EAS Journal of Parasitology and Infectious Diseases
Abbreviated Key Title: EAS J Parasitol Infect Dis
ISSN 2663-0982 (Print) & Open Access
Published By East African Scholars Publisher, Kenya

OPEN ACCESS

Volume-1 | Issue-1 | Jan-Feb-2019 |

Research Article

Screening for Extracellular *Protease* Production and Antimicrobial Activity in Culture Filtrate of Marine *Actinomycetes*

Fiddausi Yusuf Bello

Department Of Microbiology (Alumni), Umaru Musa Yar'adua University Katsina, Nigeria

*Corresponding Author
Fiddausi Yusuf Bello

Abstract: Actinomycetes are classified as vast group of Gram positive bacteria which have filamentous appearance and widely present in both terrestrial and aquatic environments. This study examined the extracellular protease production and antimicrobial activity in culture filtrate of marine actinomycetes. A total of four actinomycetes namely OAI, 28d, 6a and K3 were isolated and screened in pure culture form and their potential of protease production. Among four actinomycetes studied, none of them showed protease activity. Similarly, testing actinomycetes for antimicrobial activity against some pathogens including S. flexneri, S. aureus, C. albicans, MRSA, G. vaginalis, P. aeroginosa, K. pneumonia, S. typhi and S. epidermidis shows a significant activity against OAI, 28d, 6a and K3. The study recommends that, different methods should be employed to test and confirmed the finding of this study.

Keywords: Actinomycetes, culture filtrate, S. flexneri

INTRODUCTION

Actinomycetes are classified as vast group of gram positive bacteria which have filamentous appearance and widely present in both terrestrial and aquatic environments. *Streptomycetes* are the major class of soil *actinomycetes* that produce numerous natural metabolites such as antibiotics and enzymes (Chiani *et al*., 2010). They produce several enzymes, degrading complex organic materials in soil or sediments such as protease, cellulases, amylase, gelatinase, lectinases and ureases (Gluve and Desmukh, 2012). Amylase is one of the commonly used enzymes in different starch industries. It has many industrial applications in the production of syrup made from oligosaccharide and monosaccharide. In textile industries, amylase enzyme is used for resizing of clothing materials (Tonkova, 2006).

Actinomycetes are good decomposers of organic materials and Production of amylases was reported by some strains of actinomycetes (Kar and Ray, 2008). *Actinomycete cellulases* are inducible extracellular enzymes that can be produced during their growth on cellulosic materials. Thus, introduction of cellulolytic microorganisms is a beneficial microbiological tool for recovery of bioenergy from degraded cellulose (Balamurugan *et al*., 2011) and have gained significant attention due to their wide applicability in various industrial processes including pulp and paper, textile, laundry, biofuel production, food and feed industry, brewing and agriculture (Kuhad *et al*., 2011).

One of the most important groups of enzymes that produced commercially and for industrial purposes are Proteases (Deng *et al*., 2010). They have extensive applications in a range of industrial products and processes including detergents, food, pharmaceuticals and leather (Ribitscha *et al*., 2010). In spite of considering *Actinomycetes* to be among the most important producers of antibiotics (Avitha *et al*., 2010), The present knowledge concerning proteases of *actinomycetes* to be the most important group of secondary metabolites that are widely exploited (Limkhada *et al*., 2010).

Generally, actinomycetes are widely distributed in aquatic ecosystems, where they play an essential role in recycling refractory biomaterials by decomposing complex mixtures of polymers in dead plants, animals and fungal materials. Thus, presence of many enzymes which acts as catalysts in many biochemical reactions both in microorganisms, plants and animals activities, which are produced by many

Quick Response Code

Journal homepage:
http://www.easpublisher.com/easjpid/

Article History
Received: 06.02.2019
Accepted: 15.02.2019
Published: 28.02.2019

marine associated microorganisms, as such looking forward to have a cheaper way of isolating such enzymes in developing the biotechnological advancement seems plausible. Protease enzyme is no exception (Ibrahim, 2007).

