Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 1863-1870
Received: 04-01-2019
Accepted: 07-02-2019

Arjun Karmakar
Sam Higginbottom University of Agriculture Technology and Sciences SHUATS, Prayagraj, Uttar Pradesh, India

Jyotsna Kiran Peter
Sam Higginbottom University of Agriculture Technology and Sciences SHUATS, Prayagraj, Uttar Pradesh, India

Dr. Ankit Singla
Regional Center of Organic Farming, Ministry of Agriculture & Frames Welfare, Bhubaneswar, Odisha, India

Anita Raisagar
Sam Higginbottom University of Agriculture Technology and Sciences SHUATS, Prayagraj, Uttar Pradesh, India

Correspondence
Arjun Karmakar
Sam Higginbottom University of Agriculture Technology and Sciences SHUATS, Prayagraj, Uttar Pradesh, India

Production and characterization of PPBS from *Brevundimonas aurantiaca* KY231210 and *Enterobacter cloacae* KY231211

Arjun Karmakar, Jyotsna Kiran Peter, Dr. Ankit Singla and Anita Raisagar

Abstract
The aim of present study was production of biosurfactant from selected microorganisms i.e. *Brevundimonas aurantiaca* KY231210 and Enterobacter cloacae KY231211 and after extraction characterization of PPBS via oil displacement assay, blood haemolysis assay, emulsification index with vegetable oils in different incubation (0, 24, 48, 72 and 96h) and estimation of carbohydrate, protein and lipid content. Mustard oil and sunflower oil were used for carbon source for production. Higher biosurfactant production was obtained with mustard oil. *Brevundimonas aurantiaca* KY231210 proved as better producer of biosurfactant production than Enterobacter cloacae KY231211 and recorded 4.31g/l yield. PPBS was obtained by solvent extraction and drying process. PPBS of *Brevundimonas aurantiaca* KY231210 showed highest zone of oil displacement than PPBS of Enterobacter cloacae KY231211 in both concentration (10µl and 20µl). PPBS of both bacterial cultures showed highest hemolytic zone on blood agar plates than culture and crude. PPBS of Enterobacter cloacae KY231211 showed β heamolysis whereas PPBS of *Brevundimonas aurantiaca* KY231210 showed α haemolysis. Emulsification index was decreased after each incubation. PPBS of Enterobacter cloacae KY231211 showed highest Emulsification index with soybean oil in 0h incubation while sunflower oil gave highest Emulsification index with PPBS of *Brevundimonas aurantiaca* KY231210. Presence of carbohydrate, protein and lipid was recorded in PPBS. PPBS of Enterobacter cloacae KY231211 showed higher amount of carbohydrate while protein content was higher in PPBS of *Brevundimonas aurantiaca* KY231210.

Keywords: Biosurfactant, partially purified biosurfactant (PPBS), *Brevundimonas aurantiaca* KY231210, *Enterobacter cloacae* KY231211, haemolysis, emulsification index

Introduction
Biosurfactants are extracellular amphiphilic compounds that exhibit pronounced surface and emulsifying activities, produced by bacteria, yeast and fungi, especially when they are grown on hydrophobic substrates. Biosurfactants are surface active compounds produced by microorganisms. They reduce surface and interfacial tension by accumulating at the interface of immiscible fluids and thus increase the solubility, bioavailability and subsequent biodegradation of the hydrophobic or insoluble organic compounds (Van Hamme *et al.*, 2006) [31].
Biosurfactants are classified on the basis of diversity in their structure and their microbial origin. They contain a hydrophilic group, that contain an acid, peptide cations, or anions, mono-, di- or polysaccharides and a hydrophobic group of unsaturated or saturated hydrocarbon chains or fatty acids. Biosurfactants produced by a variety of microorganisms mainly bacteria, fungi and yeasts are diverse in chemical composition and their nature and the amount depend on the type of microbes producing a particular biosurfactant. (Lourith and Kanlayavattanakul, 2009) [18]. Major classes of biosurfactants include polysaccharide-protein complexes, glycolipids, phospholipids and fatty acids, neutral lipids, lipopeptides/lipoproteins, polymeric surfactants and particulate surfactants (Desai and Desai, 1993; Banat *et al.*, 2000; Banat, *et al.*, 2010; Franzetti *et al.*, 2010) [7, 4, 3, 11].
Biosurfactants have many potential applications related to the oil industries include enhanced oil recovery, crude oil drilling lubricants, surfactant-aided bioremediation of water-insoluble pollutants; related to the food industry as emulsifiers, foaming, wetting, solubilizers [19], anti-adhesive and antimicrobial agents (Singh and Cameotra, 2004; Rodrigues *et al.*, 2006) [29, 24]. Other developing areas of biosurfactant use are in cosmetic and soap formulations, foods, and dermal as well as transdermal drug delivery systems. Biosurfactants also have potent antimicrobial applications including antifungal, antibacterial, antimycoplasmal and antiviral activities (Rodrigues *et al.*, 2006) [24]. Therefore, medically relevant uses of biosurfactants include their role as anti-adhesive agents to pathogens, making them useful for treating many

