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Screening of Biosurfactant Producers from Bacillus subtilis and Study of its Antimicrobial Potential against Clinical Isolates

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ABSTRACT
The Current study was carried out to check the antimicrobial potential of produced biosurfactant against clinical isolates. Total 10 samples were collected during the study and further processed for isolation and identification. The predominant organisms were B.subtilis, P.aeruginosa, S.aureus and Micrococcus. The study was continued with the B.subtilis. The Biosurfactant was produced from B.subtilis. Biosurfactants production ability was confirmed with different screening methods such as Phenol Sulphuric Acid Method,Haemolysis Method and different Analytical methods such as Oil Spreading Test, Emulsification Index. The most predominant clinical isolates was P.aeruginosa, E.coli, K.pneumonia, S.aureus and S.typhi. The antimicrobial activityof produced biosurfactant checked against clinical isolates..
Keywords: Biosurfactant,Bacillus subtilis, Clinical isolates, Antimicrobial activity.

I. INTRODUCTION

Most of these microorganisms are bioactive and survive at the top few inches of the agriculturalsoils (Foster JW et al.,2010). Microbial degradation process aids the elimination of spilledoil from the environment after critical removal of large amounts of the oil by various physical and chemical methods (Ijah and Okang, 1993). This is possible because microorganisms have enzyme systems to degrade and utilize diesel oil as a source of carbon and energy (Ijah and Antai, 1988; Ezeji et al., 2005; Antai and Mgbomo, 1993).

The term "biosurfactants''refers to a class of modern surfactants that are derivedfrommicroorganisms and are equally diverse in terms of structure and function. They are attracting more attent;on due to their biodegradability and environmentally favourable qualities.(Shekhar et al., 2015). Biosurfactant producing microorganisms universally inhabit fresh water, soil, sediment, and sludge (Perfumo et al.,2010).Bacteria strain such as Bacillus, that can survive in harsh environments for a long time, are generally known as biosurfactants producers (Mulligan CN 2005).

II. MATERIAL AND METHODS

Collection of samples :
The soil sample was used for this study were collected from various Automobile garrages in Akola region.

Isolation and identification of bacteria from oil contaminated sample :

The sample were enrich using inoculating in sterile mineral salt medium. 1 ml of sample was inoculated in 100 ml of mineral salt medium. The medium was inoculated for 24 hours and were selected based on the colony morphology on the nutrient agar and selected microbes were gram scoloed. The selected isolates were screened for the production of biosurfactants and using the following screening methods (Nayarisseri A et al., 2018).

Screening and Production of Biosurfactant by using isolated microorganisms :

Bacteria were cultivated aerobically in a 500 ml Erlenmeyer flask with 100 ml mineral salt medium.Flasks containing sterilised mineral salt medium were inoculated with a loopful of bacterial culture is kept in a shaker at 200 rpm and 30°C for 7 days.After 7 days of incubation, each flask's culture broth was centrifuged for 15 minutes at 6000 rpm and 4°C, and the supernant was filtered through 0.45m pore size filter paper (Millipore).The drop collapse essay, oil spending assay, emulsification assay, BATH assay and surface tension assay were all performed using this cell – free culture broth (Nayarisseri A et al., 2018).

Phenol Sulphuric Acid Method :

In phenol Sulphuric acid method 1 ml of 5% phenol was added to the supernant and to this

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added 5 ml of concentrated Sulphuric acid drop by drop the colour changes from yellow to orange indicates the biosurfactant production (Kappeli and Finnetry, 1980).

Haemolytic activity :

The blood agar plates containing 5% v/v blood were used to seed the desired isolate, which was incubated at 37°C for 48 hours.Then, the plates were examined to form the clear zone around bacteria colonies, and the size of the clear zone was measured(Dhasayan Aet al., 2015).

Oil Spreading Test :

10 ul of crude oil is added to the surface of 40 ml of distilled water in a petridishto form a thin oil layer.Then, 10 ul of culture or culture supernant are gently placed on the centre of the oil layer. If biosurfactant is present in the supernant, the oil is displaced and a clearing zone is formed (Morikawaet al., 2000).

Emulsification test (E24) :

Several colonies of pure culture were suspended in test tubes containing 2 mL of mineral salt medium after 48 h of incubation, 2 mL hydrocarbon (oil) was added to each tube.Then, the mixture was vortexed at high speed for 1 min and allowed to stand for 24 h. The emulsion index (E24) is the height of the emulsion layer (cm) divided by total height (cm), multiplied by 100 (Bodouret al., 2004).

Collection of Clinical Isolates :

The clinical samples was used for this study were collected from Government Medical College, Akola.

