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PERSEVERANCE OF MARINE ACTINOMYCETES ISOLATED FROM GULF MANNAR EAST COSTAL REGION AND ITS ANTIMICROBIAL ACTIVITY

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ABSTRACT

The objective of present work is to explore marine Actinomycetes and its antibacterial effect against Multidrug resistant pathogens. Sample of marine sediments collected from Gulf Mannar East costal region, Kayalpatnam located at Tuticorin district of Tamil nadu, India. A Total of 19 Actinomycetes strains isolated from marine sediments. Actinomycetes are characterized by both macroscopic and microcopy studies. Analysis of morphological, physiological and cultural characterization suggested that *Actinomycetes* isolates were belongs to the genus of *Streptomyces sp, Micromonospora sp, Micropolyspora sp, Strptoverticilium sp* and *Intrasporangium sp*. Among these isolates *Stereptomyces sp* were exhibited dominant level followed by *Micromonospora sp*. The frequency of isolated marine Actinomycetes were found to be 53% *Sterptomycetes sp*, 32% *Micromonospora sp followed by 5% of Micropolyspora sp, Strptoverticilium sp* and *Intrasporangium sp.* Among the 19 tested Actinomycetes, two *Sterptomycetes sp* designated as KPMS 5 and KPMS 14 showed potent antimicrobial activity against Multidrug resistant *E.coli, Klebsiella pneumoniae, Proteus mirabilis* and *Staphylococus aureus.* The result of this investigation revealed that the marine sediments can be useful for the isolation of novel species of Actinomycetes producing potent source of novel bioactive compounds. Further investigations are needed in order to determine the active metabolites of these isolates.

KEYWORDS: Actinomycetes, Compounds, Sediment and Metabolites.

INTRODUCTION

Microbial appearance play a significant role for biologically related Industries as it offers unlimited idea to execute biochemical pathway for the production of biologically potent active compounds and other useful molecule. Among microbial groups, Actinomycetes are a group of intermediates character which belongs to the bacteria and fungi that are found in the environment and are widely distributed in their population [1]. Actinomycetes are aerobic, spore forming, free living, Gram positive bacteria which are characterized by aerial and substrate mycelial growth and belong to the order Actinomycetales [2]. Based on mycelial studies either be aerial or may spread on the substrate on which the Actinomycetes are growing [3]. In some cases, the mycelia may break to form rod or coccoied shape. In general, many genera form spores; may be called as sporangia or spore cases are found on the aerial mycelia or hyphae. Actinomycetes has a high G+C ration of the DNA in their DNA about>55-75%. The phylum Actinobacteria have a complex form of lifecycle that represents one of the largest taxonomic units among the 18 major lineages currently recognized within the domain bacteria [4].

The name Actinomycetes was derived from Greek “atkis” (a ray) and “mykes” (fungus) found in the various natural environments to provide a valuable resource for novel products of Industrial interest [5, 6]. Actinomycetes are found in soil, fresh water and marine water and its sediment. Marine Actinomycetes are of considerable interests as a new and promising source of biologically active compound. They produce a variety of metabolites which can be used for drug development. Increase in number of many Actinobacteria exists in marine environments especially marine sediments has been a fruitful area of research in the past and present decades [7]. Actinomycetes provided many important bioactive compounds of high commercial value and screened for new bioactive substances. Around 23000 bioactive secondary metabolites produced by microorganism have been reported. Over 10000 of these compounds are produced by Actinomycetes representing 45% of all bioactive microbial screening of antibacterial producing Actinomycetes. Of these compounds, antibiotics are predominating in therapeutic and commercial importance [8]. It had been emphasized that Actinomycetes from marine sediments might be valuable for the isolation of novel strains which potentially yield a broad spectrum of secondary metabolites [9]. For example Rifamycin produced by *Micromonospora* sp and Marinopyrroles from S*terptomyces* sp [10].Traditionally, Actinomycetes have been isolated from terrestrial sources although, the first report of mycelium forming Actinomycetes being from marine sediments appeared several

decades ago [11]. The marine sediments of Actinomycetes not only have several new species but also have plenty novel structures with potent bioactive compound that have different mechanism of action in worldwide [12].Reports from East coast of India, suggests that soil is a major source of Actinomycetes [13]. Correspondingly, the kayalpatnam marine ecosystem is largely exploited and may provide the rich source for the discovery of new species and it produced effective novel bioactive compounds. The present study was aimed to isolate and characterize Actinomycetes from marine sediments. Investigation can possibly reveal Actinomycetes species that produce novel antibiotics.

