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ANALYSIS OF *STAPHYLOCOCCUS* SP. FOR SECONDARY METABOLITE PRODUCTION

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ABSTRACT

Several samples were collected from a variety of sources such as surface water, deep-sea water, sediments, sea animals and plants from Arabian Sea. The samples were analyzed both qualitatively & quantitatively for the presence of bacteria. Their resistance markers were studied and two Staphylococcus sps. were screened for the production of secondary metabolites. Analytic techniques and electron microscopic observations have revealed the presence of important compounds such as cadmium binding compound, oleic acid, palmatic acid, SMHS1 and 3. These have potential for commercial exploitation.

Keywords: Marine bacteria, secondary metabolites, cadmium binding compound.

INTRODUCTION

The ocean is a vast body of salt water that covers about three-quarters of the earth's surface. The Arabian Sea lies in the northwestern section of the Indian Ocean. Deep water reaches close to the bordering lands except in the northeast, off Pakistan and India. Pakistan spans a remarkable number of the world's broad ecological regions. These ranges from the coastal areas to the spectacular mountain top. Pakistan has a coast line of 527nautical miles (nm). This coastal area of Pakistan offers a rich repository of marine organisms while microbial flora in these marine environment forms an integral part of this unique ecosystem (Ahmed and Yasmeen, 1988 and Jamil et al., 1999). The marine microorganisms, including bacteria, fungi, and microalgea, have received increasing attention over the past ten years (Davidson, 1995). Jensen and Fenical in 1994 have introduced marine bacteria as a new biomedical source, as well as commenting on both the chemical and ecological perspective of pursuing marine bacteria as a source of new secondary metabolites. Many microbiologists around the world have attempted to isolate bacteria that produce novel products such as antibiotics, enzymes, biologically active substances, polysaccharides, emulsifiers etc. (Horikoshi, 1995).Common sources of metabolite producing bacteria are sea water, sponges, vertebrates, invertebrates, and sea sediments. Examples of such metabolites include Okadanthin, a new C50 carotenoid pigment is produced by a *Pseudomonas sp*. (Miki *et al*., 1994). Patel and Hou (1993) have recovered a surfactant, from the sea water isolates of *Acinetobacter*. This surfactant is used as cleaning agent for oil tankers and oil storage tanks. It is also capable of forming complex with uranium metal, so may be useful for the recovery of uranium from waste material.

It has been reported that marine bacteria have interesting genetic markers, such as markers for biodegradation of complex compounds and resistance to heavy metals and antibiotics etc.. Several plasmids which have been isolated from marine bacteria i.e. TOL-palsmid and NAH-Plasmids, are responsible for the production of enzymes which degrade complex compounds like toluene and naphthalene respectively (Saunders, 1977). The genetics of production of few secondary metabolites has been studied such as tetracenomycin (Tcm) C. It is potent inhibitor of the growth of other *streptomycetes* and has moderate cytotoxicity towards some tumor cells. TcmC production is determined by a cluster of 12 genes contained with in an operon 13kb region of the *S. glancescens* (Hutchinson, 1992). There is a possibility of creating novel hybrid secondary metabolites by transferring genes from one bacterium to another.

The present study was conducted in three phases. In the first phase, after the isolation and purification of bacterial strains, they were characterized for biochemical and genetic characters. In second phase, selected bacterial strains were analyzed for the production of secondary metabolites under different cultural media by using different chromatographic and spectroscopic techniques. Finally the cellular morphology and metabolites was studied by using scanning electron microscopy.

MATERIALS AND METHODS

Phase-1: Characterization of bacterial strains

Sampling sites

Samples were collected at four different stations along Karachi coast. These stations were Sandspit (around 25.20^{o}N 66.8^{o}E), Clifton (around 24.71^{o}N 67.17^{o}E), Lyari outfall (around 47.21^{o}N 67.10^{o}E), Manora (around 24.87^{o}N 66.81^{o}E), Capemonze (around 24.5^{o}N, 66.4^{o}E), Gaddani (around 25.5^{o}N, 66.7^{o}E) and Dockyard.

Sample collection

Samples for free bacteria were collected from seven different sites of Arabian sea which were Manora, Dockyard, Capmounze, Sandspit, Clifton, Gadani and lyari outfall. From these sites surface sea water was collected in sterilized 100ml glass containers. Attached bacteria were collected from Fish, Crab, Bivalve, shells, stones and sediments from Lyari outfall (Figure-1). Lyari outfall sediments were collected from 2m depth at the time of sample collection tides was low.

The pH of the samples was determined by using Whatman pH paper strip at the time of sampling.

