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RESEARCH ARTICLE

Isolation And Characterization Of Pseudomonas aeruginosa From Waste Soybean Oil As Biosurfactants Which Enhances Biodegradation Of Industrial Waste With Special Reference To Kosmi Dam, Betul District, (M.P.)

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Abstract

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INTRODUCTION

Pseudomonas aeruginosa is a gram-negative, rod-shaped, asporogenous, and monoflagellated bacterium that has an incredible nutritional versatility. It is a rod about 1-5 µm long and 0.5-1.0 µm wide. P. aeruginosa is an obligate respirer, using aerobic respiration (with oxygen) as its optimal metabolism although can also respire anaerobically on nitrate or other alternative electron acceptors. P. aeruginosa can catabolize a wide range of organic molecules, including organic compounds such as benzoate. This, then, makes P. aeruginosa a very ubiquitous microorganism, for it has been found in environments such as soil, water, humans, animals, plants, sewage, and hospitals (Lederberg et al.,2000). In all oligotropic aquatic ecosystems, which contain high-dissolved oxygen content but low plant nutrients throughout, P.aeruginosa is the predominant inhabitant and this clearly makes it the most abundant organism on earth (Costerton et al., 1994).

P.aeruginosa is an opportunistic human pathogen. It is "opportunistic" because it seldom infects healthy individuals. Instead, it often colonizes immunocompromised patients, like those with cystic fibrosis, cancer, or AIDS (Botzenhardt et al., 1993). It is such a potent pathogen that firstly, it attacks up two thirds of the critically-ill hospitalized patients, and this usually portends more invasive diseases. Secondly, P.aeruginosa is a leading Gram-negative opportunistic pathogen at most medical center, carrying a 40-60% mortality rate. Thirdly, it complicates 90% of cystic fibrosis deaths; and lastly, it is always listed as one of the top three most frequent Gram-negative pathogens and is linked to the worst visual diseases (Fick, R et al.,1993).

Furthermore, P.aeruginosa is a very important soil bacterium that is capable of breaking down polycyclic aromatic hydrocarbons and making rhamnolipids, quinolones, hydrogen cyanide, phenazines, and lectins . It also exhibits intrinsic resistance to a lot of different types of chemotherapeutic agents and antibiotics, making it a very hard pathogen to eliminate (Lederberg et al., 2000). P. aeruginosa was first described as a distinct bacterial species at the end of the nineteenth century, after the development of sterile culture media by Pasteur. In 1882, the first scientific study on P. aeruginosa, entitled "On the blue and green coloration of bandages," was published by a pharmacist named Carle Gessard. This study showed P. aeruginosa's characteristic pigmentation: P. aeruginosa produced water-soluble pigments, which, on exposure to ultraviolet light, fluoresced blue-green light. This was later attributed to pyocyanine, a derivative of phenazine, and it also reflected the organism's old names: Bacillus

pyocyaneus, Bacterium aeruginosa, Pseudomonas polycolor, and Pseudomonas pyocyaneus (Botzenhardt et al., 1993). P. aeruginosa has many strains, including Pseudomonas aeruginosa strain PA01, Pseudomonas aeruginosa PA7,Pseudomonas aeruginosa strain UCBPP-PA14, and Pseudomonas aeruginosa strain 2192. Most of these were isolated based on their distinctive grapelike odor of aminoacetophenone, pyocyanin production, and the colonies' structure on agar media (Gilardi et al., 1985).

P. aeruginosa, as well as many other Pseudomonades, can degrade aromatic hydrocarbons such as methylbenzenes, which are the by-products of petroleum industries and are commonly used as solvents for enamels and paints as well as in the production of drugs and chemicals. Methylbenzenes are considered as environmental contaminants that are present in the atmosphere, underground and soils, and in surface water (Pieper et al., 1992). P. aeruginosa can break down toluene, the simplest form of methylbenzene. P. aeruginosa degrades toluene through the oxidation of the methyl group to aldehyde, alcohol, and an acid, which is then converted to catechol. Hence, P. aeruginosa can be used in pollution control (Johnson et al., 1997).