Isolation and Identification of Clinical Isolates :

The collected samples were inoculated on various selective media. All the plates were incubated at 37°C for 24 hrs.After incubation different cultural were identified on the basis of Cultural, Morphological and Biochemical Characterisation.

Antibiotics Sensitivity Test :

Antibiotic susceptibility tests were carried out by disc diffusion method (Bauer WA etal., 1966).Disc fusion method was used for the evaluation of antibiotic resistance by culturing the isolates on the Mueller-Hinton agar medium. After incubating the plates for 18-24 hours at 37 °C, the diameter of the growth inhibition zone was investigated.

Antimicrobial activity of produced biosurfactant against clinical isolates :

The antibacterial activity of crude biosurfactant was tested using the agar well diffusion method.The crude biosurfactant was tested on S.typhi, Aspergillusniger,S.aureus and E.coli along with control was used as 5% ciprofloxacin. The plates were incubated for 24 hours at 37°C. The presence of a clear zone suggests that the crude biosurfactant has antibacterial action (S.O. Adebajo et al., 2020).

III. RESULT AND DISCUSSION

Table No.1:- Frequency Distribution of Bacteria found in soil sample

Sr. No.	Name of organisms	No. Of isolate (out of 22)	Percentage (%)
1	B.subtilis	10	45.4
2	P.aeruginosa	6	27
3	S.aureus	4	18
4	Micrococcus	2	4

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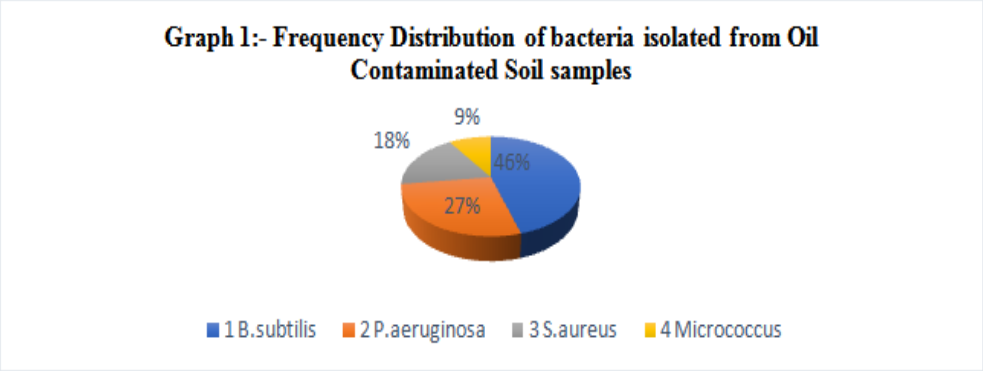

Table No.2 :- Conformation of Biosurfactant producer organism on the basis of different tests Phenol Sulphuric Acid Method, Haemolysis Method and Oil Spread Test

Sr.No.	Isolate	Phenol Sulphuric Acid Method	Haemolysis Method	Oil Spreading Test
1	B1	+	-	+
2	B2	+	+	+
3	B3	+	-	-
4	B4	+	+	+
5	B5	+	+	+
6	B6	+	-	-
7	B7	+	-	+
8	B8	+	-	-
9	B9	+	+	+
10	B10	+	+	+

Key :- +ve – Positive , -ve – Negative

Table No.3:- Emulsification Index

Sr.No	Isolate	Organisms	EI
1	B1	P.aeroginosa	53.84
2	B2	E.coli	60.8
3	B3	S.typhi	52

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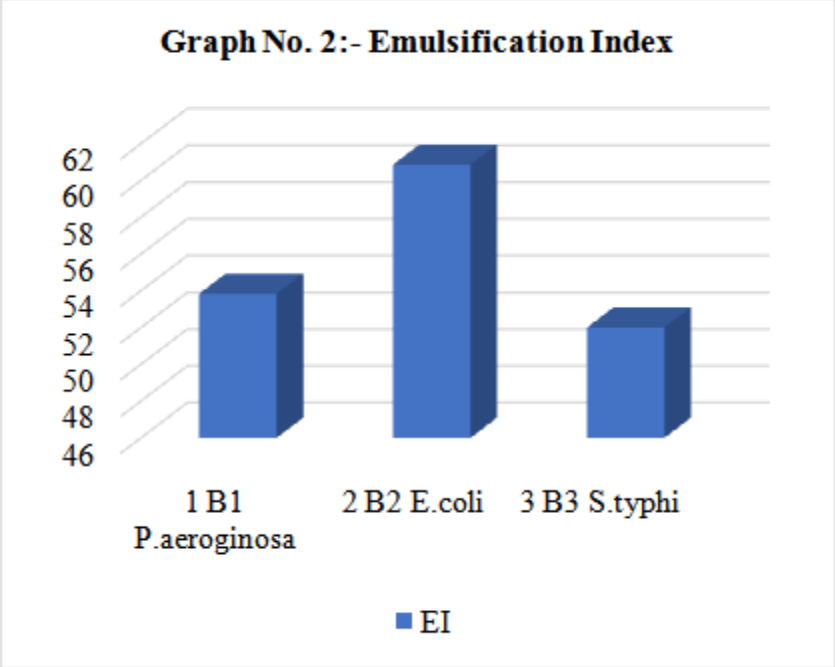

