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RESEARCH ARTICLE

STUDY ON SIDEROPHORE PRODUCING BACTERIA AND ITS APPLICATIONS

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ABSTRACT

The health of biosphere is seriously affected by many factors including human activities.The major factors include toxicity caused by heavy metals in soil and aquatic life along with accumulation of plastic waste and chemical surfactants. It is observed that Bacteria are often iron limited and hence produce extracellular iron-scavenging siderophores. Unlike iron-siderophore complexes which can be taken up by species specific receptor cells, siderophore bound to other heavy metals do not enter the cell efficiently. Hence siderophore production may contribute to a heavy metal bioremediation. In the present study, siderophore was extracted from fluorescence species of Pseudomonas isolated from spinach root surface. Indication of siderophore-metal complex was determined by spectral scan and peak changes between extracted metal free siderophore and metal siderophore complexes. Apart from this, during starvation period, obtained isolate accumulatedPoly Hydroxy Butyrate (PHB) granules intracellularly in the presence of waste fried oils as a carbon source. PHB has attracted commercial interest as a new biodegradable polymer. Acceptable amount of PHB granules were obtained by growing cells in Mineral Salt Medium (MSM) with waste fried oils as a carbon source. Along with accumulating PHB granules intracellularly this bacteria also synthesize biosurfactantextracellularly as a precipitate in the same MSM medium containing carbon source. This provides an alternative to production of chemical surfactants. The best results were obtained when using waste fried olive oil as carbon source. Thus present study provided a biological platform by exploiting abilities of a single bacterium to combat major Environmental issues.

INTRODUCTION

Planet earth is a unique piece of universe. But this planet is constantly threatened by many human activities (Indu Nair, 2013). The major factors responsible are presence of heavy metals in sea water that affect the aquatic life and also the important soil microbes if present in the soil at higher levels. Along with this, accumulation of plastic waste is also contributing to deterioration of biosphere. Plastics are strongly recalcitrant, as they have long polymeric chains and specialized groups associated with them.

Many microorganisms are unable to secrete suitable enzymes required for the decomposition of such compounds. As it is very difficult to subject the plastic wastes to degradation, alternative sources are always a better choice (Indu Nair, 2013). Apart from this, the application of synthetic surfactants for the treatment of hydrocarbon-contaminated soil improves the restoration however; they get accumulated in the ecological system leading to severe environmental damage. The biosurfactants or the surface active component synthesized by the microorganisms appears to be an excellent alternative approach for the synthetic surfactants. In the present scenario, the major drawback that prevents the widespread use of microbial biosurfactants is economic viability of the production costs. The alternative low cost production media has been focused for biosurfactants production (Varadharajan *et al*, 2013).

Pseudomonas species has been shown to produce siderophores which is able to chelate any available iron. Some siderophores can bind, besides iron, other essential metals (K, Mg,) that serve as micronutrients, used for redox processes and regulation of osmotic pressure. Moreover, microorganisms able to produce siderophores can protect themselves by binding toxic metals (Al, Pb, Cd,) (Mehri Ines el al 2012).This property of organism can be exploited for Bioremediation of heavy metals. On the other hand to overcome the hazards of plastic, bacterial polymer poly hydroxyalkanoates (PHAs) is promising alternative for synthetic plastic. PHA can serve as a carbon or energy source for microorganisms during starvation periods. When the supply of the limiting nutrient is restored, PHA can be degraded by intracellular PHB degrading polymerase and subsequently metabolized as carbon and energy source. The main advantage of PHB is their complete degradation to water

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and carbon dioxide (Indu Nair 2013). The use of cost effective substrates is an important factor which has affected the wide acceptance of bioplastic. Thus *Pseudomonas fluorescens* species can be screened for accumulation of PHB in the presence effective substrates such as waste fried oils. Similarly to provide alternative to chemical surfactants, M. Abouseod, 2007 had shown that microbe synthesizes biosurfactants during their growth on water immiscible substrates.

The present study aims to provide a new biological platform by exploiting abilities of a single bacterium to solve major issues related to the environment. This includes removal of heavy metals, use of cheaper, economical and selective carbon substrates as an alternative to expensive carbon substrates for production of PHB and Biosurfactant.

