Asian Journal of Research in Medicine and Medical Science

1(1): 18-24, 2019; Article no.AJRMMS.78

A Review of the Production of Biosurfactant and Pigmented Prodigyosin by Bacteria *Serrassia marsseñes*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Systematic Review Article

Received 16 March 2019
Accepted 23 May 2019
Published 29 May 2019

ABSTRACT

Surfactants or active agents are widely used in various industries. Biosurfactants are produced by a large group of microorganisms and capable of emulsifying hydrocarbons. *Serrawettin* biosuccinate and Prodigyosin pigment are produced by *Serrasia marssness* officinalis bacteria.

Object: Prodigyosin has a wide range of properties, including antibacterial, fungal, malaria, antibiotic properties, immunosuppressive and anticancer drugs, and many useful uses in the medical, pharmaceutical, dyeing and fields. In this review, the production of biosurfactant and pigmented Prodigiocin by bacteria *Serrazia Marseñas* and their applications in biotechnology are discussed.

Results: Due to the large volume of use, the full dependence on the import of this material, the high cost of chemical production and its side effects in order to obtain the maximum production of productivia, is necessary to optimize the conditions for growth and production. Therefore, achieving optimum conditions for the production of biosurfactant as the primary metabolite associated with the growth of the biomass can improve the production and utilization of this substance.

Keywords: Prodigyosin; biosurfactant; Serrasia marssness; serrawettin.

1. INTRODUCTION

Surfactants are active agents that can reduce surface tension in very low concentrations. These materials are amphiphile or duplex, Due to this molecular property, they can be placed between two levels of incompatibility, can reduce the intermediate level stresses and can be called

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surface activators. In addition, they have properties such as mildew formation and dissolution in non-polar materials [1]. Surfactants are classified into polymer, biosurfactant and nanosurfactant. biosurfactants according to the structure and method of production. They are also classified into anionic, cationic, non-ionic and amphoteric groups based on ionic behavior in aqueous solution by polar heads [2]. Anionic surfactants are the most common type [3].

Biosurfactants are a group of active molecules with a very diverse structure that are produced by some externally secreted bacteria, fungi and yeasts and act as a detergent, which causes the cell surface hydrophobicity well as micro-emulsion and dissolution of hydrocarbons by decreasing the surface tension of the liquids *Serratia marcescens* is a gram-negative bacterium and a member of *Enterobacteriaceae* family, which can live in water, soil, insects and vertebral gastrointestinal tract and on plant surface [1,4,5].

2. THE IMPORTANCE OF BIOSUR-FACTANTS

Biosurfactants are classified according to their type of glycolipid, lipopeptide, lipoprotein and polymer. Low molecular weight glycophosphate biosurfactant is the most famous type of biosurfactant. Ramenolipids, terpulopids and sepharolipids are among the most famous glycolipids that have important medical and environmental implications [6,7,8]. Clinging is the most important feature of biosuccinate, which is an important mechanism for the cell growth and survival causes the bacteria to stick to and grow in insoluble hydrocarbons in water and reduces the interaction of bacteria [9]. With the aid of biosurfactant, sulfur in the raw oils can be removed and sulfated. Bacteria are all-eaten, they can oxidize the sulfide in the oil under aerobic conditions and convert it to sulfate. Genetically manipulated, you can create specific strains of these bacteria that only have sulfurization activity and do not decompose the oil. Biosurfactants are also useful in cleaning the environment from toxic heavy metals such as cadmium, lead and uranium [10]. Biosafety can be a good alternative to antibiotics and can be used as antimicrobial agents and effective therapeutic agents or drugs. Biosurfactants prevent the adhesion of pathogenic bacteria to solid surfaces and infections such as surfactin, which reduces the formation of biofilm by *Salmonella typhimurium*, *Escherichia coli*, etc. [5]. The most notable microorganisms of the biosurfactant producers are shown in Table 1 [11].

