International Journal of Science and Research (IJSR)
ISSN (Online): 2319-7064
Index Copernicus Value (2015): 78.96 | Impact Factor (2015): 6.391

# Screening of Microorganism from Rhizosoheric Soil for Biosurfactant Production using Cheap Raw Material

**Sneha S. Paraskar[1], Swati N. Zodpe[2]**

P.G. Department of Microbiology, Shri Shivaji College of Arts, Commerce and Science, Akola (M.S.) India – 444003

**Abstract:** ***Biosurfactants or surface active compounds are produced by microorganisms. These molecule reduced surface tension both aqueous solutions and hydrocarbon mixtures. In this study isolation and identification of biosurfactant producing bacteria were assessed. The samples were collected from different rhizospheric soil & 15 strains were isolated. To confirm the ability of isolates in biosurfactant production, were tested by Erythrocyte Haemolysis Method and Phenol Sulphuric Acid Method, & Analytical Method i.e. Emulsification Index and Oil Displacement Area. In the present study we produced biosurfactant from different cheap raw materials i.e. Whey, Sewage & Tea Waste. The Antibiotic sensitivity/ resistance pattern is cheaked & the Antimicrobial activity of produced biosurfactant against Oral floral pathogen was studied.***

**Keywords:** Biosurfactants, Cheap raw material, Antibiotic activity, Antimicrobial activity

## 1. Introduction

Soil is excellent cultural medium for the growth of many type of organism. A narrow zone of soil affected by the presence of plant roots is defined as rhizosphere. The rhizosphere is known to be a hot spot of microbial activities. This is caused by an increased nutrient supply for microorganisms, since roots release a multitude of organic compounds (e.g., exudates and mucilage) derived from photosynthesis and other plant processes (Brimecombe *et al*., 2007).

**Biosurfactant**

Biosurfactant or surface-active compounds are a heterogeneous group of surface active molecules produced by microoaganisms, which either adhere to cell surface or are excreted extracellulary in the growth medium (Fietcher 1992; Zajic and Stiffens, 1994; Makker and Cameotra, 1998). Nowadays, biosurfactants are produced using co- and by-products of different technologies as a carbon source for microorganisms (molasses, glycerol, whey, frying oil, animal fat, soapstock and starch-rich wastes e.g. potato wastes) (Maneerat 2005; Makkar and Cameotra, 2002).

Current society is characterised by an increase in expenditures, the need to reuse materials and environmental concerns. Consequently, greater emphasis has been given to recovery, recycling and reuse. Indeed, the need for environmental preservation has led to the reuse of different industrial wastes. This is particularly valid for the food production industry, the waste products, effluents and by-products of which can be reused (Banat *et al.*, 2014).

**Whey:-** The immune potential of biosurfactant their used still remains limited, mainly due to their high production and extraction costs, low yield in production process. the vaeiety of cheap raw material including whey have been support for production of biosurfactant (Rodrigues L.R. And Teixeira J.A. et.al., 2008)

**Sewage:-** The use of industrial or municipal waste waters and sewage water, rich in organic pollutants, to achieve a double benefit of reducing the pollutants while producing useful products (Kosaric, 1992 )

**Tea waste:-** In recent year, tea waste is also gaining grounds due to its potential. The cell wall of tea consist of cellulose and hemicelluloses, lignin and structural proteins. In short tea waste have a good potential as a raw material

**Oral flora:-**The oral cavity is comprised of many surfaces, each coated with a plethora of bacteria, the proverbial bacterial biofilm. Some of these bacteria have been implicated in oral diseases such as caries and periodontitis, which are among the most common bacterial infections in humans (Albandar *et al.*, 1999)

**Antimicrobial activity against oral flora:-** Several biosurfactant have strong antibacterial, antifungal and antiviral activity. Other medically relevant used of biosurfactant include their role as anti-adhesive agent to pathogens, making them useful for treating many diseases and as therapeutic and probiotic agents.

## 2. Materials and Methods

**Collection of soil sample:-** The samples used for this study were obtained from different near rhizosphere soil in Akola region this include soil sample from various plant such as *Azadirachta indica*, *Ocimum tenuiflorum*, *Aloe vera*, Curry tree, and *Hibiscus rosa* sinusis.

**Isolation & Identification of biosurfactant producing strains:-** All the chemicals and media used during the experimentation was from Ranboxy and Himedia laboratory respectively.

**Cultural characteristics:-** The isolated colonies on various selective agar were further identified on the basis of heamolysis and colony characters

**Biochemical test:-** The strains were studied for its biochemical characterization by adopting standard method for IMViC, Enzymes & Sugars. All the tentatively conformed isolates were further screened for biosurfactant production ability.