MATERIALS AND METHODS

Isolation of Siderophore producing bacteria

Isolation was carried out as given by H. Manjunatha *et al*, 2013 with slight modification. Spinach roots with its surface soil are taken as a sample (location: Kalidas, Mulund West, Mumbai). Healthy plant was obtained by uprooting the plant along with its root. These roots with its surface soil was cut from the plant and transferred into conical flask containing 100ml of sterile distilled water and incubated on shaker at RT for 6-7 hrs.1ml of this suspension was serially diluted up to10^{-6} and about 0.1ml from last three dilutions was surface spread on King's B medium to isolate the colonies. The plates were incubated at 37^{o}c for 48 hr. The colonies showing yellow pigmentation on King's B medium were picked up based on the pigment formation. This was followed by biochemical and morphological characterization such as Gram staining and motility along with Sugars fermentation, Gelatin hydrolysis, Citrate, Oxidase, Nitrate test. Organism was identified on the basis of Bergey's manuals.

Screening for siderophore production

Siderophore production was studied according to R.Z. Sayyed*et al*, 2005 with modification. In the present study, Succinate Medium (SM) was used consisting of following components: 1L of distilled water contains :- (4 g) K_2HPO_4, (6 g) KH_2PO_4,Succinic acid(3g), $(NH_4)_2SO_4$ (1g), $MgSO_4$(0.2 g) and pH 7. 250ml flask containing succinate medium was inoculated with 0.1ml of inoculum and incubated on shaker for 48 h at 28^{o}c.

Bioremediation of heavy metals

Method for Bioremediation was according to Mehri Ines, 2012 with modification.1000ppm stock of Zinc sulphateand lead acetate were prepared and autoclave. These stock solutions were incorporated in autoclaved SM media at a final metal ions concentration of 60ppm. In order to determine the threshold level of metals at which growth and/or siderophore biosynthesis are stimulated or repressed, the selected isolate were monitored in sterile SM medium as a function of increasing amount of metal ions i.e. Zn, Cd, Pb from 20-200ppm concentration. After incubation the broth was centrifuged at 3000rpm at 4^{o}c and the supernatant was spectrophotometrically analysed for maximum absorbance.

Production of PHB granules

Method adopted for production of PHB granules was given by DarshanMarjadi *et al*, 2012. Organism under study were grown on sterile Mineral Salt basal Medium (MSM) with the following composition(g/l) :Na_2HPO_4(2.2), KH_2PO_4(1.4), $MgSO_4.7H_2O$(0.6), $FeSO_4.7H_2O$(0.01), NaCl(0.05), $CaCl_2$(0.02), yeast extract (0.02) and 0.1ml of trace element solution containing (g/l): (2.32g)$ZnSO_4.7H_2O$, (1.78g)$MnSO_4.4H_2O$, (0.56g)H_3BO_3, (1.0g)$CuSO_4.5H_2O$, (0.39g)$Na_2MoO_4.2H_2O$, (0.42g) $CoCl_2.6H_2O$, (1g) EDTA, (0.004g), $NiCl_2$. 2O and (0.66g) KI. This media was supplemented with waste fried oil such as groundnut oil, sunflower oil, coconut oil and olive oil (1%w/v) as a sole carbon source; pH of the medium was adjusted to 7.0.This was followed by incubation on shaker at RT for 72hrs. Culture was smeared on glass slide, heat-fixed and stained with Sudan Black B (Loba) to detect the presence of intracellular granules. At the same time cultures was spread on Tributyrin agar plate for the detection of lipase production.

Extraction and estimation of PHB granules

At the end of the cultivation (72 hrs.), cells were harvested by centrifugation at 10,000g for 10 min. The cell pellet was then resuspended in 100 ml of hexane by vortexing and then centrifuged again at 10,000g for 5 min to remove the remaining oils. Culture broth was centrifuged at 10,000 g for 10 minutes and supernatant was stored. The pellet was suspended in 2.5 ml of 4 % sodium hypochlorite for digestion and 2.5 ml of hot chloroform and was incubated at 37°C for 1 hour. The suspension was centrifuged at 1500 g for 10 minutes .The upper phase contains hypochlorite solution and the middle phase contains chloroform with cell debris. The bottom phase containing PHB with chloroform was collected and further followed by extraction with hot chloroform and precipitated with ethanol and acetone (1:1).The precipitate was allowed to evaporate for dryness at 30^{o}C to obtain PHB crystals and weighed. The amount of PHB in the extracted samples was determined spectrophotomertrically at 235 nm with a standard graph of 3-hydroxy butyric acid. Optimization of PHB at different pH of 5, 6,7,8,9 and temperature of 4, 27,37,45,55(^{o}c)was carried out (DarshanMarjadi *et al*, 2012).

