2016), and our preceding work was the first using both them for this purpose (Montero-Rodríguez et al., 2016). As shown in Table 2, the maximal yield of red pigment (34 g/kg dry solid) was achieved in condition 2 of FFD, using 10 g of WB supplemented with an impregnating solution containing 5 % WSO. This result was better than that obtained by Xu et al. (2011), who reported the production of 4.16 g/kg of waste kitchen, and it was near to the yield achieved by Xia et al. (2016), 40.86 g kg^{-1} dry solid using bagasse as inertial matrix and glycerol and soy peptone as carbon and nitrogen sources, respectively. Our previous work showed prodigiosin yield of 23.86 g/kg dry solid using 10 g of WB supplemented with an impregnating solution containing 3 % WSO (Montero-Rodríguez et al., 2016). Then, the results obtained here confirm the suitability of these residues for prodigiosin production.

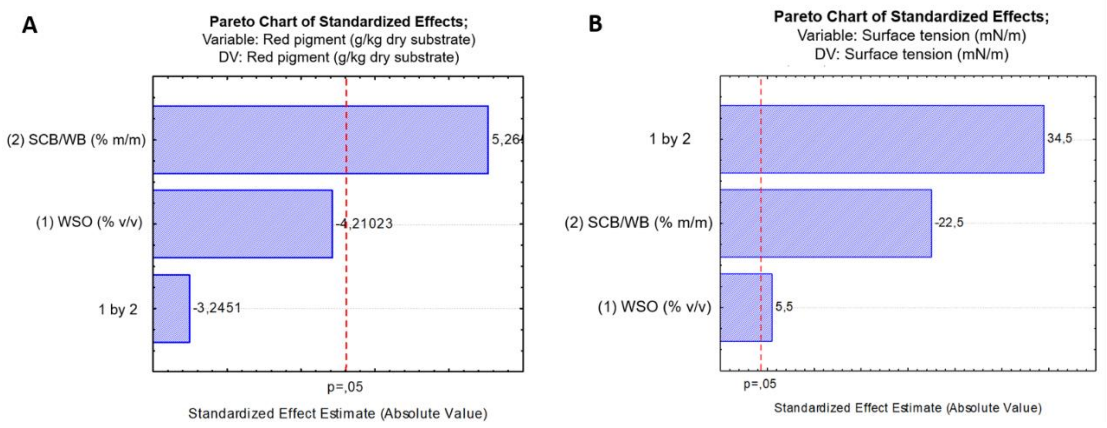


Figure 1: Pareto Charts of standardized effects of (1) WSO and (2) mixture of SCB/WB on yield of red pigment (A) and surface tension (B) obtained for the 2^2 FFD. The point at which the effect estimates were statistically significant (p = 0.05) is indicated by dashed line.

3.2 Characterization of red pigment

The crude pigment extract extracted from *S. marcescens* biomass was purified by TLC and the Rf (0.9) corresponding to prodigiosin, in according to results obtained by Araújo et al. (2010), Priya et al. (2013) and Phatake and Dharmadhikari (2016). In addition, the maximum UV absorbance at 536 nm confirmed the presence of prodigiosin as active compound (Patil et al., 2011; Suryawanshi et al., 2014) (Fig. 2).

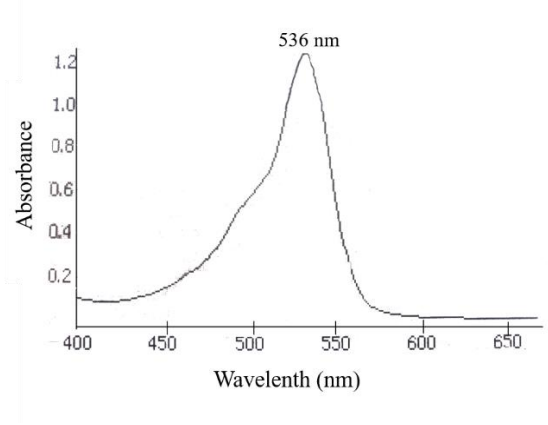


Figure 2: Absorbance spectrum of red pigment produced by S. marcescens UCP/WFCC 1549 in condition 2 of the FFD.

3.3 Production of biosurfactant

Most of the literature has reported the biosurfactant production using SmF. This method poses problem with foam formation during the production, hence SSF could be an alternate method to obtain these biocompounds. (Brumano et al., 2016). There are very few reports on the production of biosurfactant by SSF and only one using *Serratia* genus (Nalini and Parthasarathi, 2014). This study is the first involving production of biosurfactant by *S. marcescens* using agro-industrial byproducts through SSF. SCB and WB showed as excellent substrates for biosurfactant production, presenting decrease of surface tension to 30.7 and 27.9 mN/m, respectively (Table 2).
The Pareto chart illustrated in Figure 1B shows that both independent variables WSO and mixture of SCB/WB as well as the interaction between them had significantly influenced the surface tension. However, only the mixture of SCB/WB influenced negatively, in a statistically significant way, the increase in surface tension, leading to lower surface tension, suggesting the production of biosurfactant in the culture medium.

4. Conclusions

Despite the considerable attention that prodigiosin and biosurfactants have received in several industrial areas, the simultaneous production of them in co-fermentation as one strategy for reducing the relative high production cost of both products has still not been investigated in the literature. The results obtained in this study confirm the suitability of SSF to convert cheap and under-utilized agro-industrial byproducts into both industrially relevant biocompounds.

Acknowledgments
This work was financially supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) under process No. 311373/2014-3, FACEPE (Fundação de Amparo à Ciência e Tecnologia de Pernambuco) under process No. APQ-0291-2.12/15, and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). The authors are grateful to CAPES for the Ph.D. fellowship and to Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, PE-Brazil, for the use of laboratories.

References

Abu Yazid N., Barrena R., Komilis D., Sánchez A., 2017, Solid-State Fermentation as a Novel Paradigm for Organic Waste Valorization: A Review, Sustainability, 9, 224.

Almansoory A.F., Hasan H.A., Idris M., Abdullah S.R.S., Anuar, N., 2017, Biosurfactant production by the hydrocarbon-degrading bacteria (HDB) *Serratia marcescens*: Optimization using central composite design (CCD), J Ind Eng Chem, 47, 272-280.

Araujo H.W.C., Andrade R.F., Montero-Rodríguez D., Santos V.P., Maia P.C.S., Costa Filho C.F., Silva C.A.A., Campos-Takaki G.M., 2017, Biochemical identification of and molecular newly isolated pigmented bacterium and improved production of biosurfactant, Afr J Microbiol Res, 11, 945-954.

Araújo H.W.C., Fukushima K., Takaki G.M.C., 2010, Prodigiosin production by *Serratia marcescens* UCP 1549 using renewable-resources as a low cost substrate, Molecules, 15, 6931-40.

Arivizhivendhan K.V., Mahesh M., Regina Mary R., Sekaran G., 2015, Bioactive prodigiosin isolated from *Serratia marcescens* using solid state fermenter and its bactericidal activity compared with conventional antibiotics, J Microb Biochem Technol, 7, 305-312.

Camilios-Neto D., Bugay C., Santana-Filho A.P., Joslin T., Souza L.M., Sassaki G.L., Mitchell D.A., Krieger N., 2011, Production of rhamnolipids in solid-state cultivation using a mixture of sugarcane bagasse and corn bran supplemented with glycerol and soybean oil, Appl Microbiol Biotechnol, 89, 1395-1403.

Ferreira-Leitao V., Gottschalk L.M.F., Ferrara M.A., Nepomuceno A.L., Molinari H.B.C., Bon E.P., 2010, Biomass residues in Brazil: availability and potential uses, Waste Biomass Valorization, 1, 65-76.

Fleuri L.F., de Oliveira M.C., Arcuri M.D.L.C., Capoville B.L., Pereira M.S., Delgado C.H.O., Novelli P.K., 2014, Production of fungal lipases using wheat bran and soybean bran and incorporation of sugarcane bagasse as a co-substrate in solid-state fermentation, Food Sci Biotechnol, 23, 1199-1205.

Granada S.D., Bedoya-Pérez J.C., Scherlach K., Hertweck C., 2016, *Serratia marcescens* metabolites are promising candidates for biocontrol of avocado pathogens, Planta Medica, 81(S 01), P598.

Kumar A., Vishwakarma H.S., Singh J., Dwivedi S., Kumar M., 2015, Microbial pigments: production and their applications in various industries, IJPCBS, 5, 203-12.

Kumari A., Satyanarayana T., Singh B, 2016, Mixed Substrate Fermentation for Enhanced Phytase Production by Thermophilic Mould *Sporotrichum thermophile* and Its Application in Beneficiation of Poultry Feed, Appl Biochem Biotechnol, 178, 197-210.

Kuyukina M.S., Ivshina I.B., Philp J.C., Christofi N., Dunbar S.A., Ritchkova M.I., 2001, Recovery of *Rhodococcus* biosurfactants using methyl tertiarybutyl ether extraction. J Microbiol Methods, 46, 149-156.

Lapenda J.C., Silva P.A., Vicalvi M.C., Sena K.X.F.R., Nascimento S.C., 2015, Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398, World J Microbiol Biotechnol, 31, 399-406.

Lins J.L., Maciel C.C.S., Xavier H.S., da Silva C.A., Campos-Takaki G. M., 2014, Production and toxicological evaluation of Prodigiosin from *Serratia marcescens* UCP/WFCC1549 on Mannitol Solid Medium, Int J Appl Res Nat Prod, 7, 32-38.

Lizardi-Jiménez M.A., Hernández-Martínez R., 2017, Solid state fermentation (SSF): diversity of applications to valorize waste and biomass, 3 Biotech, 7, 44.

Montero-Rodríguez D., Andrade R.F.S., Rubio-Ribeaux D., Lima R.A., Ribeiro D.L.R., Silva G.K.B., ... & Campos-Takaki G.M., 2016, A low-cost solid fermentation medium for potential prodigiosin production by *Serratia marcescens* UCP/WFCC 1549, In: Microbes in the Spotlight: Recent Progress in the Understanding of Beneficial and Harmful Microorganisms, 312.

Nalini S., Parthasarathi R., 2014, Production and characterization of rhamnolipids produced by *Serratia rubidaea* SNAU02 under solid-state fermentation and its application as biocontrol agent, Bioresour Technol, 173, 231-238.

Patil C.D., Patil S.V., Salunke B.K., Salunkhe R.B., 2011, Prodigiosin produced by *Serratia marcescens* NMCC46 as a mosquito larvicidal agent against *Aedes aegypti* and *Anopheles stephensi*, Parasitol Res, 109, 1179-1187.

Phatake Y.B., Dharmadhikari S.M., 2016, Physical parameters optimization for enhancement of prodigiosin production by using *Serratia* spp., World J Pharm Med Res, 2, 40-48.

Priya K.A., Satheesh S., Ashokkumar B., Varalakshmi P., Selvakumar G., Sivakumar N., 2013, Antifouling activity of prodigiosin from estuarine isolate of *Serratia marcescens* CMST 07. In Microbiological research in agroecosystem management (pp. 11-21). Springer India.

Suryawanshi R. K., Patil C.D., Borase H.P., Salunke B.K., Patil S.V., 2014, Studies on production and biological potential of prodigiosin by *Serratia marcescens*, Appl Biochem Biotechnol, 173, 1209-1221.

Thomas L., Larroche C., Pandey A., 2013, Current developments in solid-state fermentation, Biochem Eng J, 81, 146-161.

Varjani S.J., Upasani V.N., 2017, Critical Review on Biosurfactant Analysis, Purification and Characterization Using Rhamnolipid as A Model Biosurfactant, Bioresour Technol.

Xia Y., Wang G., Lin X., Song X., Ai L., 2016, Solid-state fermentation with *Serratia marcescens* Xd-1 enhanced production of prodigiosin by using bagasse as an inertia matrix, Ann Microbiol, 66, 1239-1247.
Xu F., Xia S., Yang Q., 2011, Strategy for obtaining inexpensive prodigiosin production by *Serratia marcescen*. In 3rd International Conference on Chemical, Biological and Environmental Engineering.

CAPÍTULO 4

ARTIGO III

Promising application as textile and candle dye of prodigiosin produced by *Serratia marcescens* UCP 1549 through solid-state fermentation

[*] Manuscrito submetido para publicação ao periódico: **Biology**

Article

Promising application as textile and candle dye of prodigiosin produced by *Serratia marcescens* UCP 1549 through solid-state fermentation

Dayana Montero-Rodríguez [1,2], Rosileide F. S. Andrade [2], Davi L. Cavalcanti [1,2], Daylin Rubio-Ribeaux [1,2], Amanda A. Alencar [2,3], Tayane C. D. Mendes-Silva [1,4], Hélvia W. C. Araújo [5] and Galba M. Campos-Takaki [2,*]

[1] Center of Biosciences, Federal University of Pernambuco, 50670-420, Recife, Pernambuco, Brazil; E-mails: dayanamontero87@gmail.com (D.M.R.); davicavalcanti25@gmail.com (D.L.C.); drubioribeaux@gmail.com (D.R.R.); tayanecassiamendes@gmail.com (T.C.D.M.S.)

[2] Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, 50050-590, Recife, Pernambuco, Brazil; E-mails: rosileide_fontenele@yahoo.com.br (R.F.S.A); galba_takaki@yahoo.com.br (G.M.C.T.)

[3] Northeast Network for Biotechnology Post-graduation Program, Federal Rural University of Pernambuco, 52171-900, Recife, Pernambuco, Brazil; E-mail: amandaalencar@yahoo.com.br (A.A.A.)

[4] Laboratory of Bioprocessing, Center of Strategic Technologies of Northeastern, 50740-540, Recife, Pernambuco, Brazil.

[5] Chemistry Department, State University of Paraíba, 58429-500, Campina Grande, Paraíba, Brazil; E-mail: hwcasullo@hotmail.com (H.W.C.A).

* Correspondence: galba_takaki@yahoo.com.br ; Tel.: + 55-81-2119-4017

Academic Editor: name
Received: date; Accepted: date; Published: date

Abstract: Advances in microbial pigment over the past decades encourages consumers to use safer and cheaper colorants with enhanced potency and applications. Prodigiosin is a red pigment synthetized mainly by *Serratia marcescens*, which possess several biological properties and have gained increasing importance in industrial markets such as drugs, cosmetics, textile dyeing, etc. However, limitations like high cost production and low yield make unprofitable its large-scale production. The present study aimed to use an economic medium for prodigiosin production by *S. marcescens* UCP 1549 through solid-state fermentation. The pigment was characterized by UV-Vis spectrophotometry, TLC, HPLC and LC-MS and its potential as natural dye was evaluated. The results confirmed the suitability of wheat bran as low-cost substrate to obtain the red pigment (39.81 g/kg of dry solid). The pigment was extracted from biomass and identified as prodigiosin by preliminary test. The maximum absorbance at 537.1 nm, Rf of 0.89 and molecular mass of 323.199 *m/z* verified the presence of prodigiosin. The bacterial pigment was successfully applied as textile and candles dye, suggesting its great potential as natural colorant. The use of cheap agro-industrial byproducts as alternative of expensive conventional medium could expedite large-scale production of useful microbial metabolites such as prodigiosin.

Keywords: *Serratia marcescens*, prodigiosin, solid-state fermentation, textile dyeing, candle colouring.

1. Introduction

In recent years, natural dyes have gained worldwide interest because of their better biodegradability and compatibility with the environment, as well as lower toxicity and allergic reaction, when compared with the synthetic ones [1,2]. Microbial pigments stand out as the most potential choice for obtain natural colorants due to their short production cycle, independence from

weather conditions and high productivity [3,4]. Therefore, the production of microbial pigments is now one of the emerging fields of research to show its potential for application in diverse areas, such as the food, pharmaceutical, cosmetic and textile industries [5,6].

Prodigiosin is a red pigment typically produced in the stationary phase by some strains of *Serratia marcescens* and other bacteria [7,8]. This secondary metabolite has a pyrrolyl pyrromethene skeleton with a molecular mass of 323.44 Da and have been extensively studied due to its wide range of biological properties [9-11]. Antimicrobial, antimalarial, antiproliferative, immunosuppressive and cytotoxic activities of this pigment make it one of most powerful candidate for promising use in pharmaceutical industry [12-15]. In addition, the potential of prodigiosin to dye of textile materials, paper, candles and soap, and also, as biodegradable ink, have been demonstrated [3,16-18].

Despite of their promising industrial applications, the large-scale production of prodigiosin is limited due to the high production cost, mainly caused by the expensive conventional growth media [19-20]. Cheap agro-industrial byproducts and residues have been used as alternative substrates to guarantee a profitable process of pigment production [3-4, 21].

Another strategy that have gained researchers' attention in order to obtain cost-effective industrial bioprocess is solid-state fermentation (SSF) [22,23]. This is a promising technology that has many advantages over traditional submerged fermentation, including higher production yield, more effectiveness, more eco-friendly and easy recovery of product [24,25]. In this sense, several studies have been carried out for production of microbial pigments through SSF in last decades [26,27].

In this context, *S. marcescens* UCP 1549 is a bacterium with demonstrated ability to produce prodigiosin using various growth media [28-30]. The present study aimed to obtain prodigiosin in suitable medium proposed in our previous work (data not published yet), and also, carry out the characterization of pigment as well as its application as dye.

2. Materials and Methods

2.1. Microorganism

S. marcescens UCP 1549, originally isolated and identified by Araujo et al. [31] was kindly provided by the Culture Collection of the Catholic University of Pernambuco, Recife, Brazil. The bacterium was maintained in Luria Bertani (LB) solid medium (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L and agar 15 g/L) at 5°C. Stored cultures were transferred first to LB medium and incubated for 18 h at 28ºC. Then, two colonies were transferred to 50 mL of LB broth and incubated at 28ºC and 150 rpm in an orbital shaker. Once the optical density at 600 nm reached 0.8-1.0, this culture was used as inoculum.

2.2. Agro-industrial substrates

The solid substrate used for prodigiosin production was wheat bran (WB), bought at a local market in city of Recife, Pernambuco, Brazil. It was sieved and the fraction used was either that retained between 16- and 32- mesh sieves (opening of 1.0 and 0.5 mm, respectively). In addition, it was used waste soybean oil (WSO), kindly supplied by a local restaurant in the city of Recife, Pernambuco, Brazil.

2.3. Production of prodigiosin

Production for prodigiosin was performed through solid-state fermentation, in Erlenmeyer flasks containing medium composed by WB and impregnating solution (IS) containing 5% of WSO, as described by Montero-Rodríguez et al. [30]. Briefly, ten grams of WB and 100 ml of IS were autoclaved separately at 121°C for 15 min. Then, IS was inoculated with seed culture at 5 % and 20 ml of this were mixed into WB. The amount of IS used was equal to the liquid absorption capacity of the substrate, defined as the amount of medium (ml) that can be added to 10 g of dry substrate without the appearance of free liquid [32]. The inoculated flasks were incubated at 28 °C for 10 days.

2.4. Extraction and quantification of biomass

After the fermentation period, 100 mL of distilled water was added to each Erlenmeyer flask and contents were agitated for 1 h at 200 rpm and 30 °C on an orbital shaker. Then, the suspensions were filtered using cheesecloths and the liquid excess was squeezed out manually [30]. This procedure was done three times and the extracts were collected and centrifuged for 20 min at 10000 rpm. The pellet obtained were washed twice with distilled water by centrifugation for 20 min at 10000 rpm and the biomass was quantified by dry weight [30].

2.5. Extraction and quantification of pigment

The pigment produced was extracted from the biomass using the method proposed by Bóna-Lovász et al. [34] with modifications. Briefly, 2 ml of methanol and 4 ml of hexane were added to 1 g of biomass, and the mixture was vortexed for 15 min. Subsequent phase separation was achieved by the addition of 1 ml of water to the mixture, followed by vortexing for 15 min, and centrifugation for 15 min at 10000 rpm and 10ºC. The upper supernatant phase was filtered with PES membranes with a pore size of 0.45 μm (syringe filters - K18-430, Kasvi, PR, Brazil) and then, the pigment was air dried and quantified. Every step of extraction and the storage of pigment were carried out in the dark.

2.6. Characterization and identification of pigment

Preliminary identification of prodigiosin was carried out as follows: the pigment was dissolved in 95% ethanol and the coloured solution was divided into two portions. One part was acidified with a drop of concentrated HCl and the other was alkalinized with a drop of concentrated ammonia solution [35]. Red or pink color in acidic condition and yellow or tank color in alkaline condition confirmed a positive presumptive test for prodigiosin [36-37].

The pigment extracted in methanol was submitted to thin layer chromatography (TLC). The sample was applied to an aluminium foil sheet covered with silica gel and placed in glass cube containing the mixture chloroform–methanol (9:1, v/v) as mobile phase [28,38] (Araújo et al., 2010; Priya et al., 2013). The retention factor (R*f*) was calculated according to the formula R*f*: distance travelled by the compound/ distance travelled by the solvent front [16] and then, it was compared to the standard prodigiosin R*f* referred in the literature.

Methanol extract of pigment was further analysed by high-performance liquid chromatography (HPLC). Chromatographic separation was performed on a reverse-phase column Sunfire, Waters C18 (4.6 x 150 mm) at a temperature of 30°C and was run isocratically for 20 min, with a mobile phase consisting of acetonitrile/methanol/ethyl acetate (10:50:40), flow-rate 0.6 mL/min. The detection was performed at 536 nm using a UV-Vis detector (Waters 2998, Rio de Janeiro, Brazil).

The molecular mass of the pigment was determined by Liquid chromatography–mass spectrometry (LC-MS) on an Acquity UPLC® (Waters) system, model HSS T3 C18 (2.1 × 100 mm, 1.7 μm particle size). The LC-MS analysis was carried out in positive mode ionization using acetonitrile/ethyl acetate/methanol as mobile phase.

2.7. Application of pigment as natural dye

The potential application of pigment as natural dye of textile materials and candles was evaluate using the methanol extract containing the red pigment.

For textile dyeing, white pieces (2 cm^2) of cotton poplin, silk, polyester, viscose and satin were soaked in ten milliliters of methanol extract of prodigiosin and incubated at room temperature (28 ± 2°C) for 24 h [16,17]. Then, dyed pieces were dried at room temperature and washed with 1% detergent solution to remove any physically absorbed colorant on the surface [39]. For all cloths, white pieces without dye was taken as control.

For candle coloring, commercial candles were placed in a beaker and heated until melted before the addition of 5 ml of methanol extract. The mixtures were homogenized and poured into the molds, after greased then with mineral oil. The wicks were immediately placed into the center of the molds

and the candles were left to cool at room temperature (28 ± 2°C) for 1 h [3]. One control candle was prepared which does not contain the pigment.