TableNo.4:- Frequency Distribution of Bacteria found in Clinical Samples

Sr.No.	Name of Organisms	No. Of Isolates (Out of 30)	Percentage (%)
1	P.aeroginosa	7	23.33
2	Escherichia coli	5	16.66
3	Klebsiella pneumonia	5	16.66
4	Staphylococcus aureus	6	20.00
5	Salmonella typhi	7	23.33

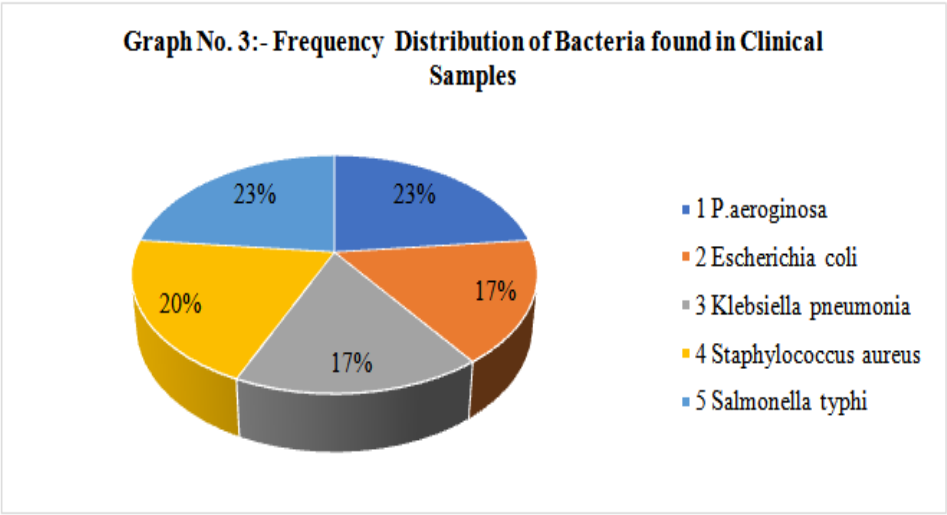

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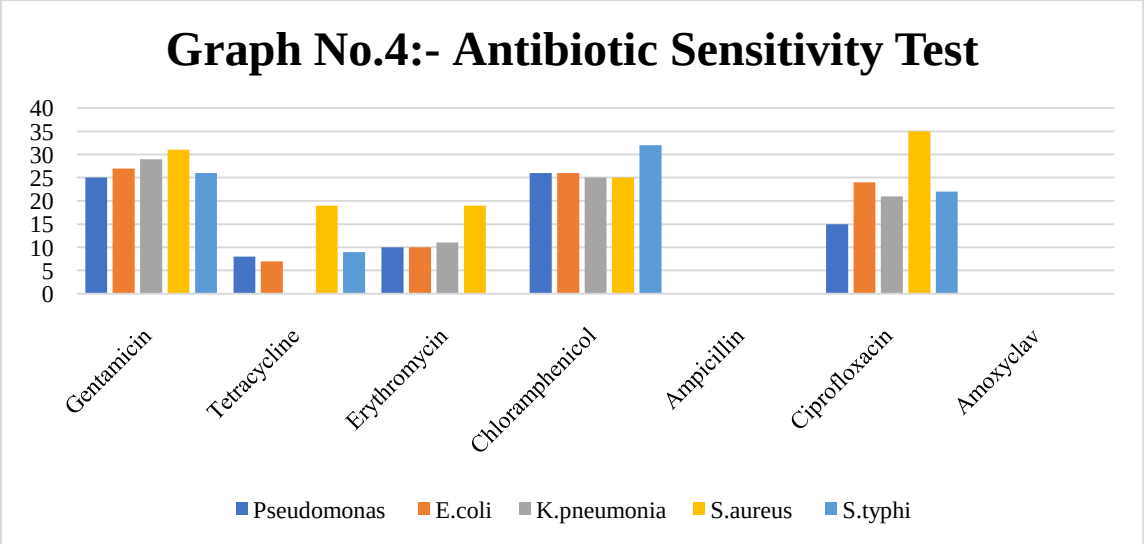
Table No. 5:-Antibiotic Sensitivity/Resistance test