**Screening of biosurfactant production:-** All the isolates tentatively detected as *Pseudomonas species, Azotobacter species and Rhizobium species* were inoculated in 10 ml nutrient broth and incubated at $37^0$ C for 4 days. Followed by incubation all the tubes were subjected to centrifugation at 3000 rpm 30 minutes. The supernatant obtained was separated and further used to screen biosurfactant producers. The screening of biosurfactant was done by adopting the Phenol sulphuric acid method & Erythrocyte haemolysis method whereas, different analytical methods used were Oil Spread Method and Emulsification Index respectively. The strain showing both the test positive were considered as biosurfactant producers.

- **Phenol sulphuric acid method:-** In phenol sulphuric acid method 1ml of 5% phenol was added to the supernanat and to this added 5ml of concentrated sulphuric acid drop by drop the colour changes from yellow to orange indicates the biosurfactant production (Kappeli and Finnerty, 1980).
- **Erythrocyte Homomlysis Method:-** In this method, to the 10ml of supernatant zinc chloride was added and precipitation appearance was seen. To this precipitation 10ml of sodium phosphate buffer was added and extracted it with diethyl ether and allow to evopurate to dryness after dryness, powder was remain and powder was mixed with 100 ml of methanol from that solution 10µl was stopped on filter paper disc and kept it on blood agar plates. Plates were incubated at room temperature for 2 days. Zone of haemolysis indicate positive test (Rodrigues *et al.*, 2006).
- **Oil displacement area (ODA) test:-** This method indicates the production of biosurfactant by crude oil to surface of water. A thin film of oil was formed when 20 µl of CFS was added in center of plate. Clear zone displacing the oil was measured in cm (Rodrigues *et al.*, 2006)
- **Emulsification Index (E24):-** The emulsifying capacity of biosurfactant was analyzed by emulsification index according to Cooper and Goldberg. In this method 2 ml of kerosene was added to 2 ml of the cell-free broth in a graduated tube, vortex at high speed for 2 min and allowed to stand for 24h, afterwards,the E24 index of sample was calculated .(Cooper, 1987)

$$E24 = \frac{height\ of\ emulsion}{total\ height\ of\ solution} \times 100$$

**Production of biosurfactant by using cheap raw material:-**Biosurfactant was carried out utilizing three different raw materials i.e whey, sewage and tea waste. The surfactant producing strains of *Pseudomonas*, *Azotobacter* and *Rhizobium* was asceptically inoculated in each medium**.** The inoculate medium were further incubated on rotary shaker at room temperature for about 2 days

**Extraction of biosurfactant :-**The extraction of biosurfactant was done by subjecting the enriched broth to centrifuged to 3000 rpm for 30 minutes. The supernatant thus obtained was separated using micropipette and further used to estimate the amount of surfactant produced. Simultaneously the optical density of enriched culture was measured at 540 nm on eight day of incubation.

**Antibiotic Susceptibility testing :-** Antibiotic sensitivity testing was performed by disc diffusion method to determine the sensitive and resistance bacteria to antibiotic by measuring the diameter of inhibition zone by mm and then compared with standard diameter that standard scales.

**Antimicrobial activity of produced biosurfactant against oral flora:-** The bacterial strain were used for this activity are *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Streptococcus mutant* were taken. All the samples are then transferred in sterilized nutrient broth for enrichment and incubated at 37°C for 24 hrs. After incubation of each enriched culture was add in the wells of nutrient agar plate and incubate at 37°C for 24 hrs.

## 3. Results and Discussion

**Table 1:** Conformations of biosurfactant producer organism on the basis of different tests

| Isolates | Phenol sulphuric acid method | Blood haemolysis method | Oil spread method | Biosurfactant producer organism |
|---|---|---|---|---|
| 1 | + ve | - ve | - ve | - ve |
| P2 | + ve | + ve | + ve | + ve |
| P3 | + ve | - ve | - ve | - ve |
| P4 | + ve | + ve | + ve | + ve |
| P5 | + ve | + ve | + ve | + ve |
| A1 | + ve | + ve | + ve | + ve |
| A2 | + ve | + ve | + ve | + ve |
| A3 | + ve | + ve | + ve | + ve |
| A4 | + ve | - ve | - ve | - ve |
| A5 | + ve | + ve | + ve | + ve |
| R1 | + ve | + ve | + ve | + ve |
| R2 | + ve | + ve | + ve | + ve |
| R3 | + ve | + ve | + ve | + ve |
| R4 | + ve | + ve | + ve | + ve |
| R5 | + ve | - ve | - ve | - ve |