3. Results and discussion

3.1. Production of pigment by solid-state fermentation

Various differential and selective media have been used for growth of prodigiosin-producing microorganism [40-42]. However, due to the high cost of synthetic medium, there is a need to design new and inexpensive medium to enhance the biosynthesis of this pigment. The use of agro-industrial wastes would provide a profitable alternative to reducing substrate costs [1,5,28]. Several agricultural products and byproducts such as corn steep liquor, cassava wastewater, pineapple waste and peanut oil cake were successfully utilized for prodigiosin production [3,19,28,43]. In addition, SSF offers a suitable strategy for develop low-cost process, but few researches involving this technology for production of red pigment are still reported [44-46].

In present study, *S. marcescens* UCP 1549 was cultivated through SSF using wheat bran as substrate, according to the selection of better medium from full factorial design carried out in our previous work (data non published). The yield of red pigment extracted from the biomass was 39.81 g/kg of dry solid. This result was better that we obtained using 10 g of WB supplemented with an impregnating solution containing 3 % WSO (23.86 g/kg dry solid) [30]. Also, it was similar to the yield achieved by Xia et al. [46] (40.86 g/kg of dry solid) using bagasse as inertial matrix and glycerol and soy peptone as carbon and nitrogen sources. Previously, Xu et al. [44] reported the prodigiosin production of 4.16 g/kg of waste kitchen by SSF.

Hence, this study confirm the suitability of SSF to convert cheap and under-utilized agro-industrial by-products such as WB into industrially relevant prodigiosin.

3.2. Identification of pigment

Presumptive test for prodigiosin was carried out for red pigment produced by *S. marcescens* UCP 1549. As shown in Figure 1, a more intense pink color in the acidified solution while a yellow color in the alkaline solution were observed, indicating a positive result for prodigiosin production. Similar results were previously obtained by other researchers [36,37,42,47] when identified pigment produced by *Serratia* sp. strains.

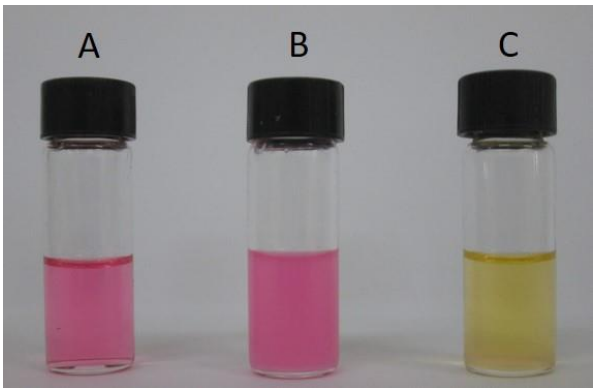


Figure 1. Presumptive test for prodigiosin using ethanol extract of red pigment produced by *S. marcescens* UCP 1549: (A) Ethanol extract of pigment before test (control), (B) acidified and (C) alkalinized ethanol extract of pigment.

The methanol extract of red pigment produced by *S. marcescens* UCP 1549 was subjected to TLC and showed a single and pink coloured band with R*f* value of 0.89. This R*f* value is similar to those

previously reported for prodigiosin using the chloroform-methanol mixture as mobile phase in TLC [9,28,38,47].

The HPLC analysis of pigment revealed a major peak with retention time of 6.574 min. This peak was analysed by UV-Visible spectrophotometer and showed a maximum absorbance at 537.1 nm (Figure 2). According to several studies of prodigiosin from *S. marcescens*, this pigment have a maximum absorbance at 534-537 nm [2,15,41,48].

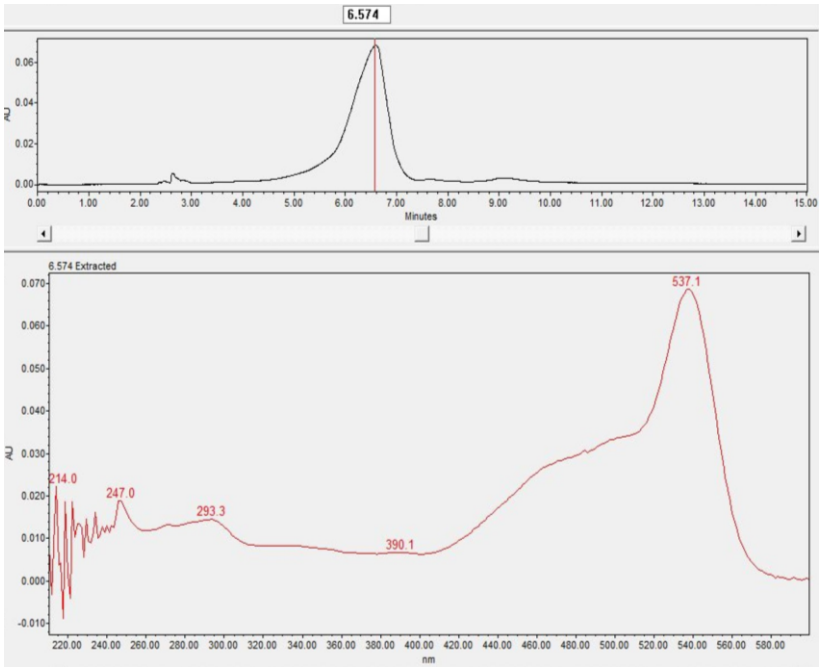


Figure 2. HPLC profile of pigment produced by *S. marcescens* UCP 1549 (A) and UV-Visible absorbance spectrum of peak obtained at retention time of 6.574 min (B).

The LC-MS analysis of the methanol extract of the pigment shows a main peak at retention value 0.42 min (Fig. 3). The molecular mass was determined as 323.199 *m*/z, which corresponds to the molecular weight of prodigiosin [41,49,50].

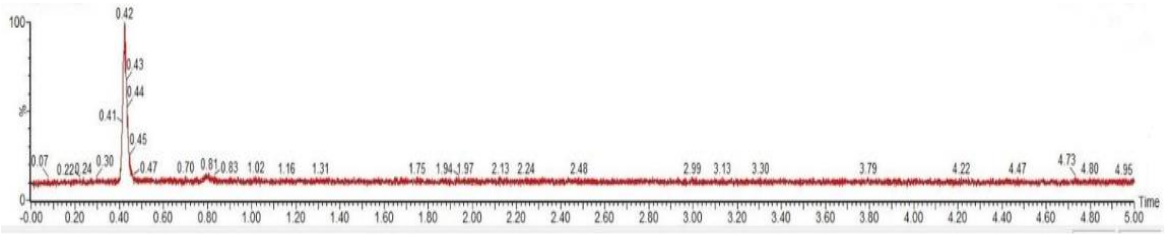


Figure 3. LC-MS analysis of methanol extract of pigment produced by *S. marcescens* UCP 1549.

Results obtained from these analyses clearly confirmed that pigment produced by *S. marcescens* UCP 1549 is prodigiosin.

3.3. Application of pigment as natural dye

There is an increased interest in the use of natural dyes for textile applications because of the prohibition of use some synthetic dyes with demonstrated carcinogenicity of the precursor or product, as well as the toxic effect of their industrial wastes in the ecosystems [1,51]. Lower toxicity and allergic

reactions of natural pigments make them more compatible for human use, due to the reduction of exposure to harmful chemicals for both textile workers and wearers of the cloths [6,49].

In present study, prodigiosin produced by *S. marcescens* UCP 1549 was successfully used as dye for different types of textiles, including silk, satin, cotton poplin, polyester and viscose (Figure 4). Viscose was the material that visually exhibited better coloration, confirming that dyeing performance depending of the type of fiber [1]. Similar textile-dyeing ability was also reported by prodigiosin produced by *Vibrio* spp. and *S. marcescens* [3,17,39].

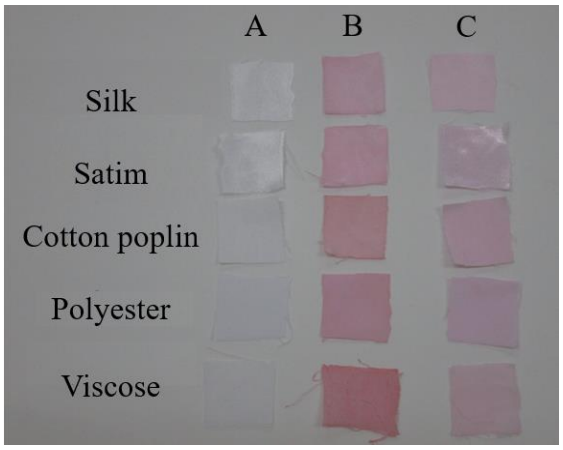


Figure 4. Application of prodigiosin produced by *S. marcescens* UCP 1549 in coloring textile materials. Cloths without dye (control) (A) and dyed with methanol extract of prodigiosin before (B) and after (C) wash with detergent solution.

However, it was evidenced a loss of pigment from all cloth materials after wash with detergent solution. This indicates that the use of a mordant or stabilizer agent during the dyeing process is required to guaranties the fixation of the pigment [52]. Krishna et al. [16] subjected different fabrics i.e. cotton, chiffon, poplene, pure silk, century cotton, polyester and nylon to dyeing with prodigiosin produced by *Serratia* sp. BTWJ8 and observed that the loss of pigment from the same textile materials was less when used thiourea as mordant.

Moreover, the potential of prodigiosin produced by *S. marcescens* UCP 1549 as natural dye was evaluated for candle coloring. Figure 5 shows that red pigment extracted in methanol colored successfully candle, confirming its dyeing ability.

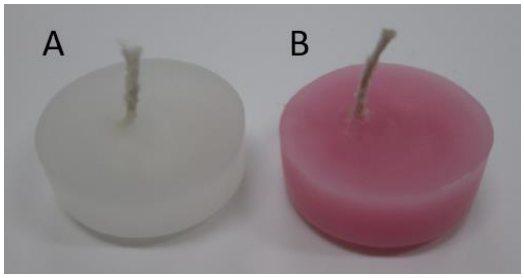


Figure 5. Application of prodigiosin produced by *S. marcescens* UCP 1549 in candle coloring. Control candle (A) and candle colored with methanol extract of prodigiosin (B).

4. Conclusions

Present study showed the suitability of wheat bran for prodigiosin production by *Serratia marcescens* UCP 1549 through solid-state fermentation. The red pigment was identified as prodigiosin by preliminary test and confirmed by UV-Visible spectrophotometry, TLC, HPLC and LC-MS. The

pigment demonstrated promising potential for be used as natural dye for textile and candles, as alternative to synthetic dyes.

Acknowledgments: This work was financially supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) under process No. 311373/2014-3, FACEPE (Fundação de Amparo à Ciência e Tecnologia de Pernambuco) under process No. APQ-0291-2.12/15, and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). The authors are grateful to CAPES for the Ph.D. fellowship and to Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, PE-Brazil, for the use of laboratories.

Author Contributions: D.M.R. and G.M.C.T conceived and designed the experiments; D.M.R., R.F.S.A. and D.R.R. performed the experiments; D.L.C. and A.A.A. supported to application tests and contributed to results analysis; A.A.A., H.W.C.A. and G.M.C.T. contributed reagents/materials; T.C.D.M.S. performed HPLC and LC-MS analyses and contributed to discussion of the results; D.M.R. and R.F.S.A. wrote the paper; H.W.C.A and G.M.C.T contributed to discussion of results and revised the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Venil, C. K.; Zakaria, Z. A.; Ahmad, W. A. Bacterial pigments and their applications. *Proc. Biochem.*, **2013**, *48*, 1065-1079.
2. Ren, Y.; Gong, J.; Fu, R.; Li, Z.; Li, Q.; Zhang, J.; Cheng, X. Dyeing and antibacterial properties of cotton dyed with prodigiosins nanomicelles produced by microbial fermentation. *Dyes Pigm.*, **2017**, *138*, 147-153.
3. Ahmad, W. A.; Ahmad, W. Y. W.; Zakaria, Z. A.; Yusof, N. Z. Application of bacterial pigments as colorant. In *Application of Bacterial Pigments as Colorant*. Springer Berlin Heidelberg Publishers, New York, 2008; pp. 57-74,
4. Panesar, R.; Kaur, S.; Panesar, P. S. Production of microbial pigments utilizing agro-industrial waste: a review. *Curr Opin Food Sci*, **2015**, *1*, 70-76.
5. Kumar, A.; Vishwakarma, H. S.; Singh, J.; Dwivedi, S.; Kumar, M. Microbial pigments: production and their applications in various industries, *Int. j. pharm. chem. biol. Sci.*, **2015,** *5*, 203-212.
6. Rao, M. P. N.; Xiao, M.; Li, W. J. Fungal and bacterial pigments: secondary metabolites with wide applications. *Front. microbiol.*, **2017**, *8*. 1-13.
7. Wang, F.; Luo, H.; Song, G.; Liu, C.; Wang, J.; Xu, J.; Ma, X. Y. Prodigiosin found in *Serratia marcescens* y2 initiates phototoxicity in the cytomembrane. *Electron. J. Biotechnol*., **2013**, *16*, 7-7.
8. Chávez-Castilla, L.; Aguilar, O. Effect of mineral supplementation and type of starch on the production of prodigiosin from a culture of *Serratia marcescens* BS303. *Rev. Mex. Ing. Quím.* **2015**, *14*, 641-652.
9. Samrot, A.V.; Chandana, K.; Senthilkumar, P.; Narendrakumar, G. Optimized production of prodigiosin from *Serratia marcescens* SU-10 grown as batch culture and evaluation of bioactivity of produced prodigiosin. *Int. J. Medicobiol. Research*. **2011**, *1*, 145-150.
10. Wang, S. L.; Wang, C. Y.; Yen, Y. H.; Liang, T. W.; Chen, S. Y.; Chen, C. H. Enhanced production of insecticidal prodigiosin from *Serratia marcescens* TKU011 in media containing squid pen. *Proc. Biochem*., **2012**, *47*, 1684-1690.
11. Darshan, N.; Manonmani, H.K. Prodigiosin and its potential applications. *J. Food Sci. Technol.*, **2015**; 52, 1-15.
12. Papireddy, K.; Smilkstein, M.; Kelly, J.X.; Shweta, S.S.M.; Alhamadsheh, M.; Haynes, S.W.; Challis, G.L.; Reynolds, K. Antimalarial activity of natural and synthetic prodiginines. *J Med Chem*, **2011**, *54*, 5296–306.
13. Sumathi, C.; MohanaPriya, D.; Swarnalatha, S.; Dinesh, M. G.; Sekaran, G. Production of prodigiosin using tannery fleshing and evaluating its pharmacological effects. *The Sci. World J.*, **2014**.
14. Lins, J. C. L.; De Melo, M. E. B.; Do Nascimento, S. C.; Adam, M. L. Differential Genomic Damage in Different Tumor Lines Induced by Prodigiosin. *Anticancer research*, **2015**, *35*, 3325-3332.
15. Suryavanshi, M. V.; Waghmode, S. R.; Bharti, N.; Choudhari, P. B.; Hingamire, T. B.; Shouche, Y. S. Isolation and virtual screening of antimicrobial prodigiosin pigment from oxalotrophic *Serratia marcescens* OX_R strain. *J. App. Pharm. Sci.*, **2016**, *6*, 052-058.
16. Krishna, G. J.; Basheer, S. M.; Elyas, K. K.; Chandrasekaran, M. Prodigiosin from marine bacterium: production, characterization and application as dye in textile industry. *Int J Biotechnol Biochem*, **2011**, *7*, 155-191.

17. Shaikh, Z. Biosynthesis of prodigiosin and its applications. *J. Pharm. Biol. Sci.,* **2016,** *11*, 1-28.
18. Venil, C. K.; Wahidin, M. A. B.; Aruldass, C. A.; Ahmad, W. A. Production of bacterial pigments in low cost medium and formulation of biodegradable ink. *Indian J. Exp. Biol.* ***2017***, *55*, 441-447.
19. Naik, C.; Srisevita, J.M.; Shushma, K.N.; Farah, N.; Shilpa, A.C.; Muttanna, C.D.; Darshan, N.; Sannadurgappa, D. Peanut oil cake: a novel substrate for enhanced cell growth and prodigiosin production from *Serratia marcescens* CF-53. *J. Res. Biol*., **2012**, *2*, 549-557.
20. Aruldass, C.A.; Venil, C.K.; Zakaria, Z.A.; Ahmad, W.A. Brown sugar as a low-cost medium for the production of prodigiosin by locally isolated *Serratia marcescens* UTM1. *Int. Biodet. Biodegrad.* **2014,** *95,* 19-24.
21. Carvalho, J.C.; Cardoso, L.C.; Ghiggi, V.; Woiciechowski, A.L.; Souza, V.L.P.; Soccol, C.R. *Microbial pigments. In: Biotransformation of waste biomass into high value biochemical*. Springer: New York; 2014. pp. 73-97.
22. Thomas, L.; Larroche, C.; Pandey, A. Current developments in solid-state fermentation. *Bioch. Eng. J.,* **2013**, *81*, 146-161.
23. Soccol, C. R.; Da Costa, E. S. F.; Letti, L. A. J.; Karp, S. G.; Woiciechowski, A. L.; De Souza V. L. P. Recent developments and innovations in solid state fermentation. *Biotechn. Res. Innov*., **2017**,
24. El-Bakry, M.; Abraham, J.; Cerda, A.; Barrena, R.; Ponsá, S.; Gea, T.; Sánchez, A. From wastes to high value added products: Novel aspects of SSF in the production of enzymes, *Proc. Bioch*., **2015**, *29*, 109-117.
25. Lizardi-Jiménez, M.A.; Hernández-Martínez, R. Solid state fermentation (SSF): diversity of applications to valorize waste and biomass, *3 Biotech*, **2017**, *7*, 2-9.
26. Singhania, R. R.; Patel, A. K.; Soccol, C. R.; Pandey, A. Recent advances in solid-state fermentation. *Bioch. Eng. J.*, **2009**, *44*, 13-18.
27. Abu, Y. N.; Barrena, R.; Komilis, D.; Sánchez, A. Solid-State Fermentation as a Novel Paradigm for Organic Waste Valorization. *Sustainability*, **2017**, *9*, 1-28.
28. Araújo, H.W.C.; Fukushima, K.; Takaki, G.M.C. Prodigiosin production by *Serratia marcescens* UCP 1549 using renewable-resources as a low cost substrate. *Molecules.* **2010**, *15*, 6931-6940.
29. Lins, J. L.; Maciel, C. C. S.; Xavier, H. S.; Da Silva, C. A.; Campos-Takaki, G. M. Production and toxicological evaluation of Prodigiosin from *Serratia marcescens* UCP/WFCC1549 on Mannitol Solid Medium. *Int. J. App. Res. Nat. Prod*., **2014**, 7, 32-38.
30. Montero-Rodríguez D.; Andrade R.F.S.; Rubio-Ribeaux D.; Lima R.A.; Ribeiro D.L.R.; Silva G.K.B.; Campos-Takaki, G.M. A low-cost solid fermentation medium for potential prodigiosin production by *Serratia marcescens* UCP/WFCC 1549, In *Microbes in the Spotlight: Recent Progress in the Understanding of Beneficial and Harmful Microorganisms*, 2016.
31. Araujo, H.W.C.; Andrade, R.F.; Montero-Rodríguez, D.; Santos,V.P.; Maia, P.C.S.; Costa Filho, C.F.; Silva C.A.A.; Campos-Takaki G.M. Biochemical identification of and molecular newly isolated pigmented bacterium and improved production of biosurfactant, *Afr. J. Microbiol. Res*., **2017**, *11*, 945-954.
32. Camilios-Neto, D.; Bugay, C.; Santana-Filho, A.P.; Joslin, T.; Souza, L.M.; Sassaki, G.L.; Mitchell, D.A,.; Krieger, N. Production of rhamnolipids in solid-state cultivation using a mixture of sugarcane bagasse and corn bran supplemented with glycerol and soybean oil. *App. Microb. Biotechn.* **2011**, *89*, 1395-1403.
33. Nalini, S.; Parthasarathi, R. Production and characterization of rhamnolipids produced by *Serratia rubidaea* SNAU02 under solid-state fermentation and its application as biocontrol agent. *Biores. Techno*., **2014**, *173*, 231-238.
34. Bóna-Lovász, J.; Bóna, A.; Ederer, M.; Sawodny, O.; Ghosh, R. A rapid method for the extraction and analysis of carotenoids and other hydrophobic substances suitable for systems biology studies with photosynthetic bacteria. *Metabolites*, **2013**, *3*, 912-930.
35. Gerber, N. N.; Lechevalier, M. P. Prodiginine (prodigiosin-like) pigments from *Streptomyces* and other aerobic *Actinomycetes*. *Can. J. Microbio.*, **1976**, *22*, 658-667.
36. Sathishkumar, T.; Aparna, H. Anti-fouling activity of prodigiosin, a pigment extracted from *Serratia marcescens*. *Int J Curr Microbiol Appl Sci.*, **2014**, 712-725.
37. Mansi, M.; Gaurav, S. Extraction of pigment from *Serratia marcescens* and its application in candle industry. *Adv. Appl. Res*., **2015**, 7, 1-4.
38. Priya, K.A.; Satheesh, S.; Ashokkumar, B.; Varalakshmi, P.; Selvakumar, G.; Sivakumar, N. Antifouling activity of prodigiosin from estuarine isolate of *Serratia marcescens* CMST 07. In Microbiological research in agroecosystem management (pp. 11-21). Springer India, 2013.

39. Alihosseini, F.; Ju, K.-S.; Lango, J.; Hammock, B.D.; Sun, G. Antibacterial Colorants: Characterization of Prodiginines and Their Applications on Textile Materials. *Biotechnol Prog.*, **2008**, 24, 742–747. http://doi.org/10.1021/bp070481r
40. Borić, M.; Danevčič, T.; Stopar, D. Prodigiosin from *Vibrio* sp. DSM 14379; a new UV-protective pigment. *Microb Ecol.*, **2011**, 62, 528.
41. Lapenda, J.C.; Silva, P.A.; Vicalvi, M.C.; Sena, K.X.F.R.; Nascimento, S.C. Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398. *World J Microbiol Biotechnol*, **2015**, 31, 399-406.
42. Rakh, R.R.; Dalvi, S.M.; Musle, B.B.; Raut, L.S. Production, Extraction and Characterization of Red Pigment Produced by *Serratia rubidaea* JCM 1240T isolated from Soil. *Int. J. Curr. Microbiol. App. Sci*, **2017**, 6, 143-154.
43. Giri, A.V.; Anandkumar, N.; Muthukumaran, G.; Pennathur, G. A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia* marcescens isolated from soil. *BMC Microbiol.*, **2004**, 4, 11.
44. Xu, F.; Xia, S.; Yang, Q. Strategy for obtaining inexpensive prodigiosin production by *Serratia marcescens*. Proceedings of the 3rd International Conference on Chemical, Biological and Environmental Engineering, 2011; Singapore.
45. Arivizhivendhan, K.V.; Mahesh, M.; Regina, M.R.; Sekaran, G. Bioactive prodigiosin isolated from *Serratia marcescens* using solid state fermenter and its bactericidal activity compared with conventional antibiotics. *J Microb Biochem Technol*, **2015**, 7, 305-312.
46. Xia, Y.; Wang, G.; Lin, X.; Song, X.; Ai, L. Solid-state fermentation with *Serratia marcescens* Xd-1 enhanced production of prodigiosin by using bagasse as an inertia matrix. *Ann Microbiol*, **2016**, 1-9.
47. Faraag, A.H.; El-Batal, A.I.; El-Hendawy, H.H. Characterization of prodigiosin produced by *Serratia marcescens* strain isolated from irrigation water in Egypt. *Nat Sci*, **2017**, 15, 55-68.
48. Song, M.J.; Bae, J.; Lee, D.S.; Kim, C.H.; Kim, J.S.; Kim, S.W.; Hong, S.I. Purification and characterization of prodigiosin produced by integrated bioreactor from *Serratia* sp. KH-95. *J Biosci Bioeng.*, **2006**, 101, 157-161.
49. Siva, R.; Subha, K.; Bhakta, D.; Ghosh, A.R.; Babu, S. Characterization and enhanced production of prodigiosin from the spoiled coconut. *Appl Biochem Biotechnol*, **2012**, 166, 187-196.
50. Fei, Y.A.N.G.; Wei, H.Y.; Li, X.Q.; Li, Y.H.; Li, X.B.; Yin, L.H.; Pu, Y.P. Isolation and characterization of an algicidal bacterium indigenous to Lake Taihu with a red pigment able to lyse *Microcystis aeruginosa*. *Biomed Environ Sci*, **2013**, 26, 148-154.
51. Yusuf, M.; Shabbir, M.; Mohammad, F. Natural Colorants: Historical, Processing and Sustainable Prospects. *Nat Prod Bioprospect*, **2017**, 1-23.
52. El-Bialy, H.A.; El-Nour, S.A.A. Physical and chemical stress on *Serratia marcescens* and studies on prodigiosin pigment production. *Ann Microbiol*, **2015**, 65, 59-68.