Sr.No	Antibiotic	Name Clinical isolates (Zone in mm)									
		P.aeroginosa		E.coli		K.pneumoniae		S.aureus		S.typhi	
1	Gentamicin	25	S	27	S	29	S	31	S	26	S
2	Tetracycline	8	R	7	R	-	R	19	I	9	R
3	Erythromycin	10	I	10	I	11	R	19	R	-	R
4	Chloramphenicol	26	S	26	S	25	S	25	S	32	S
5	Ampicillin	-	R	-	R	-	R	-	R	-	R
6	Ciprofloxacin	15	S	24	S	21	I	35	S	22	I
7	Amoxyclav	-	R	-	R	-	R	-	R	-	R



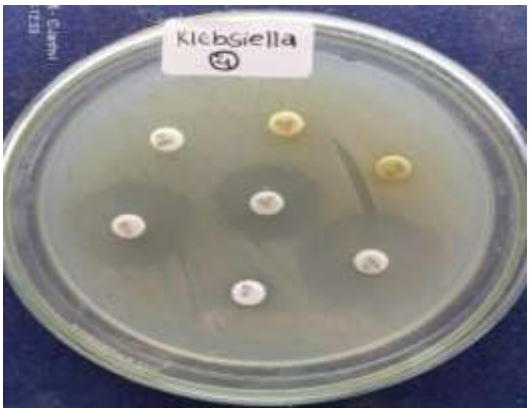


Table No.6:- Antimicrobial Activity of produced Biosurfactant from Bacillussubtilisand its activity against Clinical isolates

Sr.no	Isolate	Zone of Inhibition in mm				
		P.aeruginosa	E.coli	K.pneumonia	S.aureus	S.typhi
1	B1	31	16	15	16	13
2	B2	26	17	16	36	15

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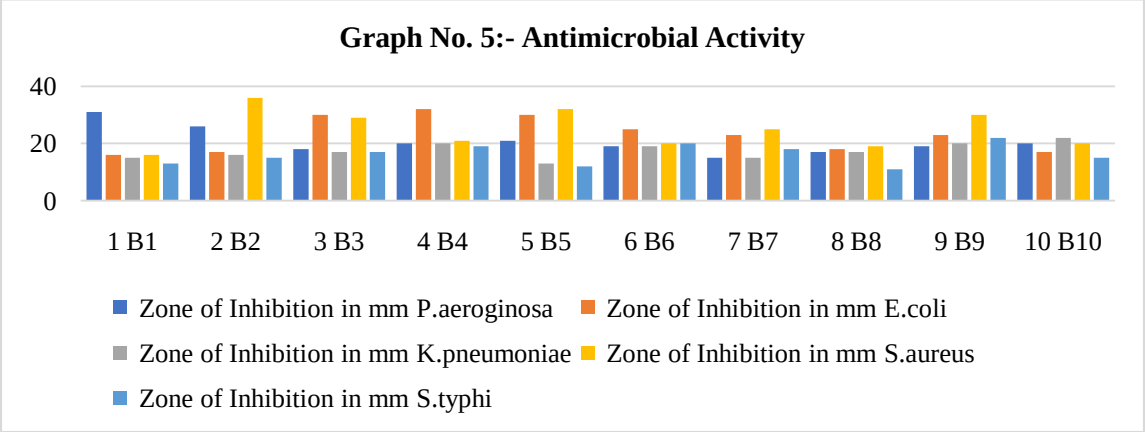
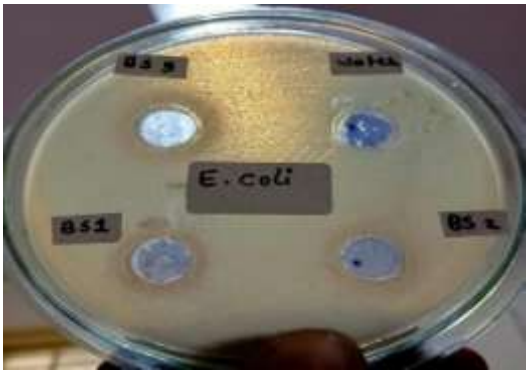
3	B3	18	30	17	29	17
4	B4	20	32	20	21	19
5	B5	21	30	13	32	12
6	B6	19	25	19	20	20
7	B7	15	23	15	25	18
8	B8	17	18	17	19	11
9	B9	19	23	20	30	22
10	B10	20	17	22	20	15