Isolation and Characterization of P.aeruginosa : Pseudomonas aeruginosa has very simple nutritional requirements. It is often observed "growing in distilled water", which is evidence of its minimal nutritional needs. In the laboratory, the simplest medium for growth of Pseudomonas aeruginosa consists of acetate as a source of carbon and ammonium sulfate as a source of nitrogen. P. aeruginosa possesses the metabolic versatility for which pseudomonades are so renowned. Organic growth factors are not required, and it can use more than seventy-five organic compounds for growth. Its optimum temperature for growth is 37 degrees, and it is able to grow at temperatures as high as 42 degrees. It is tolerant to a wide variety of physical conditions, including temperature. It is resistant to high concentrations of salts and dyes, weak antiseptics, and many commonly used antibiotics. Pseudomonas aeruginosa has a predilection for growth in moist environments, which is probably a reflection of its natural existence in soil and water. These natural properties of the bacterium undoubtedly contribute to its ecological success as an opportunistic pathogen. They also help explain the ubiquitous nature of the organism and its prominence as a nosocomial pathogen. "P. aeruginosa isolates may produce three colony types. Natural isolates from soil or water typically produce a small, rough colony. Clinical samples, in general, yield one or another of two smooth colony types. One type has a fried-egg appearance which is large, smooth, with flat edges and an elevated appearance. Another type, frequently obtained from respiratory and urinary tract secretions, has a mucoid appearance, which is attributed to the production of alginate slime". The smooth and mucoid colonies are presumed to play a role in colonization and virulence. P. aeruginosa strains produce two types of soluble pigments, the fluorescent pigment pyoverdin and the blue pigment pyocyanin. The latter is produced abundantly in media of low-iron content and functions in iron metabolism in the bacterium. Pyocyanin (from "pyocyaneus") refers to "blue pus", which is a characteristic of suppurative infections caused by Pseudomonas aeruginosa.

Biosurfactants: Surfactants are amphipathic molecules with both hydrophilic and hydrophobic moieties that partition preferentially at the interface between fluid phases with different degrees of polarity and hydrogen bonding, such as oil/water or air/water interfaces (Cirigliano and Carman, 1985). Surfactants display properties, for example, detergency, emulsification, foaming, and dispersion (Back et al., 2004; Cirigliano and Carman, 1985; Gerhardt et al., 1981). Biosurfactants (microbial surfactants) are surface active compounds produced extracellularly or as part of the cell membrane by several bacterial and fungal species. They have the unique property of reducing the surface and interfacial tension of liquids. Almost all surfactants currently in use are chemically synthesized from petroleum, however, interest in microbial surfactants has been steadily increasing in recent years, as biosurfactant have numerous advantages compared to chemical surfactants, including lower toxicity, higher biodegradability (Zajic et al., 1977), better environmental compatibility (Banat, 1995; Georgiou et al., 1992) and higher specific activity at extreme temperature, pH levels and salinity (Kretschner et al., 1982). Biosurfactants have applications in the field of agriculture, petroleum, microbial enhanced oil recovery, biomedical sciences, cosmetics, food processing and pharmaceuticals. The global biosurfactants market has grown gradually. Regardless of their greater biodegradability and reduced toxicity, cost competitiveness still remains the major concern for biosurfactant production. However, recombinant or metabolically engineered hyper producing strains combined with optimized cultivation conditions have made it possible for many companies to reap the benefits of 'green' biosurfactant technology.

Rhamnolipid biosurfactant from P. aeruginosa showed significant antiproliferative activity against cancer cell lines (Thanomsub et al., 2007). Here, we will isolate a strain from waste soybean oil. The strain produced new biosurfactant and it has very strong emulsification activity for crude oil. In addition, we purified and characterized the biosurfactant and its properties were compared with those of chemically synthesized surfactants.