CAPÍTULO 5

ARTIGO IV

Low-cost coproduction of prodigiosin and biosurfactant by *Serratia marcescens* UCP 1549 and promising applications of both biomolecules

---

[*] Manuscrito submetido para publicação ao periódico: **International Journal of Molecular Sciences**

Article

Low-cost coproduction of prodigiosin and biosurfactant by *Serratia marcescens* UCP 1549 and promising applications of both biomolecules

Dayana Montero-Rodríguez [1,4], Rosileide F. S. Andrade [4] Daylin Rubio-Ribeaux [1,4], Davi L. Cavalcanti [1,4], Amanda A. Alencar [2,4], Marcos A. C. Luna [2,4], Hélvia W. C. Araújo [3] and Galba M. Campos-Takaki [4,*]

[1] Center of Biosciences, Federal University of Pernambuco, 50670-420, Recife, Pernambuco, Brazil; E-mails: dayanamontero87@gmail.com (D.M.R.); drubioribeaux@gmail.com (D.R.R.); davicavalcanti25@gmail.com (D.L.C.);

[2] Northeast Network for Biotechnology Post-graduation Program, Federal Rural University of Pernambuco, 52171-900, Recife, Pernambuco, Brazil; E-mail: amandaalencar@yahoo.com.br (A.A.A.); macluna@bol.com.br (M.A.C.L.)

[3] Chemistry Department, State University of Paraíba, 58429-500, Campina Grande, Paraíba, Brazil; E-mail: hwcasullo@hotmail.com (H.W.C.A).

[4] Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, 50050-590, Recife, Pernambuco, Brazil; E-mails: rosileide_fontenele@yahoo.com.br (R.F.S.A); galba_takaki@yahoo.com.br (G.M.C.T.)

* Correspondence: galba_takaki@yahoo.com.br ; Tel.: + 55-81-2119-4017

Academic Editor: name
Received: date; Accepted: date; Published: date

Abstract: This study focused the simultaneous production of prodigiosin and biosurfactant by *Serratia marcescens* UCP 1549, in order to obtain these industrially relevant biocompounds by inexpensive process. For this, six agro-industrial substrates were tested for formulation of low-cost media and corn bran showed better results for coproduction of both biomolecules after 72 h. Further fermentation were carried out and the red pigment was extracted and identified as prodigiosin by maximum absorbance at 535 nm and R*f* of 0.9 in TLC. The biosurfactant produced was extracted in ethanol (2.15 g/l) and was characterized as anionic compound, able to reduce surface (29.3 mN/m) and interfacial (9.4 mN/m) tension. The biosurfactant showed stability in a wide range of pH, temperature and NaCl concentration, suggesting its potential use for environmental or industrial processes in extreme conditions. Also, promising application of prodigiosin as natural dye of textile and candles and the biosurfactant as bioemulsifier, dispersant and additive to increase the viscosity of vegetable oils-based lubricants, were demonstrated.

Keywords: *Serratia marcescens*, simultaneous production, prodigiosin, biosurfactant, applications

1. Introduction

Serratia marcescens is a Gram negative bacterium, classified in the large family of Enterobacteriaceae [1,2]. This microorganism have been extensively investigated because of its ability to produce the red pigment prodigiosin as secondary metabolite. Prodigiosin is an alkaloid with a unique tripyrrole chemical structure that possesses a wide variety of biological properties, including antimicrobial, antimalarial, immunosuppressive and cytotoxic activities [3-5]. The potential use of prodigiosin as powerful pharmaceutical candidate have been studied by several researchers, as well as its application as a multifunctional biocolorant in textile, food, and cosmetic industries [6,7].

Another industrially relevant metabolites produced by *S. marcescens* are the biosurfactants [8-12]. They are amphipathic molecules with ability to display a variety of surface activities that, among other roles, help solubilizing hydrophobic substrates and are the subject of intense investigation [13-15]. One of the major potential applications is their use as replacements for synthetic surfactants in many existing and future industrial and environmental applications [16,17]. Diverse functional properties, such as emulsification, wetting, foaming, cleansing, phase separation, surface activity and reduction in viscosity of crude oil, makes it feasible to utilize them for numerous environmental, food, pharmaceutical, medical, cleaning and other industrial application purposes [16-19].

Despite of their promising industrial applications, the large-scale production of prodigiosin and biosurfactants is limited due to their high production cost, mainly caused by the expensive conventional growth media [20,21]. Cheap agro-industrial byproducts and residues have been used as alternative substrates to guarantee a profitable process of pigment and biosurfactant production [22-24].

There are few studies that focus on the simultaneous production of microbial pigments and surfactants [8,25,26]. This would be an additional strategy for reducing the relative high production costs of both biomolecules and improving large-scale processes. Thus, this research aimed to the simultaneous production of prodigiosin and biosurfactant by *Serratia marcescens* UCP 1549 using low-cost substrates. Also, applications of both metabolites were investigated in order to propose them as suitable alternative for their synthetic counterparts.

2. Results and Discussion

2.1. Screening of agro-industrial substrates for simultaneous production of prodigiosin and biosurfactant

S. marcescens UCP 1549 is a bacterium with demonstrated ability to produce biosurfactant using different agro-industrial substrates [27-30], and also, it is a good producer of prodigiosin [5,22,31]. However, there are not studies involving this strain in concomitant production of these compounds, which is a limited feature for some microorganism.

In present study, the growth of S. *marcescens* was accompanied by measurement of absorbance at 600 nm of culture broths after 72 h of fermentation. The relative concentration of prodigiosin produced was evaluated by measurement of absorbance at 535 nm of ethanol extracts of pellets [32,33]. The results are shown in Table 1 and demonstrated that all production media were able to support the growth of *S. marcescens*. Only the medium containing SCB showed a little value of absorbance, suggesting that this substrate requires a treatment in order to transform the complex polysaccharides into simple sugars that can be assimilated by the microorganisms (Karp et al., 2013). Results in Table 2 exhibited the elemental composition of agro-industrial substrates used for formulation of production media.

Table 1. Growth, prodigiosin production and surface tension values obtained by *Serratia marcescens* UCP 1549 after fermentation in media containing agro-industrial residues for 72 h.

Production media containing agro-industrial substrates	Growth ($Abs_{600\ nm}$)	Prodigiosin ($Abs_{535\ nm}$)	Surface tension (mN/m)
Sugarcane bagasse	0.171	0.054	41.2
Wheat bran	0.877	0.066	40.4
Corn bran	0.902	0.490	27.9
Pineapple peel	1.013	0.063	38.7
Pineapple crown	1.023	0.101	42.5
Tangerine peel	0.925	0.099	36.9

Table 2. Elemental composition of agro-industrial substrates used for formulation of culture media for simultaneous production of prodigiosin and biosurfactant by *Serratia marcescens* UCP 1549.

Residues	Elemental composition (%)			
	Carbon	Hydrogen	Nitrogen	Sulfur
Sugarcane bagasse	37.28	4.76	0.59	0.08
Wheat bran	38.27	7.50	2.45	0.18
Corn bran	41.76	7.61	1.91	0.13
Pineapple peel	34.48	7.75	0.91	0.10
Pineapple crown	37.52	6.80	1.35	0.05
Tangerine peel	34.68	7.52	1.27	0.10

In contrast, the maximum production of prodigiosin was detected in medium containing CB, followed by PAC and TP. Not significant production of prodigiosin was exhibited for SCB, WB and PAP. Giri et al. (2004) and Araujo et al. (2010) also tested various agro-industrial substrates for pigment production by *S. marcescens* with different prodigiosin yield between them. From the results obtained here, it is possible to deduce that the most probable reason for enhanced bacterial growth and/or lower pigment production is related to the carbon sources and the final pH of the medium.

Prodigiosin is a secondary metabolite appears only in the later stages of the bacterial growth, and its production can be affected by several environmental factors, inorganic phosphate availability, media composition, temperature and pH (Harris et al., 2004; Williamson et al., 2005; Darshan and Manonmani, 2015). Production media composed of essential nutrients and an adequate carbon source, such as the mannitol medium and peptone–glycerol medium, favor the synthesis of prodigiosin (Abdullateef et al. 2012). According to Siva et al. (2012), alternative media composed of peanut flour, sesame seeds and corn, soybean or coconut oil are excellent substrates for the synthesis of prodigiosin. In present study, maximum production of prodigiosin was observed in medium containing CB, indicating its suitability as low-cost and available substrate to obtain this pigment.

Ethanol extract of pigment produced by *S. marcescens* UCP 1549 was submitted to UV-visible spectrophotometry in the range of 400-700 nm and maximum peak was exhibited at 535 nm, characteristic of prodigiosin, in according to Krishna et al. (2011), Vaidyanathan et al. (2012) and Andreyeva and Ogorodnikova (2015).

Additionally, the production of biosurfactant in agro-industrial media was evaluated by measuring the surface tension of cell-free metabolic liquids. Surface tension which is an important parameter for biosurfactants effectiveness measures the force of attraction between the molecules of liquids, which significantly decreases when the surfactant concentration in an aqueous medium is increased and micelles are formed (Satpute et al., 2010). As shown in Table 1 the medium containing CB exhibited better results in production of biosurfactant, with decrease of surface tension to 27.9 mN/m. Thus, this medium was selected for further fermentation, in order to obtain enough volume of culture broth to isolate and characterize both prodigiosin and biosurfactant.

2.2 Isolation and characterization of prodigiosin and biosurfactant

2.2.1. Identification of prodigiosin

The red pigment extracted from *S. marcescens* biomass was subjected to TLC and the Rf (0.90) corresponding to prodigiosin, in according to results obtained by Araújo et al. (2010), Priya et al.

(2013) and Phatake and Dharmadhikari (2016). In addition, the maximum UV absorbance at 535 nm confirmed the presence of prodigiosin as active compound (Giri et al., 2004; Khanafari, 2006; Siva et al., 2012) (Figure 1).

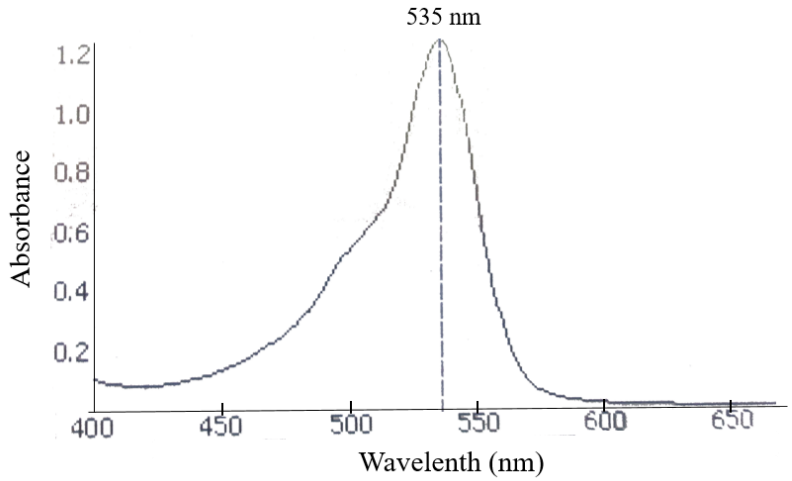


Figure 1. Absorbance spectrum of red pigment produced by *Serratia marcescens* UCP 1549 in salt medium containing waste soybean oil and extracted in acidified ethanol.

2.2.2 Analysis of biosurfactant

- Surface and interfacial tension

The ability to reduce the surface and interfacial tension is a key parameter for detecting the production of surface-active compounds (Andrade Silva et al., 2014; Marchant and Banat, 2017).

Corn bran showed suitability for production of surface-active compound in screening of agro-industrial substrates, with reduction of surface tension to 27.9 mN/m, in 100 ml of production medium. A further fermentation was carried out in 300 ml of medium to obtain higher volume of culture broth for biosurfactant analysis. The surface tension of the medium showed a slight increase (29.3 mN/m) but was still considered efficient, considering that Mulligan (2005) and Mesbaiah et al. (2016) affirm that good biosurfactants are capable of reducing surface tension to values below 30 mN/m. *S. marcescens* UCP 1549 have been previously studied due to its ability to produce efficient biosurfactant using low-cost substrates, such as cassava wastewater (Montero-Rodriguez et al. 2014; 2015; Araujo et al., 2017), sugarcane bagasse (Figuereido et al., 2015) and pineapple peels (Teixeira et al., 2017).

Also, the biosurfactant produced showed a reduction of interfacial tension to 9.4 mN/m with n-hexadecane. Similar results were obtained by Aguiar et al. (2010), who informed that the chemical surfactant Sodium Dodecyl Sulfate (SDS) was able to reduce interfacial tension from 52 to 10 mN/m in an oil/water system. Thus, the biosurfactant of *S. marcescens* demonstrates similar action in the reduction of tension between biphasic systems when compared to SDS, which is the most used in the formulation of industrial products, and could be used as an eco-friendly substitute.

- Stability studies

The suitability of biosurfactants for application in diverse industrial areas depends on its stability against extreme or varying conditions of temperature, pH and salinity (Jain et al., 2013). Figure 2 illustrates the effects of temperature, pH, and NaCl concentration on the surface tension of the biosurfactant produced by S. *marcescens* UCP 1549.

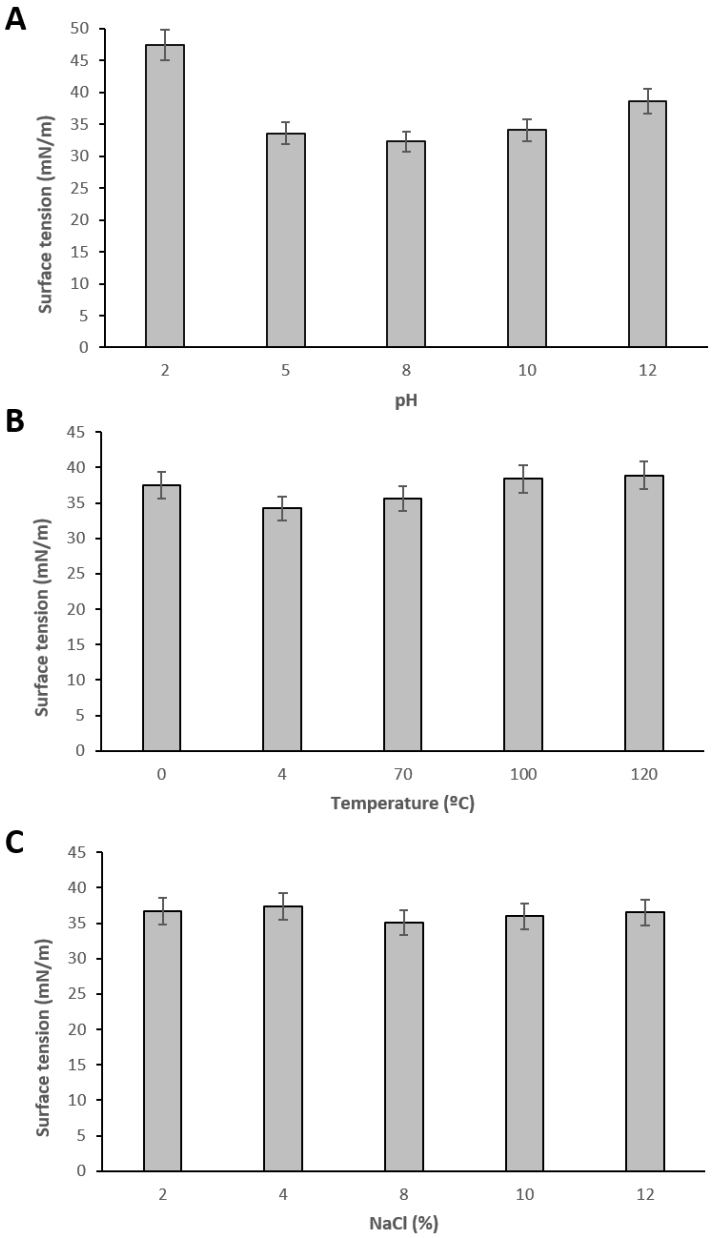


Figure 2. Stability of surface tension of biosurfactant produced by *Serratia marcescens* UCP 1549 using corn bran. Influence of pH (A); temperature (B); and sodium chloride concentrations (C) on surface tension stability.

The results demonstrated that the biosurfactant produced by *S. marcescens* was stable on alkaline or neutral conditions, but the surface tension was strongly affected by decreasing the pH value from basic to an acidic region. This result may be due to partial precipitation of the biosurfactant (Abouseoud et al., 2008; Khopade et al., 2012). In contrast, the surface tension remained practically uniform at all values of temperature and NaCl concentration, indicating that these parameters had no appreciable effects on the activity of the biosurfactant.

Previously, *S. marcescens* UCP 1549 showed similar results for biosurfactant produced in medium containing cassava wastewater and waste soybean oil (Montero-Rodríguez et al., 2016). Biosurfactant produced by other *S. marcescens* strains have also displayed stability in a wide range of pH, temperature and salinity, make them potential candidates for environmental or industrial processes in extreme conditions (Anyanwu et al., 2011; Ibrahim et al., 2013).

- Biosurfactant isolation and yield

For many biotechnological products, the downstream processing costs account for 70%–80% of the total production costs (Santos et al., 2016). Several conventional methods for the recovery of biosurfactants, such as acid precipitation, solvent extraction, ammonium sulphate precipitation and centrifugation and foam fractionation have been widely reported (Mukherjee et al., 2006; Shah et al., 2016). Often a single downstream processing technique is not enough for product recovery and purification. In such cases, a multistep recovery strategy, using a sequence of concentration and purification steps, is more effective in such a multi-step recovery for biosurfactants; it will be possible to obtain the product at any required degree of purity (Baker and Chen, 2010; Karadi et al., 2014).

In this study, three methods of recovery of biosurfactant were tested (See Biosurfactant isolation in Materials and methods section). Ethanol extraction obtained better biosurfactant yield (2.62 g/L) followed by the combination of both methods (2.15 g/L). Little amount of the biosurfactant was recovered by acid precipitation (0.544 g/L). Despite ethanol extracted higher amount of the biosurfactant, the obtained compound exhibited red coloration suggesting that prodigiosin was also extracted. In contrast, acid precipitation followed of ethanol extraction recovered a brown compound and the pigment was observed in supernatant after centrifugation. Then, last method was considered as the most effective for biosurfactant isolation without pigment.

- Ionic charge

The zeta potential determines the ionic charge of the particle, which serves to predict and control the stability of colloidal suspensions and emulsions. Higher values of zeta potential indicate good stability of the suspension, due to the repulsion between hydrophilic particles, according to the literature (Satpute et al., 2010; Andrade Silva et al., 2014).

According to the analyses using a Zeta Potential Meta 3.0+, the biosurfactant produced by *S. marcescens* showed an anionic character (-23.5 mV). Other biosurfactants produced by *Serratia* sp. also display an anionic character (Pruthi and Cameotra, 2000; Nalini and Parthasarathi, 2013; 2014). The anionic surfactants are extensively used in household cleaners and cosmetics (Karray et al., 2016; Lima et al., 2017).

2.3. Applications of prodigiosin and biosurfactant produced by S. marcescens UCP 1549

There is an increased demand for natural dyes and surfactants because of harmful effects of some of their synthetic analogues (Venil et al., 2013; Santos et al., 2016; Rao et al., 2017). Microbial pigments and biosurfactants provide eco-friendly alternatives due to their short production cycle, independence from weather conditions and high productivity. Therefore, the production of these natural compounds is now one of the emerging fields of research to show its potential for application in diverse areas, such as the food, pharmaceutical, cosmetic and textile industries (Ahmad et al., 2012; Campos et al., 2013; Tuli et al., 2015; Bhattacharya et al., 2017).

2.3.1. Application of prodigiosin as natural dye

- Textile dye

In present study, it was evaluated the potential of application as cloths dye of prodigiosin produced by *S. marcescens* UCP 1549, either in culture broth or in methanol extract. As shown in Figure 3, culture broth containing prodigiosin was more efficient dyeing the textile materials when compared with methanol extract (Figure 4). It is important to consider that the use of the culture broth represents a considerable reduction in cost of pigment application, due to avoid recovery-related costs, as well as the risks of solvents extraction.

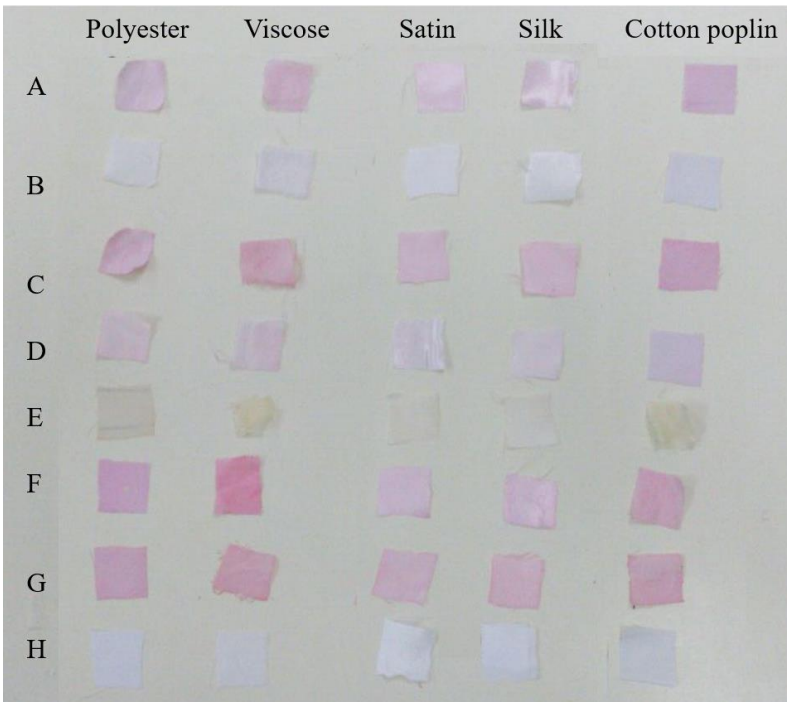


Figure 3. Application of culture broth containing prodigiosin produced by *Serratia marcescens* UCP 1549 in coloring textile materials. Dyed cloths after treatment with hot water (A), hot water + detergent (B), cold water (C), cold water + detergent (D), 10% NaOH (E), 10% HCl (F) and without treatment (G). Cloths without dye were taken as controls (H).