Hence, due to increased occurrence of disease and the drop in efficacy of existing drugs, couple with high cost and level of poverty resulting in an increase in search for more active compounds .This study aimed at assessing bioactive compound for protease secreting and production of active compound which could have more activity and accessibility.

It is in the wake of this background, this paper examined extracellular protease production and antimicrobial activity in culture filtrate of marine *actinomycetes*.

REVIEW OF LITERATURE

- ***Actinomycetes* in Marine Environment**

Of the total sea surface, only 7% to 8% is coastal area and the rest is deep sea, of which again 60% is covered by water of more than 2000 m deep (Bull et *al*., 2000). The deep sea is a unique and extreme environment characterized by high pressure, low temperature, lack of light and variable salinity and oxygen concentration. Though the geographical area of deep sea is vast, our knowledge, understanding and studies about the deep-sea microorganisms are meagre. However, it is a good source of novel organisms for microbiologists and biotechnologists (Bull et *al*., 2000). However, it has been shown to be a good source of novel microorganisms for the discovery of new antibiotics (Bull et *al*., 2000). *Actinobacteria* isolated from deep-sea sediments in earlier studies however poorly characterized. More recently, culture-independent studies have shown that indigenous marine *actinomycetes* certainly exist in the oceans. These include members of the genera *Dietzia*, *Rhodococcus*, *Streptomyces* (Deng *et al*., 2010), the newly described genera *Salinispora* and *Marinispora* , both of which require seawater for growth and have marine chemotype signatures; and *Aeromicrobium marinum* which also has an obligate requirement for salt. Another recently characterized genus, *Salinibacterium*, can tolerate up to 10% NaCl but does not have a salt requirement for growth (Deng *et al*., 2010). The recently reported *Verrucosispora* strain AB-18-032 (Ahmad *et al*., 2005) also might qualify as an indigenous marine *actinobacterium*. Some of these species were found to produce unique compounds, such as *salinosporamides*, that are now in clinical trials as potent anticancer agents.

The *actinomycetes* are active components of marine microbial communities and form stable, persistent populations in various marine ecosystems. The discovery of several new marine *actinomycete* taxa with unique metabolic activity in their natural environments (Deng *et al*., 2010), and their ability to form stable populations in different habitats and produce novel compounds with various biological activities (Al-saadi *et al*., 2013) clearly illustrate that indigenous marine *actinobacteria* indeed exist in the oceans and are an important source of novel metabolites.

- **Proteases**

Marine *actinomycetes* have a diverse range of enzyme activities and are capable of catalyzing various biochemical reactions. Different commercial enzymes viz. α-amylase, protease, cellulase, chitinase, *keratinase* and xylanase have been obtained from the marine *actinobacteria* (Balangar *et al*., 1997).

Proteases, also known as peptidyl-peptide hydrolases, are important industrial enzymes, which are responsible for approximately 60% of all enzyme sales, and are utilized extensively in a variety of industries, including detergents, meat tenderization, cheese-making, dehairing, baking, brewery, the production of digestive aids, and the recovery of silver from photographic film. The use of these enzymes as detergent additives stimulated their commercial development and resulted in a considerable expansion of fundamental research into these enzymes. In addition to detergent and food additives, alkaline proteases have substantial utilization in other industrial sectors such as leather, textile, organic synthesis, and waste water treatment. As a consequence of this kind of researches, alkaline proteases with novel properties have become the focus of renewed research interest. Alkaline proteases are generated by a wide range of organisms, including bacteria, actinobacteria molds, yeasts, and mammalian tissues.

Several studies have been made on the proteolytic enzymes of mesophilic actinomycetes. (Collins *et al*., 1995) isolated thermophilic *Streptomyces* (six strains) from soil using an enrichment technique with feathers as the sole carbon and nitrogen source and on strain which was tentatively identified as *Streptomyces thermonitrificans* showed maximum protease activity. Recently, alkaline protease from *Nocardiopsis* spp. NCIM 5124 has been purified and characterized. An extracellular protease-producing actinomycete, *Streptomyces nogalator* strain Ac 80, isolated from soil. Fulzale (2011) isolated *Streptomyces* sp. producing thermophilic proteases from Brazilian cerrado soil.