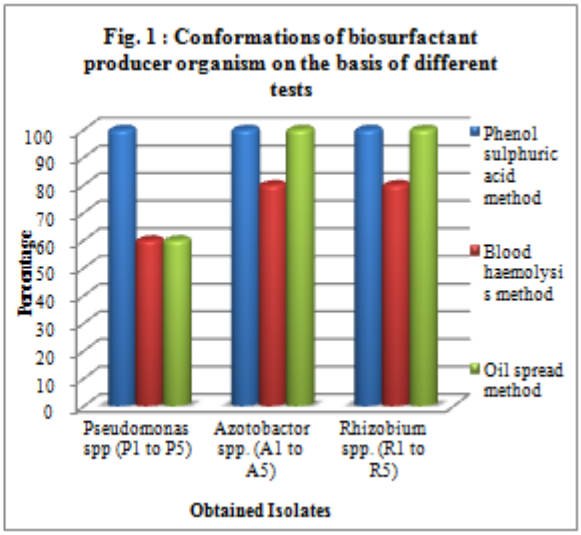


**Table 2:** Biosurfactant production by using cheap raw material

| Raw materials | Whey | Sewage | Tea waste |
|---|---|---|---|
| P2 | 0.254 | 0.228 | 0.425 |
| P3 | 1.284 | 0.184 | 0.494 |
| A1 | 0.375 | 0.316 | 0.281 |
| A2 | 0.198 | 0.333 | 0.194 |
| R1 | 0.241 | 0.872 | 0.259 |
| R2 | 0.341 | 0.284 | 0.236 |

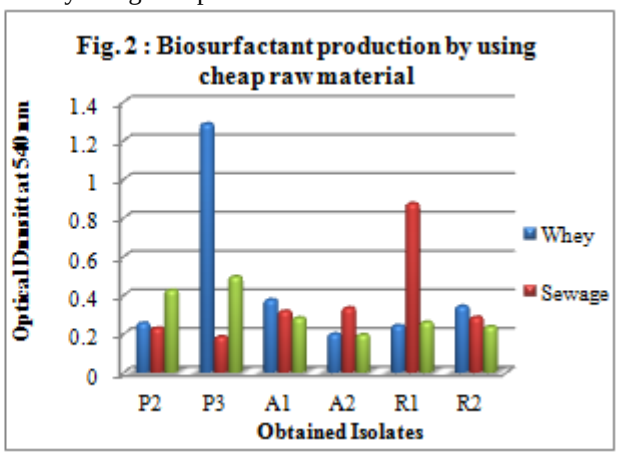



**Table 3:** Emulsification index

| Raw material | P2 | P3 | A1 | A2 | R1 | R2 |
|---|---|---|---|---|---|---|
| whey | 11.52 | 34.28 | 11.76 | 37.14 | 11.42 | 22.85 |
| Sewage | 17.14 | 11.76 | 17.14 | 48.57 | 51.42 | 34.28 |
| Tea waste | 34.28 | 14.70 | 34.28 | 34.28 | 11.42 | 34.28 |

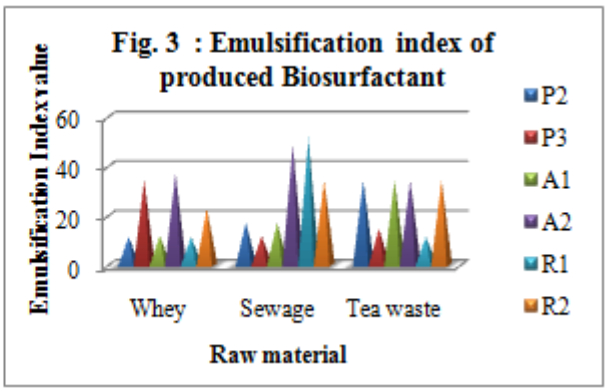



**Table 4:** Antibiotic sensitivity test

| Antibiotic | *P. aeuroginosa* | | *E. coli* | | *S. aureus* | | *M. lutis* | | *Lactobacillus* | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | S/R | | S/R | | S/R | | S/R | | S/R |
| Tetracycline | 20 mm | S | 17 mm | S | 18 mm | S | 15 mm | I | 17 mm | S |
| Amoxyclav | -- | R | -- | R | -- | R | -- | R | -- | R |
| Ciprofloxacin | 27 mm | S | 25 mm | S | 29 mm | S | 23 mm | S | 26 mm | S |
| Erythromycin | 16 mm | I | 12 mm | R | 21 mm | S | 22 mm | S | 17 mm | S |
| Gentamicin | 20 mm | S | 20 mm | S | 21 mm | S | 18 mm | S | 19 mm | S |
| Chloramphenicol | 21 mm | S | 19 mm | S | 24 mm | S | 14 mm | R | 21 mm | S |
| Ampicillin | -- | R | -- | R | -- | R | -- | R | -- | R |

