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Potential of *Stenotrophomonas maltophilia* in green biosurfactant production

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ABSTRACT

The literature describes several studies focused on the application of S. maltophilia in different areas of biotechnology. Due to its ease of interaction with numerous environments and living beings, this bacterium has an excellent adaptability, being able to convert different substrates into products of high environmental and technological interest. Stenotrophomonas maltophilia is a gram-negative, aerobic, ubiquitous, rod-shaped bacterium belonging to the class of gammaproteobacteria and can be found in various natural and anthropogenic environments, e.g. soil, water, sediments or in association with plants. Biosurfactants are an alternative, compatible with the environment, for the use of chemically synthesized surfactants, since it is a bioproduct obtained through the metabolism of microorganisms and plants. Like their synthetic counterparts, biosurfactants comprise a wide variety of chemical structures and also excellent surfactant properties. The literature presents a wide range of studies focused on the production of biosurfactant by S. maltophilia using several substrates, temperature conditions, pH, salinade and fermentation times. In addition, there is also a huge variety of application of these biosurfactants produced, which drives the incentive to study this microorganism in the production of biosurfactants.

Keywords: Biodegradation, Biosurfactant, Environment, Bacteria.

1 INTRODUCTION

Surfactants are chemical molecules with diverse functions and properties, including surface tension reduction, emulsification, oil displacement, and micelle formation (Drakontis; Amin, 2020). They can be of synthetic or biological origin. Synthetic types are derived from petrochemistry and classified into five groups: anionic, non-ionic, cationic, amphoteric, and polymeric (Mohanty et al., 2021). Synthetic surfactants are expensive and their toxic effect can persist in the environment. These negative impacts have increased the search for alternative surfactants of biological origin, "biosurfactants". Biosurfactants are non-toxic, biodegradable and compatible with the environment. The market value of commercially available biosurfactants is expected to reach $2.6 billion by 2023, gaining more than 8% and generating 540 kilotons of supply by 2024 (Gaur et al., 2022b, Kumar et al., 2021). Biosurfactants are classified according to their biological origin, mode of action, and molecular weight and generally have unique properties that make them better alternatives to synthetic surfactants (Mgbechidinma et al., 2022).

An integrated green economy for the production of biosurfactants overcomes the challenges associated with dispersed waste and the entire production chain (Johann et al., 2022). An integrated production system replaces the "take, make and dispose" structure with the concept of "resource, recovery and recycling" using natural by-products. Green production delays the use of virgin resources and reduces the extra production of waste from various sectors (Awasthi et al., 2022).

Industrial agro residues rich in fat and oil are raw materials of bioprocesses suitable for the production of biosurfactants, examples are the hydrolysate of animal fat and the residues of the palm oil refinery. There are valuable substrates for a multitude of fermenting microbes, including cooking oil (Shi et al., 2021), rice bran oil residues (Mishra et al., 2021), and plant-derived oils (sunflower, soybean, rapeseed, corn, and babassu oils) (Joy et al., 2019; Patowary et al., 2016; Radzuan et al., 2017). Oily residues offer low-cost lipid substrates with precise lipid-carbohydrate balance for microbial growth (Ibrahim et al., 2021; Mgbechidinma et al., 2023; Sohail; Jamil, 2020). Generally, the nutrient content in food waste is heterogeneous, containing about 40 to 60% starch; 5–10% protein; 0.84–1.38% salt; and 10-40% fatty or oily content (Demichelis et al., 2017). Due to their large volumes, low costs and sustainable sources of carbon and nitrogen for microbial growth, agro-industrial residues are considered green substrates for the production of biosurfactants. In addition to assisting in the management of environmental waste, the use of agri-food waste as a substrate also reduces the costs of producing biosurfactants (Das; Kumar, 2018; Mohanty et al., 2021; Sondh et al., 2022).

In this sense, the present study addresses several types of research focused on the production of biosurfactants obtained from the bacterium *Stenotrophomonas maltophilia*. In addition to presenting studies focused on the application of these biomolecules of high biotechnological, industrial and environmental interest.