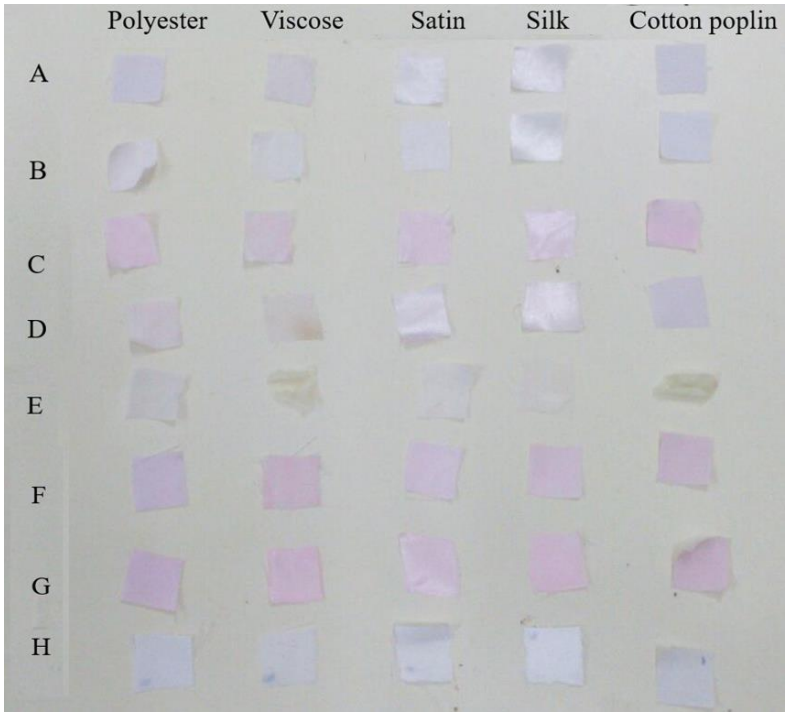


Figure 4. Application of methanol extract of prodigiosin produced by *Serratia marcescens* UCP 1549 in coloring textile materials. Dyed cloths after treatment with hot water (A), hot water + detergent (B), cold water (C), cold water + detergent (D), 10% NaOH (E), 10% HCl (F) and without treatment (G). Cloths without dye were taken as controls (H).

Prodigiosin contained in culture broth has the ability to color pink all cloths tested, but they visually exhibited different degrees of dyeing, in the following order: viscose>cotton poplin>polyester>silk>satin, (Figure 3), evidencing that dyeing performance depending of the type of fiber (Venil et al., 2013). Different levels of dyeing were also described by El-Bialy and El-Nour (2015) after coloring different textiles (cotton, nylon and polyester) as well as for materials used for medical purpose (rubber and muslin). Earlier, Ahmad et al. (2012) used culture broth of *S. marcescens* to color five types of fabric namely acrylic, polyester microfiber, polyester, silk and cotton and the pigment was able to dye both the natural and synthetic fibers. However, different color intensity was obtained due to the nature of each fiber, which requires certain types of dye materials to produce intense coloration (Vickerstaff, 1954)

In relation to the effects of wash performance in dye retention, it was observed that pink color was quite retained for all textile in cases of acid and cold water treatments, and it was even intensified in case of viscose after acid treatment (10% HCl) (Figure 4). Alkali treatment (10% NaOH) had a bad impact in the dyeing process for all studied materials and particularly for viscose and cotton poplin, it severely dandified the cloths to the point of disintegrating the fibers. A small amount of discoloration resulted after treatment with hot water and the addition of commercial detergent, on either cold or hot water, resulted in the decrease of prodigiosin dyeing. Similar results were obtained by Gulani et al. (2012) and El-Bialy and El-Nour (2015), who suggested that the discoloration accompanying detergents can be controlled by adding suitable mordant or stabilizer during the dyeing process.

In case of dyeing experiments using methanol extract of prodigiosin, it was able of dye slightly all the cloths tested (Figure 4). Acid and cold water treatment little affected the intensity of the coloration, mainly for cotton poplin and viscose, but the other treatments resulted in intense discoloration.

- Candle dye

The potential of prodigiosin produced by *S. marcescens* UCP 1549 as natural dye was also evaluated for candle coloring. Figure 5 shows that the red pigment colored successfully candles, either in culture broth or in methanol extract, confirming its dyeing ability. However, as occurred with cloths, culture broth containing prodigiosin was more efficient dyeing candle than red pigment extracted in methanol, which results in avoiding the expensive processes of extraction and purification of the pigment.

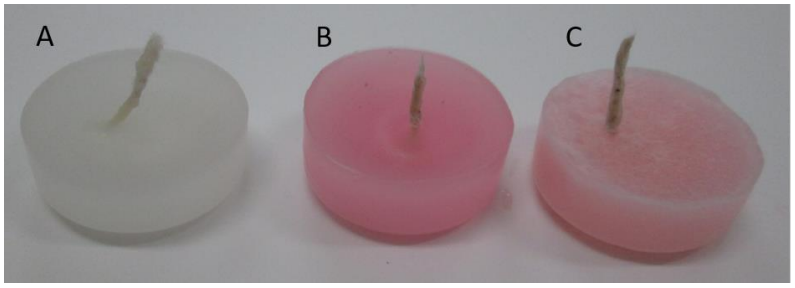


Figure 5. Application of prodigiosin produced by *Serratia marcescens* UCP 1549 in candle coloring. Control candle (A), candle colored with culture broth containing prodigiosin (B) and candle colored with methanol extract of prodigiosin (C).

Mansi and Gaurav (2015) extracted prodigiosin pigment from *S. marcescens* and applied for dyeing candles, obtaining a similar coloration to the coloured candles available in the market. These researchers suggested that the synthetic dyes could be replaced by natural pigments and applied in candle industry.

2.3.2. Applications of biosurfactant

- Bioemulsifier

One of the most important characteristics to be considered in a biological surface-active compound is its ability to form stable emulsions (Falode et al., 2017). Figure 6 presents the EI_{24} (%) of cell-free metabolic liquid containing the biosurfactant produced by *S. marcescens* UCP 1549, against different hydrophobic substrates. The results revealed that waste soybean oil was particularly a good substrate for emulsification after 24 h (91.67%), followed by kerosene (50%), olive oil (45.46%) and burned motor oil (45%). However, the biosurfactant was less efficient to emulsify vegetable oils (soybean, corn and canola oils), n-hexadecane and diesel. These evidences suggest that the ability to emulsify hydrophobic compounds depends on the type of substrate with which microbial surfactants may interact. This fact is related to the biosurfactant conformational structure and the hydrocarbon, which will allows the stabilization or not of the microscopic droplets (Silva et al., 2014).

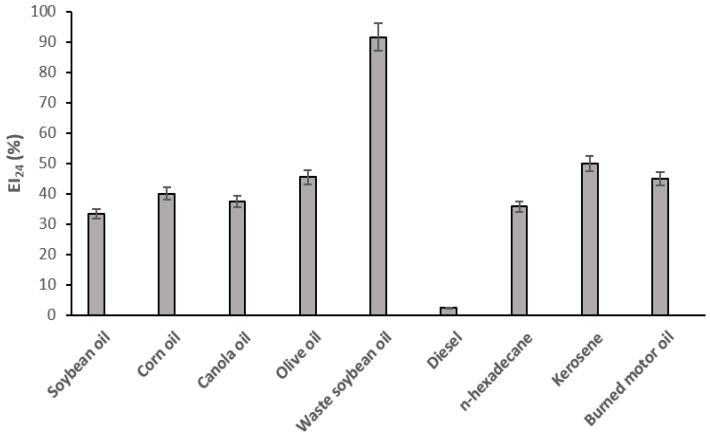


Figure 6. Emulsification index (%EI_{24}) of the biosurfactant produced by *Serratia marcescens* UCP 1549 against different hydrophobic substrates.

In contrast, Rita de Cássia et al. (2017) tested the ability of a biosurfactant produced by *P. cepacia* CCT6659 in low-cost medium using several hydrocarbon and vegetable oil for emulsion activity. These authors observed was able to emulsify 90% motor oil compared to vegetables oil, which not showed a suitable emulsification. In addition, Morales-Guzmán et al. (2017) evaluated bacterial populations from uncontaminated soils or soils contaminated and their emulsifying and diesel-degrading properties. In this study, among the isolates, the strain identified as *S. marcescens* C7S3A exhibited the highest diesel emulsification (74.2%). Amiriyan et al. (2004) suggested that emulsifier activity depends on the affinity of bioemulsifier for hydrocarbon substrates, which involves a direct interaction with itself rather than an effect on surface tension of the medium.

Biosurfactants exhibit ideal properties for incorporation in detergent formulations, such as reduction in surface tension and their emulsification potential, especially edible oil emulsification (Sharma et al., 2016). Bafghi and Fazaelipoor (2012) studied the application of rhamnolipid in the formulation of a detergent and showed that the biosurfactant was effective in oil removal from the samples and the formulation presented was comparable to commercial powders in terms of the stain removal. Similarly, biosurfactants produced by *P. aeruginosa* and *Yarrowia lipolytica* were successfully tested as biodetergents (Barbosa et al., 2013).

Furthermore, biosurfactants are emulsion stabilizing agents, incorporated in food products to maintain the consistency, texture, solubilisation of fat globules, improve aroma, foaming and dispersing properties (Campos et al., 2013). Bioemulsifier is a natural food ingredient, which can be used to enhance the rheology of dough, increase the volume and emulsification of fat, thus finds further applications in bakery and meat processing industry (Kourkoutas et al., 2004). Shepherd et al. (1995) showed that bioemulsifier from *Candida utilis* has potential for use in salad cream as well as

Campos et al. (2015) employed the biosurfactant produced by *C. utilis* UFPEDA 1009 in the formulation of a mayonnaise. Recently, Kiran et al. (2017) demonstrated that incorporation of bioemulsifier from *Nesterenkonia* sp. in muffin preparation shows improved softness and its organoleptic quality.

Also, the emulsifier function is probably the most important property of biosurfactants in the formulation of cosmetics, because emulsions have considerable advantages over other types of preparations (Vecino et al., 2017). Cosmetic and personal care products including toothpaste, shampoo, creams, makeup, among others, are usually formulated with petroleum-based surfactants, although in the last years the consume trend for "green" products is inducing the replacement of surface-active agents in these formulations by natural surfactants (Lukic et al., 2016; Bhattacharya et al., 2017). In addition to their surfactant capacity, many biosurfactants can act as good emulsifiers, which is an extra advantage in the preparation of green cosmetic products (Vecino et al., 2017). Ferreira et al. (2017) used a biosurfactant produced by *Lactobacillus paracasei* as a stabilizing agent in oil-in-water emulsions containing essential oils and natural antioxidant extract.

Considering the increased use of bioemulsifiers in several industries, identification of new compounds with low or no toxicity and high emulsifying property has become essential (Kiran et al., 2017).

- Viscosity modifier

The viscosity of hydrophobic substrates was determined before and after the addition of solution of biosurfactant produced by *S. marcescens,* in order to investigate its influence in this parameter. The results shown in Table 3 evidenced a significant increase of the viscosity in all substrates tested after the addition of the biosurfactant.

Table 3. Influence of the biosurfactant produced by *Serratia marcescens* UCP 1549 in the viscosity of hydrophobic substrates.

Hydrophobic substrates	Viscosity before addition of biosurfactant (cP)	Viscosity after addition of biosurfactant (cP)
Soybean oil	56.1	406.0
Corn oil	23.5	332.3
Canola oil	62.9	276.0
Waste soybean oil	50.3	535.7
Burned motor oil	170.0	581.7

Andrade Silva et al. (2014) also reported the increase of the viscosity of soybean (from 472.8 to 970.1 cP) and corn (from 47.6 to 404.0 cP) oils, after adding biosurfactant produced by *Cunninghamella echinulata*. Vegetable oils have some excellent properties for their potential use as lubricants, but some inconveniences should be technologically improved, i.e. limited range of viscosities available. Consequently, environmental friendly viscosity modifiers should be included in the lubricant formulation (Quinchia et al., 2010; García-Zapateiro et al., 2013). Therefore, the biosurfactant produced by *S. marcescens* could be used as an additive to increase the viscosity of vegetable oil-based lubricants, which are being actively demanded for many green industrial activities (McNutt, 2016).

In relation to the viscosity of waste soybean and burned motor oil, similar results were obtained by Andrade Silva et al. (2014), who observed viscosity increased for these substrates, from 380.1 to 556.3 cP and 148.9 to 210.7, respectively. Earlier study performed by Jara et al. (2013) using biosurfactant produced by *Geobacillus stearothermophilus* UCP 986 reported an increase of viscosity of vegetable fat post-frying, from 51.3 to 122.2 cP, and engine burning oil from 170 cP to 423.6 cP. These authors considered that non-ionic properties of the biosurfactant were donors of consistence, and consequently, the viscosity of these hydrophobic substrates was increased.

- Dispersing agent

Dispersing agents are mixed as additives to various products in order to form a uniform mixture and prevent from setting and clumping. They are formed by chemical surfactants and solvents and are extensively used in diverse industrial applications such as paints & coatings, oil & gas, construction, pharmaceuticals, pulp & paper, agricultural, detergents, and others (Song et al., 2013). However, chemical dispersant usage may cause some degree of environmental harm due to toxicity and non-biodegradability of some compound of its formulation (Kleindienst et al., 2015; Lv et al., 2016). Therefore, there is a growing demand for environmentally friendly and cost-effective dispersants and biosurfactants are considered a candidate for such dispersant formulations (Song et al., 2013; Rongsayamanont et al., 2017).

In this study, oil displacement test was carried out to investigate the dispersing ability against burned motor oil of the biosurfactant produced by *S. marcescens* UCP 1549. As shown in Figure 7, the biosurfactant produced demonstrated good dispersant capacity (70%), with the formation of the halo with 63 mm diameter. This result was better than obtained by others *S. marcescens* biosurfactants, which showed clear zones with diameter between 11-56 mm (Roldan Carrillo et al., 2011; Ibrahim et al., 2013; Elemba et al., 2015). In our previous works, biosurfactants produced by *S. marcescens* UCP 1549 with dispersion rates of 72-78% (Montero-Rodríguez et al., 2015; Figuereido et al., 2015; Teixeira et al., 2017), confirming its potential application as dispersant agent in oil spills or industrial formulations.

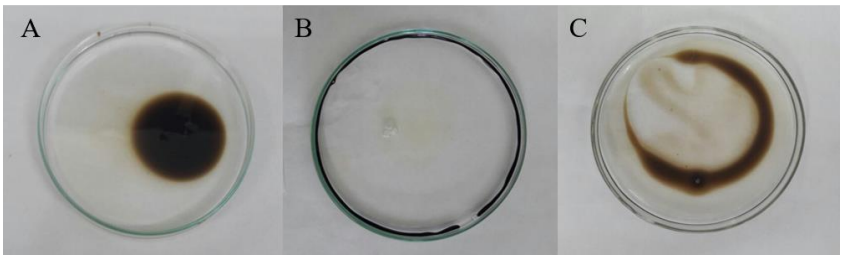


Figure 7. Oil displacement activity of crude biosurfactant produced by *S. marcescens* UCP 1549. Burned motor oil droplet without application of surfactant (A) and after application of commercial detergent (B) and crude biosurfactant produced by *S. marcescens* (C).

3. Materials and Methods

3.1. Microorganism

S. marcescens UCP 1549, originally isolated from the semi-arid soil of the state of Pernambuco, Brazil, and identified by Araujo et al. (2017), was kindly provided by the Culture Collection of the Catholic University of Pernambuco, Recife, Brazil. The bacterium was maintained in Luria Bertani (LB) solid medium (tryptone 10 g/L, yeast extract 5 g/L, NaCl 10 g/L and agar 15 g/L) at 5°C. Stored cultures were transferred first to LB medium and incubated for 18 h at 28ºC. Then, two colonies were transferred to 50 mL of LB broth and incubated at 28ºC and 150 rpm in an orbital shaker. Once the optical density at 600 nm reached 0.8-1.0, this culture was used as inoculum.

3.2. Screening of agro-industrial substrates for simultaneous production of prodigiosin and biosurfactant

3.2.1. Substrates

Six different agro-industrial substrates were used in formulation of culture media for simultaneous production of prodigiosin and biosurfactant: sugarcane bagasse (SCB), wheat bran (WB), corn bran (CB), pineapple peels (PAP), pineapple crown (PAC) and tangerine peels (TP). SCB

was kindly donated by Usina Japungu, Santa Rita (Paraíba, Brazil) and WB, CB, pineapples and tangerines were bought at a local market in city of Recife (Pernambuco, Brazil). WB and CB did not receive any kind of pretreatment. SCB was maintained at -4°C until it use, then it was thawed at room temperature, oven-dried at 70°C for 24 h and ground in a blender. Pineapples and tangerines were washed and the wastes were separated from the edible pulps, oven dried at 70°C for 72 h and ground in a blender. Then, all substrates were sieved separately in order to obtain a homogeneous powder which facilitated dissolution in the culture medium. The fraction used was either that retained between 16- and 32- mesh sieves (opening of 1.0 and 0.5 mm, respectively).

Elemental analysis (C, H, N and S) was carried out at Perkin-Elmer Series II2400 CHNS/O elemental analyzer to determine the carbon, nitrogen, hydrogen and sulfur present in one gram of each substrates as was used to formulation of production media.

3.2.2. Submerged fermentation using agro-industrial substrates

Erlenmeyer flasks containing one gram of the respective substrate were completed to 100 ml of the salt solution used previously by Montero-Rodríguez et al. (2016). Then, they were autoclaved at 121°C for 15 min, inoculated (5%, v/v) and incubated at 28ºC and 150 rpm in an orbital shaker for 72 h.

3.2.3 Quantification of prodigiosin

At the end of the fermentation, the bacterial cell absorbance of the culture broth was measured at 620 nm in a Libra S32 UV/Visible Spectrophotometer (Biochrom Ltd). The relative concentration of prodigiosin produced by liquid grown cultures was quantified as follows: 1 ml sample was harvested by centrifugation at 10000 rpm for 10 min. The supernatant was discarded and the pellet resuspended in acidified ethanol (4% 1 M HCl in ethanol 95%) to extract prodigiosin from the cells. Cell debris was removed by a second centrifugation step and the supernatant transferred to a cuvette for measurement of absorbance at 535 nm (Slater et al., 2003).

The ethanol extracts of pellets containing pigment were analyzed by scanning in a Libra S32 UV/Visible Spectrophotometer (Biochrom Ltd), for detecting the maximum absorbance (λ_{max}). The scanning range selected was 400-600 nm and absorbance at the λ_{max} was measured.

3.2.4 Measurement of surface tension

In order to investigate the production of biosurfactant in culture media, the surface tension was determined on cell-free metabolic liquids obtained by centrifugation and subsequent filtration of cultures, using a tensiometer model Sigma 70 (KSV Instruments Ltd., Finland) by the Du Nouy ring method at room temperature (± 28°C). Measurements of surface tension from distilled water were used as control (Kuyukina et al., 2001).

3.3. Isolation and characterization of prodigiosin and biosurfactant

3.3.1 Submerged fermentation

Culture medium with better results for both prodigiosin and biosurfactant production was selected for further fermentation, in other to obtain enough volume of culture broth to isolate and characterize these biomolecules. Fermentation was carried out in triplicate in Erlenmeyer flasks containing 300 ml of culture medium, inoculated (5%, v/v) and incubated at 28ºC and 150 rpm in an orbital shaker for 72 h.

At the end of fermentation, the contents of Erlenmeyer were centrifuged for 15 min at 10000 rpm and the pellet and supernatant were separated and used in the analysis described below.

3.3.2 Extraction and identification of prodigiosin

The pigment produced was extracted from the pellet using the method proposed by Bóna-Lovász et al. [34] with modifications. Briefly, 1 ml of methanol and 2 ml of hexane were added to pellet, and

the mixture was vortexed for 15 min. Subsequent phase separation was achieved by the addition of 1 ml of water to the mixture, followed by vortexing for 15 min, and centrifugation for 15 min at 10000 rpm and 10ºC. The upper supernatant phase was filtered with PES membranes with a pore size of 0.45 μm (syringe filters - K18-430, Kasvi, PR, Brazil) and then, the methanol extract of pigment was submitted to thin layer chromatography (TLC). The sample was applied to an aluminium foil sheet covered with silica gel and placed in glass cube containing the mixture chloroform–methanol (9:1, v/v) as mobile phase [28,38] (Araújo et al., 2010; Priya et al., 2013). The retention factor (R*f*) was calculated according to the formula R*f*: distance travelled by the compound/ distance travelled by the solvent front [Krishna] and then, it was compared to the standard prodigiosin R*f* referred in the literature.

The pigment were resuspended in ethanol 95% and analysed by scanning in a Libra S32 UV/Visible Spectrophotometer (Biochrom Ltd) for detecting the maximum absorbance (λmax). The scanning range selected was 400-600 nm and absorbance at the λmax was measured.

3.3.3. Analysis of biosurfactant

- Determination of surface and interfacial tension

The production of biosurfactant was monitored by measuring the surface tension of the cell-free metabolic liquid obtained after centrifugation, by the Du Nouy ring method at room temperature (±28 °C), using a Tensiometer model Sigma 70 (KSV Instruments Ltd., Helsinki, Finland) (Kuyukina et al. 2001). Measurements of surface tension from distilled water and from the conventional medium were used as control.

Interfacial tension was also determined in the cell-free metabolic liquid containing biosurfactant, using n-hexadecane as apolar compound to form immiscible phases and then measured in a Tensiometer model Sigma 70 (KSV Instruments Ltd., Helsinki, Finland) (Darvishi et al., 2011; Jara et al.,2013).

- Stability

Studies of stability were carried out using 25 ml of cell-free metabolic liquid at different temperatures (0, 5, 70, 100 and 120°C), for 1 h, and cooled to room temperature, after which the surface tension was measured. The effect of pH on surface tension was evaluated after adjustment of the metabolic liquid pH to 2, 5, 8, 10 and 12 with 2 M HCl or NaOH. The effect of NaCl concentrations (2, 4, 8, 10 and 12%, w/v) on the activity of the biosurfactant were also determined (Montero-Rodríguez et al., 2015).