Jarallah (2014), isolated alkaliphilic acinomycete from the soil and crude components such as molasses, wheat flour, and wheat bran were found to be effective for growth and protease production. The high level of enzyme production using agro-industrial by-products is commercially significant due to cheap nature of these sources. (Jiang and Xu, 1996) isolated *S. gulbargensis* DAS 131 from soil samples and multiple proteases were produced and partially purified from an

alkali-thermotolerant novel species of *S. gulbargensis* after 48 h of growth at 45 °C. Kafitha *et al*., (2010) isolated 191 different marine actinobacteria from 256 different marine samples (sediments from seashore, deep sea, lake and mangrove, seawater, brackish water from lake and mangrove, marine animals and marine algae) collected from the Bay of Bengal and its associated Pulicat lake and Pichavaram mangrove, India. Among them, 157 produced caseinase, 113 produced gelatinase and 108 produced both the protease enzymes. (Mane and Deshmukh, 2009) isolated marine actinobacteria from the flesh content of the Mud crab, *Scylla serrata* and the mutation is one of the good methods for strain development to increase the efficiency of the actinobacteria for protease production. Mohan and Vijayakumar, (2007), isolated actinomycetes (46 strains) from soil samples of Northern Himalayas and study their culture characterization, protease production and cytotoxic effects on cancer cell line.

- **Antimicrobial Activity**

Antimicrobial susceptibility testing can be used for drug discovery, epidemiology and prediction of therapeutic outcome. In this review, the study focused on the use of antimicrobial testing methods for the *in vitro* investigation of extracts and pure drugs as potential antimicrobial agents. After the revolution in the "golden era", when almost all groups of important antibiotics (tetracyclines, *cephalosporins*, *aminoglycosides* and macrolides) were discovered and the main problems of chemotherapy were solved in the 1960s, the history repeats itself nowadays and these exciting compounds are in danger of losing their efficacy because of the increase in microbial resistance. Currently, its impact is considerable with treatment failures associated with multidrug-resistant bacteria and it has become a global concern to public health. For this reason, discovery of new antibiotics is an exclusively important objective. Natural products are still one of the major sources of new drug molecules today. They are derived from prokaryotic bacteria, eukaryotic microorganisms, plants and various animal organisms. Microbial and plant products occupy the major part of the antimicrobial compounds discovered until now. Plants and other natural sources can provide a huge range of complex and structurally diverse compounds. Recently, many researchers have focused on the investigation of plant and microbial extracts, essential oils, pure secondary metabolites and new synthetized molecules as potential antimicrobial agents (Pradham *et al*., 1975).

However, when we reviewed the published articles on the antimicrobial effect of these natural products, the comparison between results is often difficult, because of the use of different non-standardized approaches inoculum preparation techniques, inoculum size, growth medium, incubation conditions and endpoints determination. The fact that a plant extracts exhibits antimicrobial activity is of interest, but this preliminary part of data should be trustworthy and allow researchers to compare results, avoiding work in which researchers use the antimicrobial activity investigation only as a complement to a phytochemical study. A variety of laboratory methods can be used to evaluate or screen the *in vitro* antimicrobial activity of an extract or a pure compound. The most known and basic methods are the disk-diffusion and broth or agar dilution methods. Other methods are used especially for antifungal testing, such as poisoned food technique. To further study the antimicrobial effect of an agent in depth, time-kill test and flow cytofluorometric methods are recommended, which provide information on the nature of the inhibitory effect (bactericidal or bacteriostatic) (time-dependent or concentration-dependent) and the cell damage inflicted to the test microorganism. Owing to the new attraction to the properties of new antimicrobial products like combating multidrug-resistant bacteria, it is important to develop a better understanding of the current methods available for screening and/or quantifying the antimicrobial effect of an extract or a pure compound for its applications in human health, agriculture and environment (Rifaat, 2003).