Table 1. Some microorganisms producing biosurfactants [12]

Biosurfactant	Microorganisms (s)
Serrawettin	*Serratia marcescens*
Cellobiose lipids	*Ustilago maydis*
Rhamnolipids	*Pseudomonas aeruginosa, Pseudomonas chlororaphis, Serratia rubidaea*
Peptide lipids	*Bacillus licheniformis*
Aminoacids lipids	*Bacillus sp.*
Ornithinelipids	*Pseudomonas sp., Thio Bacillus thiooxidans, Agrobacterium sp.*
Viscosin	*Pseudomonas fluorescens,Leuconostocmesenteriods*
Trehalose lipids	*Rhodococcus erythropolis, Nocardia erythropolis, Corynebacterium, Mycobacterium, Arthrobactrer*
Polyol lipids	*Rhodotorula glutinis, R. graminis*
Carbohydrate-lipid	*P. fluorescens, Debaryomyces polymorphus*
Lichenysin	*Bacillus licheniformis, B. sobtilis*
Sobtilisin	*Bacillus sobtilis*
Surfactin/Iturin	*Bacillus sobtilis, B. amyloliquefaciens*

3. COMMON TESTS TO IDENTIFY AND ISOLATE BACTERIA PRODUCING BIOSURFACTANTS

Hemolytic Activity: Hemolysin test is a simple and fast method used as the first screening test to identify and isolate biosurfactant producing bacteria. For this purpose, fresh colonies of the bacterium are agar-agarated on line and incubated at 30°C for 72 hours. If positive, hemolysis appears as hallia. Hemolytic activity bacteria can be biosurfactant [13]. This method was first reported by Banat in 1970 to produce biosurfactants by bacteria *Bacillus subtilis* [14]. In 1984, Mulligan et al. Introduced the hemolysis method on Bladagar's environment as a primary screening method for production of biosurfactant [15].

Oil Spill Test: This test is performed according to Morikawa et al. For this purpose, add 20 ml of water to a10 cm plate and add 10 ml of oil to the equilibrium. In the presence of a biosurfactant, oil is displaced on the surface of the plate and shifted to the margin. Then, the diameter of this area is measured and the area of the transparent area is calculated [16].

Drop Decomposition Method: This test is done according to Bodormeyer'method. First, spread some of the oil on the lamina and exposeit to the laboratory temperature for one hour. Then, place a drop of centrifuge medium on the lamina surface and after a minute, the droplet shape is examined with a 10-microscope lens. Non-sterile water is used for negative control [17].

Surface Tensile Measurement: The reduction of surface tension in the growth medium is the main criterion for the proof of biosurfactant. Because surface tension is subject to the temperature of the environment, surface tensile of all specimens is measured in the same temperature conditions by tensiometer. Studies in this area were carried out by Bennett in 1991 and Tabatabaei in 2005 to isolate the bacterial generators of biosurfactants [18,19].