- Biosurfactant isolation

Three different methods of isolation of biosurfactant were tested, using 50 ml of cell-free metabolic liquid, as follows:

Method 1: The cell-free metabolic liquid was acidified with 2 M HCl to pH 2.0 and was allowed to stand for 24 h at 4ºC (Shah et al., 2016).

Method 2: The cell-free metabolic liquid was subjected to liquid-liquid extraction with ethanol 70% (1:1, v/v) and was allowed to stand for 24 h at 4ºC (Ribeiro et al., 2012;).

Method 3: The cell-free metabolic liquid was acidified with 2 M HCl to pH 2.0 and was subjected to liquid-liquid extraction with ethanol 70% (1:1, v/v). Then, it was allowed to stand for 24 h at 4ºC (Almeida et al., 2015).

After stand at 4ºC, all metabolic liquids were centrifuged at 10000 rpm for 15 min at 5ºC and the supernatants were discarded. The isolated biosurfactants were washed twice with distilled water and dried at 70ºC for 3 h. Biosurfactant yields was expressed as g/l and compared in order to select the more efficient method of biosurfactant isolation (Max et al., 2012).

- Ionic charge

The ionic charge of the crude biosurfactant was determined using a DG-ZM3 meter, model Zeta Meter system 3.0+, with direct images to the video of the Zeta Meter, San Francisco, CA, USA. (Andrade Silva et al., 2014; Lima et al., 2017).

3.4. Applications of prodigiosin and biosurfactant

3.4.1. Applications of prodigiosin as natural dye

The potential application of prodigiosin as natural dye of textile materials and candles was evaluate using either the bacterial culture broth or methanol extract containing the red pigment.

For textile dyeing, white pieces (2 cm^2) of cotton poplin, silk, polyester, viscose and satin were soaked in ten milliliters of pigmented solution (bacterial culture broth or methanol extract) and incubated at room temperature (28 ± 2°C) for 24 h (Ahmad et al. 2012; Shaikh, 2016). Then, dyed pieces were dried at room temperature and treated separately with acid (HCl 10%), alkali (NaOH 10%) and commercial detergent either in the presence of cold of hot water. All the treatments were performed for 1 h and then, the pieces were dried at room temperature (Gulani et al, 2012; El-Bialy and El-Nour, 2015). The retention of the pigment after wash was evaluated visually by comparison with white pieces without pigment as well as dyed pieces without any treatment.

For candle coloring, commercial candles were placed in a beaker and heated until melted before the addition of 5 ml of pigmented solution (bacterial culture broth or methanol extract). The mixtures were homogenized and poured into the molds, after greased then with mineral oil. The wicks were immediately placed into the center of the molds and the candles were left to cool at room temperature (28 ± 2°C) for 1 h (Ahmad et al., 2012; Shaikh, 2016). One control candle was prepared which does not contain the pigment.

3.4.2. Applications of biosurfactant

The potential application of biosurfactant as bioemulsifier was evaluate by determination of the emulsification index (%EI_{24}) according to Nitschke and Pastore (2004). Hydrophobic substrates (soybean, corn, canola, olive and waste soybean oil, n-hexadecane, diesel, kerosene and burned motor oil) were added separately to the cell-free supernatant in test tubes, in a ratio 2:2 (v/v). Then they were vortexed for 2 min and allowed to stand for 24 h. %EI_{24} was calculated using following equation (Nalini and Parthasarathi, 2014):

$$\%EI_{24} = \frac{\text{Height of emulsified zone}}{\text{Total height of liquid (sum of aqueous, oil and emulsified zone)}} \times 100$$

The effect of the biosurfactant on the viscosity of different hydrophobic compounds (soybean, corn, canola, waste soybean and burned motor oil) was investigated according to Andrade Silva et al. (2014). The viscosity of each oil (6 ml placed in test tubes) was measured at 25ºC in an automatic viscometer (Brookfield (Middleboro, MA, USA) TC 500), using spindles No. 41 at 100 rpm. Then, 2 mL of 1% biosurfactant solution (w/v) were added to each test tubes and the mixtures were vortexed for 1 min. After 30 min of stand, the viscosity of each oil was measured again (Lima et al., 2017). The viscosity results were expressed in centipoise (cP).

The potential application of biosurfactant as dispersant agent was determined by oil displacement test (Andrade Silva et al., 2014). This test was carried out by slowly dropping 1 mL of burned motor oil onto the surface of 30 mL of distilled water layer contained in a Petri dish (9 cm in diameter). This was followed by the addition of 500 μL of the cell-free metabolic liquid (crude biosurfactant) on the center of the surface of the oil layer. The average value of the diameters of the clear zones of triplicate experiments was measured and calculated as percentage of the Petri dish diameter (Ali Diab and El Din, 2013; Montero-Rodríguez et al., 2015). Commercial detergent and was used as control.

4. Conclusions

The use of cheap agro-industrial byproducts such as alternative of expensive conventional complex medium should expedite large-scale production of useful bacterial metabolites. This study demonstrated the suitability of corn bran as low-cost substrate for the simultaneous production of prodigiosin and biosurfactant by *S. marcescens* UCP 1549. The red pigment was identified as prodigiosin by UV-Vis spectrophotometry (λ_{max}= 535 nm) and TLC (R*f*= 0.9) and successfully applied as natural dye of textile and candle. Biosurfactant was characterized as anionic compound, able to reduce surface (29.3 mN/m) and interfacial (9.4 mN/m) tension, and stable at different values of pH, temperature and NaCl concentration. The promising application of the biosurfactant as bioemulsifier and dispersant agent was demonstrated for waste soybean oil and engine motor oil, respectively. Also, promising use as additive to increase the viscosity of vegetable oils-based lubricants was suggested.

Acknowledgments: This work was financially supported by CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico) under process No. 311373/2014-3, FACEPE (Fundação de Amparo à Ciência e Tecnologia de Pernambuco) under process No. APQ-0291-2.12/15, and CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior). The authors are grateful to CAPES for the Ph.D. fellowship and to Nucleus of Research in Environmental Sciences and Biotechnology, Catholic University of Pernambuco, PE-Brazil, for the use of laboratories.

Author Contributions: D.M.R. and G.M.C.T conceived and designed the experiments; D.M.R., R.F.S.A. and D.R.R. performed the experiments and contributed to discussion of the results; D.L.C., A.A.A. and M.A.C.L. supported to application tests and contributed to results analysis; A.A.A., H.W.C.A. and G.M.C.T. contributed reagents/materials; D.M.R. and R.F.S.A. wrote the paper; H.W.C.A and G.M.C.T contributed to discussion of results and revised the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Giri, A.V.; Anandkumar, N.; Muthukumaran, G.;Pennathur, G. A novel medium for the enhanced cell growth and production of prodigiosin from *Serratia* marcescens isolated from soil. *BMC Microbiol.*,**2004**, *4*, 1-10.
2. Khanafari, A.; Assadi, M. M.; Fakhr, F. A. Review of prodigiosin, pigmentation in *Serratia marcescens*. *Onl. J. Biol. Sci.* **2006**, *6*, 1–13.
3. Papireddy, K.; Smilkstein, M.; Kelly, J.X.; Shweta, S.S.M.;Alhamadsheh, M.; Haynes, S.W.; Challis, G.L.; Reynolds, K. Antimalarial activity of natural and synthetic prodiginines. *J Med Chem*, **2011**, *54*, 5296–306.
4. Lapenda, J.C.; Silva, P.A.; Vicalvi, M.C.; Sena, K.X.F.R.; Nascimento, S.C. Antimicrobial activity of prodigiosin isolated from *Serratia marcescens* UFPEDA 398. *World J. MicrobiolBiotechnol*, **2015**, *31*, 399-406.
5. Lins, J. C. L.; De Melo, M. E. B.; Do Nascimento, S. C.; Adam, M. L. Differential Genomic Damage in Different Tumor Lines Induced by Prodigiosin. *Antic. Research.*, **2015**, *35*, 3325-3332.
6. Stankovic, N.; Senerovic, L.; Ilic-Tomic, T.; Vasiljevic, B.; Nikodinovic-Runic, J. Properties and applications of undecylprodigiosin and other bacterial prodigiosins. *Appl. Microbiol. Biotechn*, **2014**, *98*, 3841-3858.
7. Darshan, N.; Manonmani, H.K. Prodigiosin and its potential applications. *J. Food Sci. Technol.*, **2015**; *52*, 1-15.
8. Matsuyama, T.; Murakami, T.; Fujita, M.; Fujita, S.; Yano, I. Extracellular vesicle formation and biosurfactant production by *Serratia marcescens*. *Microbiology*, **1986**, *132*, 865-875.
9. Anyanwu, C. U.; Obi S.K.C.; Okolo B. N. Lipopeptide biosurfactant production by *Serratia marcescens* NSK-1 Strain isolated from petroleum contaminated soil. *J. Appl. Sci. Res.***2011**, *7*, 79-87
10. Ibrahim, M.L.U.J; Ijah, S.B.; Manga, L.S.; Bilbis U.S. Production and partial characterization of biosurfactant produced by crude oil degrading bacteria. *Int. Biodet. Biodegrad*. **2013,** *81*, 28-34.
11. Almansoory A.F.; Hasan H.A.; Idris M.; Abdullah S.R.S.; Anuar, N. Biosurfactant production by the hydrocarbon-degrading bacteria (HDB) *Serratia marcescens*: Optimization using central composite design (CCD*), J. Ind.Eng. Chem*., **2017,** *47*, 272-280.

12. Araujo, H.W.C.; Andrade, R.F.; Montero-Rodríguez, D.; Santos,V.P.; Maia, P.C.S.; Costa Filho, C.F.; Silva C.A.A.; Campos-Takaki G.M. Biochemical identification of and molecular newly isolated pigmented bacterium and improved production of biosurfactant, *Afr. J. Microbiol. Res.*, **2017**, *11*, 945-954.
13. Marchant R.; Banat I.M.Biosurfactants: a sustainable replacement for chemical surfactants? *Biotechnol.Lett.* , **2012**, *34*, 1597–1605
14. Marchant R.; Banat I.M. Microbial biosurfactants: challenges and opportunities for future exploitation. *Trends in Biotechnol* , **2012**, *30*, 558–565
15. Marchant, R.; Banat, I. M. Protocols for measuring biosurfactant production in microbial cultures. Hydrocarbon and Lipid Microbiology Protocols, Activities and Phenotypes, **2017**, 119-128.
16. Banat, I.M.; Franzetti, A.; Gandolfi, I.; Bestetti, G.; Martinotti, M.G.; Fracchia, L.; Smyth, T.J.P.; Marchant, R. Microbial biosurfactants production, applications and future potential. *Appl. Microbiol. Biotechnol.* **2010**, *87*, 427-444
17. Kapadia, S.G.; Yagnik, B.N. Current trend and potential for microbial biosurfactants. *Asian J. Exp. Biol. Sci.* **2013**, *4*, 1–8.
18. Bhattacharya, B.; Ghosh, T.K.; Das, N. Application of Bio-Surfactants in Cosmetics and Pharmaceutical Industry, *Sch. Acad. J. Pharm*., **2017,** 6, 320-329
19. Mujumdar, S., Bashetti, S., Pardeshi, S.; Thombre, R. S. Industrial applications of biosurfactants. Industrial Biotechnology, Sust. Prod. Bior. Util., **2017**, *81*,
20. Panesar, R.; Kaur, S.;Panesar, P. S. Production of microbial pigments utilizing agro-industrial waste: a review. *CurrOpin Food Sci*, **2015**, *1*, 70-76.
21. Brumano, L. P.; Soler, M. F.; DA SILVA, S. S. Recent advances in sustainable production and application of biosurfactants in Brazil and Latin America. *Ind. Biotechn*, **2016,** *12*, 31-39
22. Araújo, H.W.C.; Fukushima, K.;Takaki, G.M.C. Prodigiosin production by *Serratia marcescens* UCP 1549 using renewable-resources as a low cost substrate. *Molecules*.**, 2010**, *15,* 6931-6940.
23. Naik, C.; Srisevita, J.M.; Shushma, K.N.; Farah, N.; Shilpa, A.C.; Muttanna, C.D.; Darshan, N.; Sannadurgappa, D. Peanutoilcake: a novel substrate for enhanced cell grow and prodigiosin productionfrom*Serratia marcescens* CF-53. *J. Res. Biol*., **2012**, *2*, 549-557.
24. Gudiña, E. J.; Rodrigues, A. I.; Freitas, V.; Azevedo, Z.; Teixeira, J. A.; Rodrigues, L. R. Valorization of agro-industrial wastes towards the production of rhamnolipids. *Bior. Technol*., **2016**, *212*, 144-150.
25. Rahman, P. K.; Pasirayi, G.; Auger, V.; Ali, Z. Production of rhamnolipid biosurfactants by *Pseudomonas aeruginosa* DS10-129 in a microfluidic bioreactor. *Biotechn. Appl. Biochem*.**, 2010**, *55*, 45-52.
26. Das, S.; Das, P. Effects of cultivation media components on biosurfactant and pigment production from *Pseudomonas aeruginosa* PAO1. *Braz. J. Chem. Eng*., **2015**, *32*, 317-324.
27. Montero-Rodríguez, D. ; Andrade, R. F. S. ; Ribeiro, D. L. R. ; Lima, R. A. ; Araujo, H. W. C. ; Campos-Takaki, G. M. Ability of *Serratia marcescens* UCP/WFCC 1549 for biosurfactant production using industrial wastes and fuels biodegradation. Industrial, medical and environmental applications of microorganisms: current status and trends. A. Méndez-Vilas (Ed.), Wageningen Academic Publishers, The Netherlands, 2014. p 22-27.
28. Montero-Rodríguez, D.; Andrade, R. F.S.; Ribeiro, D. L. R.; Rubio-Ribeaux, D.; Lima, R. A.; Araújo, H. W.; Campos-Takaki, G. M. Bioremediation of Petroleum Derivative Using Biosurfactant Produced by *Serratia marcescens* UCP/WFCC 1549 in Low-Cost Medium. *Int. J. Curr. Microbiol. App. Sci*, **2015**, *4*, 550-562.
29. Figuereido, A. L. ; Texeira, L. M. ; Alves, T. A. ; Silva, A. P. B. ; MonteroRodríguez, D. ; Andrade, R. F. S. ; Araujo, H. W. C. ; Ferreira, W. B. ; CampoS-Takaki, G. M. Utilization of organic residues for biosurfactant production by *Serratia marcescens* UCP/WFCC 1549. In: VI International Conference on Environmental, Industrial and Applied Microbiology BioMicroWorld 2015, 2015, Barcelona. Book of Abstracts BioMicroWorld 2015, 2015. v. 1. p. 234-234
30. L. M. Teixeira, A. P. B. Silva, D. Montero-Rodríguez. R. F. S. Andrade, D. Rubio-Ribeaux, T. A. L. Silva, G. K. B. Silva, G. M. Campos-Takaki and H. W. C. Araújo. Pineapple peels and animal fat as low-cost substrates for biosurfactant production by *Serratia marcescens* UCP 1549 In: VII International Conference on Environmental, Industrial and Applied Microbiology BioMicroWorld 2017, 2017, Madrid. Book of Abstracts BioMicroWorld 2017, 2017. v. 1. p. 163
31. Montero-Rodríguez D.; Andrade R.F.S.; Rubio-Ribeaux D.; Lima R.A.; Ribeiro D.L.R.; Silva G.K.B.; Campos-Takaki, G.M. A low-cost solid fermentation medium for potential prodigiosin production by *Serratia*

marcescens UCP/WFCC 1549, In *Microbes in the Spotlight: Recent Progress in the Understanding of Beneficial and Harmful Microorganisms*, 2016.

32. Slater, H.; Crow, M.; Everson, L.; Salmond, G. P. Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem, in *Serratia* via both quorum-sensing-dependent and-independent pathways. *Molec. Microb*., **2003**, *47*, 303-320.
33. Krishna, G. J.; Basheer, S. M.; Elyas, K. K.; Chandrasekaran, M.Prodigiosin from marine bacterium: production, characterization and application as dye in textile industry. *Int J BiotechnolBiochem*, **2011**, *7*, 155-191.
34. Karp, S. G., Woiciechowski, A. L., Soccol, V. T., & Soccol, C. R. (2013). Pretreatment strategies for delignification of sugarcane bagasse: a review. Brazilian archives of biology and technology, 56(4), 679-689.
35. Harris AKP,Williamson, NRH,Cox HA, Abbasi S, Slater FI, Simonsen HT,Leeper FJ, Salmond GPC. 2004. The Serratia gene cluster encoding biosynthesis of the red antibiotic, Prodigiosin , shows species – and strain –dependent genome context variation. Microbiol 150:3547 – 3560.
36. Williamson NR, Simonsen HT, Ahmed RAA, Goldet G, Slater H, Woodley l,Leeper FJ, Salmond GPC. 2005. Biosynthesis of the red antibiotic, Prodigiosin, in Serratia: Identification of a novel 2-methyl-3-n-amyl-pyrrole (MAP) assembly pathway, definition of the terminal condensing enzyme, and implications for undecyl Prodigiosin biosynthesis in Streptomyces. Mol Microbiol, 56, 4:971-989.
37. Abdullateef AA, Ahmad AAR, Hani AAS, Jalal AB, Mohammad IA, Ghada BS (2012) Documentation of vancomycin-resistant *Staphylococcus aureus* (VRSA) among children with atopic dermatitis in the Qassim region, Saudi Arabia. Acta Dermatovenerol 21:51–53
38. Siva, R.; Subha, K.; Bhakta, D.; Ghosh, A.R.; Babu, S. Characterization and enhanced production of prodigiosin from the spoiled coconut. *Appl Biochem Biotechnol*, **2012**, 166, 187-196.
39. Vaidyanathan, J., Bhathena-Langdana, Z., Adivarekar, R. V., & Nerurkar, M. (2012). Production, partial characterization, and use of a red biochrome produced by *Serratia sakuensis* subsp. nov strain KRED for dyeing natural fibers. Applied biochemistry and biotechnology, 166(2), 321-335.
40. Andreyeva, I. N., & Ogorodnikova, T. I. (2015). Pigmentation of *Serratia marcescens* and spectral properties of prodigiosin. Microbiology, 84(1), 28-33.
41. Satpute, S. K., Banpurkar, A. G., Dhakephalkar, P. K., Banat, I. M., & Chopade, B. A. (2010). Methods for investigating biosurfactants and bioemulsifiers: a review. *Critical reviews in biotechnology*, *30*(2), 127-144.
42. Phatake Y.B., Dharmadhikari S.M., 2016, Physical parameters optimization for enhancement of prodigiosin production by using *Serratia* spp., World J Pharm Med Res, 2, 40-48.
43. Mulligan CN (2005). Environmental applications for biosurfactants. Environ. Pollut. 133(2):183-198
44. Mesbaiah FZ, Eddouaouda K, Badis A, Chebbi A, Hentati D, Sayadi S, Chamkha M (2016). Preliminary characterization of biosurfactant produced by a PAH-degrading Paenibacillus sp. under thermophilic conditions. Environ. Sci. Pollut. Res. 23:14221-14230.
45. AGUIAR, H.B.; BEER, A.G.F.; STRADER,M.L.; ROKE, S. The Interfacial Tension of Nanoscopic Oil Droplets in Water Is Hardly Affected by SDS Surfactant. J. AM. CHEM. SOC. 2010, 132, 2122–2123.
46. Jain, R.M., K., Mody, N. Joshi, A. Mishra and Jha, B. 2013. Effect of unconventional carbon sources on biosurfactant production and its application in bioremediation. International Journal of Biological Macromolecules 62, 52-58.
47. Abouseoud M., Maachi R., Amrane A., Boudergua S., Nabi A. Evaluation of different carbon and nitrogen sources in production of biosurfactant by *Pseudomonas fluorescens*. Desalination. 2008;223:143–151.
48. Khopade A., Biao R., Liu X., Mahadik K., Zhang L., Kokare C. Production and stability studies of the biosurfactant isolated from marine *Nocardiopsis* sp. B4. Desalination. 2012;3:198
49. Santos, D. K. F., Rufino, R. D., Luna, J. M., Santos, V. A., & Sarubbo, L. A. (2016). Biosurfactants: multifunctional biomolecules of the 21st century. International journal of molecular sciences, 17(3), 401.
50. Mukherjee, S., Das, P., & Sen, R. (2006). Towards commercial production of microbial surfactants. TRENDS in Biotechnology, 24(11), 509-515.
51. Baker, S. C., & Chen, C. Y. (2010). Enrichment and purification of lipopeptide biosurfactants. In Biosurfactants (pp. 281-288). Springer New York.
52. Karadi, R. V., Lokesh, K. N., Sadanandan, B., & Venkatrangana, M. (2014). Standardization of biosurfactant enrichment process by factorial design and elucidating its physico-chemical and structural characteristics. Journal of Biochemical Technology, 3(5), 161-166.