Biosurfactant production: To obtain predominant bacteria degrading crude oil, some bacteria were isolated from waste soybean oil. Isolated bacterial strain had a marked tributyrin (C4:0) degrading activity as developed clear zone around the colony after incubation for 24h at 37 degrees C (Lee SC et al., 2008). The samples were serially diluted and pour plated in the nutrient agar plates. Three organisms were isolated and screened for the biosurfactant production. To confirm the ability of isolates in biosurfactant production, different screening methods including Emulsification test, emulsification index E24, drop collapse method, oil spreading test and blood haemolysis test were assessed. This study suggested that Pseudomonas sps showed the maximum biosurfactant production(Padmapriya B et al., 2012). The isolated microorganisms can be identified by Gram staining and biochemical test (sneha et al., 2012) and analysis of 16S rRNA gene(Lee SC et al., 2008).

Extraction of Biosurfactant: Crude biosurfactant was extracted from the culture supernatant of Klebsiella sp. Y6-1 by organic solvent (methanol: chloroform: 1-butanol) after vacuum freeze drying and the extracted biosurfactant was purified by silica gel column chromatography. When the purified biosurfactant dropped, it formed degrading zone on crude oil plate (Lee SC et al., 2008). Crude biosurfactant was recovered from the culture of BU03 by extraction with n-hexane, and its properties were investigated (Zhao Z et al., 2009). Soybean oil biodegradation using pseudomonas aeruginosa among suspended growth, attached growth onto porous ceramic and polyurethane foam (puf) microbial carriers, was investigated. Cooking oil in wastewater, simulated by artificial substrate, could be degraded (>99 %) after incubation using batch treatment (Terng-Jou Wan et al., (2012). Biosurfactant production through a fermentation process involving the biodegradation of soybean oil refining wastes was studied. Pseudomonas aeruginosa MR01 was able to produce extracellular biosurfactant when it was cultured in three soybean oil refinement wastes; acid oil, deodorizer distillate and soapstock, at different carbon to nitrogen ratios (Maryam Partovi et al., 2013).

Consequently, applying soybean oil soapstock as a substrate for the production of biosurfactant with commercial value has the potential to provide a combination of economical production with environmental protection through the biosynthesis of an environmentally friendly (green) compound and reduction of waste load entering the environment. Moreover, this work inferred spectrophotometry as an easy method to detect rhamnolipids in the biosurfactant products. (Maryam Partovi et al., 2013). In the present work, the production of rhamnolipid from residual soybean oil (RSO) from food frying facilities was studied using a strain of Pseudomonas aeruginosa of contaminated lagoon, isolated from a hydrocarbon contaminated soil (de Lima CJ et al., 2007). The rhamnolipids produced by Pseudomonas aeruginosa strains are often a mixture of several homologues. Up to seven ($R_2C_{10}C_{10}$ + $R_1C_{10}C_{10}$ + $R_2C_{10}C_{12}$ + $R_1C_{10}C_{12}$ + $R_1C_{12:1}C_{10}$ + $R_1C_{12:2}$ + $R_1C_{8:2}$) have been identified in cultures of P. aeruginosa AT10 from soybean oil refinery wastes (Abalos et al., (2001). Biosurfactant is a structurally diverse group of surface-active molecule, synthesized by microorganisms. It has the capability of reducing surface and interfacial tension with low toxicity and high specificity and biodegradability (Padmapriya B et al., 2012). To confirm the ability of isolates in biosurfactant production, different screening methods including Emulsification test, emulsification index E24, drop collapse method, oil spreading test and blood haemolysis test were assessed (Padmapriya B et al., 2012).