IV. DISCUSSION :

A total 10 oil contaminated soil samples were collected from different automobile garages, Akola city .In that soil samples different types of bacteria obtained (Table No.1). Frequency distribution of isolated oil contaminated soil samples was taken B.subtilis is found in 10 samples (45.40%), P.aeruginosais found in 6 samples (27.00%), S.aureuswas found in 4 samples (18.00%) and Micrococcus found in 1 sample (4.00%) (Table No.2). The oil contaminated soil samples were further subjected for isolation and identification. For Conformation of the isolates B.subtilis further study was carried out with Biochemical Test are carried out. The all the test confirmed the isolates B.subtilis.

The Bacillus subtilis screened for the biosurfactant production ability. To confirmed the ability of isolate for biosurfactant production, different screening methods used were Phenol Sulphuric Acid Method and Haemolysis Method. Where, as different Analytical methods used were Oil Spread Method and Emulsification Index. The biosurfactant production ability was checked for 10 isolates, it was noticed that 7 isolates shows the positive result for phenol sulphuric acid method ,5

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isolates shows positive result for Blood Haemolysis Method which were considered as the biosurfactant confirmatory test and 7 isolates shows positive test for Oil Spread Test (Table No.3). This result is accordance with the result of Yousself et al., (2004) reported that the oil displacement technique is more suitable than the drop collapse method in detecting low levels of biosurfactant production.Total 3 isolates were tested for emulsification index.The emulsification index for the biosurfactant produced from E.coli the isolate B2 shows strong emulsification index of 60.86. The emulsification index for biosurfactant produced from Pseudomonas aeruginosa the isolates B1 shows 53.84 emulsification index. The emulsification index for biosurfactant produced from S.typhi the isolates B3 shows 52 emulsification index (Table No.4 and Graph No.2). This result is accordance with Patowary et al., (2017) reported that the biosurfactant maximum emulsification activities 100% for crude oil, followed diesel, kerosene and engine oil.

A total 15 clinical samples were collected from Government Medical College, Akola. Isolation and identification of bacteria and were tentatively confirmed on the basis of Bergey's Manual of Determinative Bacteriology (Table No.5).

Frequency distribution of clinical isolates studied and it was noticed that P.aeruginosa was found in 7 samples(23.33%), Escherichia coli is found in 5 samples (16.66%), Klebsiellapneumonia is found in 5 (16.66%), Staphylococcusaureus is found in 6 samples (20%) and Salmonellatyphi is found in 7 samples (22.33%) (Table No.6 and Graph 3).

The detail study of Morphological and Cultural characteristics of each clinical isolates. These isolates were obtained from GMC, Akola and different isolates such as P.aeruginosa , S.typhi, S.aureus, K.pneumonia and E.coli were obtained.

Antibiotic Sensitivity / Resistance pattern was studied against clinical. During the course of study it was notice that Gentamicin , Ciprofloxacin and Chloramphenicol shows sensitivity to all the isolates. Where as E.coli is resistance for Tetracycline, Klebsiella is resistance to Erythromycin. Most surprising result was obtained in case of Amoxyclav and Ampicillin which shows no zone of inhibition (Table No.7and Graph 4). Our result is in accordance with the result of Coates et al., (2011) and Gudina et al., (2016) reported the Antibiotic therapies is the main choice for treatment of most human bacterial infections.

Antimicrobial activity of produced biosurfactant from isolates was checked and it was noticed that all the biosurfactant produced from oil contaminated soil shows excellent antibacterial activity to controlling the clinical isolates showing zone of inhibition ranging from 8mm - 39mm (Table No.8 and Graph5).Where as very least activity was shown by biosurfactant produced from isolate against E.coli.Pseudomonas aeruginosa is having strong antibacterial activity against clinical isolates.Our result is correlates with Patowary et al., (2017) reported that B.subtilis, S.aureus , K. Pneumonia and E.coli were susceptible to the purified biosurfactant where it showed antibacterial properties that inhibited both Gram +ve and Gram -ve strains.

V. CONCLUSION

Biosurfactant producer are present in oil contaminated soil also. Biosurfactant produced from Bacillus subtilis isolated from soil shows best activity in controlling the isolates from clinical microflora. For commercial production of biosurfactants optimization study with respect to oil contaminated soil flora at biological and engineering level is needed. Optimization study for those of biosurfactant should be carried out. New genera among the biosurfactant producer by using various clinical samples should be investigated.

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