MATERIALS AND METHODS

Sample Collection

In a systematic screening program for isolation of Actinomycetes marine sediments sample were collected from Gulf Mannar Costal Region, Kayalpatnam, located at Tuticorin district, Tamil nadu, India. Nearly five marine sediments collected from kayalpatnam East coastal region were randomly collected by zig zag manner between 200 M at a depth of 5M using a core sampler. The central portion of the marine soil were aseptically transferred to the sterile bottles during June 2013 and brought up to laboratory with help of ice bag. The sediments sample was blackish brown colour and of a sandy texture.

Isolation of Pure Actinomycetes

The all marine sediments were air dried to minimize bacterial contaminants. One gram sediments were serially diluted up tp10^{-6} dilution. One ml of diluted sample was permitted in to the petriplate followed by Starch casein nitrate agar (SCNA) medium supplemented with cyclohexamide 50µg/ml and nystatin50µg/ml. After solidification, all the plates were incubated at 28^{o}C for 7-15 days until the colonies were developed.

Maintenance of Actinomycetes Culture

The powdery form Actinomycetes were isolated and sub cultured. The pure strain of Actinomycetes were Maintained on Starch casein nitrate agar and 25% v/v glycerol stocks at 4°c.

Microscopic study of Isolated Actinomycetes

Slide Culture Method

The slide culture method is used to observe the morphological characteristics of mold and conidiogenous cell without disturbing the arrangement of spores. The sterile starch casein

nitrate agar blocks were prepared and the setup was kept in moist chamber. The isolated strains were inoculated in to the every corner of the agar blocks. The set up was incubated at 28^0C for 5 days. After incubation, the colonies of Actinomycetes strains were examined under high power magnification and the type of mycelium was noted with respect to aerial and substrate mycelium, branching and the nature of colony.

Spore Morphology

A drop of culture were placed over the 0.1% tryphan blue stain and mixed partially without spreading. Cover slip was placed over the culture stain gently. The slide were examined under bright filed microscope and the spore were noted.

Gram Staning

The Actinomycetes isolate smear was made on the glass slide followed by heat treatment. Smear was flooded with Crystal violet for 30-60 sec, Gram's iodine for 30-60 sec and decolorized by alcohol. Finally, the smear was stained with safranin as a counter stain for 2 minutes. After, washes with water and followed by air drying, the slide was focused on microscope under100X magnification.

Biochemical Characterization

After preliminary studies, the isolates were selected for biochemical analysis. Biochemical analysis are indole production, Methyl red and Voges-proskauer test, Citrate utilization test, Catalase test, Oxidase test, Starch hydrolysis and Urea hydrolysis

Utilization of Trible Sugar Iron Agar

TSI Agar is used to determine the carbohydrate fermentation and hydrogen sulphide production. Observation of various carbohydrate utilization patterns of isolated Actinomycetes were determined by TSI Agar medium test. The medium consist of three sugars such as lactose, sucrose and glucose which allows for detection of the utilization of the substrate only. The acid base indicators were also incorporated in to the medium. After incubation, the carbohydrate utilization of Actinomycetes was detected.

Screening of Isolated Actinomycetes

Isolation of test pathogen was obtained from a hospital who admitted hospital in at after 24 hours. Clinically impact source of mid stream urine specimens were collected. The isolated *E.coli, Klebsiella pneumoniae, Proteus mirabilis* and *Staphylococus aureus.* were exposed to

33 antibiotics. Finally, the multidrug resistant *E.coli*, *Klebsiella pneumoniae*, *Proteus mirabilis* and *Staphylococus aureus* were used in this antimicrobial study. The antibacterial activity all isolated Actinomycetes was done by agar well diffusion method on Muller Hinton agar medium.