Methodology for Extraction of Biosufactants : Biosurfactant can be extracted from the whole cell-free culture broth. The bacterial cells can be removed by centrifugation at 9000 rpm at 4ºC for 30 minutes. The supernatant can be adjusted to pH 2 using sulphuric acid,H_2SO_4 (1M) prior to biosurfactant extraction using equal volume of chloroform-methanol (2:1) mixture. The organic phase can be separated and extracted. The solvent was evaporated to concentrate the crude biosurfactant. The crude biosurfactant was then dried at 60ºC to a constant weight prior to get the quantity of biosurfactant produced(N. Samadi et al.,2007).

The potential of an alkaliphilic bacterium Klebsiella sp. strain RJ-03, to utilize different carbon sources for the production of an extracellular biosurfactant can be evaluated. The crude biosurfactant production was done using starch, sucrose, xylose, galactose and glucose containing medium that exhibited significantly high viscosity, emulsification activity and maximum reduction in surface tension as compared to those obtained from fructose and maltose. The carbon source has significant effect on the quantity as well as the quality of biosurfactant production. The chemical characteristics of purified biosurfactant was compared by NMR, FT-IR, SEM, GPC, MALDI TOF-TOF MS, GC-MS, TG and DSC analysis, indicating variation in the functional groups, bonds, elements, monosaccharide composition, molecular mass and thermo stability (Jain RM et al., 2013).

Sophorolipids are carbohydrate-based, amphiphilic biosurfactants that are of increasing interest for use in environmentally benign cleaning agents. Sophorolipid production was tested for 26 strains representing 19 species of the Starmerella yeast clade, including Starmerella bombicola and Candida apicola, which were previously reported to produce sophorolipids. Five of the 19 species tested showed significant production of sophorolipids: S.

bombicola, C. apicola, Candida riodocensis, Candida stellata and a new species, Candida sp. NRRL Y-27208. A high-throughput matrix-assisted laser desorption/ionization-time of flight MS assay was developed that showed S. bombicola and C. apicola to produce a lactone form of sophorolipid, whereas C. riodocensis, C. stellata and Candida sp. NRRL Y-27208 produced predominantly free acid sophorolipids. Phylogenetic analysis of sequences for the D1/D2 domains of the nuclear large subunit rRNA gene placed all sophorolipid-producing species in the S. bombicola subclade of the Starmerella clade (CP Kurtzman et al., 2010). Pseudomonas aeruginosa J4, isolated from wastewater of a petrochemical factory located in southern Taiwan, was used to produce rhamnolipid from a variety of carbon substrates, including hydrophilic substrates, vegetable oils, and mineral oils (Yu-Hong Wei[a] et al., 2005). Mass spectrometry and NMR analysis indicate that the purified product contained two types of commonly found rhamnolipids: L-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (RL1) and L-rhamnosylL-rhamnosyl-β-hydroxydecanoyl-β-hydroxydecanoate (RL2) (Yu-Hong Wei[a] et al., 2005). The High-Level Production of Rhamnolipid Biosurfactants is a Unique Feature of Pseudomonas Aeruginosa and is strictly regulated in response to environmental conditions (Urs A. Ochsner et al., 1995). Rhamnolipid production by Pseudomonas aeruginosa ATCC 9027 with waste frying oil as sole carbon source was studied using response surface method (Zhi Luo et al., 2013). Biosurfactants have applications in the field of agriculture, petroleum, microbial enhanced oil recovery, biomedical sciences, cosmetics, food processing and pharmaceuticals. The global biosurfactant market has grown gradually. Regardless of their greater biodegradability and reduced toxicity, cost competitiveness still remains the major concern for biosurfactant production. However, recombinant or metabolically engineered hyper producing strains combined with optimized cultivation conditions have made it possible for many companies to reap the benefits of 'green' biosurfactant technology (Kamaljeet Kaur Sekhon et al., 2012). Biosurfactants produced by an isolated thermophilic strain Acinetobacter calcoaceticus BU03 were demonstrated to be effective in enhancing the solubility of polycyclic aromatic hydrocarbons (PAHs) (Jonathan W.C. Wong et al., 2010). A thermophilic bacterial strain, Acinetobacter calcoaceticus BU03, with a biosurfactant-producing capability, was isolated from petroleum-contaminated soil with an improved procedure which employed the solubilization of polycyclic aromatic hydrocarbons (PAHs), i.e. naphthalene in agar plate, as a selection criterion (Zhao Z et al., 2009). Biosurfactants from A. calcoaceticus BU03 have potential to enhance the removal of PAHs from contaminated sites (Zhao Z et al., 2009). Biosurfactants (BS) are produced by a variety of microorganisms from renewable resources, and have unique properties compared to chemical surfactants(Takahashi M et al., 2011). Sophorolipids are carbohydrate-based, amphiphilic biosurfactants that are of increasing interest for use in environmentally benign cleaning agents. Sophorolipid production was tested for 26 strains representing 19 species of the Starmerella yeast clade, including Starmerella bombicola and Candida apicola, which were previously reported to produce sophorolipids (cP Kurtzman et al., 2010). Rsan-ver, a strain of Pseudomonas aeruginosa isolated at this department, was used for the development of a continuous process for biosurfactant production. The active compounds were identified as rhamnolipids(L Guerra-Santos et al., 1984). The biosurfactant produced by B. subtilis JK-1 displayed highest emulsification activity on soybean oil and crude oil (Myeong Hoon Joo et al., 2013).