MATERIALS AND METHODS

- **Study Area**

The study was conducted at Microbiology departmental laboratory in Umaru Musa Yar'adua University Katsina state of Nigeria. Katsina is an old city of Northern Nigeria 160 miles East of the city of Sokoto, and 84 m. N.W. of Kano, close to the border with Niger. Latitude 13 N., Long. 7.41'E. Katsina lies on the river of the same name, which is a tributary of the River Benue. Katsina is capital of Katsina State and the centre of an agricultural region producing groundnuts, cotton, hides, millet and guinea corn.

- **Screening of *Actinomycetes* for protease production**

The sample was obtained from I.A. Adeleye Laboratory in Lagos. *Actinomycetes* isolates were grown on skim milk media which consist of skim milk powder 10g, peptone 5g, NaCl 0.03g and agar 20g. These components were dissolved in 1 liter of distilled water, then pH was adjusted to 7.0. The plates were streaked by isolates and incubated at 37^0C for 4 days. The formation of clear zone around *Actinomycetes* colonies represented positive results (Kavya *et al*., 2012).

- **Cross Streak Method**

Preliminary screening for antimicrobial activity of *actinomycetes* isolates were performed by cross streak plate method on Muller Hinton Agar (MHA). The *actinomycetes* isolates were inoculated in a straight line on MHA plates and incubated at 37°C for 7 days. The plates were then inoculated with the test organisms by a single streak to the *actinomycetes*

strains and incubated at 37 ^{0}C overnight for bacteria and at 28±2^{0}C for 96 h for fungus. Antagonism was observed by the inhibition of test organism.

- **Agar-Well Diffusion method**

Antibacterial activities were assessed using agar-well diffusion method. Petri plates were prepared with 20 ml of sterile Muller Hinton Agar (MHA). The test cultures (100 µl of suspension containing 108 CFU/ml bacteria) were spreaded on the top of the solidified media and allowed to dry for 10 min followed by well was made using sterile cork borer and filled with 0.05 ml of supernatant. The plates were left for 30 min at room temperature for supernatant diffusion. The plates were incubated for 24 h at 37°C. Zone of inhibition was recorded in millimetres and the experiment was repeated twice.

RESULTS AND DISCUSSION

This section presents the results of the lab work as follows:

Table 1: Code Identification and Names of Actinomycetes

Codes used for Identification	Name of Actinomycetes
6a	*Streptomyces albus*
31b	*Streptomyces albus*
G2231	*Micromonospora spp*
K3	*Streptomyces avermitilis*
28d	*Streptomyces pratensis*
Ma30	*Streptomyces spp*
Ma230	-
K10	*Streptomyces spp*
OAI	-
Act2	*Streptomyces pratensis*
19b	*Streptomyces fulvissimus*
030	*Micromospora spp*

Source: Researcher Work.

Table 1, shows the name and identification for *actinomycetes* used in this study for easy description as the case may be.

Table 2: Result for antimicrobial screening using (mm) agar well diffusion method

Organism	***S.aureus***	***K.pneumoniae***	***S.epidermidis***	***S.flexineri***	***S.typhirium***	***MRSA***	***G.vaginalis***	***P.aeroginosa***	***C.albicans***
6a	21.5	27	18	20.5	20.5	19	12	25	19
31b	20	15	14	-	19	15.5	16	17	12
G2231	25	17.5	16.5	28	-	25	10	22.5	-
K3	21	26.5	26	25	20	16.5	19	21	22
28d	27	39	21.25	27	17.5	20	17.5	23	14
Ma30	18.5	28	-	-	15	19	-	21	23
Ma230	22.5	27	21	27	17.5	-	20.5	22	16
K10	16	34	18.5	18	17.5	25.5	19	17.25	17
OAI	20	-	25	22.5	22	26	-	23	15
Act2	-	15	-	21.5	-	-	-	24	21
19b	29	33	12	19	13	20	-	21	-
030	19	22.5	-	-	14.5	-	16	18.5	25

Source: Researcher's Labwork.