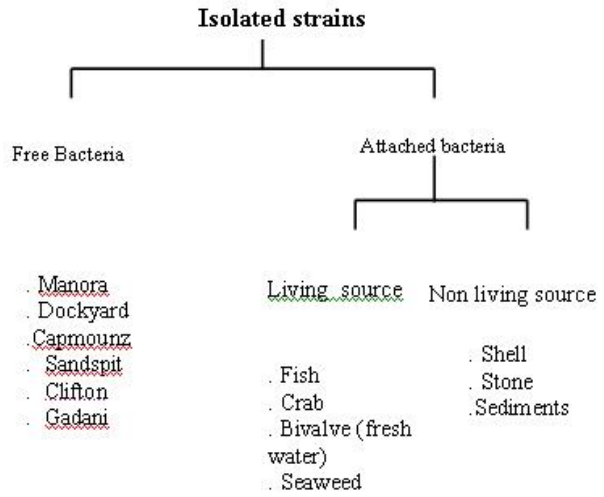

Figure-1. Classification of Isolated Strains on the basis of their origin.

Isolation, purification and identification

The seawater samples were serially diluted with 10mM $MgSO_4$ up to 10^{-10} dilution and spread on agar plates of BHI (Brain heart infusion, 2216E, ASW (Artificial sea water) and nutrient agar (Merck) plates containing 0.5M NaCl for viable counts and purification. These plates were incubated at 30^{o}C for 24hrs.

Attached bacterial strains were isolated in two ways i.e. after cleaning the samples, they were washed with sterile nutrient broth containing 0.5M NaCl to isolate attached bacteria from outside surface of living organisms and to isolate attached bacteria from inside the living organisms (crab, fish and bivalve) they were dissected and inoculated to sterile nutrient broth containing 0.5M NaCl. The resulting broths were incubated at 30^{o}C for 48hrs at 100rpm in shaker. After 48hrs broths were serially diluted with nutrient broth containing 0.5M NaCl up to 10^{-10} dilution and spread on nutrient agar (Merck) plates containing 0.5M NaCl, 2216E and BHI. These plates were incubated at 30^{o}C for 48hrs.

Morphologically different colonies were picked and restreaked on respective plates for several days for purification. Bacterial strains were identified on the basis of colonial, cellular morphology, Gram reaction, biochemical tests and confirmed by using API kit. Isolated bacterial strains were maintained on agar stabs containing 0.5M NaCl, at 4^{o}C.

Estimation of maximum tolerable concentrations of NaCl (MTC)

Salt tolerance of bacterial strains was checked against varying concentrations of NaCl in nutrient agar (Merck). The conc. of NaCl checked were 0.0 M (control), 0.5M, 1.7M and 3.4M. The cultures were streaked on nutrient agar plates containing varying concentrations of salt. The plates were incubated at 30^{o}C for 48hrs.

Study of genetic markers

Genetic markers such as resistance to heavy metals and antibiotic resistance of the isolates were studied. Resistance to five heavy metals i.e. $CuSO_4$, $CdCl_2$, $CoCl_2$, $NiCl_2$ and CrO_3 were determined on artificial sea water (23.5g NaCl, 5g $MgCl_2$, 1.1g $CaCl_2$, 0.66g KCl, 26mg H_3BO_3, 24mg $SrCl_2$, in 1Lit. of distilled water, supplemented with 2g trypone and 2g glucose (after autoclave), pH=7.0) (Suwanoh, 1991) supplemented with various concentrations (0.5, 1, 2.3mM) of heavy metals. The plates were streaked and incubated at 30^{o}C and growth was observed after 24 to 48 hrs. Stock solutions of metal salts were prepared at concentration of 1M in sterilized distilled water and added after the sterilization of media.

Resistance to antibiotics like streptomycin, ampicillin, kanamycin and tetracycline were tested on 2216E (0.1g yeast extract, 0.5g peptone, 0.01g $FePO_4$ in 100ml seawater, 1.5%agar, pH=7) plates containing 0.5M NaCl supplemented with various concentrations (ug/ml) of antibiotics. Antibiotic stock solutions were prepared as described by Maniatis et. al. (1982). The plates were incubated at 30^{o}C and growth was observed after 24 to 48 hrs.

Phase-2: Analysis for the production of secondary metabolite

Selection of the organisms

1. *Staphylococcus saprophyticus* (isolated from sea shell), a G+ve cocci (Figure-7A) showing good growth was selected to be analyzed for the presence of metabolites.
2. *Staphylococcus aureus* (isolated from fish), a G+ve cocci (Figure-7B) showing good growth was selected to be analyzed for the presence of metabolites.

1. *Staphylococcus saprophyticus*

Fermentation

Fermentation of broth was performed in two stages. In stage I, *Staphylococcus saprophyticus* were grown in 500ml conical flask containing 250ml of BHI

(Merck) for 24hrs. at 100rpm in room shaker, these broths were used as pre-cultures. Stage II culture was prepared in 20lit. BHI broth by inoculating stage I cultures, so as to get 0.05 OD (Optical density at 600nm). Broths were incubated in room shaker at 100 rpm, at 30°C for 96hrs.