2 *STENOTROPHOMONAS MALTOPHILIA*

Stenotrophomonas maltophilia is a gram-negative, aerobic, ubiquitous, rod-shaped bacterium belonging to the class of gammaproteobacteria (Chauviat; Meyer; Favre-Bonté, 2023). It is found in various natural and anthropogenic environments, e.g. soil, water, sediments or in association with plants (Ryan et al., 2009). *S. maltophilia* is also found in clinical settings where it can cause a wide variety of infections (Denton et al., 1998). The main studies about this bacterium are related to the clinical area. This opportunistic pathogen has low virulence but is of particular interest due to its natural and intrinsic phenotype of antibiotic resistance (Sanz-García et al., 2021; Deredjian et al., 2016).

S. maltophilia plays important ecosystem functions in sulfur and nitrogen cycles, in the degradation of complex compounds and pollutants, and in promoting plant growth and health. *Stenotrophomonas* can also colonize extreme artificial niches in hospitals, space shuttles, and clean rooms. *S. maltophilia* has emerged as a global opportunistic human pathogen, which generally does not infect healthy hosts, but is associated with high morbidity and mortality in severely immunocompromised and debilitated individuals (Na; Berg, 2018).

2.1 OCCURRENCE OF *S. MALTOPHILIA IN THE* ENVIRONMENT

2.1.1 Soils, water, aquatic environments, sediments

S. maltophilia is found in several soils around the world (Youenou et al., 2015; Abdelrazek et al., 2020; Furlan et al., 2019; Javaid et al., 2020; Shreya et al., 2020). Their presence has been established in soils polluted with various types of contaminants such as heavy metals and antibiotics (Youenou et al., 2015), heavy metals from a mine (Liaquat et al., 2020), metals and industrial minerals from a mineral mine (Arulazhagan et al., 2017). It has also been found in the soil of a gold mine (Nangia et al., 2009) and in soils polluted with zinc and lead from garbage piles (Oleńska et al., 2020), cadmium and chromium (Chien et al., 2007; Chen et al., 2022) and copper (Andreazza et al., 2012). It has been recovered in soils contaminated with crude oil, transformer oil, and sulfurous oil (Elufisan et al., 2019; 2020; Somaraja et al., 2013), soils contaminated with fuel, diesel, gasoline, hydrocarbons and oil (Shahzad et al., 2016) and chemical plants contaminated soil (Lara-Moreno et al., 2021). It has been found in soils containing industrial wine residues (Snellinx et al., 2003), pesticides and surfactants of alkylphenol polyethoxylate (Kumar et al., 2007), an area of e-waste dismantling (Chen et al., 2013) or even plastic debris (Frederico et al., 2021).

The bacterium *S. maltophilia* has been found in soils where the presence of a contaminant is not specified, including bulk soil (Juhnke et al., 1989), unforested dunes of the Merzouga desert (Chemao-Elfihri et al., 2020), drought-prone ecosystems (Haque et al., 2020), beach sand (Singh et al., 2011), soil of an island (Park et al., 2013), soil on the banks of a river (Dubey et al., 2013), soil near a glacier (Kuddus; Ramteke, 2009) muddy soil (Miyaji et al., 2005) and slaughterhouse soil (Waghmare et al., 2015). It is present in soils associated with the use of agricultural land, such as cultivated and altered soils, but also in alterations (Patil et al., 2016; Chauviat; Meyer; Favre-Bonté, 2023).

S. maltophilia has been identified in water, aquatic environments and sediments, its presence has been demonstrated in several natural aquatic environments such as rivers (Li, et al., 2019), sediments and water from rivers and streams (Ochoa-Sánchez; Vinuesa, 2017) on the surface of a lake (De La Rosa-García et al., 2007), in droplets of water from a cave (Enyedi et al., 2020), swamp water (Singh et al., 2011), seawater (Dewi et al., 2020), polluted coastal water (Mançano et al., 2020), groundwater (Martin et al., 2018), and biofilms in natural and artificial aquifers (Jägevall; Rabe; Pedersen, 2011).

