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RESEARCH ARTICLE

STUDY ON THE PRODUCTION AND ANTIBACTERIAL ACTIVITIES OF BIOSURFACTANT PRODUCED FROM SOME BACTERIAL SPECIES.

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Abstract

Biosurfactant are produced by some micro organisms. Kerosene was used as substrate to enhance the production of biosurfactant by *Staphylococcus aureus*, *Bacillus* sp., *Pseudomonas aeruginosa*, *Proteus* sp. and *Corynebacterium*sp. *Staphylococcus aureus* produced the highest 0.5 g, *Bacillus* sp., *Pseudomonas aeruginosa* and *Proteus* sp. 0.2 g, *Corynebacterium* sp. the least 0.1 g. The biosurfactant demonstrated antibacterial activity against the test bacteria (*Staphylococcus* sp. and *Pseudomonas aeruginosa*). The biosurfactant produced by *Pseudomonas aeruginosa* gave the highest zone of inhibition against *Staphylococcus aureus*, *Bacillus* sp., *Corynebacterium*sp., (25 mm). The biosurfactant produced by Staphylococcus aureus gave the highest zone of inhibition against *Pseudomonas aeruginosa* (39 mm), *Corynebacterium*sp., and *Proteus* sp. (30 mm), *Bacillus* sp. the least zone (25 mm). The production of biosurfactantand antibacterial efficacy can thus be promising for use in medical, therapeutics, pharmaceuticals, cosmetcs, food and beverages for treatment and control of diseases caused by micro organisms.

Introduction:-

Biosurfactants are amphiphilic compounds that contain a hydrophobic portion and a hydrophilic group. They are produced by yeast or bacteria growing on various substrates e.g., sugars, oils, alkanes and wastes (Jinfeng*et al.*, 2005). Biosurfactants are grouped as glycolipids (e.g., rhamnolipids, trehalolipids, sophorolipids), lipopeptides (e.g., surfactin, iturin, lichenysin), phospholipids, fatty acids and neutral lipids (e.g., Corynomycolic acid, Spiculisporic acid, Phosphati-dylethanolamine), polymeric and particulate compounds (e.g., Emulsan, Alasan, Biodispersan, Liposan, Mannoprotein) (Calvo*et al.*, 2009). Most of these compounds are either anionic or neutral. Only a few are cationic such as those containing amine groups. The hydrophobic part of the molecules is based on long-chain fatty acids, hydroxyl fatty acids or α-alkyl- α-hydroxy fatty acids. The hydrophilic portion can be a carbohydrate, amino acid, cyclic peptide, phosphate, carboxylic acid or alcohol. The various types are produced by different organisms, rhamnolipids (*Pseudomonas* spp.), trehalolipids (*Rhodococcus erythropolis, Arthrobacter* sp., *Nocardia* sp., *Corynebacterium* spp.), sophorolipids (*Candida* spp., *Torulopsis* spp.); surfactin, iturin, lichenysin (*Bacillus* spp.); Corynomycolic acid (*Corynebacterium lepus*); Spiculisporic acid (*Penicillium spiculisporum*); Phosphati-