In the same way inoculation was done to 20 lit. of 2216E broths.

Extraction procedure

Extraction of fermented and non fermented broths (BHI and 2216E) were done by hexane, chloroform and ethyl acetate separately (Figure-2). In the same way extraction of non fermented broths was done. Extracts were concentrated to dry under vacuum.

Purification and identification of the compounds

Hexane, Ethyl acetate and chloroform extracts of fermented and non-fermented broths of BHI and 2216E were run on TLC plates.

Gas chromatography

Purified samples from ethyl extract of 2216E media were analyzed by GC-MS. Samples (containing 25nmol nor-leucine (Sigma- Aldrich Co. Ltd, Poole, UK) added as an internal standard) were re-dissolved in pyridine (20ul), derivatised using BSTFA (Butylsilyl-N-methyltrifluroacetamide) and gently mixed and heated to 70°C for 30 minutes. One micro liter of each sample was injected into a VG organic GC-MS MD800 and separated on a BPX5 capillary column and analysed directly by the mass spectrometer. The samples were scanned and compared with the known standards (Sigma).

2. *Staphylococcus aureus*

Staphylococcus aureus was grown in Artificialsea water (ASW) for 10 days. Supernatant of fermented broths were analyzed by pulsed polarography (METROHAM) for metal binding compounds in the supernatant. Reaction mixture contained 20ml $NaNO_3$ (2M), 1000uM cadmium nitrate and different volumes of supernatant.

Phase-3: Scanning electron microscopy (SEM)

48hrs. old cultures of both isolates were centrifuged. Cell pallets were fixed in 2% (vol/vol water) triple-distilled glutaraldehyde in ASW pH 7.0 for 2hrs at room temperature, and then washed four times with ASW (15min/wash). Dehydration was through a 25-100% (by vol.) ascending series of ethanol in distilled water, samples being left for an hour at each stage. Three transfers were made in absolute ethanol. The samples were coated for 5min using a Polaron E5100 series II 'cool' sputter coater fitted with a Au/Pd target. The samples were examined at a voltage of 15 to 25kV using a JEOL JSM-35 SEM.

RESULTS AND DICUSSION

The importance of marine bacteria has been recently realized with a view of exploiting them for the production of novel compounds. Since they remain relatively unexplored as a source of novel metabolites. This study was carried out to screen the bacteria from Arabian Sea for metabolite production. Samples (from seven coastal areas of Karachi) from the sea shore and deep sea were analysed quantitatively and qualitatively for the presence of bacteria (Table-1).

Table-1. Quantitative analysis of marine bacteria on different media.

Samples	2216E	ASW	BHI	3% NaCl NA
Fish	$4356x10^{10}$	$108x10^{10}$	$2500x10^{10}$	$3002x10^{10}$
Crab	$3997x10^{10}$	$515x10^{10}$	$2570x10^{10}$	$2560x10^{10}$
Unio	$3560x10^{10}$	$444x10^{10}$	$560x10^{10}$	$2x10^{10}$
Shells	$560x10^{10}$	$99x10^{10}$	$4060x10^{10}$	-
Stones	$1702x10^{10}$	$268x10^{10}$	$2050x10^{10}$	$3x10^{10}$
Clifton Seawater	$670x10^{10}$	$303x10^{10}$	$1160x10^{10}$	$300x10^{10}$
Manora Seawater	$9x10^{10}$	-	-	$16x10^{10}$
Sandspit	$2560x10^{10}$	$2560x10^{10}$	$2560x10^{10}$	$314x10^{10}$
Lyari outfall	$2560x10^{10}$	$2560x10^{10}$	$3112x10^{10}$	$360x10^{10}$

These bacteria were classified into free and attached bacteria according to their origin (Figure-1). Those bacteria attached to any surface (living or nonliving) referred as attached bacteria while those, which are free in water, are referred to free living bacteria. It was reported that greatest bacterial population was found when phytoplanktons were in abundant. So in natural aquatic environments, a proportion of the bacteria was attached to surface. Both gram positive and gram-negative bacteria are found in marine environment (Wood, 1975).

A total of 193 bacterial strains were isolated and purified using different media. Their morphological characters and Gram's reaction was studied; 121 were found to be gram negative (88 attached and 33 free) and 72 were gram positive (17 attached and 55 free). These results correspond to Zobell and Upham (1949) who suggested that 80% of the marine bacteria are Gram negative. The isolated bacterial strains were preserved at -20°C for long time storage.

The bacterial strains showed better growth on nutrent agar (NA) medium containing increased concentrations of NaCl (0.5M) because a large number of the bacterial flora of the ocean have a specific requirement for sodium ion for growth (Ahmed and Yasmeen, 1988). When the tolerance of sodium chloride, which is the property of marine bacteria (Aamir et. al., 1996) was checked most of the strains showed tolerance to 1.7M NaCl.