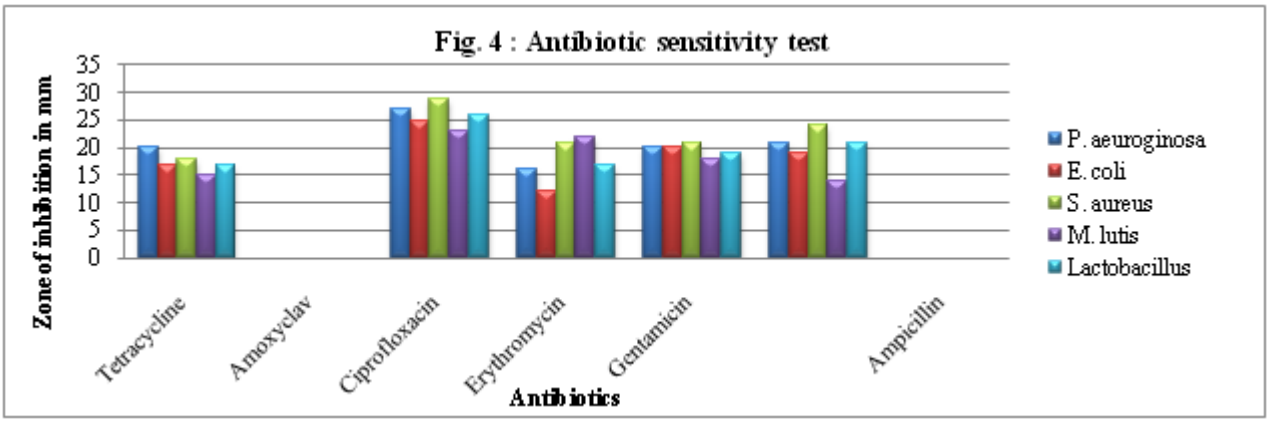


**Table 5 (a):** Antibacterial activity of produced biosurfactant from P2 isolate against oral flora

| Oral flora of P2 | Whey | Sewage | Tea waste |
|---|---|---|---|
| *P. aeuroginosa* | 16 mm | 9 mm | 17 mm |
| *E. coli* | 14 mm | 17 mm | 19 mm |
| *S. aureus* | 14 mm | 14 mm | 14 mm |
| *M. lutis* | 17 mm | 19 mm | 18 mm |
| *Lactobacillus* | 14 mm | 16 mm | 16 mm |

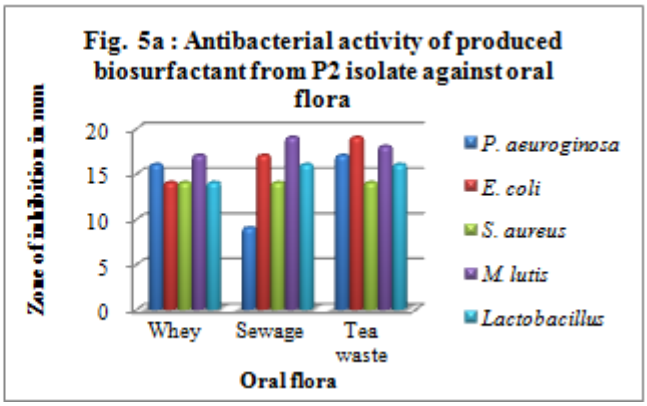



**Table 5 (b):** Antibacterial activity of produced biosurfactant from P3 isolate against oral flora

| Oral flora of P3 | Whey | Sewage | Tea waste |
|---|---|---|---|
| *P. aeuroginosa* | 15 mm | 22 mm | 21 mm |
| *E. coli* | 16 mm | 22 mm | 22 mm |
| *S. aureus* | 21 mm | 22 mm | 18 mm |
| *M. lutis* | 19 mm | 20 mm | 21 mm |
| *Lactobacillus* | 25 mm | 24 mm | 22 mm |

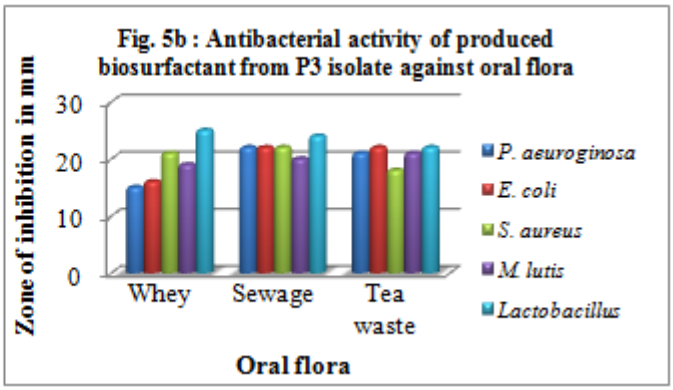



**Table 5 (c):** Antibacterial activity of produced biosurfactant from A1 isolate against oral flora

| Oral flora of A1 | Whey | Sewage | Tea waste |
|---|---|---|---|
| *P. aeuroginosa* | 21 mm | 22 mm | 22 mm |
| *E. coli* | 19 mm | 23 mm | 21 mm |
| *S. aureus* | 12 mm | 13 mm | 17 mm |
| *M. lutis* | 17 mm | 19 mm | 22 mm |
| *Lactobacillus* | 14 mm | 20 mm | 20 mm |