Production of Biosurfactant

Production of biosurfactant were carried out in same MSM medium along with the same incubation conditions and waste fried oil as a carbon source, as biosurfactant is produce extracellularly.

Biosurfactant recovery and characterization

According to M. Abouseoud *et al*, 2007 the culture broth was centrifuged (10000 g, 15 min) to remove the cells and supernatant thereafter sterilized with Millipore membrane

filter. The clear sterile supernatant served as the source of the crude biosurfactant. The biosurfactant was recovered from the cell free culture supernatant by cold acetone precipitation. Three volumes of chilled acetone was added and allowed to stand for 10 h at 4°c. The precipitate was collected by centrifugation and evaporated to dryness to remove residual acetone after which it was re-dissolved in sterile water.

Structural characterization of biosurfactant

Rhamnose test

The presence of carbohydrate groups in the biosurfactant molecule was assayed by rhamnose test. A volume of 0.5 ml of cell supernatant was mixed with 0.5 ml of 5% phenol solution and 2.5 ml of sulfuric acid, and incubated for 15 min before measuring absorbance at 490 nm.

Emulsification index (E24)

E24 of culture samples was determined by adding 2 ml of hydrocarbons (gasoil) to the same amount of culture, mixing with a vortex for 2 min, and leaving to stand for 24 hours. The E24 index is given as percentage of height of emulsified layer (mm) divided by total height of the liquid column (mm).This was followed by optimization of biosurfactant at different pH and temperature range similar to that used for optimizationation PHB granules.

RESULTS

Isolation and characterization of obtained isolate

Siderophore producing bacterial colonies were isolated from the surface of spinach root sample and it was observed that, three colonies as compare to other colonies produced diffusible yellow green florescent pigment around them on Kings B (KB) Agar (specific for pyoverdine).Pure cultures of all the three isolate was prepared and maintained.

These were screened for siderophore production in sterile iron free succinate medium. The result showed that,all the three isolate were able to produce diffusible yellow green siderophore but out of these three, one isolate were visually producing intense yellow green diffusible siderophore of pyoverdine type. This was also compared with the control as shown in the Figure 1. Presence of non-pigmented colonies on Kings A (KA) agar (specific for pyocynin) further confirmed siderophore of pyoverdine type (Fig 2).Thus, this isolate obtained was selected for further study. On the basis of morphological, biochemical and structural characterization, isolate was found to be gram negative short rods which were positive for Gelatin hydrolysis, Oxidase,Citrate,Nitrate along with the growth at 4^0c. These are the characteristic features of *Pseudomonas fluorescens* according to Bergey's manuals 9th edition. Table 1 shows the biochemical characterization of different Pseudomonas species. Thus, according to results obtainedand by comparing the obtained characteristics with standards for Pseudomonades in Bergey's manuals, obtained isolates was identified as ***Pseudomonas fluorescens***

Table 1 Standard biochemical characterization of different Pseudomonas species.

Characteristics	*P.aeruginosa*	*P.fluorescens*	*P. Putida*	*P.Stutzeri*
Oxidase	+	+	+	+
Growth at 41°C	+	-	-	+/-
Pyoverdin (Fluorescein)	+	+		
Pyocyanin	+	-	-	-
Gelatinase	+	+	-	-

Figure 1 Siderophore in succinate media(right), control (left)

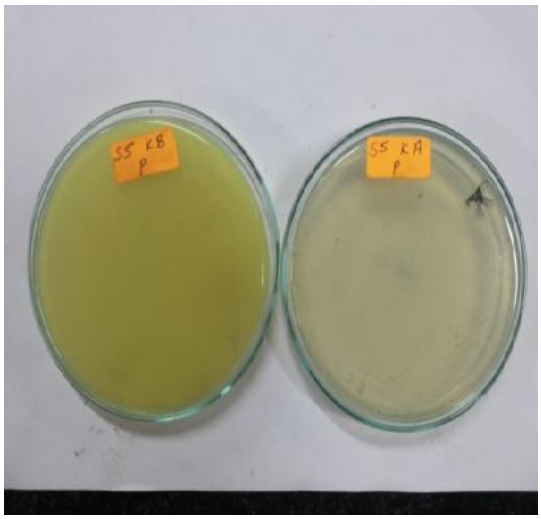

Figure 2 *Pseudomonas fluorescens* showing diffusiblepigment on KB agar and non-pigmented on KA agar