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dylethanolamine (*Acinetobacter* spp., *Rhodococcus erythropolis*); Emulsan (*Acinetobacter calcoaceticus* RAG-1); Alasan (*Acinetobacter radioresistens* KA-53); Biodispersan (*Acinetobacter calcoaceticus*A2); Liposan (*Candida lipolytica*); Mannoprotein (*Saccharomyces cerevisiae*) (Pacwa-Plociniczak*et al*., 2011). *Pseudomonas aeruginosa* can produce rhamnolipids from substrates including C_{11} and C_{12} alkanes, succinates, pyruvate, citrate, fructose, glycerol, olive oil, glucose and mannitol (Sifour *et al*., 2007). The composition and yields depend on the fermentor design, pH, nutrient composition, substrate and temperature used (Joseph and Joseph, 2009). Biosurfactants can be potentially as effective with some distinct advantages over the highly used synthetic/chemical surfactants. Biosurfactants have high specificity, biodegradability, biocompatibility and less toxicity e.g., glycolipids from *Rhodococcccus* species 413A were 50% less toxic than Tween 80 in naphthalene solubilization tests (Christofi and Ivshina, 2002). Among the genus *Bacillus* spp., *Bacillus subtilis* produces a broad spectrum of bioactive lipopeptides which have a great potential for biotechnological and biopharmaceutical applications. The characteristic structure of lipopeptides is a fatty acid combined with an amino-acid moiety. Several lipopeptides have potent antibiotic activity and have been the subject of several studies on the discovery of new antibiotics. The surfactin, produced by *B. subtilis*, is the most powerful of biosurfactant known to date. These compounds have many pharmacological activities: antibacterial, antifungal, antiviral, and antimycoplasma properties; inhibition of the fibrin clot formation and hemolysis; formation of ion channels in lipid bilayer membranes (Gudina*et al*., 2010); antitumour activity against Ehrlich's ascites carcinoma cells; and inhibition of the cyclic adenosine 3,5-monophosphate phosphodiesterase (Fernandes *et al*., 2007). Lipopeptides have a broad spectrum of action, including antimicrobial activity against microorganisms with multidrug-resistant profiles (Gudina *et al*., 2010). Some biosurfactants are able, even in low concentrations, to destabilize the microorganism's membranes, killing them or disabling their growth (Calvo *et al*., 2009; Carrilo *et al*., 2003).

The microbially produced surfactants are alternatives to chemical surfactants whose effects have been reported variously by authorities. The effects of surfactants on the human body are divided into effects on the skin and in the body. The main ingredients of modern life detergents are surfactants, long-term use cause skin irritation effect and lead to some degree of damage. After the surfactants enter into the human body, they damage the enzyme activity and thus disrupt the body's normal physiological function. Surfactants have some toxicity and may accumulate in the human body, so it is difficult to degrade (Venhuis and Mehrva, 2004). In general, nonionic surfactants are not electrically charged, not combined with protein. They have minimal irritation to the skin. The toxicity of cationic surfactants is the biggest, and the toxicity of anionic surfactants is between that of non-ionic surfactants and cationic surfactants. Prolonged exposure of skin to surfactants can cause chafing because surfactants (e.g. soap) disrupt the liquid coating that protects skin and other cells. There have been the reports that SDBS (sodium dodecyl benzene sulfonate) is absorbed through the skin, they damage the liver and cause narrowing and other chronic symptoms, as well as teratogenic and carcinogenic (Toll *et al*., 2000). It is based on these effects that this study was carried out to determine the biosurfactant producing ability of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Bacillus* sp., *Corynebacterium*sp., *Proteus* sp. and ascertain their antimicrobial prorperties for applications in medical, therapeutics, pharmaceuticals, cosmetcs, food and beverages for treatment and control of diseases caused by micro organisms.

Materials and methods:-

The materials used included peptone water, stock cultures of *Staphylococcus aureus*, *Bacillus* sp., *Corynebacterium* sp., *Pseudomonas aeruginosa*, and *Proteus* sp., 0.2 M H_2S0_4, chloroform, methanol, centrifuge, glass Petri-dish, Pasteur pipette, measuring scale, pH meter.

Production of biosurfactant:-

Biosurfactant was produced using stock cultures of *Staphylococcus aureus*, *Bacillus* sp., *Corynebacterium* sp, *Pseudomonas aeruginosa*, and *Proteus* sp. A loopful of each of the isolates was placed in 5 ml of sterile peptone water in a test tube and 0.1 ml of hydrocarbon (kerosene) was added to enhance the growth of the bacterial species. The suspensions were then allowed to stand for 48 h. After the 48 h incubation at room temperature, the biosurfactant produced by each microorganism was extracted as described by Anandaraj and Thivakaran, (2010); Okore *et al.*, (2017a; 2017b).