In Table 2, results for anti-microbial screening were presented. However, it is noteworthy that the result was augmented from agar well diffusion method. Meanwhile, the zone of inhibition was measured in mm using ruler.

Table 3: ANOVA Test for Significant Difference among the selected five (5) Isolates

Isolates	F	P-value
6a	4.487**	.004
31b	5.238**	.003
G2231	3.989*	.021
K3	4.473**	.004
28d	3.090*	.022

Source: Researcher's computations using SPSS v.21
***** (*) indicates significance at 1% and 5% levels respectively.***

Table 3, presents the result of Analysis of Variance (ANOVA), in which case, it was revealed that, there exists a statistically significant difference between the test isolates under study as their respective p-values are statistically less than 0.05 at α=5% level of significance.

Table 4: Anti-bacterial activity of Actinomycetes using Cross-Streak Method

Test isolates	*Escherichia coli*	*Staphylococcus aureus*	*Salmonella typhi.*
ULK_{10}	+	+	+
$UL31_b$	+	-	-
UL_{030}	+	+	-
$ULMA_{230}$	-	+	+
$ULMA_{30}$	+	-	+
$UL19_b$	+	-	+
ULK_3	+	+	+
$UL6_a$	+	+	+
ULOAI	+	+	+
ULAct2	+	+	+
ULG_{2231}	+	+	+
$UL28_d$	+	+	+

Source: Researcher's Labwork.

Table 4, presents the anti-bacterial activity of *actinomycetes*. The 12 *actinomycetes* isolates used in testing the anti-bacterial activity showed important bio-activity against the test organisms i.e. *Escherichia coli, staphylococcus aureus* and *salmonella typhi.* The result was presented using (+/-) based on the type of activity shown.

Table 5: Screening for Extracellular Protease Production from Marine Actinomycetes

Strain Code	Production of Extracellular enzyme (Protease)
OAI	**-**
28d	**-**
6a	**-**
K3	**-**

Source: Researcher's Labwork, 2017.

Table 5, presents the result for the screening of extracellular protease production from marine *actinomycetes* for which the result depicts negative, which implies that all the 4 *actinomycetes* failed to produce protease activity extracellularly. However, they cannot be regarded as non-protease producers unless they are studied with different protein substrates. Alternatively all the 12 *actinomycetes* could be studied simultaneously such that overall inferences could be drawn. This finding went consistent with the findings of (Deng *et al*., 2010), Mohan and Vijayakumar (2007) who found some of the isolates negative whilst others positive in their study in India.

Discussions of Findings

The marine environment is the largest habitat on Earth, representing more than 70% of the surface of our planet. Oceans include the greatest extremes of temperature, light and pressure encountered by life (Deng *et al*., 2010; Ahmed, 2007). Adaptation of marine bacteria to the harsh environments has led to a rich biological and genetic diversity. Marine bacteria are attracting attention as new biotechnological resources. This study was aimed at finding the potential of extracellular enzymes produced by the organisms in test. The antibacterial activity produced by these organisms in table 1 which shows that, *Streptomyces albus*, *Micromonospora spp*, *Streptomyces avermitilis*, *Streptomyces pratensis* inhibited the pathogenic bacteria ranging from *S.flexneri*, *S. aureus*, *C.albicans*,

MRSA, *G.vaginalis*, *P.aeroginosa*, *K.pneumonia*, *S.typhi* and *S.epidermidis.*

The result of Analysis of Variance (ANOVA) revealed that, there exists a statistically significant difference between the test isolates under study as their respective p-values are statistically less than 0.05 at α=5% level of significance.

However, all the four (4) tested *actinomycetes* from this study also revealed their inability to produce protease enzymes as the organisms does not complied with the previous studies conducted by (Mane and Deshmukh 2009; Tawiah, 2012; and Ahmed 2007).