Confirmation of siderophore produced

Iron free Succinate medium was used for further confirmation of siderophore. Siderophoreproduced by *Pseudomonas fluorescens*showed florescence under UV light (Fig 3).This was further confirmed by maximum absorbance at 410 nm. This was similar with the research reported by Aditi Bhattacharya, 2010 where the presence of siderophore, a polar substance showed bands of absorption at different wavelength such as 260 nm and 402 nm, 448 nm, Maximum absorbance was observed at 365 and 410 nm for pyoverdins. The production of

reddish brown precipitate on addition of iron further confirms the yellow green diffusible pigment as siderophore. This is because of iron acquisition by siderophore molecule (Bholay A. D *et al*, 2012).

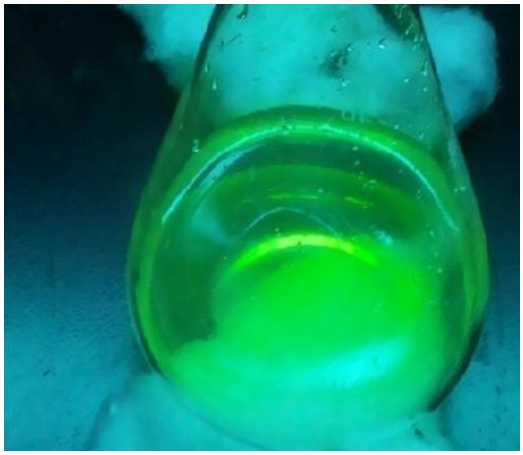

Figure 3 Fluorescens of siderophore under UV light.

Bioremediation of heavy metals

Spectrophotometric analysis of the supernatant from the control i.e. SM medium without metal ions and succinate medium containing lead ionsshowed an absorption area between 380 to 450 nm with a sharp peak at about 410nm.Absorbance maxima of supernatant containing lead metal ions and siderophores was lower than the control (media without metal ions) (Fig.4).

Thus by keeping 410nm as constant wavelength, growth and siderophore production was screened for different metal ion concentration ranging from 20-200ppm. Results showed that for zinc ions *Pseudomonas fluorescens* were able to grow till 140ppm zinc ions concentration with increasing intensity of siderophores production having maximum absorbance peak at 120ppm then drops at 140ppm. No growth and siderophore produce above 160ppm zinc ion concentration (Fig 5). Growth and siderophore synthesis for lead ion concentration was observed till 200ppm. Siderophore with increasing intensity were produced giving maximum absorbance peak at 140ppm of lead ion concentration (Fig 6-7).

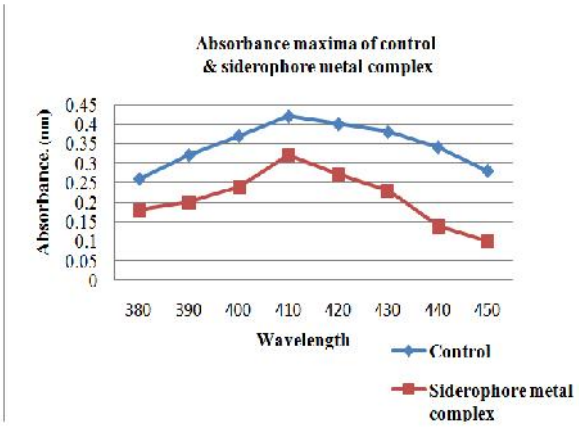

Figure 4 Absorbance maximum of metal free siderophore and siderophore metal complex

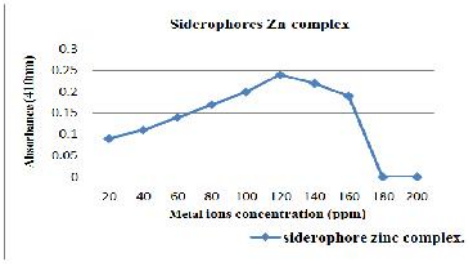

Figure 5-Graph of siderophores -Zn complex.