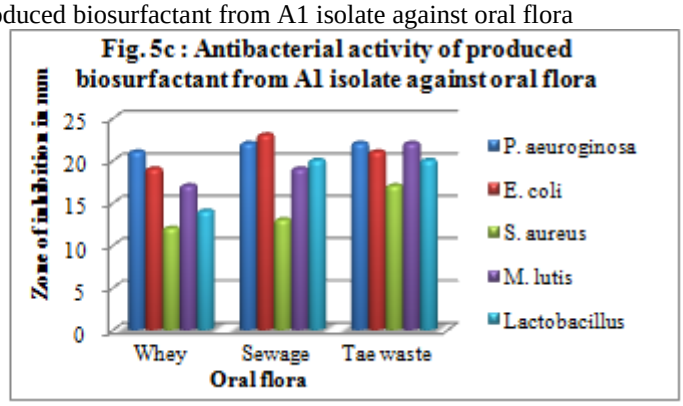



**Table 5 (d):** Antibacterial activity of produced biosurfactant from A2 isolate against oral flora

| Oral flora of A2 | Whey | Sewage | Tea waste |
|---|---|---|---|
| *P. aeuroginosa* | 17 mm | 18 mm | 22 mm |
| *E. coli* | 20 mm | 18 mm | 18 mm |
| *S. aureus* | 18 mm | 15 mm | 22 mm |
| *M. lutis* | 17 mm | 20 mm | 17 mm |
| *Lactobacillus* | 17 mm | 19 mm | 18 mm |

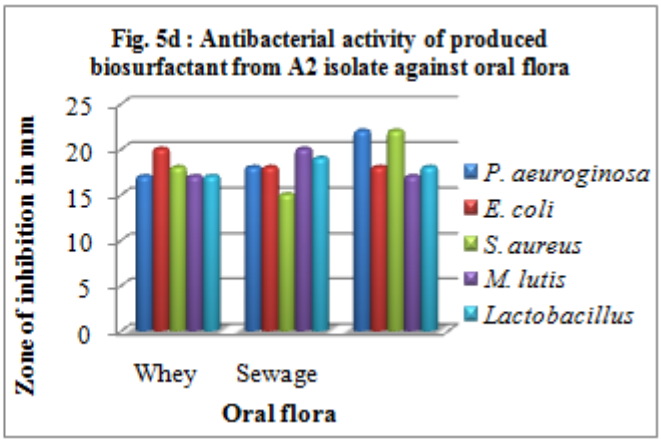


**Table 5 (e):** Antibacterial activity of produced biosurfactant from R1 isolate against oral flora

| Oral flora of R1 | Whey | Sewage | Tea waste |
|---|---|---|---|
| *P. aeuroginosa* | 21 mm | 17 mm | 16 mm |
| *E. coli* | 7 mm | 19 mm | 17 mm |
| *S. aureus* | 17 mm | 18 mm | 12 mm |
| *M. lutis* | 19 mm | 20 mm | 13 mm |
| *Lactobacillus* | 16 mm | 20 mm | 15 mm |

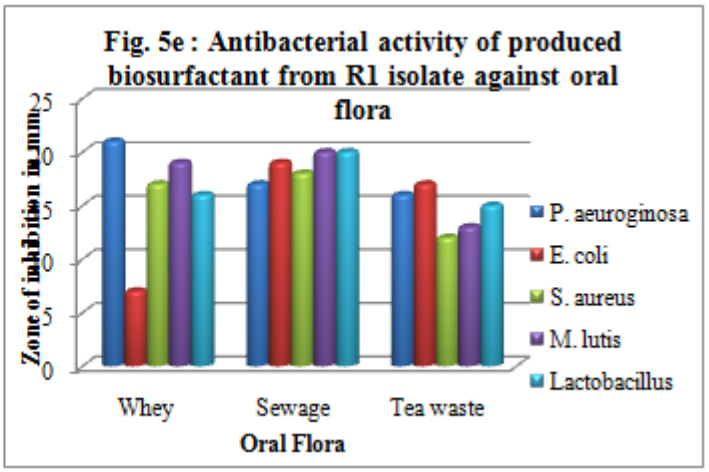
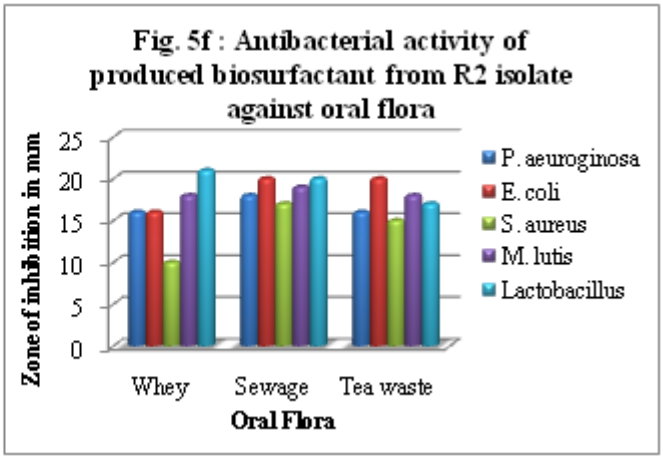