diseases and as therapeutic and probiotic agents (Rodrigues *et al.,* 2004) [26]. Biosurfactants have been used for gene transfection, as ligands for binding immunoglobulins, as adjuvants for antigens and also as inhibitors for fibrin clot formation and activators of fibrin clot lysis (Rodrigues *et al.,* 2006) [25]. In addition, there is other interesting market for biosurfactants that includes emulsion polymerization for paints, paper coatings and industrial coatings (Layman, 1985)[16].
The name *Brevundimonas* derives from Latin adjective *brevis,* short; Latin feminine gender noun *unda,* a wave; Latin feminine gender noun *monas,* nominally meaning a unit but in effect meaning a bacterium; new Latin feminine gender noun *Brevundimonas,* bacteria with short wavelength flagella (Euzeby, 1997) [9]. *Brevundimonas aurantiaca* is a gram negative, non-fermenting rods 1 to 4 µm in length and 0.5 µm in width, aerobic bacteria. Genus *Brevundimonas* belongs to the alphaproteobacteria class and Caulobacteraceae family with a DNA G+C content 65% to 68% (Segers *et al.,* 1994) [28]. It is motile by one short wavelength flagellum and grows at 30 ºC. The *Brevundimonas* species are ubiquitous in the environment. It is mostly found in the rhizosphere region of the soil (Lee *et al.,* 2011) [17].
Enterobacter cloacae is a significant Gram- negative, facultative-anaerobic, rod shaped bacterium, bears peritrichous flagella, oxidase negative, catalase positive, frequently grown at 30ºC on nutrient agar or broth or at 35 ºC in tryptic soy broth (Dalben *et al.,* 2008) [6]. *Enterobacter cloacae* is a member of the normal gut flora of many humans and is not usually a primary pathogens (Keller *et al.,* 1998) [14]. *Enterobacter cloacae* have been used in a bioreactor based method for the biodegradation of explosives and in the biological control of plant diseases (Pudge *et al.,* 2003) [22].
Biosurfactants have unique properties such as biodegradability, biocompatibility, low toxicity, relative ease of preparation, digestibility, widespread applicability, easily availability of raw material etc. makes it useful for different purposes and it may be used as an alternative approach to chemical surfactant. The present study demonstrates production and characterization of biosurfactant from bacteria (*Enterobacter cloacae* and *Brevundimonas aurantiaca*).

Material and Methods

Place of Work

The present study was conducted under lab in the Department of Industrial Microbiology, Jacob Institute of Biotechnology and Bioengineering SHUATS, Prayagraj, Uttar Pradesh (India) during the year 2015-2016.

Procurement and Maintenance of Test organisms

Brevundimonas aurantiaca KY231210 and *Enterobacter cloacae* KY231211 were used in the present study and routinely subcultured in Nutrient agar medium at 15-20 days interval incubated at 30 °C for 24 h and stored in refrigerator at 4°C for further use.

Production of biosurfactant

Bushnell Haas broth was used as the production medium for the biosurfactants. 100 ml of the Bushnell Haas broth was prepared in 250 ml capacity Erlenmeyer flask and autoclaved at 121 °C at 15 lbs/inch2 pressure for 15 minutes. The sterilized medium was seeded with 24-48h old starter culture of *Enterobacter cloacae* KY231211 and *Brevundimonas aurantiaca* KY231210 that were prepared in Nutrient broth medium (5ml) under aseptic condition and mustard oil and sunflower oil was added as carbon source. An uninoculated flask was also used to cross check any contamination. The inoculated and uninoculated flasks were kept in a shaking incubator maintained at 35 °C for 10 days interval at 160 rpm. After 10 days the broth contained the biosurfactant that was further extracted at two sub stages *viz.* crude extraction of biosurfactants and solvent extraction of biosurfactants (partially purified biosurfactants; PPBS).

Extraction of Biosurfactant

Crude extraction of biosurfactant

The broth culture was centrifuged at 10,000 rpm for 30 minutes at 4 °C and supernatant was collected. The pellet was discarded that contained the bacterial cell fractions. The supernatant was collected as crude biosurfactant and was further purified through solvent extraction method.

Solvent extraction (Chloroform: methanol) to obtain partially purified biosurfactants (PPBS)

The supernatant was subjected to acid precipitation by adding concentrated HCl (drop wise) to achieve a final pH of 2.0 and kept at 4 °C overnight. The biosurfactant was extracted with mixed solvent system *i.e.* chloroform: methanol in 2:1 ratio. Chloroform: methanol (2:1) solvent solution was added to the acid precipitated supernatant and centrifuged at 10,000 rpm for 30 minutes to recover the biosurfactant from the supernatant as a precipitated layer between the aqueous and organic solvent phases. The pellet was recovered through careful decantation of liquid and the pellet was collected and dried using a water bath set at 40 °C to obtain the dry mass of biosurfactant and stored in a clean screw capped bottle for further use.

Characterization of solvent extracted partially purified biosurfactants (PPBS) Determination of dry weight of biosurfactants PPBS

Sterilized petriplate was taken and the weight of the plate was measured in grams. Now the sediment was poured on the plate and placed in the hot air over for drying at 100°C for 30 minutes. After drying the plates were weighed (Anandraj and Thivakaran, 2010) [1]. The dry weight of the biosurfactants was calculated using the following formula

Dry weight of biosurfactants = weight of the plate after drying-weight of the empty plate

Oil displacement assay

30ml of distilled water was taken in a Petri-plate. 1ml of Sunflower oil was added to the centre of the plates containing distilled water. Then 20µl of the PPBS was poked into the oil drop. An uninoculated oil drop in another petri-plate served as a negative control. PPBS displace the oil (increases in diameter) and spread in water (Anandraj and Thivakaran, 2010) [1].