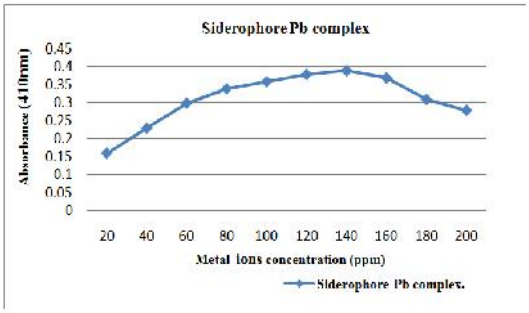

Figure 6 Graph of siderophores-Pb complex.

Figure 7 Intensity of Siderophore production with increasing concentration of lead

PHB production and extraction

For PHB production, cost of carbon substrates is major problem faced. Thus to minimize the cost of production media, different low cost carbon substitutes were evaluated for the growth and intracellular PHB production in*Pseudomonas fluorescens*. Different waste fried oils such as groundnut oil, sunflower oil, olive oil, coconut oil in minimal media proved to be the most effective which showed visible growth turning the media milky. For confirming PHB production intracellularly, sudan black B staining was done. Dark black granules confirmed the presence of PHB with different carbon substrates used. Further confirmation was done by presence of zone of hydrolysis on Tributyrin agar plate. This indicates the secretion of lipase enzyme. Extraction of PHB was done by sodium hypochlorite digestion process and intracellular PHB granules were released. Further PHB granules were precipitated with ethanol and acetone and small granules were obtained after complete evaporation(Fig 8). Further conversion of PHB to crotonic acid by concentrated sulphuric acid with maximum absorption at 235nm using UV was use to confirm the presence of PHB. A standard graph of 3-hydroxy butyric acid was used

to estimate the amount of PHB granules synthesis by bacteria (Fig-9). Maximum PHB granules 1.23grms were obtained from olive oil as a carbon source for 100ml of MSMmedium. This was followed by optimization at different pH and temperature. Results showed maximum PHB production at 37°c and pH 9 with olive oil as carbon source (Table 2&3).

Similar study was reported by Lingayya Hiremath *et al*, 2015 were different media formulation with different compositions of Molasses/Glycerol/Glucose was used for enhancement of bio-polymer synthesis. Microbes were screened for PHB production by Sudan black staining and were analysed by Spectrophotometer at 235nm using crotonic acid as standard. Ten isolates out of 16 could produce PHB.

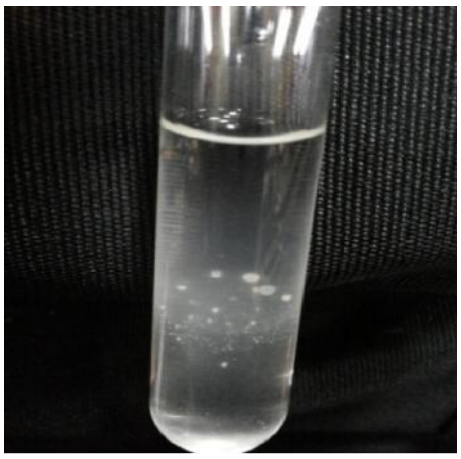

Figure 8 –PHB granules produce during cold acetone precipitation

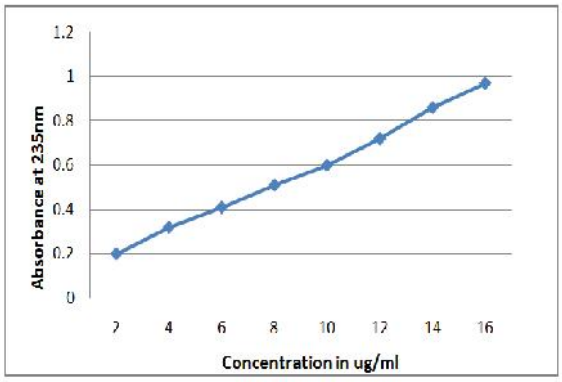

Figure 9-Standard graph of 3-hydroxy butyric acid.