Extraction of biosurfactant:-

The biosurfactant produced by each of the isolates was extracted by centrifuging at 5000 rpm for 20 min to obtain a cell-free supernatant of each of the test organisms. Then l ml supernatant of each of the suspension was taken and placed in a sterile glass Petri-dish and acidified with 1 ml of 2 M H_2SO_4 to obtain a pH of 2.0. Thereafter, the

biosurfactant produced was extracted using a mixture of chloroform and methanol in the ratio of 1:2 (1 ml of chloroform: 0.5 ml of methanol). The mixture of biosurfactant and the extracting solvents (chloroform and methanol) was allowed for 24 h to evaporate at room temperature. Then the biomass of biosurfactant produced was determined by subtracting the total weight of the Petri-dish with the biosurfactant from the initial weight before the experiment (Okore *et al.,* 2017a; 2017b).

Antibacterial activity of the biosurfactant produced:-

The disc technique as described by Osadebe and Ukwueze, (2004) was adopted for this study to evaluate the antibacterial activity of the biosurfactants. About 0.2 ml aliquot of the biosurfactants were dropped on sterile filter paper disc of about 6 mm in diameter and allowed to get absorbed before they were placed into nutrient agar plates inoculated with each of the test organisms *Staphylococcus aureus* and *Pseudomoas aeruginosa* and appropriately labelled, discs impregnated with water and ethanol were used as control in each case. The plates were then incubated at 37^{o}C for 24 h and the zones of inhibition obtained by each of the biosurfactant were measured.

Results:-

The results of the production of biosurfactant by the stock culture of *Staphylococcus aureus*, *Bacillus* sp., *Corynebacterium* sp., *Pseudomonas aeruginosa*, and *Proteus* sp. are presented in Table 1 and result for the antimicrobial activities of the biosurfactant produced on Table 2 and Table 3.

Table 1:-Weight of biosurfactant (grams) produced by the bacterial isolates.

Bacteria	Weight of Petri dish before extraction (g)	Weight of Petri dish after extraction (g)	Weight of biosurfactant (g)
Staphylococococcus aureus	40.2	40.7	0.5
Bacillus sp.	38.3	38.5	0.2
Corynebacterium sp.	50.7	50.8	0.1
Pseudomonas sp.	51.5	51.7	0.2
Proteus sp.	50.0	50.2	0.2

Table 2:-Zones of inhibition of the biosurfactant produced by *Staphylococcus aureus* on test organisms.

Test organisms	N2	N4	N22	N7
Zones of inhibition (mm) of biosurfactant by *Staphylococcus aureus*	25	30	30	39
Zones of inhibition (mm) using ethanol (control)	-	-	-	-
Zones of inhibition (mm) using water (control)	-	-	-	-

Table 3:- Zones of inhibition of the biosurfactant produced by *Pseudomonas* sp.on test organisms

Test organisms	N2	N4	N22	N1
Zones of inhibition (mm) of biosurfactant by ***Pseudomonas* sp.**	25	25	18	25
Zones of inhibition (mm) using ethanol (control)	-	-	-	-
Zones of inhibition (mm) using water (control)	-	-	-	-

Key: N1 = *Staphylococcus aureus*
N2 = *Bacillus* sp., N4 = *Corynebacterium* sp.
N7 = *Pseudomonas aeruginosa*
N22 =*Proteus* sp.
(-) = No zone of inhibition

Discussion:-

The results of the mass of biosurfactant produced by the different bacterial species as presented in Table 1 indicated that *Staphylococcus arueus* produced the highest quantity of biosurfactant (0.5 g) while *Corynebacterium* sp. the

least (0.1 g); *Bacillus* sp., *Pseudomonas* sp. and *Proteus* sp. produced 0.2 g of biosurfactant each. Anandaraj and Thivakaran (2010) equally obtained a dry weight of 0.122 g of biosurfactant from *Pseudomonas* sp. This study has confirmed that some bacterial spp. produce biosurfactant when grown on kerosene as have been documented by many authorities. Santa Anna *et al.,* (2002) investigated the production of biosurfactant from *Pseudomonas aeruginosa* PA1 isolated from oil wells grown on N-Hexadecane. Several studies (Santa *et al*., 2001; Priya and Usharani, 2009; Liu *et al*., 2011; Dhail and Jasuja, 2012; Okore *et al.,* 2013; Tambekar and Gadaki, 2013; Hassanshahia, 2014) also identified biosurfactant production by *Pseudomonas* spp. *Bacillus* spp. have equally been reported to produce biosurfactant by these studies (Ahimonu *et al*., 2001; Joshi *et al.,* 2008; Okore *et al*., 2013; 2017a; 2017b; Chakarabarti, 2015). *Corynebacterium* spp. have been identified as by works of Muthusamy *et al*., (2008); Franzetti *et al*., (2010); Sai-Ard *et al*., (2013), to produce biosurfactant.