Many marine heterotrophic bacteria are known to produce antibacterial substances which inhibit or kill other bacteria. Research studies have shown that these antibacterial compounds are not only inhibitory to terrestrial bacteria but also to indigenous bacterial strains, which is of considerable ecological significance (Deng *et al*., 2010). Many studies have concentrated on the screening of marine bacteria for antibacterial activity against human pathogenic bacteria (Blunt and Prinsep, 2006).

Thus, the screening of marine bacteria for antibacterial activity in this study has further reaffirmed and resulted in antibacterial activity. Marine bacteria including *actinomycetes* were already isolated from such intertidal sediments and studied for various biotechnological applications (Bhagat *et al.,* 2010).

CONCLUSION AND RECOMMENDATIONS

Following the findings of the study, it is concluded that most of the *actinomecytes* have bioactive compounds capable of inhibiting the growth of tested bacteria. Although, the four (4) tested *actinomecetes* were reportedly incapable of secreting protease, the research findings about the marine bacteria gave insight into the ecology of microbes in aquatic sediments in which they served as the potential candidates for pharmaceutical applications but lacking the desired attributes needed for. Following the findings of this study, it sounds plausible to recommend that:

- Different methods should be employed to test, confirmed and or further reaffirmed the finding of this study considering the fact that this study was relegated and confined to four (4) tested *actinomycetes*.
- However, a major limitation is culturing of certain strains have posed a difficulty. Once cultured, further studies can give information on the applications of these microbes. Since the marine bacteria obtained are not from deep sea levels, it proves that these microbes are easy to access and can be subjected to bioprospecting.
- Further investigation should be done to study the composition as well as to identify the bioactive compounds produced by the selected marine actinomycetes. The composition of optimized media for the production of bioactive secondary metabolites should also be studied in further details.

REFERENCES

1. Ahmad, F., Ahmad, I., & Khan, M.S. (2005). Indole acetic acid production by the indigenous isolates of *Azotobacter* and fluorescent pseudomonas in the presence and absence of tryptophan. *Turk. J. Biol, (*29), 29-34.
2. Ahmed, A. A. (2007). Production of antimicrobial agent by Streptomyces violachromogenes. Saudi J. Bio. Sci, (14), 7-16.
3. Al-Saadi, A., Hameed, N.M., Jaralla, E. M. (2013).Isolation andIdentification of Streptomyces from DifferentSample of Soils. Journal of Biology and Medical Sciences – JBMS, (1),31-36.
4. Atta, H.M. (2010). Production, Purification, Physico - Chemical Characteristics and Biological Activities of Antifungal AntibioticProduced by Streptomycesantibioticus, AZZ710. American-Eurasian J. of Sci, (5), 39-49.
5. Avitha, A.,Prabhakar, P.,Vijayalakshmi, M., & Venkateswarlu, Y. (2010). Purification and biological evaluation of the metabolites produced by Streptomyces sp. TK-VL_333.Res Microbiol, (161), 335-345.
6. Ayed, L., Cheriaa, J., Laadhari, N., Cheree, A. and Bakhrouf, A. 2009. Biodegradation of crystal violet by an isolated *Bacillus* sp. *Ann. Microbiol*, (59), 267-272.
7. Azamjon, B.S., Hosokawa, S.K., & Enomoto, K. (2011). Bioactive Pigments from marine Bacteria: Applications and physiological roles. *Evid. Based Complementary Alternative Med*,-17.
8. Azmi, W., Sani, RK. and Banerjee, U.C. 1998. Biodegradation of triphenylmethane dyes. *Enz. Microb. Technol*, (22), 185-191.
9. Barros, R.R.O.; Oliveira, R.A.; Gottschalk, L.M.F. and Bon, E.P.S. (2010). Production of cellulolytic enzymes by fungi AcrophialophoranainianaandCeratocystisparadoxausing different carbon sources. Appl. Biochem. Biotechnol,(161), 448-454.
10. Balamurugan, A., Jayanthi, R., Nepolean, P., Vidhya, P.R., & Premkumar, R. (2011). Studies on cellulose degrading bacteria in tea garden soils.Afr. J. Plant Sci, 5 (1),22–27.
11. Bredy, J.(2005).Bioactive microbial metabolites.J.Antibiot,(58),1-26.
12. Balan, S.S., Nethaji. R., Sankar, S., &Jayalakshmi, S. (2012). Production of gelatinase enzyme from Bacillus spp isolated from the sediment sample of Porto Novo Coastal sites. *Asian Pacific Journal of Tropical Biomedicine*, 1811–1816.
13. Baharum, S.N., Beng, E.K., & Mokhtar, M.A.A. (2010). Marine microorganisms: potential application and challenges. *J. Biol. Sci, (*10), 555-564.