Biosurfactant production

Pseudomonas fluorescens produced biosurfactant extracellularly which was detected in the cell free supernatant of sterile MSM media processed for PHB extraction with the same waste fried oils as carbon source. Maximum biosurfactant production was observed in 100ml of the MSM mediumwith olive oil as carbon source. This is of great advantage as two major factors responsible for environmental deterioration can be combated at a time. Crude biosurfactant was precipitated on addition of chilled acetone and recovered by centrifugation. Upon characterization of biosurfactant by rhamnose test it was found that the separated biosurfactant is a glycolipid (Fig 10). Aqueous solutions of biosurfactantalso showed good foaming stability. Total disappearance of the foam was detected after 8-10 hours. Extracted biosurfactant was also proved very effective in emulsifying different hydrocarbons used as a substrate. Kerosene was found to be the best substrates. Sunflower oil was less effective substrates for emulsification followed by Diesel oil. Emulsification activity (E24) of biosurfactant against different hydrocarbons such as Diesel oil was 34(%) Kerosene was 54 %Sunflower oil was 45%. According to M.Abouseoud *et al* 2007, in addition to surface and interfacial tension, stabilization of an oil and water emulsion is commonly used as a surface activity indicator.

Optimization results showed that, biosurfactant was stable with the temperature range of 4, 27, 37, 45(°c) but it was not stable at 55°c.Also the biosurfactant was produce at pH range of 7, 8, and 9. Table 2&3 describes the production of biosurfactant at different pH and temperature ranges respectively.

Precipitation of bio surfactant Crude bio surfactant Structural characterization

Figure 10 Production of Bio surfactants followed by its extraction and structural characterization.

Table 2 Production of biosurfactant and PHB at different pH range

Micro organism	**P.fluorescens**				
Carbon source	Olive oil				
PH	5	6	7	8	9
Weight of PHB Granules (grm)	No growth	No growth	0.75	0.92	1.23
Biosurfactant (grms)	No growth	No growth	0.92	1.23	2.10

Table 3- Production of biosurfactant and PHB at different temperature range.

Micro organism	**P.fluorescens**				
Carbon source	Olive oil				
Temperature °C	4°C	27°C	37°C	45°C	55°C
Weight of PHB Granules	0.02	0.94	1.02	No growth	No growth
Biosurfactant	No growth	0.95	1.22	1.28	No growth

DISCUSSIONS

On the basis of screening for intense siderophore producers from spinach roots surface, *Pseudomonas fluorescens*was identified as potential siderophore producers in iron free succinate medium. Results obtained after confirmation of siderophore produced shows that siderophore produced is of pyoverdine type. This was found similar with previous studies on siderophores. From graph obtained for Absorbance maximum of metal free siderophore and siderophore metal complex (Fig 4), it clearly indicates that the siderophore has bound with the metal ions forming siderophore metal complex. This results was similar with research reported by Mehri Ines *et al*, 2012 where in the supernatant from culture medium supplemented with zinc, the amount of pyoverdine

(siderophore) is lower than that excreted in control medium (no added metal). This result indicates that pyoverdins of studied strains might be able to complex zinc instead of iron. Results from the graph obtained for Siderophore zinc complex and siderophore lead complex (Fig 5&6) shows that bacteria are able to protect themselves by complexing its siderophores to heavy metal ions which would otherwise enter the cell by diffusions and kills them.

Thus this ability of the siderophore to complex with heavy metals can be exploited in bioremediation of heavymetals from environment, as siderophore are not chemicals by nature they does not deteriorate the environment.
Results from PHB production and extraction reveled that *Pseudomonas fluorescens*was able to produce PHB intracellularly during starvation period as waste fried oils served as a sole source of carbon. Sodium hypochlorite digestion process was successful in digesting the cells are releasing PHB outside in the third phase obtain after centrifugation. From Figure 8 it is a clear evident that PHB was extracted from the cells which was precipitated as granules after cold acetone addition. PHB was further confirmed from standard graph of 3-hydroxy butyric acid which gets converted to crotonic acid on addition of concentrated sulphuric acid. Similarly obtained PHB was estimated by converting it to crotonic acid.

Microorganisms utilize a variety of organic compounds as the source of carbon and energy for their growth. When the carbon source is an insoluble substrate like a hydrocarbon (CxHy) microorganisms facilitate their diffusion into the cell by producing a variety of substances, the biosurfactants. Some bacteria excrete ionic surfactant, which emulsify hydrocarbon substrates in the growth medium.