53. Pruthi, V., & Cameotra, S. S. (2000). Novel sucrose lipid produced by Serratia marcescens and its application in enhanced oil recovery. Journal of Surfactants and Detergents, 3(4), 533-537.
54. Nalini, S., & Parthasarathi, R. (2013). Biosurfactant production by Serratia rubidaea SNAU02 isolated from hydrocarbon contaminated soil and its physico-chemical characterization. Bioresource technology, 147, 619-622.
55. Karray F, Mezghani M, Mhiri N, Djelassi B, Sayadi S (2016). Scaledown studies of membrane bioreactor degrading anionic surfactants wastewater: Isolation of new anionic-surfactant degrading bacteria. Int. Biodeterior. Biodegradation 114:14-23
56. Venil, C. K.; Zakaria, Z. A.; Ahmad, W. A. Bacterial pigments and their applications. *Proc. Biochem.*, **2013**, *48*, 1065-1079.
57. Rao, M. P. N.; Xiao, M.; Li, W. J. Fungal and bacterial pigments: secondary metabolites with wide applications. *Front. microbiol.*, **2017**, *8*. 1-13.
58. Campos, J. M., Montenegro Stamford, T. L., Sarubbo, L. A., de Luna, J. M., Rufino, R. D., & Banat, I. M. (2013). Microbial biosurfactants as additives for food industries. Biotechnology progress, 29(5), 1097-1108.
59. Tuli, H. S., Chaudhary, P., Beniwal, V., & Sharma, A. K. (2015). Microbial pigments as natural color sources: current trends and future perspectives. *Journal of food science and technology*, *52*(8), 4669-4678.
60. Vickerstaff T (1954) The physical chemistry of dyeing. Oliver and Boyd, London, pp 1–110
61. Mansi, M., & Gaurav, S. (2015). Extraction of pigment from *Serratia marcescens* and its application in candle industry. Adv. Appl. Res., 7(2), 1-4.
62. Falode, O. A., Adeleke, M. A., Ogunshe, A. A. Evaluation of Indigenous Biosurfactant-producing Bacteria for De-emulsification of Crude Oil Emulsions. Microbiology Research Journal International 18(3): 1-9, 2017.
63. Silva, E.J., Rocha e Silva, N.M.P., Rufino, R.D., Luna, J.M., Silva, R.O., Sarubbo, L.A., 2014. Characterization of a biosurfactant produced by *Pseudomonas cepacia* CCT6659 in the presence of industrial wastes and its application in the biodegradation of hydrophobic compounds in soil. Colloids Surf. B: Biointerf. 117, 36–41.
64. Rita de Cássia, F., Almeida, D. G., Meira, H. M., Silva, E. J., Farias, C. B., Rufino, R. D., Luna, J. M, Sarubbo, L. A. (2017). Production and characterization of a new biosurfactant from *Pseudomonas cepacia* grown in low-cost fermentative medium and its application in the oil industry. Biocatalysis and Agricultural Biotechnology.
65. Morales-Guzmán, G., Ferrera-Cerrato, R., del Carmen Rivera-Cruz, M., Torres-Bustillos, L. G., Arteaga-Garibay, R. I., Mendoza-López, M. R., Esquivel-Cote, R., Alarcón, A. (2017). Diesel degradation by emulsifying bacteria isolated from soils polluted with weathered petroleum hydrocarbons. Applied Soil Ecology, 121, 127-134.
66. Amiriyan A., Mazaheri Assadi M., Sajadian V.A., Noohi A.A. Bioemulsan production by Iranian oil reservoirs microorganism. Iran. J. Environ. Health Sci. Eng. 2004;1:28–35.
67. Sharma, D. (2016). Applications of Biosurfactants in Food. In Biosurfactants in Food (pp. 43-80). Springer International Publishing.
68. Bafghi, M. K., & Fazaelipoor, M. H. (2012). Application of rhamnolipid in the formulation of a detergent. Journal of Surfactants and Detergents, 15(6), 679-684.
69. Barbosa, S. A., da Freitas Coêlho, D., Silveira, E., Tambourgi, E. B., & da Souza, R. R. (2013). Biosurfactant Production by *Pseudomonas aeruginosa* and *Yarrowia lipolytica* and Its Use for Detergent Formulations. Journal of Chemistry and Chemical Engineering, 7(8), 767.
70. Campos, J. M., Montenegro Stamford, T. L., Sarubbo, L. A., de Luna, J. M., Rufino, R. D., & Banat, I. M. (2013). Microbial biosurfactants as additives for food industries. Biotechnology progress, 29(5), 1097-1108.
71. Kourkoutas, Y., Bekatorou, A., Banat, I. M., Marchant, R., and Koutinas, A. A. (2004). Immobilization technologies and support materials suitable in alcohol beverages production: a review. *Food Microbiol.* 21, 377–397. doi: 10.1016/j.fm.2003.10.005
72. Shepherd, R., Rockey, J., Shutherland, I. W., and Roller, S. (1995). Novel bioemulsifiers from microorganisms for use in foods. *J. Biotechnol.* 40, 207–217. doi: 10.1016/0168-1656(95)00053-S
73. Campos, J. M., Stamford, T. L., Rufino, R. D., Luna, J. M., Stamford, T. C. M., & Sarubbo, L. A. (2015). Formulation of mayonnaise with the addition of a bioemulsifier isolated from *Candida utilis*. Toxicology Reports, 2, 1164-1170.

74. Kiran, G. S., Priyadharsini, S., Sajayan, A., Priyadharsini, G. B., Poulose, N., & Selvin, J. (2017). Production of Lipopeptide Biosurfactant by a Marine Nesterenkonia sp. and Its Application in Food Industry. Frontiers in microbiology, 8.
75. Vecino, X., Cruz, J. M., Moldes, A. B., & Rodrigues, L. R. (2017). Biosurfactants in cosmetic formulations: trends and challenges. Critical reviews in biotechnology, 1-16.
76. Lukic, M., Pantelic, I., & Savic, S. (2016). An overview of novel surfactants for formulation of cosmetics with certain emphasis on acidic active substances. Tenside Surfactants Detergents, 53(1), 7-19.
77. Ferreira, A., Vecino, X., Ferreira, D., Cruz, J. M., Moldes, A. B., & Rodrigues, L. R. (2017). Novel cosmetic formulations containing a biosurfactant from Lactobacillus paracasei. Colloids and Surfaces B: Biointerfaces, 155, 522-529.
78. Quinchia, L. A., Delgado, M. A., Valencia, C., Franco, J. M., & Gallegos, C. (2010). Viscosity modification of different vegetable oils with EVA copolymer for lubricant applications. Industrial Crops and Products, 32(3), 607-612.
79. García-Zapateiro, L. A., Franco, J. M., Valencia, C., Delgado, M. A., Gallegos, C., & Ruiz-Méndez, M. V. (2013). Viscosity modification of high-oleic sunflower and castor oils with acid oils-derived estolides for lubricant applications. European Journal of Lipid Science and Technology, 115(10), 1173-1182.
80. McNutt, J. (2016). Development of biolubricants from vegetable oils via chemical modification. Journal of Industrial and Engineering Chemistry, 36, 1-12.
81. D. Song, S. Liang, Q. Zhang, J. Wang, L. Yan, Development of High Efficient and Low Toxic Oil Spill Dispersants based on Sorbitol Derivants Nonionic Surfactants and Glycolipid Biosurfactants, J Environ Prot, 04 (2013) 16-22.
82. Kleindienst, S., Seidel, M., Ziervogel, K., Grim, S., Loftis, K., Harrison, S., ... & Dittmar, T. (2015). Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. Proceedings of the National Academy of Sciences, 112(48), 14900-14905.
83. Lv, Z., Cai, Q., Zhang, B., & Chen, B. (2016). ENV-624: A NEW HIGH-YIELDING BIO-DISPERSANT PRODUCER MUTATED FROM RHODOCOCCUS ERYTHROPOLIS STRAIN P6-4P.
84. Rongsayamanont, W., Soonglerdsongpha, S., Khondee, N., Pinyakong, O., Tongcumpou, C., Sabatini, D. A., & Luepromchai, E. (2017). Formulation of crude oil spill dispersants based on the HLD concept and using a lipopeptide biosurfactant. Journal of Hazardous Materials, 334, 168-177.
85. Roldan-Carrillo, T., X. Martinez-Garcia, I. Zapta-Penaso, G. Castrorena-Cortes, J. Reyes-Avila, M. Mayol-Castino and Olguin-Lora, P. 2011. Evaluation of the effect of nutrient ratios on biosurfactant production by *Serratia marcescens* using a boxbehuken design. Colloids and Surfaces B; Biointerfaces 86, 284-389.
86. Elemba, O. M., Ijah, U. J. J., & Chibunna, M. Isolation, characterization and meor ability of the biosurfactant produced from *Serratia marcescens* UEO15.
87. Kuyukina, M.S.; Ivshina, I.B.; Philp, J.C.;. Christofi, N.; Dunbar, S.A.; Ritchkova, M.I. Recovery of *Rhodococcus* biosurfactants using methyl tertiarybutyl ether extraction. *J. Microbiol Methods*., **2001**, *46*, 149-156.
88. Bóna-Lovász, J.; Bóna, A.; Ederer, M.; Sawodny, O.; Ghosh, R. A rapid method for the extraction and analysis of carotenoids and other hydrophobic substances suitable for systems biology studies with photosynthetic bacteria. *Metabolites*,**2013**, *3*, 912-930.
89. Priya, K.A.; Satheesh, S.; Ashokkumar, B.;Varalakshmi, P.;Selvakumar, G.;Sivakumar, N. *Antifouling activity of prodigiosin from estuarine isolate of Serratia marcescens CMST 07*. In Microbiological research in agroecosystem management (pp. 11-21). Springer India, 2013.
90. Darvishi, P.; Ayatollahi, S.; Dariush, M.; Niazi, A. Biosurfactant production under extreme environmental conditions by an efficient microbial consortium. *Colloids Surf. B –II,* **2011**, *84*, 292-300
91. Jara A.M; Andrade R.F.S.; Campos-Takaki G.M. Physicochemical characterization of tensio-active produced by *Geobacillusstearothermophilusisolated* from petroleum-contaminated soil. *Colloids Surf. Biointerf*. **2013**, *101*, 315-318.
92. Shah, M. U. H.; Sivapragasam, M.; Moniruzzaman, M.; Yusup, S. B. A comparison of Recovery Methods of Rhamnolipids Produced by *Pseudomonas aeruginosa*. *Proc. Eng*., **2016**, *148*, 494-500.
93. de Almeida, F. C. G.; Silva, T. A. D. L.; Garrard, I.; Asfora, L., Sarubbo, G. M. D. C. T.; Tambourgi, E. B. Optimization and evaluation of biosurfactant produced by *Pantoea*sp. using pineapple peel residue, vegetable fat and corn steep liquor. *J. Chem*, **2015,** *9*, 269-279.

94. Ribeiro, D. L. R.; Andrade, R. F.S.; Antunes, A. A.; Silva, A. M.; Max, F. Y.; Campos-Takaki, G. M. Biosurfactant production by *Rhodotorulaglutinis* UCP 1555 using industrial wastes. Microbes in Applied Research: Current Advantages and Challenges, **2012**, *55*,
95. Max, F. Y.; Andrade, R. F.S.; Silva, A. M.; Ribeiro, D. L. R.; Jara, A. M. A. T.; Alencar, A. A.; Gusmão, N. B. Biosurfactant production by *Rhodotorula glutinis:* Emulsifying property and stability. Microbes in Applied Research: Current Advantages and Challenges, **2012**, 358.
96. Andrade Silva, N. R.; Luna, M. A.; Santiago, A. L.; Franco, L. O.; Silva, G. K.; Souza, P. M.;Campos-Takaki, G. M. Biosurfactant-and-bioemulsifier produced by a promising *Cunninghamella echinulata* isolated from caatinga soil in the northeast of Brazil. *Int. j. Mol. Sci*., **2014**, *15*, 15377-15395.
97. Lima, R. A.; Andrade, R. F.S.; Rodriguez, D. M.; Araujo, H. W.; Santos, V. P.; Campos-Takaki, G. M. Production and characterization of biosurfactant isolated from *Candida glabrata* using renewable substrates. *Afric. J. Microb. Res*, **2017,** *11*, 237-244.
98. Ahmad, W. A.; Ahmad, W. Y. W.; Zakaria, Z. A.;Yusof, N. Z. Application of bacterial pigments as colorant. In *Application of Bacterial Pigments as Colorant.* Springer Berlin Heidelberg Publishers, New York, **2012**; pp. 57-74,
99. Shaikh, Z. Biosynthesis of prodigiosin and its applications. *J. Pharm. Biol. Sci.,***2016,** *11*, 1-28.
100. Gulani, C.; Bhattacharya, S.; Das, A. Assessment of process parameters influencing the enhanced production of prodigiosin from *Serratia marcescens* and evaluation of its antimicrobial, antioxidant and dyeing potentials. *Malays J Microbiol*, **2012,** *8*, 116-122.
101. El-Bialy, H.A.; El-Nour, S.A.A. Physical and chemical stress on *Serratia marcescens* and studies on prodigiosin pigment production. *Ann Microbiol*, **2015**,*65*, 59-68.
102. Nitschke, M.; Pastore, G. M. Biosurfactant production by *Bacillus subtilis* using cassava-processing effluent. *App. Bioch. Biotechn*, **2004**, *112*, 163-172.
103. Nalini, S.; Parthasarathi, R. Production and characterization of rhamnolipids produced by *Serratia rubidaea* SNAU02 under solid-state fermentation and its application as biocontrol agent. *Bior. Techn*., **2014**, *173*, 231-238.
104. Ali Diab, A.; El Din, S.G.. Application of the biosurfactants produced by Bacillus spp. (SH 20 and SH 26) and Pseudomonas aeruginosa SH 29 isolated from the rhizosphere soil of an Egyptian salt marsh plant for the cleaning of oil-contaminated vessels and enhancing the biodegradation of oily sludge. *Afr. J.Environ. Sci. Technol*., **2013**, *7*, 671- 679
105. Lins, J. L.; Maciel, C. C. S.; Xavier, H. S.; Da Silva, C. A.; Campos-Takaki, G. M. Production and toxicological evaluation of Prodigiosin from *Serratia marcescens* UCP/WFCC1549 on Mannitol Solid Medium. *Int. J. App. Res. Nat. Prod*., **2014**, 7, 32-38.

6. CONCLUSÕES GERAIS

- *Serratia marcescens* UCP 1549 demonstrou habilidade para utiliza substratos agroindustriais na produção simultânea de prodigiosina e biossurfactante por fermentação em estado sólido.

- O máximo rendimento de pigmento vermelho e melhor redução da tensão superficial foram obtidos no ensaio 2 do planejamento fatorial, no meio constituído de 10 g de farelo de trigo e solução umedecedora (sais e 5% de óleo de soja pós-fritura).

- O pigmento produzido por fermentação em estado sólido foi identificado como prodigiosina pelo resultado positivo ao teste presuntivo e confirmado por espectrofotometria UV-Vis, TLC, HPLC e LC-MS.

- A prodigiosina produzida por fermentação em estado sólido mostrou eficácia no tingimento de diferentes tecidos e velas, demonstrando potencialidade de ser utilizado como alternativa aos corantes sintéticos.

- *S. marcescens* UCP 1549 mostrou potencial para a produção simultânea de biossurfactante e prodigiosina por fermentação submersa em meio constituído por farelo de milho 1%.

- O pigmento produzido foi identificado como prodigiosina por espectrofotometria UV-Vis e TLC.

- O biossurfactante produzido foi caracterizado como um composto aniônico, com capacidade de reduzir a tensão superficial e interfacial.

- A prodigiosina presente no meio de cultura demonstrou maior efetividade no tingimento de diferentes tecidos e velas, quando comparado com o pigmento extraído em metanol.

- O biossurfactante mostrou estabilidade em diferentes valores de pH, temperatura e concentração de NaCl, além de excelentes propriedades para a emulsificação, dispersão e o incremento da viscosidade de diferentes substratos hidrofóbicos, o que sugere sua potencial aplicação em diversas indústrias.

7. ANEXOS

TRABALHOS PUBLICADOS DURANTE O DOUTORADO

Montero-Rodríguez, D.; ANDRADE, R. F. S. ; RUBIO-RIBEAUX, D. ; LIMA, R. A. ; RIBEIRO, D. L. R. ; SILVA, G. K. B. ; ARAUJO, H. W. C. ; CAMPOS-TAKAKI, G. M. . A low-cost solid fermentation medium for potential prodigiosin production by *Serratia marcescens* UCP/WFCC 1549. In: VI International Conference on Environmental, Industrial and Applied Microbiology BioMicroWorld 2015, 2015, Barcelona. Book of Abstracts BioMicroWorld 2015, 2015. p. 192-192.

RUBIO-RIBEAUX, D. ; ANDRADE, R. F. S. ; **Montero-Rodríguez, D.** ; LUNA, M. A. C. ; PHELE, M. A. ; ANTUNES, A. ; FRANCO, L. O. ; LIMA, M. A. B. ; CAMPOS-TAKAKI, G. M. . Biotechnological production of biosurfactant in economic medium by *Candida* spp and evaluation of biodegradation potential of petro derivates. In: VI International Conference on Environmental, Industrial and Applied Microbiology BioMicroWorld 2015, 2015, Barcelona. Book of Abstracts BioMicroWorld 2015, 2015. p. 199-199.

MELO BIONE, A. P.. ; BARBOSA LINS, A. ; CAVALCANTI SILVA, T. ; **Montero-Rodríguez, D.** ; DE SOUZA, D. G. ; REGO BARROS FILIZOLA, P. ; DE VÉRAS SOUZA MAIA, P. C. ; FÉLIX NOBREGA, H. ; CAMPOS-TAKAKI, G. M. . Production of chitin, chitosan and biosurfactant by *Cunninghamella phaeospora* through submerged fermentation using water soluble substrates. In: VI International Conference on Environmental, Industrial and Applied Microbiology BioMicroWorld 2015, 2015, Barcelona. Book of Abstracts BioMicroWorld 2015, 2015. p. 628-628.

FIGUEREIDO, A. L. ; TEXEIRA, L. M. ; ALVES, T. A. ; SILVA, A. P. B. ; **Montero-Rodríguez, D.** ; ANDRADE, R. F. S. ; ARAUJO, H. W. C. ; FERREIRA, W. B. ; CAMPOS-TAKAKI, G. M. . Utilization of organic residues for biosurfactant production by *Serratia marcescens* UCP/WFCC 1549. In: VI International Conference on Environmental, Industrial and Applied Microbiology BioMicroWorld 2015, 2015, Barcelona. Book of Abstracts BioMicroWorld 2015, 2015. v. 1. p. 234-234.

Montero-Rodríguez, D.; ANDRADE, R. F. S. ; RUBIO-RIBEAUX, D. ; RIBEIRO, D. L. R. ; LIMA, R. A. ; ARAUJO, H. W. C. ; CAMPOS-TAKAKI, G. M. . CONVERSION OF

AGRO-INDUSTRIAL WASTES BY *Serratia marcescens* UCP/WFCC 1549 INTO BIOSURFACTANT AND EVALUATION OF STABILITY AND EMULSIFICATION PROPERTIES. In: 28º Congresso Brasileiro de Microbiologia, 2015, Florianópolis. ANAIS DO 28ºCBM 2015, 2015.

FIGUEREIDO, A. L. ; TEXEIRA, L. M. ; ALVES, T. A. ; **Montero-Rodríguez, D.** ; ANDRADE, R. F. S. ; ARAUJO, H. W. C. ; CAMPOS-TAKAKI, G. M. . PRODUCTION OF BIOSURFACTANT BY *Serratia marcescens* UCP 1549 USING SUGARCANE BAGASSE AND WASTE COOKING OIL AS SUBSTRATES. In: 28 Congresso Brasileiro de Microbiologia, 2015, Florianópolis. ANAIS DO 28º CBM 2015, 2015.

DAYLIN RUBIO RIBEAUX ; Rosileide Fontenele da Silva Andrade ; **DAYANA MONTERO RODRÍGUEZ** ; Goretti Sonia Silva ; Sergio Carvalho de Paiva ; Galba María de Campos-Takaki . APLICAÇÃO DO BIOSSURFACTANTE PRODUZIDO POR *Candida* sp. NA DISPERSÃO DE POLUENTE HIDROFÓBICO EM ÁGUA. In: Arminda Saconi Messias. (Org.). TRATAMENTO DE ÁGUA: QUALIDADE, ECONOMIA E SUSTENTABILIDADE. 1ed.RECIFE: FASA, 2015, v. 1, p. 164-173.

LIMA, R. A. ; SACONI, A. ; CAMPOS-TAKAKI, G. M. ; ANDRADE, R. F. S. ; **Montero R.,** Dayana . Optimized Submerged Batch Fermentation for Co-enzyme Q Production by *Candida glabrata* Using Renewable Substrates. International Journal of Innovative Research in Engineering & Management, v. 2, p. 37-42, 2015.

D. Montero-Rodríguez; ANDRADE, R. F. S. ; RUBIO-RIBEAUX, D. ; LIMA, R. A. ; RIBEIRO, D. L. R. ; SILVA, G. K. B. ; ARAUJO, H. W. C. ; CAMPOS-TAKAKI, G. M. . A low-cost solid fermentation medium for potential prodigiosin production by *Serratia marcescens* UCP/WFCC 1549. Microbes in the spotlight: recent progress in the understanding of beneficial and harmful microorganisms. 1ed.: BrownWalker Press, 2016, v. , p. 31

RUBIO-RIBEAUX, D. ; ANDRADE, R. F. S. ; **Montero-Rodríguez, D.** ; LUNA, M. A. C. ; PHELE, M. A. ; FRANCO, L. O. ; LIMA, M. A. B. ; CAMPOS-TAKAKI, G. M. . Biotechnological production of biosurfactant in economic medium by *Candida* spp and evaluation of biodegradation potential of petro derivates. Microbes in the spotlight: recent progress in the understanding of beneficial and harmful microorganisms. 1ed.: BrownWalker Press, 2016, v. 1, p. 321-325.

MELO BIONE, A. P.. ; BARBOSA LINS, A. ; CAVALCANTI SILVA, T. ; **Montero-Rodríguez, D.** ; DE SOUZA, D. G. ; REGO BARROS FILIZOLA, P. ; DE VÉRAS SOUZA MAIA, P. C. ; FÉLIX NOBREGA, H. ; CAMPOS-TAKAKI, G. M. . Production of biosurfactant by *Cunninghamella phaeospora* in submerged fermentation using water soluble substrates. Microbes in the spotlight: recent progress in the understanding of beneficial and harmful microorganisms. 1ed.: BrownWalker, 2016, v. , p. 361-365.

Ana Carla Rocha de Carvalho ; Patrícia Nunes Santos ; **DAYANA MONTERO RODRÍGUEZ** ; DAYLIN RUBIO RIBEAUX ; Ana Lúcia Figueiredo Porto ; Rosileide Fontenele S. Andrade ; Clarissa Daisy da Costa Albuquerque ; Valdemir Alexandre dos Santos ; Galba M. Campos-Takaki. Chitosan production by a new strain of *Mucor subtilissimus* isolated from Caatinga soil (Pernambuco, Brazil). In: 1er Congreso Iberoamericano de Biotecnología BioIberoamérica2016, 2016, Salamanca. LIBRO DE RESÚMENES BIOIBEROAMERICA 2016, 2016.

SOUZA, ADRIANA ; **RODRIGUEZ, DAYANA** ; RIBEAUX, DAYLIN ; LUNA, MARCOS ; LIMA E SILVA, THAYSE ; ANDRADE, ROSILEIDE ; GUSMÃO, NORMA ; CAMPOS-TAKAKI, GALBA . Waste Soybean Oil and Corn Steep Liquor as Economic Substrates for Bioemulsifier and Biodiesel Production by *Candida lipolytica* UCP 0998. International Journal of Molecular Sciences (Online), v. 17, p. 1608, 2016.

LIMA, R. A. ; ANDRADE, R. F. S. ; **Rodríguez, D.M.** ; ARAUJO, H. W. C. ; SANTOS, Vanessa. P. ; Galba M. Campos-Takaki . Production and characterization of biosurfactant isolated from *Candida glabrata* using renewable substrates. African Journal of Microbiology Research, v. 11, p. 237-244, 2017.

HELVIA, W. CASULLO ARAUJO ; ROSILEIDE, F. S. ANDRADE ; **DAYANA, MONTERO-RODRÍGUEZ** ; VANESSA, PIMENTEL SANTOS ; PATRICIA, C. V. SOUZA MAIA ; CARLOS, F. B. COSTA FILHO ; CARLOS, A. ALVES DA SILVA ; GALBA, MARIA CAMPOS-TAKAKI . Biochemical and molecular identification of newly isolated pigmented bacterium and improved production of biosurfactant. AFRICAN JOURNAL OF MICROBIOLOGY RESEARCH[JCR], v. 11, p. 945-954, 2017.