Blood haemolysis assay by bacterial cultures, crude biosurfactant and partial purified biosurfactant (PSBS)

Hemolytic activity of culture, crude and PPBS was tested using Blood agar plate as per described by Prakash *et al.* (2016) [21]. The clear zone around the wells indicated the presence of biosurfactant producing organisms (Carrillo *et al.,* 1996) [5].

E24assay of the partially purified biosurfactant (PPBS)

Emulsification activity of the partially purified biosurfactants was performed by adding 2 ml of the Sunflower oil and

Soybean oil to 2 ml of the aqueous solution of the biosurfactant, in a screw cap tube, and vortexes at high speed for 2min. The emulsion stability was determination at 0, 24, 48, 72 and 96h of incubation. The emulsification index (E24) was calculated by dividing the measured height of emulsion layer by the mixture's total height (cm) and multiplying by 100 (Anandraj and Thivakaran, 2010) [1].

Formula: Emulsification Activity = Height of emulsion/ Total height

- E0= emulsification index at 0h
- E24= emulsification index after 24h
- E48= emulsification index after 48h
- E72= emulsification index after 72h
- E96= emulsification index after 96h

Estimation of Carbohydrate contents of partially purified biosurfactant (PPBS)

The anthrone method is the basis of a rapid and convenient method for determination of hexoses, aldopentoses and hexuronic acids either free or present in polysachharides. The blue green solution shows an absorption maximum at 620 nm. 4ml Anthrone reagent was added to one ml of a protein free carbohydrate solution and mixed rapidly. The tubes were placed in a boiling water bath for 10 minutes with covers to prevent loss of water content due to evaporation, cooled and extinction was measured at 620 nm against a reagent blank. A standard curve for glucose was made.

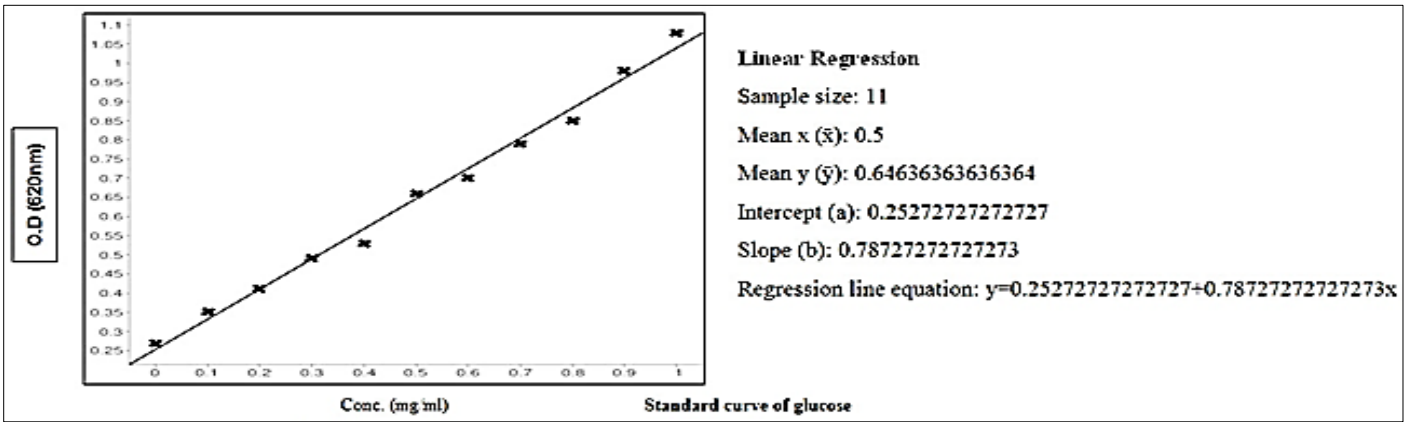


Fig 1

Estimation of lipid contents of partially purified biosurfactants (PPBS)

Olive oil was taken in different test tubes in increasing amount *i.e.* 20 µl, 40 µl, 60 µl, 80 µl and 100 µl. Just half quantity of CHCl3 was added to each test tube to give the ratio of 2:1. The tubes were kept at 600 C for 10 min to evaporate the solvent and 1 ml of water was added to the lipid standard.

Preparation of phospho-vanillin reagent: Dissolved 0.3 g vanillin in 5 ml absolute ethanol, 45ml distilled water and stirred continuously. Subsequently 200ml of phosphoric acid was added to the mixture and the resulting reagent was stored in the dark place until use.