Some examples of this group of biosurfactants are rhamnolipids which are produced by different Pseudomonas species (Laith Al-Araji*et al* 2007). Thus in the present study by providing insoluble substrates like waste fried oils *Pseudomonas fluorescens* was able to accumulate biosurfactantextracellularly. The major advantage in this study was the production of Biosurfactant and PHB at a same time in MSM medium supplemented with waste fried oils as sole source of carbon. As bacteria is under starvation the accumulate PHB intracellular and Biosurfactantextracellularly for maximum utilization of immiscible carbon substrates. Waste fried olive oil served as a better substrate for maximum PHB and Biosurfactant production. This ability of bacteria was exploited to overcome present environmental issues.

CONCLUSION

Pseudomonas fluorescens was able to solve the major issues related to the Environment. That is by extraction of siderophore which can be used for bioremediation of heavy metals, from waste fried oil such as olive oil as carbon source it synthesize Poly hydroxy Butyrate intracellularly and biosurfactantextracellularly which can overcome problems associated with accumulation of thermoplastics and synthetic surfactants. This can also serve as cost effective methods of production. This study provides biological platform by exploiting abilities of *Pseudomonas fluorescens* to overcome major environmental issues.

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References

Aditi Bhattacharya (2010). Siderophore mediated metal uptake by *Pseudomonas fluorescens* and its comparison to iron chelation .Cey.J.Sci (bio sci), 39(2):147-155.

AshrutaGawali, V.D. Nanoty, U.K. Bhalekar (2014). Studies on Bioadsorption Process for Removing Heavy Metal from aqueous effluent by a bacterial EPS, Indian Journal of Applied Research, 4(3): 17-19.

B.Sreeden, S.preethi, J.Promoda kumara (2014).Isolation production and optimization of Siderophore producing Pseudomonas from paddy soil.Int.J. Pharma.Res.Sci, 02(1): 71-88.

Bholay A. D., JadhavPriyanka.U., Borkhataria B. V., Mayuri, V. Dhalkari (2012).Fluorescent Pseudomonads as Plant Growth Promoting Rhizobacteria and their Siderophoregenesis. IOSR Journal of Pharmacy and Biological Sciences, 3(1): 27-32.

Darshan Marjadi and Nitish Dharaiya (2012).Bioprospecting and characterization of poly-beta-hydroxyalkanoate (PHAS) producing Pseudomonas species isolated from edible oil contaminated soil. Research in biotechnology, 3(5): 01-08

FekaduAlemu (2013). Isolation of *Pseudomonas fluorescens*species from rhizospheric soil of faba bean and assessment of their siderophores production, International Journal of Advanced Research, 1(8): 203-210.

H.Manjunatha and M.K.Naik (2013).Biological and molecular characteristics of fluorescens Pseudomonas isolate from crop rhizosphere soil. In.J.Sci.Res and Tech, 1(1):18-22.

Indu C Nair (2013). Screening of novel bacteria capable of accumulating intracellular PHB under phenol stress. IJPAE, 3(2): 2231-4490.

M.Abouseoud, R.Macchi and A.Amrane (2007).Biosurfactant production from olive oil by *Pseudomonas fluorescens*. Communicating current research and education topics and trends in applied microbiology.

Mehri Ines, KhessairiAmel, TurkiYousra, SaidiNiela, Daly Imen, Meyer Jean Marie and Hassen Abdennasseur (2012).Effect of dose response of zinc and manganese on siderophores induction. American Journal of Environmental Science, 8(2):143-055.

O Brien S, Hodgson DJ, Buckling A. (2014) Social evolution of toxic metal Bioremediation in *Pseudomonas aeruginosa*. Proc. R. Soc. B 281: 20140858. http://dx.doi.org/10.1098/rspb.2014.0858

Varadharayan Saravanan and SubramaniyamVijayakumar (2014). Production of biosurfactants by *Pseudomonas*

aeruginosa (PB3A) using agro industrial wastes as carbon source. Malaysian Journal of Microbiology, 10(1): 57-62.

Laith Al-Araji, Raja Noor Zaliha Raja Abd. Rahman, MahiranBasri and Abu Baker Salleh (2007).Microbial Surfactant. Asia Pacific Journal of Molecular Biology and Biotechnology, 15 (3) : 99-105.

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