4. ADVANTAGES AND DISADVANTAGES OF PRODUCING BIOSURFACTANTS

Despite the superiority of biosurfactants over surfactants, including greater degradability, resistance to severe changes in temperature, salinity, pH, high osmolarity, high defectiveness and low toxicity, their production is not economically feasible [9]. In order to reduce the production costs, strategies such as increasing the production efficiency and using cheap resources available for the growth of microorganisms and production of biosurfactants have been suggested to be taken into account [20]. *Serratia marcescens* is a gram-negative graft and a member of *Enterobacteriaceae* family, which is isolated from the other genera by producing lipase, gelatinase and DNAase enzymes [21]. Fifteencases of infection by the bacterium were reported in 1968 [22]. Optimum growth conditions of sardinia and shrubs were in short-grain medium at temperatures of -40 to 10°C, pH of 5 to 9, and salinity of 5 to 7% w / v.). Usingelectron microscope, it has been observed that some of the *Serratia marcescens* have flagellas through which theycan be transmitted and can cause infection [23]. This bacterium can produce chitinase, several proteases, nucleases, lipase and several extracellular enzymes [24]. Production of *Serrawettin* biosurfactant and pigment prodigiosin (C20H25N3O), with tradenames of Mevinolin, Monacolin K and Mevacor, are considered to be the most prominent characteristics of this bacterium [25,26]. Prodigiosin pigmentation was first detected in 1902 in *Serratia marcescens*, and its chemical structure was identified by Reapoport and Holden [27]. This pigment is produced by the microorganisms such as *Hahellachejuensi*, *Pseudovibrio denitrificans Pseudoalteromonas rubra*, *Vibriogazogenes*, *V. psychroerythreus*, *Serratia plymuthica*, *Zooshikellarubidu* [28]. PG is one of the components of the family of statin drugs that has antibacterial, antifungal, antimalarial, antibiotic, immunosuppressive, and anti-cancer effects in addition to lowering blood cholesterol [29]. Preparation of this valuable material, which is the most important bacterial bacterium in Iran, is very expensive and requires the development of inexpensive biotechnological processes for the separation and purification of this pigment [30].

The effect of environmental factors on the production of prodigiosin by *Serratia m.* production of PG is influenced by many factors, including environmental conditions such as available phosphate mineral resources, oxygen content, light composition, culture medium, temperature, pH and incubation time [31]. The conditions of its culture, composition and fermentation are precursors for the production of PG derivatives. The dissolved oxygen content is usually 40% oxygen in or above the air, so its

growth and metabolic activity are strongly dependent on the oxygen present in the culture medium [32].

4.1 Effect of Temperature on the Production of PG

Temperature is an important factor for the growth of microorganisms. Each of is capable of growing at a certain temperature range. Increasing the temperatures above 50°C have adverse effects on the growth of most microorganisms and can be fatal to bacteria. By increasing the temperature, enzymes that affect bacterial growth are degraded, which disrupts the cell metabolism. Cacace and Mazza showed that temperatures above 50 ° C could reduce the production [33].

4.2 Effect of pH on the Production of PG

PH is the most important bio-absorption parameter that depends on the chemical solubility, activity of functional groups in biomass and ionic competition. PH is an important factor in the production of PG in *Serratia marcescens*. Therefore, maintaining a stable pH is essential for the production of PG [34]. PG a light-sensitive pigment. According to reports, production of this material varies with respect to the intensity of light used for cultivation, and the optimal production of this material in *Serratia marcescens* occurs when it is cultivated in the dark [35].

4.3 Rare Elements and Production of PG

Rare elements, especially thiamine and iron are useful in the biosynthesis of PG in *Serratia m*. However, light, mineral phosphate and ribosome, according to reports, inhibit biosynthesis [36]. The mechanism of inhibiting phosphorus (Pi) is to reduce the activity of alkaline phosphatase and to inhibit the accumulation of elements such as iron and zinc. Adenosine triphosphate (ATP) is also a potent inhibitor of biosynthesis of PG this bacterium [37,38].

4.4 Effect of Carbon Source on the Production of PG

Studies have shown that secondary metabolism of bacteria is dependent on the type and concentration of carbon source due to the catabolic inhibition process. In addition, carbon sources may influence the regulation of expression of genes and activity of enzymes. The amount of carbon source should be sufficient for cell growth.Excessive increase in carbon resources leads to a sharp decrease in production. Therefore, the choice of type and concentration of carbon source is effective in the production of PG. Different concentrations of carbon sources on cell growth and production of pigment PG in *Serratia marcescensare* shown in Table 2 [39]. Mannitol is a good carbon source for cell growth and production of this pigment by *Serratia marcescens*MO-1 [40].