Antibiotic resistance against streptomycin, tetracycline, ampicillin and kanamycin were checked in 2216E agar. Most of the strains showed resistance to ampicillin (Table-2). Resistance to penicillins often results from the release of extracellular penicillinases in mixed cultures with ampicillin-sensitive strains. Due to release of extra cellular pencillinases in mixed cultures, allowed ampicillin-sensitive strains to grow in the presence of ampicillin and develop resistance to ampicillin (Ogawara, 1981). Mostly the isolates were sensitive to tetracycline however resistance was observed by few isolates (Table-2). Multiple metal resistances were observed in most of the isolated bacterial strains (Table-2).

Table-2. Identification and characterization of 36 bacterial strains.

Strains	**Origion**	**Classification Attached/Free**	**Gram reaction**	**Antibiotic resistance markers**	**Heavy metal resistance**	**MTC of NaCl**
Enterobacter aerogenes	Clifton	Crab (Attached)	-ve	Am, Tc	Co, Cu, Cr	3.4
Klebsiella oxytoca	Manora	Sea water (Free)	-ve	Am, Tc	Co, Cu, Cr	3.4
Bacillus subtillis	Dockyard	Sea water (Free)	+ve	Am,	Co, Cr	1.7
E-coli	Clifton	Shell (Attached)	-ve	Am, Sm, Tc, Km	Co, Cu,Cr	3.4
Serratia liquefacies	Dockyard	Fish (Attached)	-ve	Tc	Co, Cu,Cr	1.7
E-coli1	Lyari outfall	Sediments(Attached)	-ve	Am, Tc	CoCu,Cr	1.7
Edwardsiella hoshiae	Lyari outfall	Sediments(Attached)	-ve	Am, Sm, Tc	Co,Cu, Cr	1.7
Aeromona salmonicida	Lyari outfall	Sediments(Attached)	-ve	Am, Tc	Co	1.7
E-coli	Clifton	Bivalve (Attached)	-ve	Am, Tc	Cu,Cr	1.7
E-coli	Clifton	Bivalve (Attached)	-ve	Sm, Tc	Co,Cu,Cr	3.4
E-coli	Clifton	Bivalve (Attached)	-ve	Am, Tc	Co, Cu,Cr	1.7
E-coli	Clifton	Crab(Attached)	+ve	Am, Tc,Km	Co, Cu, Cr	3.4
Klebsiella ozaenae	Clifton	Crab (Attached)	-ve	Am, Tc	Co, Cu,Cr	1.7
Enterobacter cloacae	Lyari outfall	Sediments(Attached)	-ve	Tc	Co, Cu	1.7
Bacillus cereus	Clifton	Shell (Attached)	+ve	Am, Sm	Co, Cr	1.7
Lactobacillus acidophilus	Clifton	Shell (Attached)	+ve	Am, Tc	Co, Cu	3.4
Staphylococcus saprophyticus	Clifton	Shell (Attached)	+ve	Am, Km	Co,Cr	1.7
Micrococcus luteus	Clifton	Stone(Attached)	+ve	Am, Sm, Tc, Km	Co,Cr	3.4
Bacillus subtilis	Clifton	Stone (Atached)	+ve	Tc, Km	Co,Cu, Cr	1.7
Micrococcus luteus	Dockyard	Fish mouth (Attached)	+ve	Am, Sm, Tc, Km	Co, Cu, Cr	1.7
Bacillus subtilis	Sandspit	Red sea weed (Atached)	+ve	Am, Sm, Tc, Km	Co, CuCr	1.7
Bacillus subtilis	Sandspit	Red sea weed (Atached)	+ve	Am, Sm, Km	Co, Cu	1.7
Aotinobacillus lignieresii	Capemonz	Sea water (Free)	+ve	Am, Sm, Km	Cu,Cr	1.7
Stomatococcus mucilaginosa	Clifton	Sea water (Free)	+ve	Am, Sm	Co, Cu	1.7
Ps. Pseudomallei	Sandspit	Sea water (Free)	-ve	Am, Sm, Tc	Co, Cu	0.85
Haemophilus arphrophilus	Gaddani	Sea water (Free)	-ve	Sm, Tc	Co, Cu	1.7
Pseudomonas cepacia	Dockyard	Sea water (Free)	-ve	Am, Sm,	Co, Cu, Cd	1.7
Staphylococcus aureus	Dockyard	Fish intestine (Attached)	-ve	Am, Sm	Cu	1.7
Bacillus polymxa	Dockyard	Sea water (Free)	+ve	Am, Sm	Co, Cr	1.7
Bacillus polymxa	Dockyard	Sea water (Free)	-ve	Am, Sm	Co, Cd	1.7
Pseudomonas cepacia	Clifton	Sea water (Free)	-ve	Am, Km	Co,Cr	1.7
Serratia	Gadani	Fish (Attahed)	-ve	Am, Km	Cu,Cr	1.7
Pseudomonas	Gadani	Prawn (Attahed)	-ve	Am, Km	Co,Cu	1.7
Stenotrophomonas maltophilia	Gadani	Sea water(Free)	-ve	Am, Sm, Km	Co, Cu	1.7
Psedomonas	Gadani	Prawn (Attahed)	-ve	Sm, Km	Co,Cu, Cr	1.7
Pseudomonas aeoginosa	Lyari outfall	Sediments(Attached)	-ve	Am, Sm, Km	Co, Cu,Cr	1.7

