ISSN 0976 – 3333

Available Online at www.ijpba.info

International Journal of Pharmaceutical & Biological Archives 2012; 3(5): 1142-1149

ORIGINAL RESEARCH ARTICLE

Isolation and Screening of α-glucosidase Enzyme Inhibitor Producing Marine Actinobacteria Isolated from Pichavaram Mangrove

K.Sathiyaseelan* and D. Stella

Department of Microbiology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, Chidambaram - 608 002, Tamil Nadu, India

Received 29 May 2012; Revised 02 Oct 2012; Accepted 12 Oct 2012

ABSTRACT

Most of the bioactive microbial metabolites were isolated from Actinobacteria especially from *Streptomycetes* and also from some rare actinomycetes. During the last 20-30 years, the interest in the marine microflora increased due to the investigation of novel bioactive compounds especially antibiotics and enzymes. The sediment samples were collected from different stations of the Pichavaram mangrove ecosystem (11°24'-11°27'N and 79°46'-79°48'), situated along the Southeast coast of India, for isolation of actinomycetes, using Kuster agar medium. The isolated strains were identified using physiological and biochemical methods. Eight *Actinomycetes* sp. were isolated and tested for their antagonistic activity against human pathogens. Antagonistic activity strains were tested against α-glucosidase inhibitors activity. The isolated strains showed significant inhibitory activity against α -glucosidase enzyme. Of the 8 strains, only one strains showed significant α -glucosidase inhibitory activity.

Key words: Actinomycetes, Mangrove ecosystem, Antagonistic activity and α-glucosidase inhibitors activity.

1. INTRODUCTION

Marine microorganisms have innumerable new genes and biochemical approaches, and available enzymes, antibiotics and other useful molecules. As to biological technology, they are really a huge gold mine. Thus, the combination of biological technology and marine microorganism research will create a mutual benefit prospect. In the nearly 300 new compounds, almost 80% of them refer to bioactivities, especially some important discoveries of pharmacology and toxicology. But, the microorganisms from mangrove have not yet gained enough recognition until now. Mangrove has a special ecological status, for they locate at the transitions between marine-derived and terrene-derived plants. Some scientists evaluated that the 80% of marine microorganisms can be found in mangroves. Mangrove is a big source of diverse microorganisms and their metabolites, and will attract more and more scientists to devote time to its research [1].

Natural products or derivatives thereof are widely used in human medicine to fight numerous diseases, including bacterial, viral and fungal infections, cancer, and immune system disorders, etc. Actinomycete bacteria have proven to be a rich source of biologically active natural products, and a number of terrestrial actinomycetes, especially those belonging to the genus *Streptomyces*, are being extensively used for commercial production of different medically important compounds. As the search for producers of novel compounds continues, it becomes apparent that many terrestrial *Streptomyces* isolated from different environments produce the same compounds, probably due to frequent genetic exchange between the species. Therefore, the chance of finding genuinely new biologically active molecules while isolating and screening large libraries of actinomycetes is greatly reduced [2]. The situation is worsened by the fact that *Streptomyces* species are easy to isolate and cultivate, and they generally dominate the collections. On the other hand, evidence was being accumulated that rare actinomycetes, which are often very difficult to isolate and cultivate, might represent a unique source of novel biologically active compounds [3].

***Corresponding Author:** Dr. K.Sathiyaseelan**, Email:** drsathiyaseelan@gmail.com

At present, more than 80% of drug substances were natural products or inspired by a natural compounds. The natural products include compounds from plants, microbes and animals. They cover a range of therapeutic indications like anticancer, antimicrobial and antidiabetic [4]. The most striking feature of the genus *Streptomyces* and closely related genera is their ability to produce a wide variety of secondary metabolites. These natural products have been an extraordinary rich source for lead structures in the development of new successful drugs. In addition to the field of antimicrobials, further compounds have been approved as immunosuppressants (FK-506, rapamycin, ascomycin), as anticancer compounds (bleomycin, dactinomycin, doxorubicin, staurosporin), as antifungal compounds (amphotericin B, nystatin), as herbicides (phosphinothricin), in the treatment of diabetes (acarbose) and as anthelmintic agents (avermectin, milbemycin). Of the 12,000 secondary metabolites with antibiotic activity known in 1995, 55% were produced by *Streptomyces* and additional 11% by other actinomycetes. According to a mathematical modeling, only 3% of all antibacterial agents synthesized by *Streptomyces* have been reported [5]. This leaves a vast amount of possible new drugs to be discovered or generated by novel innovative methods.