RESULT AND DISCUSSION

The present work was carried out the existence of marine Actinomycetes isolated from Gulf Mannar Costal Region, Kayalpatnam located at Tuticorin district of Tamil nadu, India. Collected samples were analyzed by serial dilution and plating method (Table 1).The number of colonies were calculated by colony forming units(CFU/GM).Totally 19 Actinomycetes strains were isolated and identified as *Streptomycetes sp* (10), *Micromonospora sp* (6), *Micropolyspora sp* (1), *Strptoverticillium sp* (1) and *Intrasporangium sp* (1) respectively. All the isolated Actinomycetes were morphologically differ on the basis of colony color, types of mycelium, spore and pigmentation. Out of 19, five were isolated from 400 m distance of collecting site and remaining sites showed 3 to 4 isolates/ sample. From the five sampling site, *Streptomyces sp* was observed and it was predominantly isolated. The dominance of *Streptomyces sp* was reported by many workers as a rich and sustainable source in marine sediments [14, 15, 16]. Marine environments are largely untapped source for the isolation and discovery of Actinomycetes with potentially to produce active secondary metabolites with wide range of biological activity [17].

All the isolates were found to be gram positive and most of the isolates showed production of aerial and branched substrate mycelium on SCNA. Cultural characterization of Actinomycetes shows white, ash and grey powdery and rough colonies on SCNA in nature. Of these isolate 63% were showed pigmentation on SCNA slants (Table 2).The taxon of *Actinomycetes* are currently accommodates spore forming Gram positive bacteria that form extensive branching substrates and aerial mycelia [18].

Based on spore formation (Table 3), *Streptomyces sp* was identified based on the production of spiral chain of spore on substrate mycelium. Similarly *Micromonospora sp* produced monospore on aerial mycelium; *Micropolyspora sp* produced chain of spore with fragmented mycelium. Isolates of *Micropolyspora* showed straight sporophores formation and *Intrasporangium* has produced zygospore. The formation of spore analyzed by slide cultural method [19] and compared with Bergy's manual of determinative bacteriology.

All the isolated Actinomycetes were differentiated based on biochemical tests such as Indole, MR-VP, Citrate utilization test, Catalase, Oxidase, Urease and Starch hydrolysis and the results were tabulated (Table 4). All the Actinomycetes isolates were strictly Indole positive and failed to utilize Citrate. Among the *Streptomyces* only three were (KPMS 3, KPMS 6, KPMS 12) urease positive. All the Streptomyces *sp* were utilized Starch. Similarly all the six isolates of *Micromonospora* showed Urease positive and only two (KPMS1 and KPMS13) hydrolyzed Starch. Isolates belongs to *Micropolyspora sp,* and *Intrasporangium sp* unable to produce urease enzyme but hydrolyzed starch partially. Actinomycetes are tradionally classified as part of the valuable bacteria which are well exploited for secondary metabolites. Isolation and characterization of promising strain of marine Actinomycetes research is a major area for many years in worldwide [20]. Most of the isolates showed TSI positive but only two strains KPMS 7 and KPMS 8 belongs to *Streptomyces sp* showed that the production of H_2S (Table 5). Various biochemical tests were performed to identify but it was unable to identify Actinomycetes up to species level due to lack of the other test. For identification of genera and species of Actinomycetes, besides morphological and physiological properties were performed for the potent identification of Actinomycetes isolates [21, 22].

The antibiogram of purified cultures of all Actinomycetes was performed against multidrug resistant *E.coli , Klebsiella pneumoniae,Proteus mirablis and Staphylococcus aureus.* All the four test pathogens were highly sensitive to KPMS 5 and moderately sensitive to KPMS 14 which belongs to *Streptomyces* genus. The maximum zone of inhibition of KPMS 5were 24 mm against *Klebsiella pneumoniae* and *Staphylococcus aureus* followed that 21 mm against *E.coli* where as KPMS 14 were found to be 16 mm against all test pathogens (Table 6). Recent investigations indicate that the tremendous and potential marine Actinomycetes particularly *Streptomyces sp* as a useful and sustainable source of new bioactive natural products [23]. Actinomycetes represent ubiquitous groups of microbes widely distributed in diverse range of natural ecosystem especially in sediments obtained from marine or deep sea [24]. The need for the identification of effective compound is obtained from marine sediments has proved to be the single most successful strategy for the discovery of new drugs. The present study indicated that the existence of diverse group of Actinomycetes in the marine sediments which resulted in selective isolation of dominant level of *Streptomyces sp* followed by *Micromonospora sp*. But more precise work and further development in this field is required to produce novel bioactive compounds from marine sediment Actinomycetes.

Table-1. Distance of sample collection site and colony forming unit.