In addition, *S. maltophilia* has been isolated in association with wastewater and water treatment plants, in surface water, groundwater, and bioremediation reactor samples from the Niagara watershed (Nakatsu et al., 1995), water treatment and subsequent distribution systems (Hoefel et al., 2005), a drinking water treatment plant (Gomes et al., 2020), wastewater treatment plants (Permala et al., 2018), effluents from palm oil mills (Bala et al., 2018), final effluents from a pig farm (Kim; Park; Seo, 2018) wastewater from an oil reservoir (Hassanshahian et al., 2013) and in contaminated sewage (Elufisan et al., 2020). Several types of sediments containing S. maltophilia have been identified, i.e., sewage sediments from agricultural drainage ponds (Dungan et al., 2003), anaerobic and activated sludge from a wastewater treatment plant (Chen et al., 2020; URSZULA et al., 2009; Xiong et al., 2020), wastewater bioreactor sludge (Bramucci; Nagarajan, 2000) wastewater sediments in a processing unit (Al-Dhabi et al., 2021), sediment from a coal tailings solids storage facility (Siddique et al., 2007), coastal sediment from a shipyard (Patel; Patel; Datta, 2013), sediment of a channel (DUDÁŠOVÁ et al., 2014), fresh water (Jayaraman; Sigamani; Ramamurthy, 2021), seawater (Jamal et al., 2018), and polluted river (Iyer; Damania; Iken, 2019), sediments, and even microplastics from a river (ZHANG, et al. 2021).

2.1.2 Interaction with plants and animals

S. maltophilia interacts with several plants, notably in the rhizosphere. It has been isolated from the rhizospheres of landfill-growing plants (Franco et al., 2005), rice (Oryza sativa) (Mir et al., 2022), oats (Avena sativa) and plantains *(Plantago lanceolata*) (Andreazza et al., 2022), *cabbage (*Brassica oleracea), mustard (Sinapis alba*), corn (Triticum vulgare*), beetroot *(*Beta vulgaris*) (*Dunne et al., 1997), potato *(Solanum tuberosum*) (Lottmann et al., 1999), tomato plants (Solanum lycopersicum) (Haque et al., 2020), eggplant *(Solanum melongena) (Messiha et al., 2007)* and strawberry (Fragaria x *ananassa)* (Berg et *al., 2002*). Strains were recovered from moist brown forest soil (Krsek; Wellington, 2001) and grassland. *S. maltophilia* is part of the phyllosphere of green beans *(Phaseolus vulgaris*) (Leng et al., 2016), potato (Solanum tuberosum*) (*Wilson; Lindow, 1994) and rice (*Oryza sativa*) and has been described as an epiphyte of common ivy (*Hedera helix*) (Sahu et al., 2021), as well as several other plant and plant species.

S. maltophilia is found in reptiles, for example, in the oral microflora of snakes (Hejnar et al., 2007) and in prairie rattlesnakes (Fitzgerald et al., 2013). It has been blamed for chronic respiratory disease of the king python (Albini et al., 2009) and septicemia in the dwarf crocodile of West Africa (Harris; Rogers, 2001). S. maltophilia was isolated from mammals. It has been found in the feces of tapirs (Guan et al., 2008), in porcine semen (Althouse et al., 2000) and can cause mastitis in cows (Bhanderi et al., 2014), chronic respiratory disease in horses, cats and dogs.*S. maltophilia* has been detected in association with fish, including cultured yellowtail (*Seriola quinqueradiata*) (FURUSHITA et al., 2005), cultivated channel catfish (Ictalurus punctatus) (Geng et al., 2010), in the kidneys of juvenile African catfish *(Clarias gariepinus*) (*Abraham et al., 2016).* and in the spleen of rainbow trout *(Oncorhynchus mykiss*) (Duman; Saticioglu; Altun, 2019) where it causes disease. In birds, S. maltophilia has been recovered from feces on a poultry farm (Hassan et al., 2021), from the environment of poultry flocks (Shah et al., 2020), and from the gut microbiota of Condor (Jacobs et al., 2019).

3 BIOSSURFACTANTS

Environmental legislation has evolved rapidly to develop sustainable products. This has inspired modern researchers to examine natural resources for industrial and domestic applications (Othmani et al., 2022; Akcil et al., 2015). Among several synthetic products, surfactants or surfactants are widely used worldwide, with a growing trend of their synthesis and subsequent application (Inès; Dhouha, 2015). Due to their distinct properties, e.g., dispersion, emulsification, biological activity, surfactants have found several industrial applications (Shahid et al., 2021; Banat et al., 2021). Chemical surfactants, also known as surfactants, are synthetic molecules used primarily in industrial products such as washing powder, detergents, toothpastes, soaps, and shampoos (Onaizi, 2021; Zou et al., 2014; Patel; Patel, 2020.).