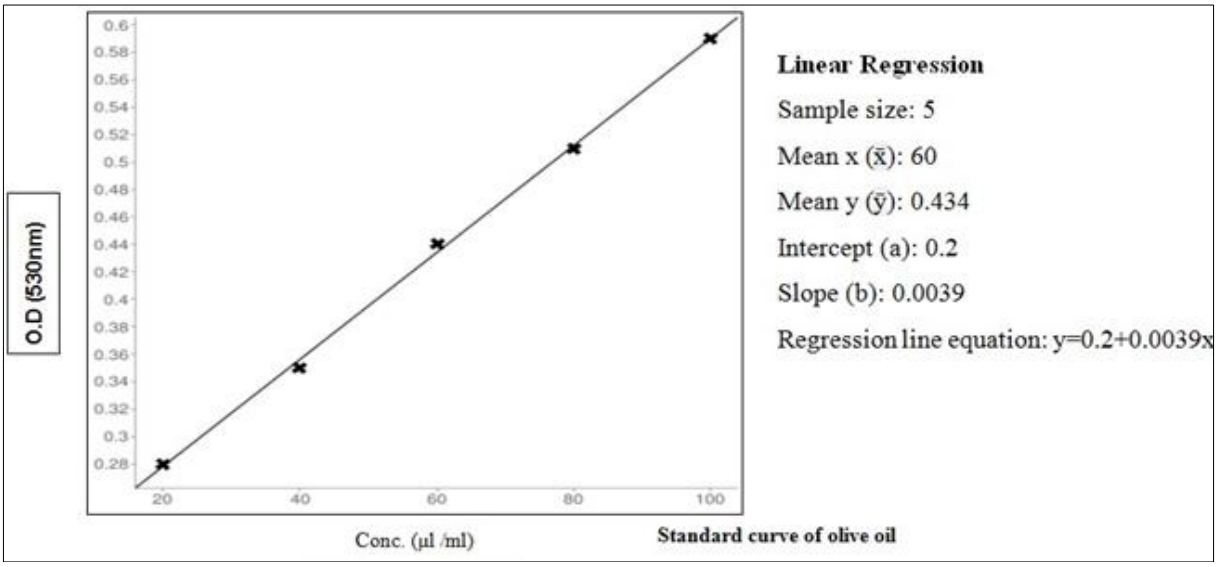


Fig 2

Estimation of protein contents of partially purified biosurfactant (PPBS)

Protein content of the sample was measured by Lowry's method. To a test tube 1 ml of 1N NaOH solution was taken and heated up to 100oC followed by addition of 1 ml of protein sample to the solution and left stand for 4-6 minutes. There after 5ml of Alkaline Copper Sulphate reagent was added and mixed and left at room temperature for 10 minutes.

After incubation 0.5 ml of Folin Ciocalteau reagent was added with immediate mixing. The mixture was further left for 30 minutes in dark at room temperature for the development of light blue color. The absorbance of the solution was read at 750nm. A standard curve of BSA was prepared where absorbance was measured at 750 nm taking 10 ml with appropriate dilution. A graph between protein concentration and absorbance was plotted. The amount of protein in the sample was calculated following the standard curve of known proteins (Lowry *et al*., 1951) [19].

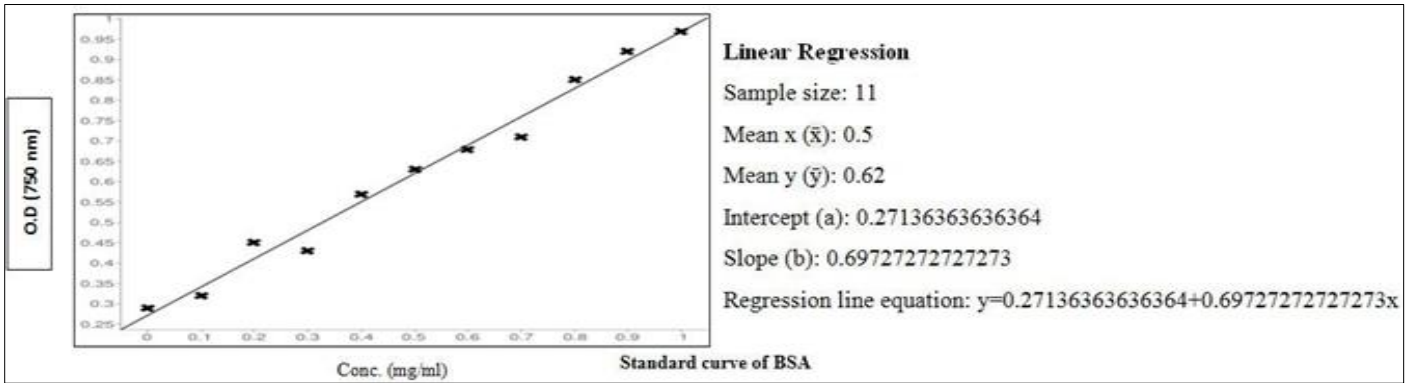


Fig 3

Formula

Y=a+bX

Where,

$$n\,x_i\,y_i - n\bar{x}\bar{y}$$

$$b = \frac{\sum_{i=1}}{n}$$

$$\sum i=1\ xi2 - nx - 2$$

$a = \bar{Y} - b\bar{X}$

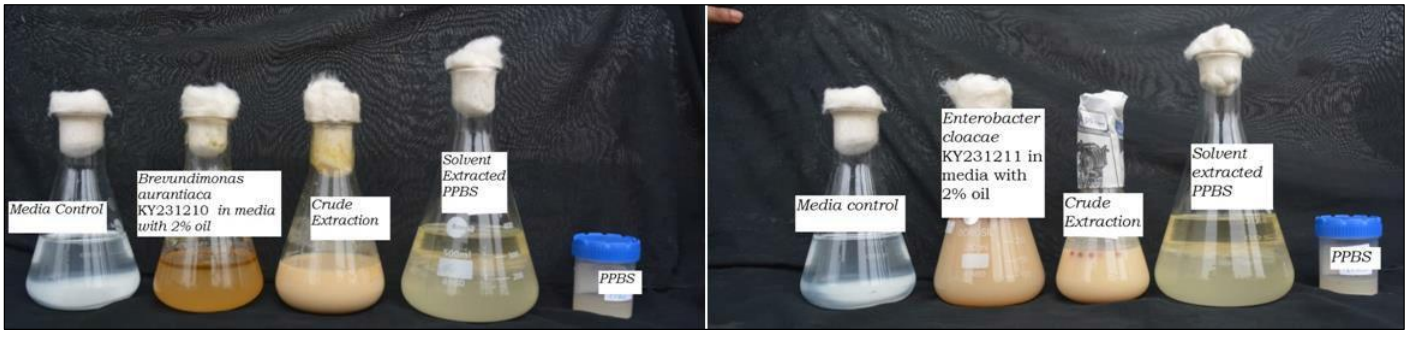
Determination of inoculums size of *Brevundimonas aurantiaca* KY231210 and *Enterobacter cloacae* KY231211