The *Staphylococcus aureus and Proteus* sp. grown on kerosene as the carbon source yielded 0.5 g and 0.2 g of biosurfactant. These two organisms have scarcely been reported in literature as biosurfactant producers, as emphases have been on the use of non pathogenic strains for biosurfactant production with specificity to the area of application.

The above Table 2 and 3, show the variation in the zones of inhibition by each of the biosurfactants produced from the bacterial species. The biosurfactant produced from *Pseudomonas aeruginosa* had the highest zones of inhibition against *Staphylococcus aureus, Bacillus* sp., *Corynebacterium* sp. (25 mm) and least on *Proteus* sp. (25 mm). The biosurfactant produced from *Staphylococcus aureus* gave the highest zone of inhibition against *Pseudomonas aeruginosa* (39 mm), followed by *Corynebacterium* sp. (30 mm) and *Proteus* sp. (30 mm), the *Bacillus* sp. gave the least (25 mm). The control (water and ethanol) did not show any zones of inhibition against the test organisms. Many researchers have demonstrated the antimicrobial activity of different *Pseudomonas* spp. Govindammal and Parthasarathi (2013), studied the antimicrobial property of *Pseudomonas flurescens* MFSO3 on *Bacillus subtilis* and *Staphylococcus aureus*. The recorded zones of inhibition ranging from 15±0.5 mm to 21±0.14 mm for *Bacillus subtilis*; 17±0.14 mm to 23±0.26 mm for *Staphylococcus aureus*; 14±0.16 mm to 18±0.23 mm for Methicillin-resistant *Staphylococcus aureus* (MRSA). Khare and Arora (2011), worked with fluorescent *Pseudomonas* against *Macrophomnina phaseolina* ARIFCC257 a plant pathogenic fungus and recorded zones of inhibitions of 42 mm and 36 mm. Abalos *et al.* (2002), investigated the inhibitory activity of biosurfactant from *Pseudomonas aeruginosa* ATIO. They recorded zones of inhibitions on *Escherichia coli, Micrococcus luteus, Alcaligenes faecalis, Serratia marcescens, Mycobacterium phlei and Staphylococcus epidermidis.* The antimicrobial activity of surfactin was tested against several microbes. All tested bacteria, except for *Bacillus. subtilis*, showed susceptibility to surfactin. *P. aeruginosa* was the most sensitive Gram-negative bacteria, while *E. coli*, *Salmonella choterasius* and *Serratia marcescens* were inhibited in a lower level. Also, the lipopeptide affected the growth of Gram-positive bacteria, especially *Micrococcus luteus* and *Bacillus cereus* (Rodrigues *et al.*, 2006a; 2006b; 2006c). These biosurfactants can be used in the production of antibiotics that are specific to the target bacteria since the biosurfactants produced will likely be specific to certain genes or genomic composition of the target bacteria.

Conclusion and recommendation:-

Biosurfactant can be produced from bacterial broth cultures supplemented with hydrocarbons (e.g. kerosene) and extracted by acidification followed by liquid liquid extraction with chloroform-methanol mixture in the ratio of 2:1. The biosurfactants also have pronounced antibacterial activities against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The *Staphylococcus aureus* is well implicated in causing various infections including wound infections and other superficial infections. This thus validates the reported medical importance of biosurfactant. It is therefore recommended that biosurfactant be massively produced as well as purified and used in the production of pharmaceutical products due to their proven antimicrobial activities.

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