The high incidences of tolerance to metal salt indicated the level of heavy metal pollution in the coastal areas of Karachi. This is perhaps due to the direct discharge of untreated industrial and domestic wastewater from Karachi city to coastal regions.

It is generally believed that bacteria attached to surface in aquatic environments are more active than free- living bacteria (Marshall, 1976). Differences in the activity of attached and free-living bacteria may be due to different conditions at the solid/liquid interface, as compared with bulk liquid. For example, charge on the substratum can affect the concentration of ions or charged molecules at the solid surface, thereby influencing pH (Hattori and Hattori, 1963, McLaren and Skujins, 1963) and substrate concentration (Hack and McFeters, 1982). Nutrients, exoenzyme metabolites or inhibitors (Estermann and McLaren,1959) may also be concentrated at solid/ liquid interface by entrapment in exopolymers (Geesey, 1982) or adsorption. More over, adsorption of large molecules may result in conformational changes, whereby potentially usable molecules become more accessible (Stotzky, 1972) or resistant (Ellison and Zisman, 1954) to microbial degradation. There should thus be differences in the specific composition of attached and free living populations, which should affect their competition for nutrition, mutualism, and antagonism. Therefore, the comparative activities of attached and free-living bacteria depend upon the physicochemical properties of the substratum and substrates and upon the characteristics and intentions of the organisms present (Bright and Fletchers, 1983).

Two strains were selected from the isolates which were *Staphylococcus saprophyticus* (isolated from sea shell) and *Staphylococcus aureus* (isolated from fish).

One of the isolates identified as *Staphylococcus saprophyticus*, a G+ve cocci showed good growth was facultative anerobic, catalase positive, Oxidase and coagulase negative, MTC for antibiotic was150ug/ml for Am, Km, MTC for $NiCl_2$, $CoCl_2$, CrO_3 and $ZnCl_2$ was 2mM. When *Staphylococcus saprophyticus* were observed under scanning electron microscope there were no extra cellular compounds observed on the surface of cells while cells were arranged in clusters (Figure-2).

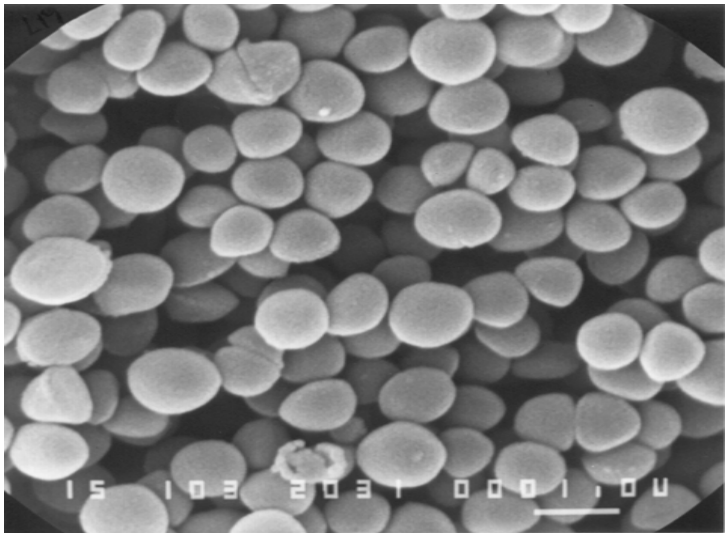

Figure-2. SEM Micrograph of Staphylococcus saprophyticus.

20 liters of the *Staphylococcus saprophyticus* culture prepared in BHI was extracted with hexane, ethyl acetate and chloroform.

The hexane extract after condensation was run on T.L.C. It showed interesting spots. This fraction when subjected to column chromatography gave the compound SMHS1 at 8% chloroform: Hexane (Figure-3).

This compound was UV active and gave brownish purple spot after spraying with ceric sulphate on TLC plates at 25 % chloroform hexane with RF 0.6. This compound was submitted for EIMS. EIMS m/Z (rel int.); 368.7 [M+] (11), 245.4 (2), 117.3 (100), 90.2 (32), 69.6 (13), 57.3 (17). HREIMS, 368.34174, (368.340261calculated for $C_{22}H_{44}N_2O_2$. The mass spectra of the compound do not match with any of the reported data.