D. Montero-Rodríguez, R. F. S. Andrade, D. Rubio-Ribeaux, T. A. L. Silva, G. K. B. Silva, H. W. C. Araújo and G. M. Campos-Takaki. Suitability of wheat bran as promising substrate for coproduction of prodigiosin and biosurfactant by *Serratia marcescens* UCP/WFCC 1549

In: VII International Conference on Environmental, Industrial and Applied Microbiology BioMicroWorld 2017, 2017, Madrid. Book of Abstracts BioMicroWorld 2017, 2017. v. 1. p. 170

L. M. Teixeira, A. P. B. Silva, **D. Montero-Rodríguez**, R. F. S. Andrade, D. Rubio-Ribeaux, T. A. L. Silva, G. K. B. Silva, G. M. Campos-Takaki and H. W. C. Araújo. Pineapple peels and animal fat as low-cost substrates for biosurfactant production by *Serratia marcescens* UCP 1549 In: VII International Conference on Environmental, Industrial and Applied Microbiology BioMicroWorld 2017, 2017, Madrid. Book of Abstracts BioMicroWorld 2017, 2017. v. 1. p. 163

Biology — Instructions for Authors

Shortcuts

Submission Checklist

Please

1. read the Aims & Scope to gain an overview and assess if your manuscript is suitable for this journal;
2. use the Microsoft Word template or LaTeX template to prepare your manuscript;
3. make sure that issues about publication ethics, research ethics, copyright, authorship, figure formats, dataand references format have been appropriately considered; and
4. ensure that all authors have approved the content of the submitted manuscript.

Manuscript Submission Overview

Types of Publications

Biology has no restrictions on the length of manuscripts, provided that the text is concise and comprehensive. Full experimental details must be provided so that the results can be reproduced. *Biology* requires that authors publish all experimental controls and make full datasets available where possible (please read the guidelines about Supplementary Materials and references to unpublished data carefully).

Manuscripts submitted to *Biology* should neither been published before nor be under consideration for publication in another journal. The main article types are as follows:

- *Articles:* research manuscripts that report new evidence or new conclusions. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides a substantial amount of new information. Authors should not unnecessarily divide their work into several related manuscripts, although short *Communications* of preliminary, but significant, results will be considered. Replications of previous studies are permitted if clearly indicated as such.
- *Reviews:* review manuscripts provide concise and precise updates on the latest progress made in a given area of research. Systematic reviews should follow the PRISMA guidelines.
- *Conference Papers:* Expanded and high quality conference papers are also considered in *Biology* if they fulfill the following requirements: (1) the paper should be expanded to the size of a research article; (2) the conference paper should be cited and noted on the first page of the paper; (3) if the authors do not hold the copyright to the published conference paper, authors should seek the appropriate permission from the copyright holder; (4) authors are asked to disclose that it is conference paper in their cover letter and include a statement on what has been changed compared to the original conference paper.

Submission Process

Manuscripts for *Biology* should be submitted online at susy.mdpi.com. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the criteria to qualify for authorship) and that they all have read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the submission website. Once you have registered, click here to go to the submission form for *Biology*. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

Accepted File Formats

Authors must use the Microsoft Word template or LaTeX template to prepare their manuscript. Using the template file will substantially shorten the time to complete copy-editing and publication of accepted manuscripts. The total amount of data for all files must not exceed 120 MB. If this is a problem, please contact the editorial office Biology@mdpi.com. Accepted file formats are:

- *Microsoft Word:* Manuscripts prepared in Microsoft Word must be converted into a single file before submission. When preparing manuscripts in Microsoft Word, the *Biology* Microsoft Word template file must be used. Please insert your graphics (schemes, figures, *etc.*) in the main text after the paragraph of its first citation.
- *LaTeX:* Manuscripts prepared in LaTeX must be collated into one ZIP folder (include all source files and images, so that the Editorial Office can recompile the submitted PDF). When preparing manuscripts in LaTeX, please use the *Biology* LaTeX template files. You can now also use the online application writeLaTeXto submit articles directly to *Biology*. The MDPI LaTeX template file should be selected from the writeLaTeX template gallery.
- *Supplementary files:* May be any format, but it is recommended that you use common, non-proprietary formats where possible (see below for further details).

Cover Letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of your paper is significant, placing your findings in the context of existing work and why it fits the scope of the journal. Please confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. Any prior submissions of the manuscript to MDPI journals must be acknowledged. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

Note for Authors Funded by the National Institutes of Health (NIH)

This journal automatically deposits papers to PubMed Central after publication of an issue. Authors do not need to separately submit their papers through the NIH Manuscript Submission System (NIHMS, http://nihms.nih.gov/).

[Return to top]

Preparation of a Manuscript

General Considerations

- **Research manuscripts** should comprise:
  - Front matter: Title, Author list, Affiliations, Abstract, Keywords
  - Research manuscript sections: Introduction, Materials and Methods, Results, Discussion, Conclusions (optional).

- Back matter: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, References.

- **Review manuscripts** should comprise the front matter, literature review sections and the back matter. The template file can also be used to prepare the front and back matter of your review manuscript. It is not necessary to follow the remaining structure. Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.
- **Abstract graphic:** Authors are encouraged to provide a graphical abstract as a self-explanatory image to appear alongside with the text abstract in the Table of Contents. Figures should be a high quality image in any common image format. Note that images displayed online will be up to 11 by 9 cm on screen and the figure should be clear at this size.
- **Abbreviations** should be defined in parentheses the first time they appear in the abstract, main text, and in figure or table captions.
- **SI Units** (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible
- **Accession numbers** of RNA, DNA and protein sequences used in the manuscript should be provided in the Materials and Methods section. Please also see the section on Deposition of Sequences and of Expression Data.
- **Equations:** If you are using Word, please use either the Microsoft Equation Editor or the MathType add-on. Equations should be editable by the editorial office and not appear in a picture format.
- **Research Data and supplementary materials:** Note that publication of your manuscript implies that you must make all materials, data, and protocols associated with the publication available to readers. Please disclose at the submission stage any restrictions on the availability of materials or information. Read the information about Supplementary Materials and Data Deposit for additional guidelines.
- **Preregistration:** Where authors have preregistered studies or analysis plans, links to the preregistration must be provided in the manuscript.
- **Guidelines and standards:** MDPI follows standards and guidelines for certain types of research. See http://www.mdpi.com/editorial_process for further information.

[Return to top]

Front Matter

These sections should appear in all manuscript types

- **Title:** The title of your manuscript should be concise, specific and relevant. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.
- **Author List and Affiliations:** Authors' full first and last names must be provided. The initials of any middle names can be added. The PubMed/MEDLINE standard format is used for affiliations: complete address information including city, zip code, state/province, country, and all email addresses. At least one author should be designated as corresponding author, and his or her email address and other details should be included at the end of the affiliation section. Please read the criteria to qualify for authorship.
- **Abstract:** The abstract should be a total of about 200 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.
- **Keywords:** Three to ten pertinent keywords need to be added after the abstract. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Research Manuscript Sections

- **Introduction:** The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance, including specific hypotheses being tested. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the main conclusions. As far as possible, please keep the introduction comprehensible to scientists working outside the topic of the paper.
- **Materials and Methods:** They should be described with sufficient detail to allow others to replicate and build on published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. Give the name and version of any software used and make clear whether computer code used is available. Include any pre-registration codes.
- **Results:** Provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.
- **Discussion:** Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible and limitations of the work highlighted. Future research directions may also be mentioned. This section may be combined with Results.
- **Conclusions:** This section is not mandatory, but can be added to the manuscript if the discussion is unusually long or complex.
- **Patents:** This section is not mandatory, but may be added if there are patents resulting from the work reported in this manuscript.

[Return to top]

Back Matter

- **Supplementary Materials:** Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.
- **Acknowledgments:** All sources of funding of the study should be disclosed. Clearly indicate grants that you have received in support of your research work and if you received funds to cover publication costs. Note that some funders will not refund article processing charges (APC) if the funder and grant number are not clearly and correctly identified in the paper. Funding information can be entered separately into the submission system by the authors during submission of their manuscript. Such funding information, if available, will be deposited to FundRef if the manuscript is finally published.
- **Author Contributions:** Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it; AND has approved the submitted version (and version substantially edited by journal staff that involves the author's contribution to the study); AND agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.
  For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "X and Y conceived and designed the experiments; X performed the experiments; Y analyzed the data; Y wrote the paper." **Authorship must include and be limited to those who have contributed substantially to the work. Please read the section concerning the criteria to qualify for authorship carefully.**
- **Conflicts of Interest:** Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. If there is no conflict of interest, please state "The authors declare no conflict of interest." Any role of the funding sponsors in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results must be declared in this section. If there is no role, please state "The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results".

- **References:** References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, ReferenceManager or Zotero to avoid typing mistakes and duplicated references. We encourage citations to data, computer code and other citable research material. Include the digital object identifier (DOI) for all references where available. If available online, you may use reference style 9. below.
- Citations and References in Supplementary files are permitted provided that they also appear in the main text and in the reference list.

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10). or [6] (pp. 101–105).

The reference list should include the full title, as recommended by the ACS style guide. Style files for Endnoteand Zotero are available.

References should be described as follows, depending on the type of work:

 Journal Articles:
1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range, DOI. Available online: URL (accessed on Day Month Year).

 Books and Book Chapters:
2. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, Year; pp. 154–196; ISBN.
3. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A.; Editor 2, B., Eds.; Publisher: Publisher Location, Country, Year; Volume 3, pp. 154–196; ISBN.

 Unpublished work, submitted work, personal communication:
4. Author 1, A.B.; Author 2, C. Title of Unpublished Work. status (unpublished; manuscript in preparation).
5.Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Abbreviated Journal Name* stage of publication (under review; accepted; in press).
6.Author 1, A.B. (University, City, State, Country); Author 2, C. (Institute, City, State, Country). Personal communication, Year.

 Conference Proceedings:
7. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In *Title of the Collected Work* (if available), Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference; Editor 1, Editor 2, Eds. (if available); Publisher: City, Country, Year (if available); Abstract Number (optional), Pagination (optional).

 Thesis:
8. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.

 Websites:
9.Title of Site. Available online: URL (accessed on Day Month Year).
Unlike published works, websites may change over time or disappear, so we encourage you create an archive of the cited website using a service such as WebCite. Archived websites should be cited using the link provided as follows:

10. Title of Site. URL (archived on Day Month Year).
See the Reference List and Citations Guide for more detailed information.

[Return to top]

Preparing Figures, Schemes and Tables

- File for Figures and schemes must be provided during submission in a single zip archive and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.
- *Biology* can publish multimedia files in articles or as supplementary materials. Please contact the editorial office for further information.
- All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).
- All Figures, Schemes and Tables should have a short explanatory title and caption.
- All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Authors should use the Table option of Microsoft Word to create tables.
- Authors are encouraged to prepare figures and schemes in color (RGB at 8-bit per channel). There is no additional cost for publishing full color graphics.

[Return to top]

Supplementary Materials, Data Deposit and Software Source Code

Data Availability

In order to maintain the integrity, transparency and reproducibility of research records, authors must make their experimental and research data openly available either by depositing into data repositories or by publishing the data and files as supplementary information in this journal.

Computer Code and Software

For work where novel computer code was developed, authors should release the code either by depositing in a recognized, public repository or uploading as supplementary information to the publication. The name and version of all software used should be clearly indicated.

Supplementary Material

Additional data and files can be uploaded as "Supplementary Files" during the manuscript submission process. The supplementary files will also be available to the referees as part of the peer-review process. Any file format is acceptable, however we recommend that common, non-proprietary formats are used where possible.

Unpublished Data

Restrictions on data availability should be noted during submission and in the manuscript. "Data not shown" should be avoided: authors are encouraged to publish all observations related to the submitted manuscript as Supplementary Material. "Unpublished data" intended for publication in a manuscript that is either planned, "in preparation" or "submitted" but not yet accepted, should be cited in the text and a reference should be added in

the References section. "Personal Communication" should also be cited in the text and reference added in the References section. (see also the MDPI reference list and citations style guide).

Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal Data also accepts submissions of data set papers.

Deposition of Sequences and of Expression Data

New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

- *New nucleic acid sequences* must be deposited in one of the following databases: GenBank, EMBL, or DDBJ. Sequences should be submitted to only one database.
- *New high throughput sequencing (HTS) datasets* (RNA-seq, ChIP-Seq, degradome analysis, …) must be deposited either in the GEO database or in the NCBI's Sequence Read Archive.
- *New microarray data* must be deposited either in the GEO or the ArrayExpress databases.The "Minimal Information About a Microarray Experiment" (MIAME) guidelines published by the Microarray Gene Expression Data Society must be followed.
- *New protein sequences* obtained by protein sequencing must be submitted to UniProt (submission tool SPIN).

All sequence names and the accession numbers provided by the databases should be provided in the Materials and Methods section of the article.

References in Supplementary Files

Citations and References in Supplementary files are permitted provided that they also appear in the reference list of the main text.

[Return to top]

Research and Publication Ethics

Research Ethics

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), revised in 2008. According to point 23 of this declaration, an approval from an ethics committee should have been obtained before undertaking the research. As a minimum, a statement including the project identification code, date of approval and name of the ethics committee or institutional review board should be cited in the Methods Section of the article. Data relating to individual participants must be described in detail, but private information identifying participants need not be included unless the identifiable materials are of relevance to the research (for example, photographs of

participants' faces that show a particular symptom). Editors reserve the right to reject any submission that does not meet these requirements.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

A written informed consent for publication must be obtained from participating patients who can be identified (including by the patients themselves). Patients' initials or other personal identifiers must not appear in an image. For manuscripts that include any case details, personal information, and/or images of patients, authors must obtain signed informed consent from patients (or their relatives/guardians) before submitting to an MDPI journal. Patient details must be anonymized as far as possible, e.g., do not mention specific age, ethnicity, or occupation where they are not relevant to the conclusions.

You may refer to our sample form and provide an appropriate form after consulting with your affiliated institution. Alternatively, you may provide a detailed justification of why informed consent is not necessary. For the purposes of publishing in MDPI journals, a consent, permission, or release form should include unlimited permission for publication in all formats (including print, electronic, and online), in sublicensed and reprinted versions (including translations and derived works), and in other works and products under open access license. To respect patients' and any other individual's privacy, please do not send signed forms. The journal reserves the right to ask authors to provide signed forms if necessary.

Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs':

- Replacement of animals by alternatives wherever possible,
- Reduction in number of animals used, and
- Refinement of experimental conditions and procedures to minimize the harm to animals.

Any experimental work must also have been conducted in accordance with relevant national legislation on the use of animals for research. For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [1].

Manuscripts containing original descriptions of research conducted in experimental animals must contain details of approval by a properly constituted research ethics committee. As a minimum, the project identification code, date of approval and name of the ethics committee or institutional review board should be cited in the Methods section.

Biology endorses the ARRIVE guidelines (www.nc3rs.org.uk/ARRIVE) for reporting experiments using live animals. Authors and reviewers can use the ARRIVE guidelines as a checklist, which can be found at www.nc3rs.org.uk/ARRIVEchecklist.

1. Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Used in Scientific Procedures. Available online: http://www.official-documents.gov.uk/document/hc8889/hc01/0107/0107.pdf.

Research Involving Plants

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana, Nicotiana benthamiana, Oriza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from 'Crown Mix' (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopis mutant lines (SALKxxxx, SAILxxxx,…) were kindly provided by Dr. XXX , institute, city, country).

Publication Ethics Statement

Biology is a member of the Committee on Publication Ethics (COPE). We fully adhere to its Code of Conductand to its Best Practice Guidelines.

The editors of this journal enforce a rigorous peer-review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *Biology* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *Biology* must abide to the following:

- Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.
- Authors should accurately present their research findings and include an objective discussion of the significance of their findings.
- Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.
- Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.
- Simultaneous submission of manuscripts to more than one journal is not tolerated.

- Republishing content that is not novel is not tolerated (for example, an English translation of a paper that is already published in another language will not be accepted).
- If errors and inaccuracies are found by the authors after publication of their paper, they need to be promptly communicated to the editors of this journal so that appropriate actions can be taken. Please refer to our policy regarding publication of publishing addenda and corrections.
- Your manuscript should not contain any information that has already been published. If you include already published figures or images, please obtain the necessary permission from the copyright holder to publish under the CC-BY license. For further information, see the Rights and Permissions page.
- Plagiarism, data fabrication and image manipulation are not tolerated.
    - **Plagiarism is not acceptable** in *Biology* submissions.

      Plagiarism includes copying text, ideas, images, or data from another source, even from your own publications, without giving any credit to the original source.

      Reuse of text that is copied from another source must be between quotes and the original source must be cited. If a study's design or the manuscript's structure or language has been inspired by previous works, these works must be explicitly cited.

      If plagiarism is detected during the peer review process, the manuscript may be rejected. If plagiarism is detected after publication, we may publish a correction or retract the paper.

    - **Image files must not be manipulated or adjusted in any way** that could lead to misinterpretation of the information provided by the original image.

      Irregular manipulation includes: 1) introduction, enhancement, moving, or removing features from the original image; 2) grouping of images that should obviously be presented separately (e.g., from different parts of the same gel, or from different gels); or 3) modifying the contrast, brightness or color balance to obscure, eliminate or enhance some information.

      If irregular image manipulation is identified and confirmed during the peer review process, we may reject the manuscript. If irregular image manipulation is identified and confirmed after publication, we may correct or retract the paper.

Our in-house editors will investigate any allegations of publication misconduct and may contact the authors' institutions or funders if necessary. If evidence of misconduct is found, appropriate action will be taken to correct or retract the publication. Authors are expected to comply with the best ethical publication practices when publishing with MDPI.

[Return to top]

Suggesting Reviewers

During the submission process, please suggest three potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper.

[Return to top]

English Corrections

To facilitate proper peer-reviewing of your manuscript, it is essential that it is submitted in grammatically correct English. Advice on some specific language points can be found here.

If you are not a native English speaker, we recommend that you have your manuscript professionally edited before submission or read by a native English-speaking colleague. This can be carried out by MDPI's English editing service. Professional editing will mean that reviewers and future readers are better able to read and assess the content of your manuscript. All accepted manuscript undergo language editing, however **an additional fee will be charged** to authors if very extensive English corrections must be made by the Editorial Office: pricing is according to the service here.

[Return to top]

Preprints

Biology accepts articles that have previously been made available as preprints, provided that they have not undergone peer review (see the article types section for information regarding conference papers).

MDPI operates *Preprints*, a preprint server, to which you can upload submitted papers directly after completing your journal submission. Note that *Preprints* operates independently of the journal and posting a preprint does not affect the peer review process. You can check the *Preprints* instructions for authors for further information.

[Return to top]

Qualification for Authorship

Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it. In addition, all authors must AND has approved the submitted version (and any substantially modified version that involves the author's contribution to the study); AND agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even those in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature. Note that acquisition of funding, collection of data, or general supervision of the research group do not, by themselves, justify authorship.Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgements.

More detailed guidance on authorship is given by the International Council of Medical Journal Editors (ICMJE).The journal also adheres to the standards of the Committee on Publication Ethics (COPE) that "all authors should agree to be listed and should approve the submitted and accepted versions of the publication. Any change to the author list should be approved by all authors including any who have been removed from the list. The corresponding author should act as a point of contact between the editor and the other authors and should keep co-authors informed and involve them in major decisions about the publication (e.g. answering reviewers' comments)." [1]. We reserve the right to request confirmation that all authors meet the authorship conditions.

1. Wager, E.; Kleinert, S. Responsible research publication: international standards for authors. A position statement developed at the 2nd World Conference on Research Integrity, Singapore, July 22-24, 2010.

In Promoting Research Integrity in a Global Environment; Mayer, T., Steneck, N., eds.; Imperial College Press / World Scientific Publishing: Singapore; Chapter 50, pp. 309-16.

[Return to top]

Editorial Procedures and Peer-Review

Initial Checks

All submitted manuscripts received by the Editorial Office will be checked by a professional in-house *Managing Editor* to determine whether it is properly prepared and whether the manuscript follows the ethical policies of the journal, including those for human and animal experimentation. Manuscripts that do not fit the journals ethical policy will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission. After these checks, the *Managing Editor* will consult the journals' *Editor-in-Chief* or the *Guest Editor* (or an *Editorial Board member* in case of a conflict of interest) to determine whether the manuscript fits the scope of the journal and whether it is scientifically sound. No judgment on the significance or potential impact of the work will be made at this stage. Reject decisions at this stage will be verified by the *Editor-in-Chief*.

Peer-Review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. A single-blind review is applied, where authors' identities are known to reviewers. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

In the case of regular submissions, in-house assistant editors will invite experts, including recommendations by an academic editor. These experts may also include *Editorial Board members* and Guest Editors of the journal. In the case of a special issue, the *Guest Editor* will advise on the selection of reviewers. Potential reviewers suggested by the authors may also be considered. Reviewers should not have published with any of the co-authors during the past five years and should not currently work or collaborate with one of the institutes of the co-authors of the submitted manuscript.

Editorial Decision and Revision

All the articles, reviews and communications published in MDPI journals go through the peer-review process and receive at least two reviews. The in-house editor will communicate the decision of the academic editor, which will be one of the following:

- *Accept after Minor Revisions*:
  The paper is in principle accepted after revision based on the reviewer's comments. Authors are given five days for minor revisions.
- *Reconsider after Major Revisions*:
  The acceptance of the manuscript would depend on the revisions. The author needs to provide a point by point response or provide a rebuttal if some of the reviewer's comments cannot be revised. Usually, only one round of major revisions is allowed. Authors will be asked to resubmit the revised paper within ten days and the revised version will be returned to the reviewer for further comments.
- *Reject and Encourage Resubmission*:
  An article where additional experiments are needed to support the conclusions will be rejected and the authors will be encouraged to re-submit the paper once further experiments have been conducted.

- *Reject*:
  The article has serious flaws, makes no original contribution, and the paper is rejected with no offer of resubmission to the journal.

All reviewer comments should be responded to in a point-by-point fashion. Where the authors disagree with a reviewer, they must provide a clear response.

Author Appeals

Authors may appeal a rejection by sending an e-mail to the Editorial Office of the journal. The appeal must provide a detailed justification, including point-by-point responses to the reviewers' and/or Editor's comments. The *Managing Editor* of the journal will forward the manuscript and relating information (including the identities of the referees) to an Editorial Board member. If no appropriate Editorial Board member is available, the editor will identify a suitable external scientist. The Editorial Board member will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage will be final and cannot be revoked.

In the case of a special issue, the *Managing Editor* of the journal will forward the manuscript and relating information (including the identities of the referees) to the *Editor-in-Chief* who will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage will be final and cannot be revoked.

Production and Publication

Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, final corrections, pagination, and, publication on the www.mdpi.com website.