A loopful culture of *Enterobacter cloacae* KY231211 and *Brevundimonas aurantiaca* KY231210 was serially diluted up to 10-7 dilution in Ringer solution. 10-6 and 10-7 dilution was pour plated in Nutrient agar medium. The media without any dilution was used as media control. Then *Enterobacter cloacae* KY231211 plates were incubated at 28 °C for 24-48 h and *Brevundimonas aurantiaca* KY231210 plates were incubated at 30 °C for 24-48 h.

Result and Discussion

Production of Biosurfactant

Biosurfactants were produced through submerged batch mode fermentation using 2% Mustard oil and Sunflower oil supplemented to Bushnell Hass broth seeded with bacterial inoculums of *Enterobacter cloacae* KY231211 and *Brevundimonas aurantiaca* KY231210 followed by incubation 30±5 ºC for 15-20 days at 160 rpm. Yield of PPBS was measured after solvent extraction and drying process. *Brevundimonas aurantiaca* KY231210 gave highest yield than *Enterobacter cloacae* KY231211. (Table 1; Fig. 1 and 2)



(a) **(b)**

Fig 4: Production, Extraction and Partial purification of (a) *Brevundimonas aurantiaca* KY231210 and (b) *Enterobacter cloacae* KY231211

Fig 5: Yield of biosurfactants in g/l of production medium

Table 1

Biosurfactants Source	Yiel (g/1)
Brevundimonas aurcintiaca	4.
KY231210	
Enterobacter	3.
cloacae	
KY231211	

Characterization of solvent extracted partially purified biosurfactants (PPBS)

Physiochemical and biochemical characterization of solvent extraction biosurfactant obtained from *Enterobacter cloacae* KY231211 and *Brevundimonas aurantiaca* KY231210 was done using with estimation of dry weight, oil displacement assay, blood haemolysis assay emulsification index of vegetable oil (Soybean and Sunflower) as E0, E24, E48, E72 and E96 h and estimation of carbohydrate, protein, lipids contents.

Determination of dry weight of partially purified biosurfactants (PPBS)

The dry weight of partial purified biosurfactants (PPBS) produced by *Enterobacter cloacae* KY231211 and *Brevundimonas aurantiaca* KY231210 was recorded as 1.02g and 1.50g respectively. *Brevundimonas aurantiaca* KY231210 was able to produced higher quantity of PPBS using 2% Mustard oil in Bushnell Hass broth medium in 15-20 days of incubation (Table 2).

Table 2: Dry weight of partially purified biosurfactants (PPBS)

Cultures name	Weight of the	Weight of	Dry weight of
Enterobacter	73.99	72.83	1.02
Brevundimonas	72.06	72.52	1.50

Oil displacement assay

Oil displacement assay is a screening technique to identify biosurfactant activity by microorganism or by biosurfactants. Displacement of oil was measured for two different concentrations *viz.*10 µl/drop and 20µl/drop oil. In each case displacement of oil was more by 20 µl PPBS/drop oil than 10 µl PPBS/drop. PPBS of *Brevundimonas aurantiaca* KY231210 showed highest zone of oil displacement in comparison with PPBS of *Enterobacter cloacae* KY231211 in all cases. (Table 3)

Table 3: Oil displacement assay by PPBS

Name of bacteria	Zone of oil displacement (mm)			
	Sunflower oil		Soybean oil	
	10 µl	20 µl	10 µl	20 µl
Enterobacter cloacae KY231211	12.5	23	17	27.5
Brevundimonas aurantiaca KY231210	22	31	25	36.5

Blood Haemolysis assay

Blood haemolysis pattern of broth culture, crude biosurfactant and PPBS of *Enterobacter cloacae* KY231211 and *Brevundimonas aurantiaca* KY231210 were examined on Sheep Blood agar medium. Broth culture (24 h old) and PPBS of *Enterobacter cloacae* KY231211 revealed β- haemolysis pattern while its crude biosurfactant showed α-haemolysis pattern. While in case of broth culture of *Brevundimonas aurantiaca* KY231210 had β-haemolysis pattern and its crude and PPBS had α-haemolysis on Blood agar plates. PPBS of both bacteria revealed highest hemolytic zone signifying blood cell lysis than culture and crude biosurfactant. (Table 4; Fig: 3)

Table 4

	Enterobacter cloacae KY231211		Erevundintonas gyrancippa KY231210	
Test emergence	Zone of haemolysis (mm)	Type of haemolysis	Zone of Haemolysi s (mm)	Type of haemolysis
Culture	5	13	5.	13
Crude	8.	a	8.	a
PPBS	11	13	10.	a