S. No	Distance of Sample Collecting Site	Number colonies in each Gram of sample(Cfu/gm)	Isolated strain
1	200 m	$4x10^{-6}$	KPMS 1- 4
2	400 m	$5x10^{-6}$	KPMS 5- 9
3	600 m	$4x10^{-6}$	KPMS 10-13
4	800 m	$3x10^{-6}$	KPMS 14-16
5	1000 m	$3x10^{-6}$	KPMS 17-19
Total		19×10^{-6}	

Table-2. Color of the Mycelial Isolates.

S. N	Isolate code	Type of mycelium	Colour of aerial mycelium	Colour of pigments
1	KPMS1	AM/SM	Greenish grey	Yellowish Green pigment
2	KPMS2	AM/SM	White	-
3	KPMS3	AM/SM	Ash	Pale yellow pigment
4	KPMS4	AM/SM	Ash	Diffusable brown pigment
5	KPMS5	AM/SM	Dark greenish ash	-
6	KPMS6	AM/SM	Grey	Blackish brown pigment
7	KPMS7	AM/SM	Sandal White	-
8	KPMS8	AM/SM	Light grey	Brown pigment
9	KPMS9	AM/SM	Dull white	Diffusable yellowish brown pigment
10	KPMS10	AM/SM	Whitesh ash	Diffusable Redwine pigment
11	KPMS11	AM/SM	Dark ash	-
12	KPMS12	AM/SM	Grey	Diffusable yellowish brown pigment
13	KPMS13	AM/SM	Dark grey	Pale yellow pigment
14	KPMS14	AM/SM	white	Pink pigment
15	KPMS15	AM/SM	Lightbrownish grey	Pale yellow pigment
16	KPMS16	AM/SM	Light grey	-
17	KPMS17	AM/SM	Whitesh grey	-
18	KPMS18	AM/SM	Pinkish brown	Pink pigment
19	KPMS19	AM/SM	white	-

Table-3. Microscopic Examination of the Actinomycetes Isolates.

S. No	Isolate code	Spore morphology	Cell wall type	Isolated Genus
1	KPMS1	Rarely branched septate hyphae with Monospore	Gram positive	*Micromonospora sp*
2	KPMS2	Spiral chain of spore	Gram positive	*Streptomyces sp*
3	KPMS3	Long chain of spore	Gram positive	*Streptomyces sp*
4	KPMS4	chain of spore on fragmented hyphae	Gram positive	*Micropolyspora sp*
5	KPMS5	Spiral chain of spore	Gram positive	*Streptomyces sp*
6	KPMS6	Moderate length of chain of spore	Gram positive	*Streptomyces sp*
7	KPMS7	Long chain of spore	Gram positive	*Streptomyces sp*
8	KPMS8	Rarely branched spiral spore	Gram positive	*Streptomyces sp*
9	KPMS9	Septate hyphae with monospore	Gram positive	*Micromonospora sp*
10	KPMS10	Septate hyphae with monospore	Gram positive	*Micromonospora sp*

11	KPMS11	Long chain of spore	Gram positive	*Streptomyces sp*
12	KPMS12	Spiral chain of spore	Gram positive	*Streptomyces sp*
13	KPMS13	Septate hyphae with monospore	Gram positive	*Micromonospora sp*
14	KPMS14	Moderate length of chain of spore	Gram positive	*Streptomyces sp*
15	KPMS15	Straight sporo spores	Gram positive	*Streptoverticillium sp*
16	KPMS16	Spiral chain of spore	Gram positive	*Streptomyces sp*
17	KPMS17	Septate hyphae with monospore	Gram positive	*Micromonospora sp*
18	KPMS18	Oval intergallery vesicle with Zygospore	Gram positive	*Intrasporangium sp*
19	KPMS19	Septate hyphae with monospore	Gram positive	*Micromonospora sp*

Table4. Biochemical properties of Isolated Actinomycetes.