Table 2. Effect of carbon on prodigiosinand biomass *S. marcescensMO-1 [41]*

Carbon	Biomass	PG
Mannitol	2.54±0.09	277.74±7.6
Glycerol	2.32±0.05	184.32±5.9
Glucose	2.07±0.11	6.35±1.6

When glucose is used as a carbon source, production of PG is reduced in *Serratia marcescensMO-1* culture medium [42]. In the case of carbon glucose source and nitrogen source of ammonium chloride, *the S. marcescens MO-1* does not produce a pigment PG [43]. To reduce the production cost of prodigiosin, cheap substrates such as vegetable oils are used. Wei et al. Used vegetable oils as a carbon source to increase the production of PG and serrawettin [44].

4.5 Effect of Nitrogen Source on Production of PG

Among the nutrients in the medium, the most important role is attributed to the sources of carbon and nitrogen, and they directly affect the composition of the biota and metabolites. These compounds, as a precursor and cofactor, play a major role in the synthesis of building blocks of biomass and production of PG. The ratio of carbon to nitrogen plays an important role in the production of various metabolites. Some reports have shown that the ratio of carbon to nitrogen is positively correlated with PG [44]. The effects of many mineral nitrogen sources, including ammonium salts such as (NH4) 2 SO4, NH4Cl NH4 NO3 have been studied on the production of pigmented pigments, which have an inhibitory effect on pigment production [45]. Research has shown that yeast extract as a source of nitrogen has high potential for producing PG. To improve the production of PG, Wei and Chen adjusted the carbon / nitrogen ratio of yeast extract and try p tone in LB modified broth [44].

4.6 Effect of Sodium Chloride Concentration on Production of PG

High salt concentration in the environment leads to cell membrane degradation and inactivation of enzymes, and these conditions can be fatal to microorganisms. The concentration of high salt in the culture medium, due to the osmotic pressure that it enters into the cell, inhibits the production of PG [44,41].

4.7 Biosurfactant and Enhancement of PG Improvement

Due to the large volume of PG derivative application, its full dependence on the import of this substance, the high cost of chemical production and its side effects in order to obtain the maximum amount of production, it is necessary to provide optimal conditions for growth and production. Therefore, achieving optimum conditions for the production of biosurfactant, as the primary metabolite associated with the growth of the biomass, can improve the production and utilization of this substance [44]. Mallick showed that *Serratia marcescens* has a large, hydrophobic cellular surface for production of PG [44]. Therefore, production of Serrawettin by this bacterium affects the production of PG. Yamashita et al. showed that increasing the carrier gel to the *Serratia m.* medium results in cell growth and production of PG and Serrawettin [44]. Wei et al. suggested that production of PG is related to the activity of extracellular surface emulsion, thus producing PG is associated with Serrawettin [44].

4.8 Production Mechanism of PG

Prodigiosin is produced by a discontinuous and semi-continuous ferromagnet. The semi-continuous conductors have higher yields than discontinuous ones, which is expected to be due to the fact that PG is produced as a secondary metabolite product. Bio-production of material can also be increased by using statistical methods such as surface response method. As many fermentation strategies for producing prodigiosin in discontinuous culture, gene expression in microorganisms is rapidly dependent on different environmental conditions such as cell proliferation, temperature, and culture and culture media. The expression of PG production gene is controlled by the quotient system [44]. The presence and measurement of the purity of PG are carried out using high performance HPLC liquid chromatography. The growth rate of microorganisms is measured by determining the intensity of optical absorption at 600 nm wavelength using a spectrophotometer. The high cost of producing,purifying and isolating the complex processes of fermentation of various microorganisms has limited the development of prodigiosin [41].

5. CONCLUSION

Due to the increasing biological risks and environmental pollution as well as the risk of increasing antibiotic resistance and various types of cancers, it is of great importance to pay attention to new biotechnology products. *Serratia m.* as one of the bacteria producing bio surfactant and prodigios in pigment can be useful. Of course, the optimal production methods and these compounds should be of great interest to the researchers.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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