**Table 5 (f):** Antibacterial activity of produced biosurfactant from R2 isolate against oral flora

| *Oral flora of R2* | *Whey* | *Sewage* | *Tea waste* |
|---|---|---|---|
| *P. aeuroginosa* | 16 mm | 18 mm | 16 mm |
| *E. coli* | 16 mm | 20 mm | 20 mm |
| *S. aureus* | 10 mm | 17 mm | 15 mm |
| *M. lutis* | 18 mm | 19 mm | 18 mm |
| *Lactobacillus* | 21 mm | 20 mm | 17 mm |




## 4. Discussion

A total of 15 samples were collected from different rhizoshpreic soil i.e soil from *Azadirachta indica*, *Ocimum tenuiflorum*, *Aloe vera*, Curry tree, and *Hibiscus rosa* sinusis rhizospheric soil, of Akola city.

The samples were further subjected for isolation and identification.

These isolates were obtained from various soil samples and different strains of *Pseudomonas species* obtained were designated as P1. P2, P3, P4, & P5, *Azotobacter species* obtained were designated as A1, A2, A3, A4, & A5. And *Rhizobium species* obtained were designated as R1, R2, R3, R4 & R5. The isolates were tentatively conformed on the basis of conventional & biochemical characteristics. The detail study of that 15 samples out of which 5 samples were screened to be *Pseudomonas species*, 5 were screend to be *Azotobacter species* & 5 samples were screened to be *Rhizobium species*.

The organisms screened for the biosurfactant production ability.

To confirmed the ability of isolates for biosurfactant production, different screening methods used were Phenol Sulphuric Acid Method & Erythrocyte Haemolysis Method. Whereas, different Analytical methods used were Oil Spread Method & Emulsification Index.

The biosurfactant production ability was checked for 15 isolates, it was noticed that 11 isolate shows the positive result for phenol sulphuric acid method and erythrocyte haemolysis method which were considered as the biosurfactant conformatery test. They are shown in (Table No.1). This result is also accordance with Deshmukh P. W.(2015) they reported that Phenol Sulphuric Acid Method and Erythrocyte Haemolysis Method from the result they noticed that out of these three *Pseudomonas* isolate strain no.1 (PS1) shows positive result for both producers. Whereas (PS2) was positive for Phenol Sulphuric Acid Method and (PS3) was positive for Erythrocyte Haemolysis Method, hence strain (PS1) are conformed as biosurfactant producer.

On the basis of Oil Spread Method out of 15 isolates11 isolates shows the clear zone at the centre of plate indicates the positive test. Whereas, other 4 isolates showed negative test. On the basis of results it was confirmed that 11 isolates were biosurfactant producer (Table No. 1). Our result is also accordance with the result of Youssef *et al.*, (2004) they reported that oil spreading assay method is a reliable technique for testing biosurfactant production. These result suggested that the other method for biosurfactant detection in the supernatant from a culture medium.

**Biosurfactant production by using cheap raw material:-**

To check the biosurfactant production ability of obtained isolates from different cheap raw materials such as Whey, Sewage and Tea Waste were used to find out which was found to be superior biosurfactant producer.

Among the 3 substrate tested, isolate P2 produced a maximum of 0.254 gm/l biosurfactant with whey followed by sewage 0.228 gm/l & tea waste 0.425 gm/l. Further study with P3 isolate showed maximum biosurfactant production of 1.284 gm/l with whey followed by sewage 0.184 gm/l & tea waste 0.494 gm/l.

Whereas, isolate A1 showed maximum biosurfactant production of 0.372 gm/l with whey followed by sewage 0.316 gm/l & tea waste 0.281 gm/l. and in case of Isolate A2 produced a maximum biosurfactant i.e. 0.198 gm/l with whey followed by sewage 0.333 gm/l & tea waste 0.194 gm/l.