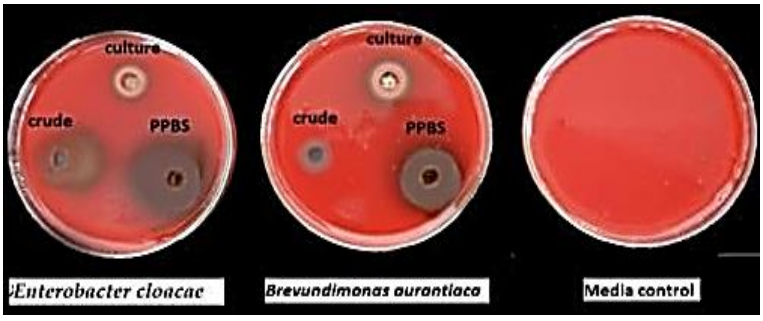


Fig 6: Blood Haemolysis assay by broth culture and their crude and (PPBS)

Emulsification index of PPBS of *Enterobacter cloacae* KY231211

PPBS produced from *Enterobacter cloacae* KY231211 performed emulsification index (%) for different vegetable oil *viz.* Sunflower oil and Soybean oil. Emulsification of PPBS was determined by adding 2ml of a vegetable oil *(*Sunflower oil and Soybean oil) to the same amount of PPBS of *Enterobacter cloacae* KY231211, mixing with vortex for 2 min and leaving at 0h, 24h, 48h, 72h and 96h for incubation. PPBS from *Enterobacter cloacae* KY231211 in both Soybean oil and sunflower oil E0 or 0h gave highest emulsification index (%) while E24, E48, E72 and E96 decreased with incubation but in comparison to Sunflower oil at 0h, Soybean oil gave highest emulsion at 0h incubation respectively (Table 5; Fig. 4)

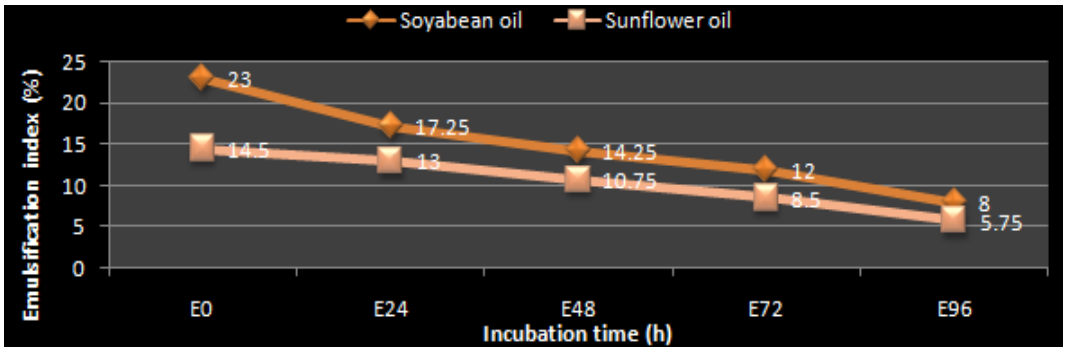


Fig 8: Emulsification index of PPBS of *Enterobacter cloacae* KY231211

Emulsification index of PPBS of *Brevundimonas aurantiaca* KY231210

PPBS produced from *Brevundimonas aurantiaca* KY231210 performed emulsification index (%) for different vegetable oil *viz.* Sunflower oil and Soybean oil. Emulsification of PPBS was determined by adding 2ml of a vegetable oil *(*Sunflower oil and Soybean oil) to the same amount of PPBS of *Brevundimonas aurantiaca* KY231210, mixing with vortex for 2 min and leaving at 0h, 24h, 48h, 72h and 96h for incubation. PPBS from *Brevundimonas aurantiaca* KY231210 in both Sunflower oil and soybean oil E0 or 0h gave highest emulsification index (%) while E24, E48, E72 and E96 decreased after incubation, but Sunflower oil gave highest emulsion at 0h incubation in comparison to Soybean oil at 0h. (Table 5; Fig. 5)

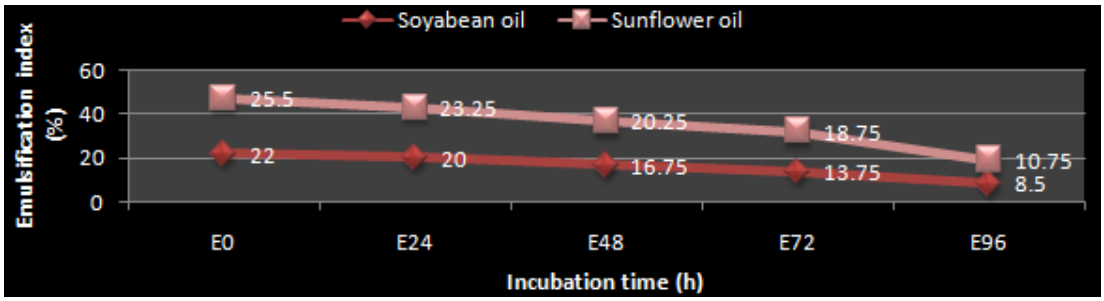


Fig 9: Emulsification index of PPBS of *Brevundimonas aurantiaca* KY231210

Table 5: Emulsification index of partially purified biosurfactants of *Enterobacter cloacae* KY231211 with Sunflower oil and Soybean oil

	***Enterobacter cloacae* KY231211**					***Brevundimonas aurantiaca* KY231210**				
Oil	**E0**	**E24**	**E48**	**E72**	**E96**	**E0**	**E24**	**E48**	**E72**	**E96**
Soybean	23	17.25	14.35	12	8	22	20	16.75	13.75	8.5
Sunflower	14.6	13	10.75	8.5	5.75	25.5	23.25	20.25	18.75	10.75