α-Glucosidase is a very important enzyme responsible for the hydrolysis of dietary disaccharides into absorbable monosaccharide in microbial system and in small intestine of animal digestive system. Glucosidases are not only essential for carbohydrate digestion but it is also very important for processing of glycoproteins and glycolipids and are also involved in a variety of metabolic disorders and other diseases such as diabetes [6].

α-Glucosidase inhibitors are useful for the control of carbohydrates dependent diseases such as diabetes and obesity. Studies on glycosidase inhibitors proved that they are the prospective therapeutic agents for the disorders caused by glucosidases. When compared to the enzyme inhibitors from animal and plants, the microbial inhibitors possess low molecular weight compounds derived from hydrolysis of macromolecular substances. The terrestrial sources of new enzyme inhibitors seem to be rare and different species of microorganisms may produce structurally identical inhibitors [7] and thereby provides limited options for enzyme inhibition activities.

An alternative approach to prevent postprandial hyperglycemia involves the use drugs that function as competitive inhibitors of small intestinal brush-border alpha-glucosidases. By inhibiting these enzymes, the digestion of non-absorbable poly- and oligosaccharides (starch, sucrose) was prevented and thus the formation of absorbable monosaccharides (glucose, fructose) was delayed. The efficacy of this approach was supported by the introduction of acarbose several years ago.

2. MATERIALS AND METHODS

2.1. Sampling:

A study was done by the collection of samples from Pichavaram Mangroves.

2.2. Sample collection:

The samples were collected in the month of February, from top 4 cm soil profile where most of the microbial activity takes place and thus where most of the bacterial population is concentrated. Soil samples (approximately 500g) were collected by using clean, dry and sterile polythene bags along with sterile spatula and other accessories. The site selection was done by taking care of the point where widely varying characteristics as possible with regard to the organic matter, moisture content, and particle size and colour of soil and to avoid contamination as far as possible. Samples were stored in iceboxes and transported to the laboratory where they were kept in refrigerator at 4^0C until analysis.

2.3. Isolation and enumeration of Actinomycetes population from collected soil samples

The soil samples collected from three different locations of mangrove ecosystem were pooled and one ml soil sample was transferred to 100ml of sterile distilled water in a 250ml Erlenmeyer flask and incubated on a rotator shaker (100rpm) for 30 minutes at 37°C. The well mixed suspension was then diluted appropriately up to 10^{-6} dilution. One ml of suspension from 10^{-4} dilution was aseptically transferred to sterile petriplates and then 10-20ml of selective Starch Casein agar medium was added and incubated at 37ºC for 7 days. Three replications were maintained for each dilution. The colonies were counted by using Qubec colony counter. The total number of colonies in the original samples was expressed as cfu g^{-1}. The isolated Actinomycetes strains were identified on the

© 2010, IJPBA. All Rights Reserved.

basis of their phenotypic characterization, physiological characteristics and biochemical characteristics. It includes some basic tests, aerial mass colour, reverse side pigment, melanoid pigments and spore chain morphology

2.3.1. Aerial mass colour:

For the grouping and identification of actinomycetes the Chromogenicity of the aerial mycelium is considered to be an important character. The colours of the mature sporulating aerial mycelium are white, gray, red, green, blue and violet. When the aerial mass colour falls between two colours series, both the colors are recorded. In the cases where aerial mass colour of a strain showed intermediate tints, then in that place both the colour series should be noted.

2.3.2. Reverse side pigments*:*

The strains are divided into two groups according to their ability to produce characteristic pigments on the reverse side of the colony, called as distinctive (+) and not distinctive or none (-).A colour with low chroma such as pale yellow, olive or yellowish brown occurs, it is included in the latter group (-).