Extracts of *(Staphylococcus saprophyticus* supernatant in 2216E) of hexane, ethyl acetate and chloroform when subjected to TLC plates as compared to control (non fermented broths), the chloroform extracts of fermented broth showed interesting spots as compared to control extract of chloroform (non-fermented broth).

From chloroform extract SMH3 was purified on T.L.C (10% Methnol:Chloroform, Rf 0.6, UV active Ceric sulfate active). The compound SMHS3 was submitted for EIMS. EMIS m/z (relint); 168.2 [M+] (36), 128.1(47), 97.2 (29), 70.2(100), 37(24). HREIMS, 168.088530 (168.089872 calculated for $C_8H_{12}N_2O_2$). The mass spectra of the compound do not match with any of the reported data. These results confirmed that production of metabolite was effected by media i.e. the compound which was purified from BHI media was more complex than the compound from 2216E because BHI was highly complex and enrich media.

Oleochemicals and Allied Products include Fatty acids, Glycerin, Alcohols, Metallic Soaps, and

Fatty Nitriles and their Derivatives. These Oleochemicals feedstock are converted into a wide range of chemical products for use in lubricants, soaps and detergents, cosmetics, pharmaceuticals, food additives, leather, paints and coatings, printing inks, rubber, plastics, metal-working and many other industries. Fatty acids were extracted from ethyl acetate extract of *Staphylococcus saprophyticus.*

Ethyl acetate extract (of fermented 2216E broth) were subjected to column chromatography, when eluted with 20% ethylacetate:hexane, Fraction 1(F1) and Fraction 2 (F2) were collected (Figure-3). Fraction 1, (Njc6) and Fraction 2 (Njc13) were purified and identified by Gas chromatography. Njc3 was identified as Oleic acid ($C_{17}H_{33}$.COOH, FW 282.47) while Njc13 was identified as Palmitic acid (C_{16} $H_{32}O_2$, FW 256.4) (Figure-4).