Estimation of Carbohydrate, Protein and Lipid contents of PPBS

Carbohydrate, protein and lipid contents of partially purified biosurfactants were estimated quantitatively. 1mg/ml concentration was used for carbohydrate and protein whereas 80µl/ml concentration was used for lipid estimation. The carbohydrate content was found highest in PPBS of *Enterobacter cloacae* KY231211 which were followed by protein and lipid contents respectively, while in PPBS of *Brevundimonas aurantiaca* KY231210, protein content was found highest followed by carbohydrate and lipid content respectively. (Table 6)

Table 6: Estimation of Carbohydrate, Protein and Lipid contents of PPBS

Culture name	Carbohydrate (mg/ml)	Protein (mg/ml)	Lipid (µl/ml)
Enterobacter cloacae KY231211	1.08	0.89	0.6
Brevundimonas aurantiaca KY231210	0.81	0.92	0.56

Determination of inoculums size

Enterobacter cloacae KY231211 and *Brevundimonas aurantiaca* KY231210 was serially diluted up to 10-7 dilution for observation of the inoculums size. *Enterobacter cloacae* KY231211 plates were incubated at 28°C for 24-48 h and *Brevundimonas aurantiaca* KY231210 plates were incubated at 30°C for 24-48 h. After 24h incubation *Enterobacter cloacae* KY231211 plate was observed and counted 52 colonies followed by 48h incubation 76 colonies. In case of *Brevundimonas aurantiaca* KY231210 plate was observed and counted 28 colonies followed by 48h incubation 49 colonies respectively. (Table 7)

Table 7: Determination of inoculums size

Culture	**106 Cfu/ml**	
	24h	**48h**
Enterobacter cloacae KY231211	52	76
Brevundimonas aurantiaca KY231210	28	49

Discussion

Biosurfactant was produced by *Enterobacter cloacae* KY231211 and *Brevundimonas aurantiaca* KY231210 and after the production extraction was done by solvent extraction method by using organic solvent solvent chloroform and methanol (2:1v/v) as earlier reported by Santhini and

Parthasarathi (2014) [27] is followed with centrifugation and sedimentation. This is the most common biosurfactant recovery methods (Kosaric, 1992; Desai and Banat, 1997) [15, 8]. The extraction method of biosurfactant by centrifugation and sedimentation was similar as reported by earlier workers (Urum *et al.*, 2004) [30]. Peter *et al.* (2014) [20] also reported the solvent extraction method for partially purification of biosurfactant and obtained highest yield of biosurfactant in *Serratia spp.* Musturd and sunflower oil were used for production as carbon source. Biosurfactant production was recorded higher with mustard oil in compared with sunflower oil. For the production of biosurfactant use of various carbon sources has been also reported earlier such as glycerol, glucose and hydrocarbons by Jayanti and Joshi (1992) [13] and waste oils like sunflower oil by Fiebig *et al*., 1997 [10]; Haba *et al.*, 2000 [12]; Raza *et al*., 2007. These types of substrates are low priced, high in purity and they can possibly enhance the production of biosurfactants. (Van Hamme *et al.*, 2006) [31]. Among different edible oils, the sun flower oil was the best carbon sources in production of biosurfactant according to Auhim *et al.* (2013) [2]. Biosurfactant causes lysis of erythrocytes. There is an association between hemolytic activity and surfactant production (Carrillo *et al.*, 1996) [5]. Positive blood haemolysis was recorded with PPBS of both test bacteria. Bacterial biosurfactant may include antigenic lipids or lipids acts as endotoxin which could destroy cells or cell membrane integrity. A biosurfactant renders lipophilic nature with hydrophobic and hydrophilic attributes and distort the membrane (living cell) integrity thus are able to disrupt RBC. Emulsification index was recorded in different incubation period with sunflower and soybean oil in which soybean oil gave highest emulsion at 0h incubation than sunflower oil. Similar finding was also observed in case of Consortium that showed highest emulsification in soybean oil by Peter *et al.* (2014) [20]. Presence of carbohydrates, proteins and lipids in biosurfactants was recorded in present study. *Brevundimonas aurantiaca* KY231210 was better biosurfactant producer as compared to *Enterobacter cloacae* KY231211 because *Brevundimonas aurantiaca* KY231210 gave highest yield than *Enterobacter cloacae* KY231211.

Conclusion

Brevundimonas aurantiaca KY231210 showed better biosurfactant production than *Enterobacter cloacae* KY231211. Produced biosurfactant also contains carbohydrates, proteins and lipids.