14. Belanger, C., Desrosiers, B. &Lee, K. (1997). Microbialextracellular enzyme activity in marine sediments: Extreme pH to terminate reaction and sample storage. *AquaticMicrobial Ecol. (*13),187-196.
15. Bhagat, J., Muthezhilan, R., Jaffar H., A. andJayaprakashvel. M. (2010a). Siderophore producing antagonistic rhizobacteria from coastal sand dune vegetation. In: National seminar on biotechnological applications in human welfare held on 26th March 2010. *Department of Biotechnology, University of Madras, Chennai, India.*
16. Bhagat, J., Muthezhilan, R., Jaffar H.A. & Jayaprakashvel. M. (2010 b). Antagonistic activity of siderophore producing rhizobacteria from coastal vegetation. In: Abstracts of the national conference on advances in plant pathology; 11-12, March 2010. Centre for Advanced Studies in Botany, University of Madras, Chennai, India. -71.
17. Blunt, J.W., & Prinsep, M.R. (2006). Marine natural products. *Nat. Prod. Rep.* 23: 26-78. 10. Bric, J.M., Bostok R.M. and Silverstone, S.A. 1991. Rapid in situ assay for indole acetic acid production by bacteria immobilized on a nitrocellulose membrane. *Appl. Environ. Microbiol*. (57), 535-538.
18. Bull, A.T., Ward, A.C., & Goodfellow, M. (2000). Search and discovery strategies for biodiscovery: The paradigm shift. *Microb. Mol. Biol Rev, (*64), 573-606.
19. Cheng, H.R., & Jiang, N. (2006). Extremely rapid extraction of DNA from bacteria and yeasts. *Biotechnology letters*, 28(1),55–9.
20. Chiani, M., Akbarzadeh, A., Farhangi, A., Mazinani, M., Saffari, Z., Emadzadeh, K.,& Mehrabi, M.R. (2010). Optimization of culture medium to increase the production of desferrioxamine B (desferal) in Streptomyces pilosus.Pak. J. Biol. Sci, (13), 546-550.
21. Collins, C.H.,P.M.Lyne and J.M.Granje. (1995).MicrobiologicalMethod.Butterworth and Heinemann Puplishers ,London.PP:129-131.
22. Deng, A., Wua, J., Zhang, Y., Zhang, G., & Wena, T. (2010). Purification and characterization of a surfactant-stable high-alkaline protease from Bacillus sp. B001.Bioresource Technol. (101), 7100-7106.
23. Fulzele R. (2011). Characterization of novel extracellular protease produced by marine bacterial isolate from the indian ocean. *Brazilian Journal of Microbiology*, 1364–1373.
24. Gulve, R.M. & Deshmukh, A.M. (2012).Antimicrobial activity of the marine Actinomycetes. Inter. Multidiscipl. Res. J, 2(3),16-22.
25. Ibrahim, A.S.S. (2007).Isolation and identification of new cellulase producing thermophilic bacteria from an Egyptian hot spring and some properties of the crude enzyme.Aust. J. Basic Appl. Sci. (1), 473–478.
26. Jarallah,I .M.(2014). Screening for Antimicrobial Agents Producing Actinomycetes Isolated from Agricultural Soils in Hilla/Iraq.IOSRJournal.Vol.9(2), 47-52.
27. Jiang C, and Xu L, (1996). Diversity of aquatic actinomycetes in lakes of the middle plateau, yunnan, china. *Applied and environmental microbiology*, *62*(1), 249–53.