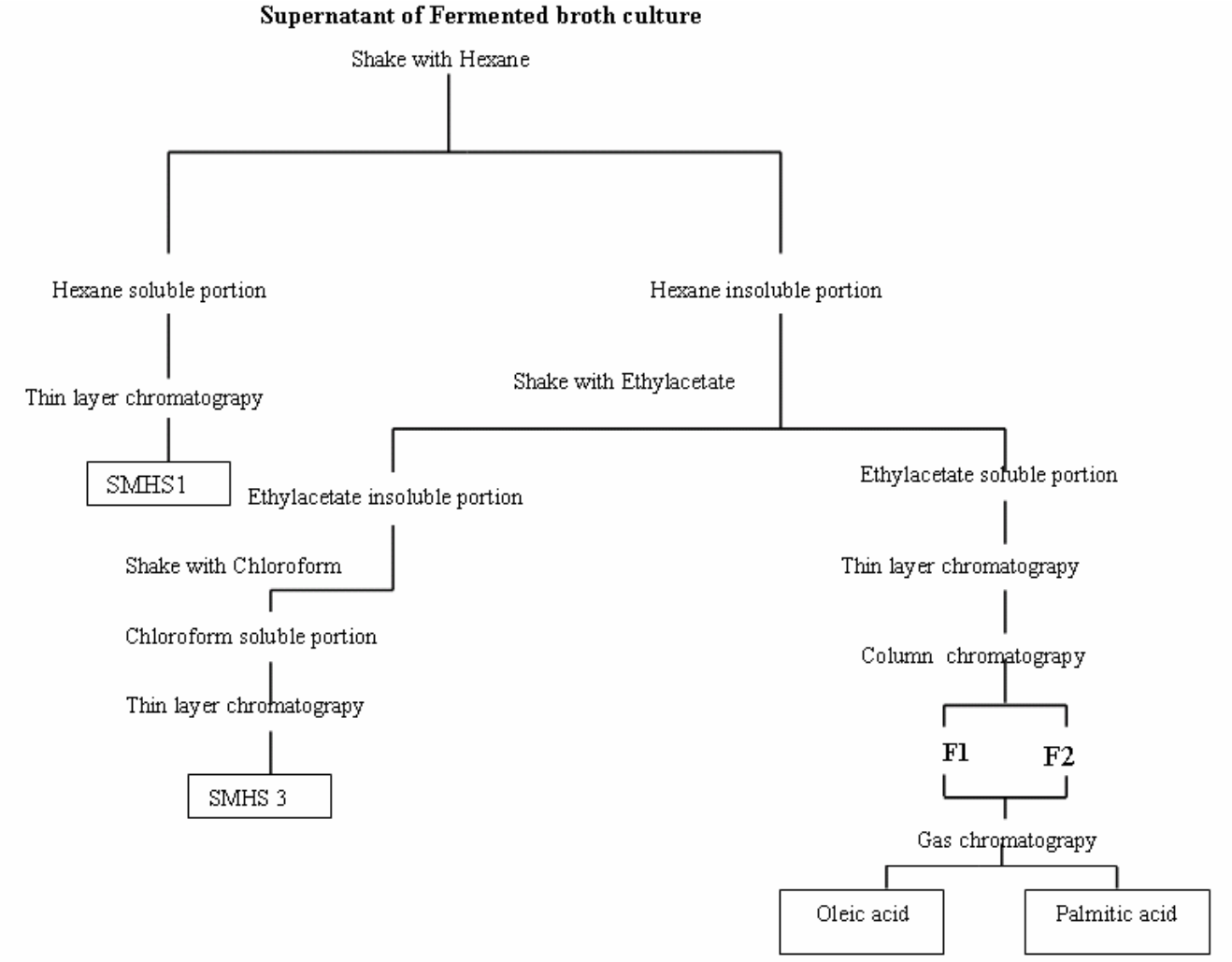

Figure-3. Flow chart for extraction.

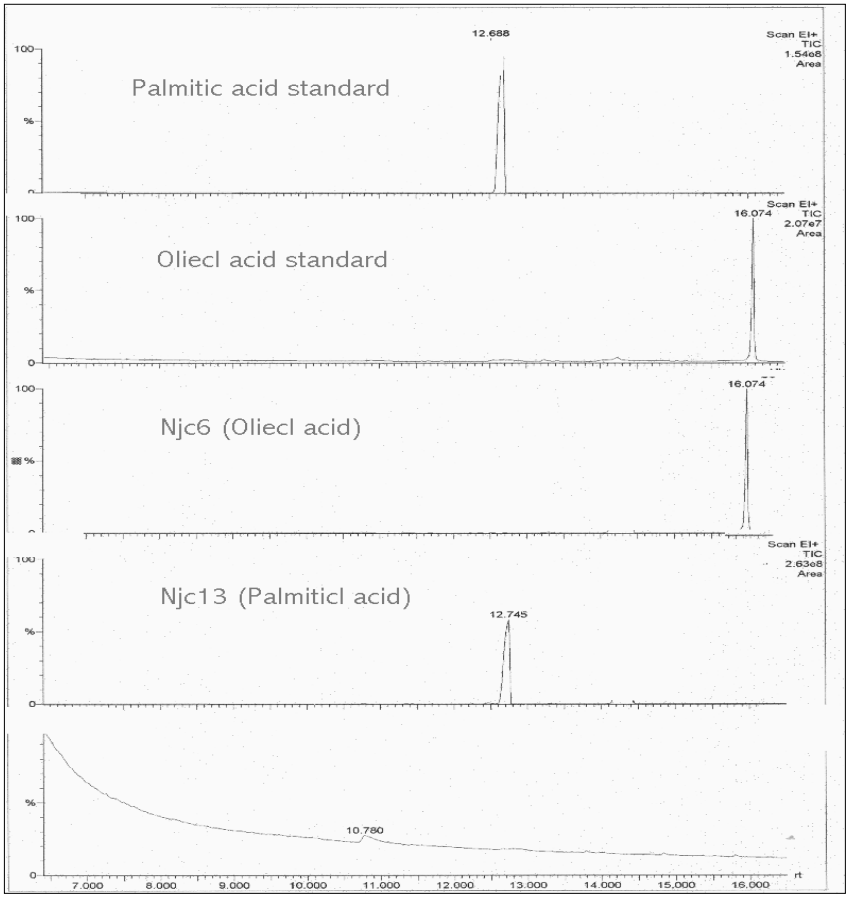

Figure-4. Profile of Gas Chromatography.

The second selected strain was *Staphylococcus aureus*. SEM analysis showed the presence of extra cellular compounds which is visible in form of aggregates on the surface of cells (Figure-5).