Surfactants are chemical compounds formed by amphipathic molecules containing hydrophilic and hydrophobic portions that divide into physical interfaces. Apolar portions are often hydrocarbon chains, while polar portions can be cationic, anionic, non-ionic, or amphoteric molecules (Sarubbo et al., 2022). This combination of hydrophobic and hydrophilic portions allows surfactants to reduce surface and interfacial stresses and form microemulsions, in which hydrocarbons are solubilized in water or vice versa (Farias et al., 2021). The most effective way to characterize a surfactant is by measuring the force of attraction between liquid molecules, thus classifying the surfactant in its ability to affect surface and interfacial tensions. Effective surfactants reduce surface tensions, facilitating interactions between molecules of different polar natures (Santos et al., 2016). Most commercially available surfactants are derived from petroleum-based organic compounds, so scientists are now eager for their possible replacement. However, its implication is associated with various environmental concerns due to its source of origin (Kashif et al., 2022). Some recent scientific evidence has suggested the use of bio-based surface active compounds as an innovative and green replacement of their counterparts (Ambaye et al., 2021; Hentati et al., 2021).

The biosurfactant is an alternative, compatible with the environment, for the use of chemically synthesized surfactants since it is a bioproduct obtained through the metabolism of microorganisms and plants. Like their synthetic counterparts, biosurfactants comprise a wide variety of chemical structures. They are produced by microorganisms grown on insoluble (oils, residues and hydrocarbons) or soluble (carbohydrates) substrates (Silva et al., 2014; Bezerra et al., 18). Replacing synthetic surfactants with biosurfactants would reduce CO2 emissions by 8%, avoiding about 1.5 million tons of CO2 released into the atmosphere (Farias et al., 2021; Rocha and Silva et al., 2021; Banat et al., 2021). Currently, biosurfactants occupy about 10% of the total world production of surfactants (approximately ten million tons per year) (Sarubbo et al., 2022). These natural surfactants have applications in the petroleum, food (as emulsifiers), pharmaceutical (formulation of moisturizers, creams and medicines), medical (antimicrobial agents), agricultural (fertilizers) and civil (waste and sewage treatment) industries (Ribeiro; War; Sarubbo, 2020).

4 APPLICATIONS OF *STENOTROPHOMONAS MALTOPHILIA*

4.1 BIOSURFACTANT PRODUCTION

The literature presents a wide range of studies focused on the production of biosurfactant by *S. maltophilia* using several substrates, temperature conditions, pH, salinade and fermentation times. In addition, there is also a huge variety of application of these biosurfactants produced, which drives the incentive to study this microorganism in the production of biosurfactants.

In a study conducted by Ferreira et al., (2022) the bacterium S. maltophilia UCP 1601 converted the low-cost renewable substrates, milhocin, residual glycerol and post-frying soybean oil into an anionic biosurfactant with surface tension of 27mN/m, in addition to stability in different environmental conditions, such as temperature, salinity and pH. All the determinations made confirm the generation of a stable and efficient bioproduct that contributes to the circular bioeconomy and the great potential for remediation in adverse environmental conditions.

Larik et al (2019) evaluated biosurfactant production by *S. maltophilia.* The bacterium showed excellent potential for the production of biosurfactant (oil displacement assay=>6 mm; surface tension=33.9 and 34.4 m N m−1) and lipase enzyme (lipolytic zone=1.8±0.1 cm and 2.2±0.3 cm).

The study conducted by Ezebuiro et al (2019) investigated the effects of nitrogen and carbon sources on the production of biosurfactant by a bacterium that uses hydrocarbons as a substrate by the bacterium, *Stenotrophomonas* sp. The surface tension obtained was 30.85 mN/m. The authors highlight that this method of biosurfactant production can be extended for industrial purposes.

4.2 BIODEGRADATION AND BIOSURFACTANT PRODUCTION

The process of biodegradation happens when the structure of contaminating compounds is transformed with the breakdown of their molecules, in a biotic way, making them, in most cases, less toxic than the original molecules (Fenner et al., 2013; Oliveira et al., 2022).