Isolated Genus	strain	Indole	MR	VP	Citrate	Catalase	Oxidase	Starch	Urease
Streptomyces sp	KPMS2	+	+	+	-	+	+	+	-
	KPMS3	+	+	-	-	-	-	+	+
	KPMS5	+	+	+	-	+	+	+	-
	KPMS6	+	+	+	-	-	+	+	+
	KPMS7	+	+	-	-	-	+	+	-
	KPMS8	+	+	-	-	+	+	+	-
	KPMS11	+	+	+	-	-	+	+	-
	KPMS12	+	+	+	-	-	+	+	+
	KPMS14	+	+	-	-	-	-	+	-
	KPMS16	+	+	-	-	-	-	+	-
Micromonospora sp	KPMS1	+	+	+	-	+	+	+	+
	KPMS9	+	+	+	-	-	-	-	+
	KPMS10	+	-	+	-	+	+	-	+
	KPMS13	+	+	+	-	-	+	+	+
	KPMS17	+	-	-	-	-	-	-	+
	KPMS19	+	+	-	-	+	-	-	+
Micropolyspora	KPMS4	+	+	-	-	-	-	±	-
Streptoverticillium	KPMS15	+	+	-	-	+	-	±	-
Intrasporangium	KPMS18	+	+	-	-	+	-	±	-

Table 5. Utilization of Triple Sugar Iron Agar Test.

Isolated Genus	Isolate code	Butt	Slant	Gas production	H_2S production
Streptomyces sp	KPMS2	Acid	Alkaline	-	-
	KPMS3	Acid	Alkaline	-	-
	KPMS5	Acid	Alkaline	-	-
	KPMS6	Acid	Alkaline	-	-
	KPMS7	Alkaline	Alkaline	-	+
	KPMS8	Alkaline	Alkaline	-	+
	KPMS11	Acid	Alkaline	-	-
	KPMS12	Acid	Acid	+	-
	KPMS14	Acid	Acid	+	-
	KPMS16	Acid	Alkaline	-	-
	KPMS1	Acid	Alkaline	-	-
	KPMS9	Acid	Acid	+	-
	KPMS10	Acid	Alkaline	-	-
	KPMS13	Acid	Alkaline	-	-

Micromonospora sp	KPMS17	Acid	Alkaline	-	-
	KPMS19	Acid	Acid	+	-
Micropolyspora sp	KPMS4	Acid	Alkaline	-	-
Streptoverticillium sp	KPMS15	Alkaline	Alkaline	-	-
Intrasporangium sp	KPMS18	Acid	Alkaline	-	-

Table 5: Antimicrobial study of isolated Marine Atnomycetes.

Isolated Genus	Isolate code	*K.pneu*	*E.coli*	*Proteus mirabilis*	*S.aureus*
Streptomyces sp	KPMS2	-	-	-	-
	KPMS3	-	-	-	-
	KPMS5	24 mm	21 mm	22 mm	24 mm
	KPMS6	-	-	-	-
	KPMS7	-	-	-	-
	KPMS8	-	-	-	-
	KPMS11	-	-	-	-
	KPMS12	-	-	-	-
	KPMS14	16 mm	16 mm	16 mm	16 mm
	KPMS16	-	-	-	-
Micromonospora sp	KPMS1	-	-	-	-
	KPMS9	-	-	-	-
	KPMS10	-	-	-	-
	KPMS13	-	-	-	-
	KPMS17	-	-	-	-
	KPMS19	-	-	-	-
Micropolyspora sp	KPMS4	-	-	-	-
Streptoverticillium sp	KPMS15	-	-	-	-
Intrasporangium sp	KPMS18	-	-	-	-

CONCLUSION

It is suggestive that marine sediments could be an interesting source collected from the different distance located the Gulf Mannar Costal region, kayalpatnam represent that the rich source of Actinomycetes. We can conclude that the present observation, Actinomycetes in the marine sediments resides the potential sources of unique bioactive metabolites. Further studies will be focused to identify the active lead molecule.

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REFERENCES

1. Kuster E. (1968) the actinomycetes.In: soil biology, eds. Burges (A) and Raw (F), Academic press, London. 111-124.
2. Lechevalier H and Lechevalier MP. (1981) Introduction to the order Actinomycetales. In:

Starr, MP, Stolp, H, Traper, HG, Balows, A., Schlegel, HG, eds. The prokaryotes.Germany: Springer-verlag Berlin. 2:1915-1922.