References

1. Anandaraj B, Thivakaran P. Isolation and production of biosurfactant producing organism from oil spilled soil. Journal of Biosciences and Technology. 2010; 1(3):120-126.
2. Auhim HS, Mohamed AI. Effect of different environmental and nutritional factors on biosurfactant production from *Azotobacter chrococcum*. International Journal of Advances in Pharmacy, Biology and Chemistry. 2013; 2(3):477-481.
3. Banat IM, Franzetti A, Gandolfi I, Bestetti G, Martinotti MG. Microbial biosurfactants production, applications and future potential. Appl Microbiol Biotechnol. 2010; 87:427-444.
4. Banat IM, Makkar RS, Cameotra SS. Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol. 2000; 53:495-508.
5. Carrillo P, Mardaraz C, Pitta-Alvarez S, Giulietti A. Isolation and selection of biosurfactant producing bacteria. World Journal of Microbiology and Biotechnology. 1996; 12:82-84.
6. Dalben M, Varkulja G, Basso M, Krebs VL, Gibelli MA, Van der Heijden I *et al*. Investigation of an outbreak of *Enterobacter cloacae* in a neonatal unit and review of the literature. The Journal of Hospital Infection. 2008; 70(1):7-14.
7. Desai J, Desai A. Biosurfactants: production, properties and applications. N., Kosaric (Ed.), Marcel Dekker, New York, 1993, 65-97.
8. Desai JD, Banat IM. Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology. 1997; 61:47-64.
9. Euzeby JP. List of bacterial names with standing in nomenclature: a folder available on the internet. Int J Syst Bacteriol. 1997; 47:590-2.
10. Fiebig RD, Schulze J, Chung C, Lee ST. Biodegradation of polychlorinated biphenyls (PCBs) in the presence of a bio-emulsifier produced on sunflower oil. Earth and Environmental Science. 1997; 8:67-75.
11. Franzetti A, Tamburini E, Banat IM. Application of biological surface active compounds in remediation technologies. Adv Exp Med Biol. 2010; 672:121-134.
12. Haba E, Espuny MJ, Busquets M, Manresa A. Screening and production of rhamnolipids by *Pseudomonas aeruginosa* 47T2 NCIB 40044 from waste frying oils. Journal of Applied Microbiology. 2000; 88:379-387.
13. Jayanti KL, Joshi SR. Surfactant production by *Pseudomonas stutzeri*. Journal of Microbiology and Biotechnology. 1992; 7:18-21.
14. Keller R, Pedroso MZ, Ritchmann R, Silva RM. Occurrence of virulence associated properties in *Enterobacter cloacae*. Infection and Immunity. 1998; 66(2):645-9.
15. Kosaric N. Biosurfactants in industry. Pure & Appl. Chem. 1992; 64:1731-1737.
16. Layman PL. Industrial surfactants set for strong growth. Chem. Eng. News. 1985; 23:23-48.
17. Lee M, Huang YT, Liao CH, Chuang TY, Lin CK, Lee SW *et al*. Bacteremia caused by *Brevundimonas* species at a tertiary care hospital in Taiwan. *European* Journal of Clinical Microbiology & Infectious Diseases. 2011; 30:1185-1191.
18. Lourith N, Kanlayavattanakul M. Natural surfactants used in cosmetics: glycolipids. Int J Cosmet. 2009; 29:225-261.
19. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin phenol reagent. Journal Biological Chemistry. 1951; 193:256-275.
20. Peter JK, Rao AK, Kumari R. Consortium based Biosurfactant development for degradation and emulsification of oils and Petroleum Hydrocarbons. International Journal of Engineering and Computer Science. 2014; 3(6):6476-6490.
21. Prakash G, Sankhwar S, Jhanwar A, Singh K. Bilateral renal angiomyolipoma presenting as tuberous sclerosis syndrome. BMJ Case Rep, 2016.
22. Pudge IB, Daugulis AJ, Dubois C. The use of *Enterobacter cloacae* ATCC 43560 in the development of a two-phase partitioning bioreactor for the destruction of hexahydro-1, 3, 5-trinitro-1, 3, 5-s-triazine (RDX). Journal of Biotechnology. 2003; 100(1):65-75.

23. Raza ZA, Rehman A, Khan MS, Khalid ZM. Improved production of biosurfactant by a *Pseudomonas aeruginosa* mutant using vegetable oil refinery wastes. Earth and Environment Science. 2006; 18:15-121.
24. Rodrigues LR, Banat IM, Teixeira JA, Oliveira R. Biosurfactants: potential applications in medicine. J Antimicrob. Chemother. 2006; 57:609-618.
25. Rodrigues LR, Teixeira JA, Van de Mei HC, Oliveira R. Isolation and partial characterization of a biosurfactant produced by *Streptococcus thermophillus* A. *Colloids and Surfaces. B: Biointerfaces*. 2006; 53:105-112.
26. Rodrigues LR, Van der Mei HC, Teixeira JA, Oliveira R. Biosurfactant from *Lactococcus lactis* 53 inhibits microbial adhesion on silicone rubber. Appl. Microbiol. Biotechnol. 2004; 66:306-311.
27. Santhini K, Parthasarathi R. Isolation and Screening of Biosurfactant Producing Microorganisms from Hydrocarbon Contaminated Soils from Automobile Workshop. International Journal of Pharmaceutical and Biological Archives. 2014; 5(2):158-167.
28. Segers P, Vancanneyt M, Pot B. Classification of *Pseudomonas diminuta* Leifson and Huge 1954 and *Pseudomonas vesicularis* Busin, Doll and Freytag 1953 in *Brevundimonas* gen. nov. as *Brevundimonas diminuta* combo. nov. and *Brevundimonas vesicularis* combo. nov. Respectively. Int J Syst Bacteriol. 1994; 44:499-10.
29. Singh P, Cameotra S. Potential applications of microbial surfactants in biomedical sciences. Trends Biotechnol. 2004; 22(3):142-146.
30. Urum K, Pekdemir T. Evaluation of biosurfactants for crude oil contaminated soil washing. Chemosphere. 2004; 57:1139-1150.
31. Van Hamme JD, Singh A, Ward OP. Physiological aspects Part 1 in a series of papers devoted to surfactants in microbiology and biotechnology. Biotechnology Advances. 2006; 24:604-620.