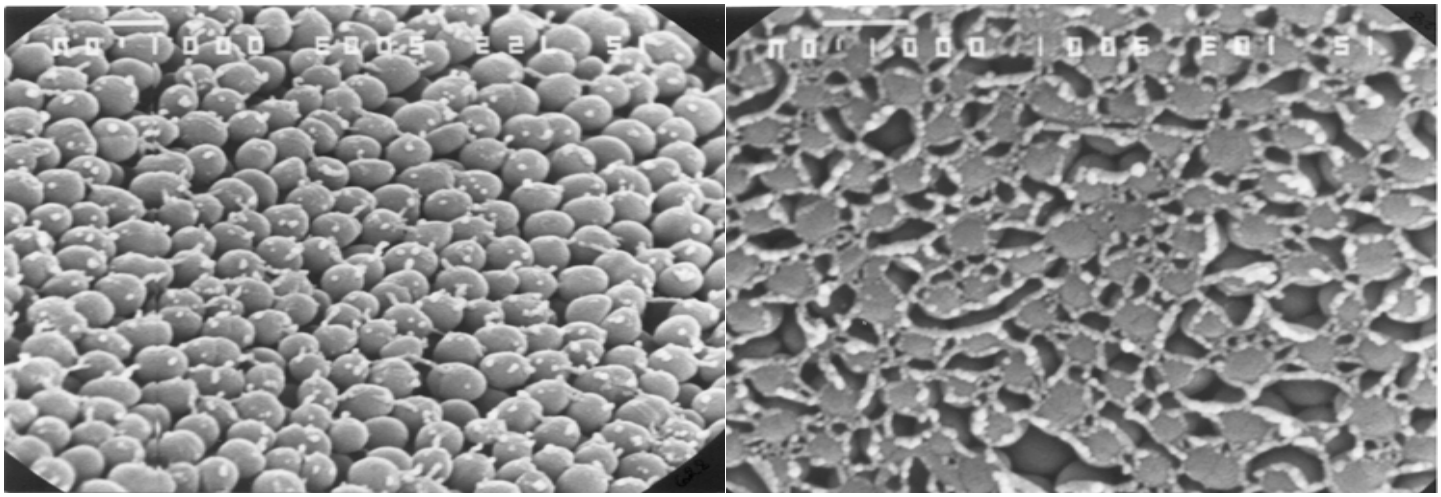

Figure-5. SEM Micrographs of *Staphylococcus aureus*.

It ferments glucose, lactose, sucrose, maltose and mannitol. This strain was analysed for secondary metabolites by pulse polarography. Polarography and stripping voltammetry are analytical techniques, within a group of methods known as voltammetry (current-voltage measurements at a desired electrode) which can be used to assess metal complexation by microbial metabolites. Supernatant of fermented broths of *Staphylococcus aureus* were analysed at different time periods. The supernatant of 10 days broth had a Cadmium binding compound in it (Figure-6). Successive additions of supernatant result in a decreased peak height of current while no shift in the evolution potential, indicating complex formation between cadmium and compound in the supernatant. Complexation of metal ions with different species like citrate can result in the formation of highly mobile species and therefore allowed transport and activity of toxic metals at a distance from their source.

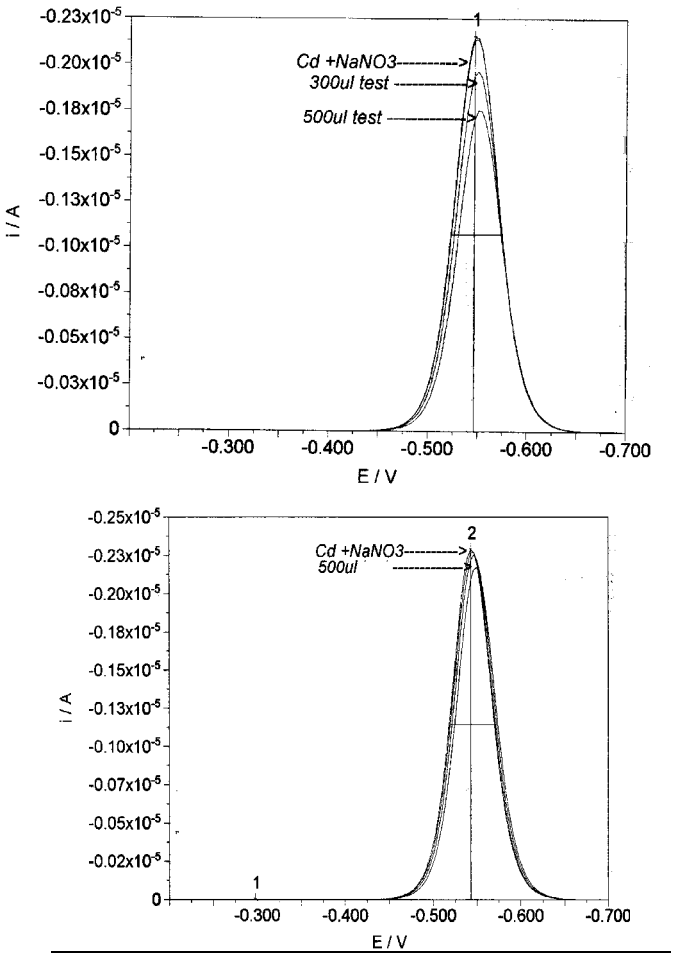

Figure-6. Polarogram's of supernatant of *Staphylococcus aureus*.

1. Complexation of 1000uM Cd^{+2} shown by polarogram obtained stepwise addition of 100ul of supernatant, obtained after 10 days growth at 30°C. The decrease in peak height in the evolution potential is indicative of complex formation.

2. Complexation of 1000uM Cd^{+2} shown by polarogram obtain stepwise addition of 100ul of control (non-fermented media). There is no decrease in peak height of evolution.

CONCLUSION

Bacterial strains of Arabian Sea have potential for the production of secondary metabolite under different nutrition conditions. It is possible to exploit bacteria for the production of metabolites of commercial importance.

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