Further study with Isolate R1 produced a maximum biosurfactant i.e. 0.241 gm/l with whey followed by sewage 0.872 gm/l & tea waste 0.259 gm/l. whereas, Isolate R2 produced a maximum biosurfactant i.e. 0.341gm/l with whey followed by sewage 0.284 gm/l & tea waste 0.236 gm/l. (Table No. 2).

Similar finding were obtained from the result of Deshmukh et al., (2015). It was observed that, the maximum surfactant was produced (97 mg/ml) when the pseudomonas was cultivated using oil as a substrate, followed whey (37 mg/ml), Triptic soy broth (24 mg/ml) and Nutrient broth (21 mg/ml). in case of inorganic salt medium as an offered substrate it was at part with whey medium in production of biosurfactant during eight days of incubation.

The emulsification index for the biosurfactant produced from whey the isolate A2 shows strong emulsification index of 37.14 followed to this isolate P3 shows 34.28, isolate R2 shows 22.85, whereas, isolate A1 shows 11.76, isolate P2 shows 11.52 & isolate R1 shows 11.42 emulsification index.

The similar result for emulsification index was observed for the biosurfactant produced from sewage, the isolate R1 shows strong emulsification index of 51.42 followed to this isolate A2 shows 48.57, isolate R2 shows 34.28 emulsification index. Whereas isolate A1 & P2 shows 17.14, and isolate P3 shows 11.76 emulsification index.

The emulsification index for the biosurfactant produced from tea waste and the isolate was P2,A1,A2 & isolate R2 shows similar emulsification index of 34.28, where as isolate P3 shows 14.70 & isolate R1 shows 11.42 emulsification index.They are shown in (Table No. 3).

Our result is similar with Tabatabaee A (2005) they seen screening of microbial isolate perform in order to check the ability of crude oil emulsifying bacteria. Among 35 strain, 23 had 70% emulsification activity.

Antibiotic sensitive/resistance pattern was studied against the oral floral pathogen. The most predominants oral flora isolated was *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Staphylococcus aureus, M. lutis & Lactobacillus.* During the cource of study it was notice that Ciprofloxin and Gentamycin shows sensitivity to all the oral floral organism. Whereas *M.lutis* is resistance to Chloremphenicol, *Escherichia coli* is resistance to Erythromycin & for *Pseudomonas aeruginosa* Erythromycin shows intermediate activity. Most surprising result was obtained in case of Amoxyclave and Ampicillin which shows no zone of inhibition towards any organisms, indicating drug resistance towards this antibiotics. presented in (Table No. 4).

The result is also similar with Maestre JR,et al., (2005) they conducted a study to analyze 261 aerobic and anaerobic bacteria isolated in 48 adult patients with periodontitis they observed that 54.1% of bacteria of the Prevotella genus, 38.9% bacteria of the Fusobacterium genus, and 30% of the Capnocytophaga genus, produced ß-lactamase. For this reason, ß-lactamics that are capable of resisting the action of these enzymes, such as the amoxicillin + clavulanic acid association, have become the antibiotic of choice for oral infections. In their study, 100% of the dental pathogenic strains (both aerobic and anaerobic) that were isolated in patients with periodontitis were sensitive to the amoxicillin + clavulanic acid association. 100% of *Streptococcus viridans* were also sensitive to aminopenicillins.

Further study is continued to checked the antibacterial activity of biosurfactant produced from cheap raw material i.e. whey, sewage & tea waste against the oral flora to control the oral infections.

Antibacterial activity of produced biosurfactant from P2 & P3 isolate was checked and it was noticed that all the biosurfactant produced from various raw material shows excellent antibacterial activity to controlling the oral pathogens showing zone of inhibition ranging from 14mm – 25mm. In case of isolate P2 biosurfactant produced from sewage shows very less activity i.e. 9mm only in controlling the *Pseudomonas aeruginosa* infection.

Overall result revealed that biosurfactant production using all these raw materials A1 & A2 isolate i.e. *Azotobacter species* is having strong antibacterial activity against oral pathogens for all the 5 isolate. It shows very excellent activity, showing zone of inhibition ranging between 12mm – 23mm. So all the cheap raw materials were found to be best biosurfactant producers.

Similar finding were obtained in case of isolate R1 & R2 i.e. *Rhizobium species* in controlling the oral pathogens, from the produced biosurfactant. The zone of inhibition against the oral pathogens were measured, the ranging in between 13mm – 21mm. Whereas very least activity was shown by whey produced from isolate R1 against *Escherichia coli.*

Our result is correlates with Das et al.,(2009) they observed a biosurfactant produced by marine *B. circulans* that had a potent antimicrobial activity against gram-positive & gram-negative pathogenic and semi-pathogenic microbial strains. Similarly Fernandes et al., (2007) investigated the antimicrobial activity of biosurfactants from *Bacillus*

*subtillis* R14 against 29 bacterial strains. Their result demonstrated that lipopeptide have a broad spectrum of action, including antimicrobial activity against microorganism with multidrug resistance profiles.