Industrial waste in Kosmi dam and why its biodegradation required? **:** Deterioration of water body is a major threat to the life of living organism. Kosmi dam and Machna River is an important source of drinking water for the people of the Betul city. Human, Industrial, Religious and various other activities taking place in and around this river are making the water of this river unsafe for human consumption. After a study it was found that water is severely polluted at some places and this polluted water when used for the irrigational purposes affects to the vegetable, crops and also on human beings because so many pathogens are present in this industrial waste. Though the causes leading to river degradation are diverse, disposal of solid and liquid waste, encroachment upon the river waterway and water extraction are some of the obvious causes of river degradation. This study was carried out with the aim of evaluating the factors and processes leading to the degradation of Machna River and the solid waste, industrial, there from and the adaptation of people depending on river for their livelihood. Due to increasing population in the region and poor management of urbanization and industrial growth, the water quality of River Machna has significantly deteriorated, particularly in the dry season. The primary sources of pollution are untreated sewage and industrial wastewater. Non-point pollution sources from religious activities at various locations along the river, agriculture and livestock as well as poor solid waste management also contributes to pollution. In addition, substantial abstraction of water, primarily for irrigation, has led to low flows and associated poor water quality in the critical middle stretch of the river. Millions of people all over the world, particularly in the developing countries are losing their lives every year from water born-diseases (Neelesh Shrivastava et al.,2012).

Study Area: Kosmi Dam is situated in Betul district, M.P. kosmi Dam is connected with Machna River and this river supply 80% of drinking water to the houses of Betul city. Kosmi dam area is covered with so many different types of industries, mainly milk and soybean oil factories. So industrial waste dumped behind factories get flow

through water from vrindavan nagar to kosmi dam and lastly to machna river. The study is undertaken to assess the impact of industrial, solid waste disposed by soybean oil factory. The five sampling stations will determine in the Machna river of Betul city which are severely polluted with respect to other sampling stations.

Conclusion:

Rhamnolipid biosurfactant from P. aeruginosa showed significant antiproliferative activity against cancer cell lines (Thanomsub et al., 2007). Here, we will isolate a P.aeruginosa strain from waste soybean oil. The strain produces new biosurfactant, we extract this biosurfactant and it has very strong emulsification activity for crude oil. In addition, we purified and characterized the biosurfactant and its properties were compared with those of chemically synthesized surfactants. That biosurfactants are than used for the biodegradation of industrial waste. This review article emphasizes on the present worldwide scenario of biosurfactant production, correlation between biosurfactant production, recent developments in this line of research and future prospects. Hence, **P. aeruginosa can be used in pollution control**.

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