[Return to top]

Clinical Trials Registration

Registration

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register or and to cite a reference to the registration in the Methods section. Suitable databases include clinicaltrials.gov, the EU Clinical Trials Register and those listed by the World Health Organisation International Clinical Trials Registry Platform.

CONSORT Statement

Biology requires a completed CONSORT 2010 checklist and flow diagram as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (http://www.consort-statement.org) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist. Meeting these basic reporting requirements will greatly improve the value of your trial report and may enhance its chances for eventual publication.

International Journal of Molecular Sciences — Instructions for Authors

Shortcuts

Submission Checklist

Please

1. read the Aims & Scope to gain an overview and assess if your manuscript is suitable for this journal;
2. use the Microsoft Word template or LaTeX template to prepare your manuscript;
3. make sure that issues about publication ethics, research ethics, copyright, authorship, figure formats, dataand references format have been appropriately considered; and
4. ensure that all authors have approved the content of the submitted manuscript.

Manuscript Submission Overview

Types of Publications

IJMS has no restrictions on the length of manuscripts, provided that the text is concise and comprehensive. Full experimental details must be provided so that the results can be reproduced. *IJMS* requires that authors publish all experimental controls and make full datasets available where possible (please read the guidelines about Supplementary Materials and references to unpublished data carefully).

Manuscripts submitted to *IJMS* should neither been published before nor be under consideration for publication in another journal. The main article types are as follows:

- *Articles:* research manuscripts that report new evidence or new conclusions. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides a substantial amount of new information. Authors should not unnecessarily divide their work into several related manuscripts, although short *Communications* of preliminary, but significant, results will be considered. Replications of previous studies are permitted if clearly indicated as such.
- *Reviews:* review manuscripts provide concise and precise updates on the latest progress made in a given area of research. Systematic reviews should follow the PRISMA guidelines.
- *Case reports:* Case reports present detailed information on the symptoms, signs, diagnosis, treatment (including all types of interventions), and outcomes of an individual patient. Case reports usually describe new or uncommon conditions that serve to enhance medical care or highlight diagnostic approaches.
- *Conference Papers:* Expanded and high quality conference papers are also considered in *IJMS* if they fulfill the following requirements: (1) the paper should be expanded to the size of a research article; (2) the conference paper should be cited and noted on the first page of the paper; (3) if the authors do not hold the copyright to the published conference paper, authors should seek the appropriate permission

from the copyright holder; (4) authors are asked to disclose that it is conference paper in their cover letter and include a statement on what has been changed compared to the original conference paper.

Submission Process

Manuscripts for *IJMS* should be submitted online at susy.mdpi.com. The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the criteria to qualify for authorship) and that they all have read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the submission website. Once you have registered, click here to go to the submission form for *IJMS*. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

Accepted File Formats

Authors must use the Microsoft Word template or LaTeX template to prepare their manuscript. Using the template file will substantially shorten the time to complete copy-editing and publication of accepted manuscripts. The total amount of data for all files must not exceed 120 MB. If this is a problem, please contact the editorial office IJMS@mdpi.com. Accepted file formats are:

- *Microsoft Word:* Manuscripts prepared in Microsoft Word must be converted into a single file before submission. When preparing manuscripts in Microsoft Word, the *IJMS* Microsoft Word template file must be used. Please insert your graphics (schemes, figures, *etc.*) in the main text after the paragraph of its first citation.
- *LaTeX:* Manuscripts prepared in LaTeX must be collated into one ZIP folder (include all source files and images, so that the Editorial Office can recompile the submitted PDF). When preparing manuscripts in LaTeX, please use the *IJMS* LaTeX template files. You can now also use the online application writeLaTeXto submit articles directly to *IJMS*. The MDPI LaTeX template file should be selected from the writeLaTeX template gallery.
- *Supplementary files:* May be any format, but it is recommended that you use common, non-proprietary formats where possible (see below for further details).

Cover Letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of your paper is significant, placing your findings in the context of existing work and why it fits the scope of the journal. Please confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. Any prior submissions of the manuscript to MDPI journals must be acknowledged. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

Note for Authors Funded by the National Institutes of Health (NIH)

This journal automatically deposits papers to PubMed Central after publication of an issue. Authors do not need to separately submit their papers through the NIH Manuscript Submission System (NIHMS, http://nihms.nih.gov/).

[Return to top]

Preparation of a Manuscript

General Considerations

- **Research manuscripts** should comprise:
    - Front matter: Title, Author list, Affiliations, Abstract, Keywords
    - Research manuscript sections: Introduction, Results, Discussion, Materials and Methods, Conclusions (optional).
    - Back matter: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, References.
- **Review manuscripts** should comprise the front matter, literature review sections and the back matter. The template file can also be used to prepare the front and back matter of your review manuscript. It is not necessary to follow the remaining structure. Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.
- **Case reports** should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.
- **Abstract graphic:** Authors are encouraged to provide a graphical abstract as a self-explanatory image to appear alongside with the text abstract in the Table of Contents. Figures should be a high quality image in any common image format. Note that images displayed online will be up to 11 by 9 cm on screen and the figure should be clear at this size.
- **Abbreviations** should be defined in parentheses the first time they appear in the abstract, main text, and in figure or table captions.
- **SI Units** (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible
- **Accession numbers** of RNA, DNA and protein sequences used in the manuscript should be provided in the Materials and Methods section. Please also see the section on Deposition of Sequences and of Expression Data.
- **Equations:** If you are using Word, please use either the Microsoft Equation Editor or the MathType add-on. Equations should be editable by the editorial office and not appear in a picture format.
- **Research Data and supplementary materials:** Note that publication of your manuscript implies that you must make all materials, data, and protocols associated with the publication available to readers. Please disclose at the submission stage any restrictions on the availability of materials or information. Read the information about Supplementary Materials and Data Deposit for additional guidelines.
- **Preregistration:** Where authors have preregistered studies or analysis plans, links to the preregistration must be provided in the manuscript.
- **Guidelines and standards:** MDPI follows standards and guidelines for certain types of research. See http://www.mdpi.com/editorial_process for further information.

[Return to top]

Front Matter

These sections should appear in all manuscript types

- **Title:** The title of your manuscript should be concise, specific and relevant. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.
- **Author List and Affiliations:** Authors' full first and last names must be provided. The initials of any middle names can be added. The PubMed/MEDLINE standard format is used for affiliations: complete address information including city, zip code, state/province, country, and all email addresses. At least one author should be designated as corresponding author, and his or her email address and other details should be included at the end of the affiliation section. Please read the criteria to qualify for authorship.

- **Abstract:** The abstract should be a total of about 200 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.
- **Keywords:** Three to ten pertinent keywords need to be added after the abstract. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Research Manuscript Sections

- **Introduction:** The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance, including specific hypotheses being tested. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the main conclusions. As far as possible, please keep the introduction comprehensible to scientists working outside the topic of the paper.
- **Results:** Provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.
- **Discussion:** Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible and limitations of the work highlighted. Future research directions may also be mentioned. This section may be combined with Results.
- **Materials and Methods:** They should be described with sufficient detail to allow others to replicate and build on published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. Give the name and version of any software used and make clear whether computer code used is available. Include any pre-registration codes.
- **Conclusions:** This section is not mandatory, but can be added to the manuscript if the discussion is unusually long or complex.
- **Patents:** This section is not mandatory, but may be added if there are patents resulting from the work reported in this manuscript.

[Return to top]

Back Matter

- **Supplementary Materials:** Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.
- **Acknowledgments:** All sources of funding of the study should be disclosed. Clearly indicate grants that you have received in support of your research work and if you received funds to cover publication costs. Note that some funders will not refund article processing charges (APC) if the funder and grant number are not clearly and correctly identified in the paper. Funding information can be entered separately into the submission system by the authors during submission of their manuscript. Such funding information, if available, will be deposited to FundRef if the manuscript is finally published.
- **Author Contributions:** Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it; AND has approved the submitted version (and version substantially edited by journal staff that involves the author's contribution to the study); AND agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.
  For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "X and Y conceived and designed the

experiments; X performed the experiments; Y analyzed the data; Y wrote the paper." **Authorship must include and be limited to those who have contributed substantially to the work. Please read the section concerning the criteria to qualify for authorship carefully.**

- **Conflicts of Interest:** Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. If there is no conflict of interest, please state "The authors declare no conflict of interest." Any role of the funding sponsors in the design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript, or in the decision to publish the results must be declared in this section. If there is no role, please state "The founding sponsors had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, and in the decision to publish the results".
- **References:** References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, ReferenceManager or Zotero to avoid typing mistakes and duplicated references. We encourage citations to data, computer code and other citable research material. Include the digital object identifier (DOI) for all references where available. If available online, you may use reference style 9. below.
- Citations and References in Supplementary files are permitted provided that they also appear in the main text and in the reference list.

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10). or [6] (pp. 101–105).

The reference list should include the full title, as recommended by the ACS style guide. Style files for Endnoteand Zotero are available.

References should be described as follows, depending on the type of work:

 Journal Articles:
1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range, DOI. Available online: URL (accessed on Day Month Year).

 Books and Book Chapters:
2. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, Year; pp. 154–196; ISBN.
3. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A.; Editor 2, B., Eds.; Publisher: Publisher Location, Country, Year; Volume 3, pp. 154–196; ISBN.

 Unpublished work, submitted work, personal communication:
4. Author 1, A.B.; Author 2, C. Title of Unpublished Work. status (unpublished; manuscript in preparation).
5.Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Abbreviated Journal Name* stage of publication (under review; accepted; in press).
6.Author 1, A.B. (University, City, State, Country); Author 2, C. (Institute, City, State, Country). Personal communication, Year.

 Conference Proceedings:
7. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In *Title of the Collected Work* (if available), Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference; Editor 1, Editor 2, Eds. (if available); Publisher: City, Country, Year (if available); Abstract Number (optional), Pagination (optional).

 Thesis:
8. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.

 Websites:
9.Title of Site. Available online: URL (accessed on Day Month Year).
Unlike published works, websites may change over time or disappear, so we encourage you create an archive of the cited website using a service such as WebCite. Archived websites should be cited using the link provided as follows:
10. Title of Site. URL (archived on Day Month Year).
See the Reference List and Citations Guide for more detailed information.

[Return to top]

Preparing Figures, Schemes and Tables

- File for Figures and schemes must be provided during submission in a single zip archive and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.
- *IJMS* can publish multimedia files in articles or as supplementary materials. Please contact the editorial office for further information.
- All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).
- All Figures, Schemes and Tables should have a short explanatory title and caption.
- All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Authors should use the Table option of Microsoft Word to create tables.
- Authors are encouraged to prepare figures and schemes in color (RGB at 8-bit per channel). There is no additional cost for publishing full color graphics.

[Return to top]

Supplementary Materials, Data Deposit and Software Source Code

Data Availability

In order to maintain the integrity, transparency and reproducibility of research records, authors must make their experimental and research data openly available either by depositing into data repositories or by publishing the data and files as supplementary information in this journal.

Computer Code and Software

For work where novel computer code was developed, authors should release the code either by depositing in a recognized, public repository or uploading as supplementary information to the publication. The name and version of all software used should be clearly indicated.

Supplementary Material

Additional data and files can be uploaded as "Supplementary Files" during the manuscript submission process. The supplementary files will also be available to the referees as part of the peer-review process. Any file format is acceptable, however we recommend that common, non-proprietary formats are used where possible.

Unpublished Data

Restrictions on data availability should be noted during submission and in the manuscript. "Data not shown" should be avoided: authors are encouraged to publish all observations related to the submitted manuscript as Supplementary Material. "Unpublished data" intended for publication in a manuscript that is either planned, "in preparation" or "submitted" but not yet accepted, should be cited in the text and a reference should be added in the References section. "Personal Communication" should also be cited in the text and reference added in the References section. (see also the MDPI reference list and citations style guide).

Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal Data also accepts submissions of data set papers.

Deposition of Sequences and of Expression Data

New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

- *New nucleic acid sequences* must be deposited in one of the following databases: GenBank, EMBL, or DDBJ. Sequences should be submitted to only one database.
- *New high throughput sequencing (HTS) datasets* (RNA-seq, ChIP-Seq, degradome analysis, …) must be deposited either in the GEO database or in the NCBI's Sequence Read Archive.
- *New microarray data* must be deposited either in the GEO or the ArrayExpress databases.The "Minimal Information About a Microarray Experiment" (MIAME) guidelines published by the Microarray Gene Expression Data Society must be followed.
- *New protein sequences* obtained by protein sequencing must be submitted to UniProt (submission tool SPIN).

All sequence names and the accession numbers provided by the databases should be provided in the Materials and Methods section of the article.

References in Supplementary Files

Citations and References in Supplementary files are permitted provided that they also appear in the reference list of the main text.

[Return to top]

Research and Publication Ethics

Research Ethics

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), revised in 2008. According to point 23 of this declaration, an approval from an ethics committee should have been obtained before undertaking the research. As a minimum, a statement including the project identification code, date of approval and name of the ethics committee or institutional review board should be cited in the Methods Section of the article. Data relating to individual participants must be described in detail, but private information identifying participants need not be included unless the identifiable materials are of relevance to the research (for example, photographs of participants' faces that show a particular symptom). Editors reserve the right to reject any submission that does not meet these requirements.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

A written informed consent for publication must be obtained from participating patients who can be identified (including by the patients themselves). Patients' initials or other personal identifiers must not appear in an image. For manuscripts that include any case details, personal information, and/or images of patients, authors must obtain signed informed consent from patients (or their relatives/guardians) before submitting to an MDPI journal. Patient details must be anonymized as far as possible, e.g., do not mention specific age, ethnicity, or occupation where they are not relevant to the conclusions.

You may refer to our sample form and provide an appropriate form after consulting with your affiliated institution. Alternatively, you may provide a detailed justification of why informed consent is not necessary. For the purposes of publishing in MDPI journals, a consent, permission, or release form should include unlimited permission for publication in all formats (including print, electronic, and online), in sublicensed and reprinted versions (including translations and derived works), and in other works and products under open access license. To respect patients' and any other individual's privacy, please do not send signed forms. The journal reserves the right to ask authors to provide signed forms if necessary.

Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs':

- Replacement of animals by alternatives wherever possible,
- Reduction in number of animals used, and
- Refinement of experimental conditions and procedures to minimize the harm to animals.

Any experimental work must also have been conducted in accordance with relevant national legislation on the use of animals for research. For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [1].

Manuscripts containing original descriptions of research conducted in experimental animals must contain details of approval by a properly constituted research ethics committee. As a minimum, the project identification code, date of approval and name of the ethics committee or institutional review board should be cited in the Methods section.

IJMS endorses the ARRIVE guidelines (www.nc3rs.org.uk/ARRIVE) for reporting experiments using live animals. Authors and reviewers can use the ARRIVE guidelines as a checklist, which can be found at www.nc3rs.org.uk/ARRIVEchecklist.

1. Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Used in Scientific Procedures. Available online: http://www.official-documents.gov.uk/document/hc8889/hc01/0107/0107.pdf.

Research Involving Plants

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana, Nicotiana benthamiana, Oriza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from 'Crown Mix' (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopis mutant lines (SALKxxxx, SAILxxxx,…) were kindly provided by Dr. XXX , institute, city, country).

Publication Ethics Statement

IJMS is a member of the Committee on Publication Ethics (COPE). We fully adhere to its Code of Conduct and to its Best Practice Guidelines.

The editors of this journal enforce a rigorous peer-review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *IJMS* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *IJMS* must abide to the following:

- Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.
- Authors should accurately present their research findings and include an objective discussion of the significance of their findings.
- Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.
- Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.
- Simultaneous submission of manuscripts to more than one journal is not tolerated.
- Republishing content that is not novel is not tolerated (for example, an English translation of a paper that is already published in another language will not be accepted).
- If errors and inaccuracies are found by the authors after publication of their paper, they need to be promptly communicated to the editors of this journal so that appropriate actions can be taken. Please refer to our policy regarding publication of publishing addenda and corrections.
- Your manuscript should not contain any information that has already been published. If you include already published figures or images, please obtain the necessary permission from the copyright holder to publish under the CC-BY license. For further information, see the Rights and Permissions page.
- Plagiarism, data fabrication and image manipulation are not tolerated.
    - **Plagiarism is not acceptable** in *IJMS* submissions.

      Plagiarism includes copying text, ideas, images, or data from another source, even from your own publications, without giving any credit to the original source.

      Reuse of text that is copied from another source must be between quotes and the original source must be cited. If a study's design or the manuscript's structure or language has been inspired by previous works, these works must be explicitly cited.

      If plagiarism is detected during the peer review process, the manuscript may be rejected. If plagiarism is detected after publication, we may publish a correction or retract the paper.

    - **Image files must not be manipulated or adjusted in any way** that could lead to misinterpretation of the information provided by the original image.

      Irregular manipulation includes: 1) introduction, enhancement, moving, or removing features from the original image; 2) grouping of images that should obviously be presented separately (e.g., from different parts of the same gel, or from different gels); or 3) modifying the contrast, brightness or color balance to obscure, eliminate or enhance some information.

      If irregular image manipulation is identified and confirmed during the peer review process, we may reject the manuscript. If irregular image manipulation is identified and confirmed after publication, we may correct or retract the paper.

Our in-house editors will investigate any allegations of publication misconduct and may contact the authors' institutions or funders if necessary. If evidence of misconduct is found, appropriate action will be taken to correct or retract the publication. Authors are expected to comply with the best ethical publication practices when publishing with MDPI.

[Return to top]

Suggesting Reviewers

During the submission process, please suggest three potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper.

[Return to top]

English Corrections

To facilitate proper peer-reviewing of your manuscript, it is essential that it is submitted in grammatically correct English. Advice on some specific language points can be found here.

If you are not a native English speaker, we recommend that you have your manuscript professionally edited before submission or read by a native English-speaking colleague. This can be carried out by MDPI's English editing service. Professional editing will mean that reviewers and future readers are better able to read and assess the content of your manuscript. All accepted manuscript undergo language editing, however **an additional fee will be charged** to authors if very extensive English corrections must be made by the Editorial Office: pricing is according to the service here.

[Return to top]

Preprints

IJMS accepts articles that have previously been made available as preprints, provided that they have not undergone peer review (see the article types section for information regarding conference papers).

MDPI operates *Preprints*, a preprint server, to which you can upload submitted papers directly after completing your journal submission. Note that *Preprints* operates independently of the journal and posting a preprint does not affect the peer review process. You can check the *Preprints* instructions for authors for further information.

[Return to top]

Qualification for Authorship

Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it. In addition, all authors must AND has approved the submitted version (and any substantially modified version that involves the author's contribution to the study); AND agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even those in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature. Note that acquisition of funding,

collection of data, or general supervision of the research group do not, by themselves, justify authorship.Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgements.

More detailed guidance on authorship is given by the International Council of Medical Journal Editors (ICMJE).The journal also adheres to the standards of the Committee on Publication Ethics (COPE) that "all authors should agree to be listed and should approve the submitted and accepted versions of the publication. Any change to the author list should be approved by all authors including any who have been removed from the list. The corresponding author should act as a point of contact between the editor and the other authors and should keep co-authors informed and involve them in major decisions about the publication (e.g. answering reviewers' comments)." [1]. We reserve the right to request confirmation that all authors meet the authorship conditions.

1. Wager, E.; Kleinert, S. Responsible research publication: international standards for authors. A position statement developed at the 2nd World Conference on Research Integrity, Singapore, July 22-24, 2010. In Promoting Research Integrity in a Global Environment; Mayer, T., Steneck, N., eds.; Imperial College Press / World Scientific Publishing: Singapore; Chapter 50, pp. 309-16.

[Return to top]

Editorial Procedures and Peer-Review

Initial Checks

All submitted manuscripts received by the Editorial Office will be checked by a professional in-house *Managing Editor* to determine whether it is properly prepared and whether the manuscript follows the ethical policies of the journal, including those for human and animal experimentation. Manuscripts that do not fit the journals ethical policy will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission. After these checks, the *Managing Editor* will consult the journals' *Editor-in-Chief* or the *Guest Editor* (or an *Editorial Board member* in case of a conflict of interest) to determine whether the manuscript fits the scope of the journal and whether it is scientifically sound. No judgment on the significance or potential impact of the work will be made at this stage. Reject decisions at this stage will be verified by the *Editor-in-Chief*.

Peer-Review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. A single-blind review is applied, where authors' identities are known to reviewers. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

In the case of regular submissions, in-house assistant editors will invite experts, including recommendations by an academic editor. These experts may also include *Editorial Board members* and Guest Editors of the journal. In the case of a special issue, the *Guest Editor* will advise on the selection of reviewers. Potential reviewers suggested by the authors may also be considered. Reviewers should not have published with any of the co-authors during the past five years and should not currently work or collaborate with one of the institutes of the co-authors of the submitted manuscript.

Editorial Decision and Revision

All the articles, reviews and communications published in MDPI journals go through the peer-review process and receive at least two reviews. The in-house editor will communicate the decision of the academic editor, which will be one of the following:

- *Accept after Minor Revisions*:
  The paper is in principle accepted after revision based on the reviewer's comments. Authors are given five days for minor revisions.
- *Reconsider after Major Revisions*:
  The acceptance of the manuscript would depend on the revisions. The author needs to provide a point by point response or provide a rebuttal if some of the reviewer's comments cannot be revised. Usually, only one round of major revisions is allowed. Authors will be asked to resubmit the revised paper within ten days and the revised version will be returned to the reviewer for further comments.
- *Reject and Encourage Resubmission*:
  An article where additional experiments are needed to support the conclusions will be rejected and the authors will be encouraged to re-submit the paper once further experiments have been conducted.
- *Reject*:
  The article has serious flaws, makes no original contribution, and the paper is rejected with no offer of resubmission to the journal.

All reviewer comments should be responded to in a point-by-point fashion. Where the authors disagree with a reviewer, they must provide a clear response.

Author Appeals

Authors may appeal a rejection by sending an e-mail to the Editorial Office of the journal. The appeal must provide a detailed justification, including point-by-point responses to the reviewers' and/or Editor's comments. The *Managing Editor* of the journal will forward the manuscript and relating information (including the identities of the referees) to an Editorial Board member. If no appropriate Editorial Board member is available, the editor will identify a suitable external scientist. The Editorial Board member will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage will be final and cannot be revoked.

In the case of a special issue, the *Managing Editor* of the journal will forward the manuscript and relating information (including the identities of the referees) to the *Editor-in-Chief* who will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage will be final and cannot be revoked.

Production and Publication

Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, final corrections, pagination, and, publication on the www.mdpi.com website.

[Return to top]

Clinical Trials Registration

Registration

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register or and to cite a reference to the registration in the Methods section. Suitable databases include clinicaltrials.gov, the EU

Clinical Trials Register and those listed by the World Health Organisation International Clinical Trials Registry Platform.

CONSORT Statement

IJMS requires a completed CONSORT 2010 checklist and flow diagram as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (http://www.consort-statement.org) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist. Meeting these basic reporting requirements will greatly improve the value of your trial report and may enhance its chances for eventual publication.