## References

[1] Albandar, J. M., J. A. Brunelle, and A. Kingman. (1999). Destructive periodontal disease in adults 30 years of age and older in the United States, 1988–1994. *J. Periodontol*. **70 :**13–29.
[2] Banat, I.M.; Satpute, S.K.; Cameotra, S.S.; Patil, R.; Nyayanit, N. (2014). Cost effective technologies and renewable substrates for biosurfactants' production. *Front. Microbiol*., 5. [CrossRef] [PubMed]
[3] Brimecombe M. J., De Leij FAAM, Lynch J. M., (2007). Rhizodeposition and microbial populations. In: R Pinton, Z Varanini, P Nannipieri (eds) The rhizosphere: biochemistry and organic substances at the soil-plant interface. CRC Press, Taylor & Francis Group, Boca Raton, London, New York, pp 73–109.
[4] Cooper D. G.and B. G. Goldenberg (1987), Surface-active agents from two *Bacillus* species," *Appl. Environ. Microbiol*, vol. 53, pp. 224-229,.
[5] Das P., A. K. Mukherjee and R. Sen (2009). Antiadhesive action of a marine microbial surfactants Colloids Surf. B. *Biointerfaces*, **71** : 2277 – 2282.
[6] Deshmukh, P. W. Kulkarni N. S. (2015). Production of Biosurfactant of Microbial Origin Using Different Raw Materials. *International Journal of Science and Research*, **5**(5) **:** 1221 – 1224.
[7] Fietcher A (1992). .Biosurfactant moving toward industrial application. *Tibtech*, **10**: 208 -17.
[8] Kappeli, O., Finnerty, W. R. (1980). Characteristics hexadecane partition by the growth medium of *Acenetobacter spp*. *Biotechnol. Bioeng.*, **22** : 495 – 503.
[9] Kappeli, O., Finnerty, W. R. (1980). Characteristics hexadecane partition by the growth medium of *Acenetobacter spp*. *Biotechnol. Bioeng.*, **22** : 495 – 503.
[10] Koch A. K., Reiser J., Kappeli, O. and Fiechter A. (1988). *Bio/Technology*, **6** : 1335 – 1339.
[11] Kosaric N. (1992). Biosurfactants in industry. Pure Appl Chem 64:1731–1737 Kuan YH, Liong MT (2008) Chemical and physicochemical characterization of agrowaste fibrous materials and residues. *J. Agric. Food Chem.*, **56** : 9252 –9257.
[12] Maestre J. R., Gimenez M. J., Bascones A. *et al.*, (2005). Odontopathogen Susceptibility to Amoxicillin/Clavulanic acid and other common antibiotic used in odontology. 7th European Coingress of Chemotherapy and Infection. Florence Italy, P 209.
[13] Makkar R. S. and Cameotra S. S. (2002). An update on the use of unconventional substrates for biosurfactants production and their new application. *Appl. Microbial. Biotechnol*., **58** : 428 – 434.
[14] Makker R S, Cameotra S S (1998). Production of biosurfactant at mesophilic and thermophilic conditions by a strain of *Bacillus subtilis*. *J Industrial Microbiol & Biotechnolo*, **20** : 48 - 52.
[15] Maneerat S. (2005). Production of biosurfactants using substrates from renewable resources. Songklanakarin. *J. Sci. Technol.*, **27**(3) : 675 - 683.
[16] Robinson D., Griffiths B., Ritz K., Wheatley R. (1989). Root-induced nitrogen mineralisation: a theoretical analysis. *Plant Soil*, **117** : 185 – 193.
[17] Rodrigues, L. R., J. A. Teixeira, H. C. van der Mei, and R. Oliveira (2006). "Physicochemical and functional characterization of a biosurfactant produced by *Lactococcuslactis* 53," *Colloid Surface B*, **49** : 79-86.
[18] Rodrigues, L. R., Teixeira, J. A., Oliveira, R. and van der Mei, H. C. (2008). *Process Biochem.*, **41** : 1 - 10
[19] Tabatabaee A., M Mazaheri Assadi, A A Noohi,V A Sajadian (2005). Isolation of Biosurfactant Producing Bacteria from Oil Reservoirs. *Iranian J Env Health Sci Eng,*, **2**(1) : 6 - 12.
[20] Youssef N. H, Duncan K. E., Nagle D. P., Savage K. N., Knapp R. M., *et al*., (2004). Comparison of methods to detect biosurfactant production by diverse microorganisms. *J Microbiol Methods*, **56** : 339-347.
[21] Zajic J. E., Stiffens W. (1994). Biosurfactants. *CRC Rev Biotechnol*, **1** :87-106.