28. Kavitha, A., Vijayalakshmi, M., Sudhakar, P., & Narasimha, G. (2010). Screening of actinomycetes strains for the production of antifungal metabolites. *African Journal of Microbiology Research*, *4*(1), 027-032.
29. Kar, S., &Ray, R. C. (2008). Statistical optimization of alpha amylase production by Streptomyces erumpens MTCC 7317 cells in calcium alginate beads using response surface methodology. Polish J. Microbiol, (57), 49-57.
30. Kavya, M., Solomon, M., & Devamma,N.(2012).Isolation and screening of Streptomycetes sp. from Coringa Mangrove for enzyme production and antimicrobial activity.Inter.J.Pharmceut.Chemical&Biological Sciences,2(1),110-116.
31. Khamna, S., Yokota, A., & Lumyong, S. (2009). L-Asparaginase production by actinomycetes isolated from some Thai medicinal plant rhizosphere soils. *Int. J. Integr. Biol.* 6(1), 22-26.
32. Kumar, M.S., Selvam, K. (2011).Isolation and purification of high efficiency L-asparaginase by quantitative preparative continuous elution SDS PAGE electrophoresis.J. Microb. Biochem. Technol, 3(5), 073-083.
33. Kuhad, R.C., Gupta, R., & Singh, A. (2011). Microbial cellulases and their industrial applications.Enzyme Res. 1-10.
34. Limkhada, J.R., Mander, P., Cho, S.S., & Yoo, J.C. (2010). A novel fibrinolytic protease from Streptomyces sp. CS684.ProcBiochem. (45), 88-93.
35. Mane, U.V., & Deshmukh, A.M. (2009). Chitin degrading potential of three aquatic actinomycetes and its optimization. *African Journal of Biotechnology*, *8*(23),6617–6620.
36. Mohan, R., & Vijayakumar, R. (2007).Isolation and characterization of marine antagonistic actinomycetes from west coast of India. *Medcine&Biology .15(1), 13-19.*
37. Pridham, T. G., &Tresner, R. D. (1975). Family VU.StreptomycetaceaeWaksman and Henrici 1943, 339.fu: RE Buchanan and NE Gibson (eds). Bergey's Manual od Determinative Bacteriology. 8thed :747-845. Wi1liams and Wilkins, Baltimore, Maryland.
38. Ribitscha, D., Karla, W., Birner-Gruenbergera, R., Grubera, K., Eiteljoerg, I., Remlera, P. (2010). C-terminal truncation of a metagenome-derived detergent protease for effective expression in E. coli.J Biotech, (150), 408-416.

39. Rifaat, H.M. (2003). The biodiversity of Actinomycetes in the River Nile exhibiting antifungal activity. *Journal of Mediterranean Ecology*, *4*(3), 5–7.
40. Rizvi, R., Kamble, L., & Kadam, A. (2012). Searching the submerged: A report on prevalence of actinomycetes in sediments of river Godavari and optimized strategy for their isolation. *Trends in Biotechnology Research*, *1*(2):16–19.
41. Savitri, N.A., & Azmi, W. (2003). Microbial L-asparaginase: A potent antitumour enzyme. Ind. J. Biotechnol, (2), 184-194.
42. Tawiah, (2012). Antibiotic producing microorganisms from River Wiwi , Lake Bosomtwe and the Gulf of Guinea at Doakor Sea Beach, Ghana. *BMC Microbiology*, (*12*), 234.
43. Tonkova, A. (2006). Microbial starch converting enzymes of the amylase family, In: Ray, C.R. and O.P. words. *Mirobial biotech.Horticult, (1), 421-472.*