3. Harvey RA. (2007). Microbiology. Baltimore: Lippicncott williams and Wilkins.
4. Ventura M, Canchaya C, Tauch A, Chandra G, Fitzgerald GF, Chater KF. (2007). Genomics of Actinobacteria:Tracing the evolutionary history of an ancient phylum. Micrbiology And Molecular Biology Reviews. 71:495-548.
5. Das S, Lyla PS, Khan SA.(2008). Distribution and generic composition of culturable marine Actinomycetes from the sediments of Indian continental slope of bay of bengal. Chinese Journal of Oceanology and Limnology. 26:166-177.
6. Tsujibo H, Kubota T, Yamamoto M, Miyamoto K, Inamori Y. (2003). Characteristics of chitinase genes from an alkaliphilic actinomycetes Nocardiopsis prasina OPC-131.Applied and EnvironmentalMicrobiology.69:894-900. doi: 10.1128/AEM.69.2.894-900.2003.
7. Mincer TJ, Jensen PR, Kauffman CA.(2002). Widespread and persistent population of major new marine Actinobacteria taxon in ocean sediments. Applied and Environmental Microbiology. 68: 5005-5011.doi:10.1128/AEM.68.10.5005-5011.2002.
8. Ouhdouch Y, Barakate M, Finanse C. (2001). Actinomycetes of moroccan habitats:isolation and screening for antifungal activities. European Journal of Soil biology. 37:69-74. http://dx.doi.org/10.1016/S1164-5563(01)01069-X
9. Maskey RP, Grun-wollny I, Laatsch H.(2003). Isolation, structure elucidation and biological activity of 8-0-methylaverufin and 1,8-0-dimethylaverantin as new antifungal agents from pencillium chrysogenum. Journal of Antibiotics. 56:459-63.
10. Hughes CC, Prieto-Davo A, Jensen PR.(2008). The marinopyrroles antibiotics of an unprecedented structure from a marine *Streptomyces* sp. Orglett. 10:629-33.
11. Weyland H. (1981). Characteristics of Actinomycetes isolated from marine sediments. Hygiene. 1(2):309-314.
12. Barsby T, Kelly MT, Gagne SM. (2001). Bogorol a produced culture by a marine *Bacillus* s reveals a novel template for cationic peptide antibiotics. Orglett. 3:437-40.
13. Sivakumar K, Sahu M, Kathiresan K. (2005). Isolation and characterization of Streptomycetes producing antibiotic from mangrove environment. Asian Journal of Microbial Biotechnology and Environmental Science. 7:457-764.
14. Jensen PR, Dwight R, Fenical W. (1991). Distribution of Actinomycetes in near shore tropical marine sediments. Applied and Environmental Microbiology. 57:1102-1108.

15. Lu Y, Dong X, Liu S. (2009). T-muurolol sesquiterpenes from the marine *Streptomyces* sp M491 and revision of the configuration of previously reported amorphanes. Journal of Natural Product. 72:99-101.
16. Hassan AA, El-Barawy AM, Mokhtar EL, Nahed M. (2011). Evaluation of biological compounds of Streptomyces sp for control of some fungal diseases. Journal of American Science. 7:752-60.
17. Bredholt H, Fjaervik E, Jhonsen G, Zotechev SB. (2008). Actinomycetes from sediments in Trondhein Fjrod, Norway, Diversity and biological activity. Journal of Marine Drugs. 6:12-24.
18. 18. Waksman SA. (1961). The *Actinomycetes:* Classification, Identification and Description of genera and species. Baltimore: Lippicncott Williams and Wilkins. 2:377-375.
19. Raja A, Prabakaran P, Gajalakshmi P, Rahman AH. (2010). A population study of psychrophilic Actinomycetes isolated from Rothang Hill-manali soil sample. Journal of Pure and Applied Microbiology. 4:847-51.
20. Laidi RF, Kansoh AL, Elshafei AM, Cheikh B. (2006). Taxonomy, Identification and biological activities of novel isolate of *Streptomyces tendae*. Arab Journal of Biotechnology. 9:427-436.
21. Kuster E. (1972). Simple working key for the classification and identification of named taxa included in the international streptomyces project. International Journal of Systematic Bacteriology. 22:139-148. doi:10.1099/00207713-22-3-139
22. Abbas IH. (2006). A biological and biochemical studies of Actinomycetes isolated from kuwait saline soil-kuwait. Journal Applied Science Research. 2:809-815.
23. Sharma D, Kaur T, Chadha BS, Manhas RK. (2011). Antimicrobial activity of Actinomycetes against Multidrug resistant Staphylococcus aureus, E. coli and various other pathogen. Tropical Journal of Pharmaceutical Research.10:801-808
24. Pathom-aree W, Stach JE, Ward AC, Horikoshi K, Bull AT, Goodfellow M.(2006). Diversity of Actinomycetes isolated from challenger deep sediment (10.898M) from the Mariana Trench. Extremophiles. 10:181-189.