A study conducted by Larik et al (2019) evaluated the biodegradation of two petrochemical hydrocarbons, namely diesel oil and motor oil used by *S. maltophilia* with blackchemical substrates as the only carbon source. The biodegradation rates of hydrocarbons were estimated in terms of % removal using UV-Vis spectrophotometer, while the characteristic spectral changes in both hydrocarbons were confirmed using infrared Fourier transform spectrometry with attenuated total reflection. Significant degradation rates of 88.5 and 71.8% (respectively) were achieved during the experiment.

Aliyu et al (2022) investigated the potential use of bacterial isolates expressing biosurfactants in the biodegradation of one of the products of the petroleum refining process (PMS- Premium Motor Spirit). Among the microorganisms tested, *S. maltophilia* showed excellent biosurfactant production results in the biodegradation process. The authors point out that the ability of these isolates to grow in mineral salt media supplemented with PMS as the only carbon source presents a true path for exploration in biotechnology, towards bioremediation mediated by biosurfactants of hydrocarbon pollutants in oil-contaminated soils.

A study by Patel, Patel (2020) evaluated the biosurfactant production capacity of *Stenotrophomonas* sp. nov. S1VKR-26, profiling its bioremediation capacity to remediate wastewater from oil refineries in a laboratory-scale bioreactor and evaluating of the phytotoxicity of bioremediated petroleum wastewater. Different PAHs (Polycyclic Aromatic Hydrocarbons) such as naphthalene (93%), phenanthrene (86%), fluoranthene (92%) and pyrene (98.3%), total petroleum hydrocarbons (72.33%) and phenolic compounds (93.06%) were significantly remediated from wastewater after treatment of the S1VKR-26 strain. In addition, the diluted petroleum effluent 1:1 treated with the S1VKR-26 strain has higher germination (100%), vigor (486) and seedling (4.86 cm) compared to the untreated effluent. Indicating an excellent potential of biosurfactant production in the biodegradation process.

Studies indicate the genus *Stenotrophomonas* as a producer of biosurfactant in the process of engine oil degradation (Gargouri et al., 201; Larik et al., 2019), crude oil degradation (Muthukamalam et al., 2017), and diesel oil degradation (Larik et al., 2019). A study by Deng et al. (2015) points to the degradation of eight organophosphate pesticides by a strain of *Stenotrophomonas maltophilia* in 24 h (Deng et al., 2015). Nogueira et al (2020) conducted a study with the bacterium *Steotrophomonas maltophilia in the* production of bioemulsifier, having post-frying soybean oil as a source of insoluble carbon, this biomolecule was characterized of polymeric nature and obtained excellent Emulsification Index with burnt motor oil of 82.74%. The biosurfactant of S. *maltophilia* was reported as an iron chelating agent in a study by Hemlata, Selvin and Tukaram (2015).

A study by Zaffar et al, (2018) described the degradation of the organochlorine pesticide endosulfan by a strain of *Steotrophomonas maltophilia* isolated from contaminated soil. The bacterium demonstrated the ability to use endosulfan as the sole source of sulfur and excellent pesticide degradation capacity.

4.3 ENZYME PRODUCTION

The literature also describes the production of enzymes by this bacterium (Muthukamalam et al., 2017; Wang et al., 2016). Aktas et al (2022) evaluated the production of chitinase by *S. maltophilia* for application as a biological control agent. The enzyme produced was effective in reducing the growth of the fungus *Fusarium oxysporum* and in destroying the chitin structure of the potato beetle.

The study by Subramanian et al (2022) summarizes the isolation of chitinase-producing *S. maltophilia* bacteria from different shrimp waste disposal sites in Parangipettai (India). After purification, the specific activity of chitinase was 5.01 (U/ml) and the protein content was 72 mg and the recovery rate was 48.06%. The results of the study cover that the use of foods enriched with chitin derivatives is taken to large-scale approaches, thus benefiting the aquaculture sector.

The bacterium capable of producing extracellular fibrinolytic protease was isolated and identified as *Stenotrophomonas maltophilia* Gd2 based on ribotyping. The in vitro fibrinolytic profile of this enzyme showed 73% fibrin clot dissolution in 4h. Further optimization of enzyme production was achieved with the implementation of the Plackett-Burman experimental design, where production levels increased to 3411FUml-1 and noted that peptone, pH, dextrose and K2HPO4 were considered significant factors. The author describes that this result reports the higher yield of fibrinolytic enzyme with *S. maltophilia* about reports in the literature.

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