2.3.3. Melanoid Pigments*:*

The grouping is made on the production of melanoid pigments (i.e. greenish brown, brownish black or distinct brown pigment modified by other colours) on the medium. The strains are grouped as melanoid pigment produced (+) and not produced (-) (6). For the melanoid pigment observation, the inoculated plates where kept under incubator for 4 to 5 days. The strains which shows cultures forming a greenish brown to brown to black diffusible pigment or a distinct brown pigment modified by other color are recorded as positive (+) total absence of diffusible pigment, are recorded as negative (-) for melanoid pigment production.

2.3.4. Spore chain morphology:

The species with spore bearing hyphae are reported to be three types:-Flexible-Rectiflexibiles (RF), Open loops-Retinaculiaperti (RA) and Spira- Spirales (S). A characteristic of the spore bearing hyphae and spore chains is determined by the direct microscopic examination of the culture area. Adequate magnification used to establish the presence or absence of spore chains and to observe the nature of spore chains is 40x. By the standard protocol of Cover slip culture technique, the plates were prepared and after the incubation of 7 to 10 days it was observed. During this method of spore morphological study, plates have to be prepared containing ISP2 medium. After solidification, by a sharp scalpel from the central portion of the plate, medium should be scooped out making a rectangular area. Then three sterile coverslips have to be placed on the hollow rectangular space. Slowly actinomycetes spores have to be inoculated at the edge of the coverslips touching the medium. The plates must be incubated at 28±2°C for 5 days and examined periodically taking out the coverslips.

2.4. Biochemical characteristics

2.4.1. Assimilation of carbon sources

The ability of different actinomycetes strains in utilizing various carbon compounds as source of energy was studied by following the method recommended in International Streptomyces Project. The Carbon utilization medium was used in this Carbon assimilation test. Stock solution of 10 sugars i.e; D-Glucose, L-Arabinose, D- Sucrose , D-Fructose, D – Xylose, D-Raffinose, D-Mannitol, Cellulose, Rhamnose and Inositol having concentration of 10x was prepared in autoclaved water and sterilized by filtering through 0.22µm pore size membrane filters and stored at 40C. Growth of actinomycetes strain was checked by taking 1% carbon source in ISP2 media. Plates were streaked by inoculation loop by Flame sterilization technique and Incubated at 37^0C for 7 to 10 days. Growth of the isolates was observed by comparing them with positive and negative control.

2.4.2. Sodium chloride tolerance

Different concentrations of sodium chloride (0, 5, 10, 15 and 20%) solution were added to the Starch casein agar medium to check the sodium chloride tolerance test. This test was very much important to understand the native nature of the marine actinomycetes isolates. The isolate was streaked on the agar medium, incubated at 37°C for 7-15 days and the presence or absence of growth was recorded on 7^{th} day onwards.

2.4.3. Degradation of cellulose

1% of Carboxy methyl cellulose (CMC) was added to the ISP 2 media. The plates were inoculated and incubated for 7-15 days. Control plate was used as standard to check the growth of actinomycetes after 7- 15 days for cellulose degradation activity which may be visually observed.

2.4.4. Hydrogen-sulphide production

The inoculated Tryptone-Yeast extract agar slants were incubated for 7 days for this test.

© 2010, IJPBA. All Rights Reserved.

Observations on the presence of the characteristic greenish-brown, brown, bluish-black or black colour of the substrate, indicative of H_2S production were recorded on 7th, 10th and 15th days. The incubated tubes were compared with uninoculated controls.

2.4.5. Gelatin liquefaction

The medium consists of nutrient supplemented with 12% gelatine this high gelatin concentration results in a stiff medium and also serves as the substrate for the activity of gelatinase. Gelatin liquefaction was studied by sub-culturing the strain on gelatin agar medium and inoculated them at 28°C. Observation was made after 7 days. The extent of liquefaction was recorded after keeping the tubes in cold conditions (5-10°C) for an hour. Cultures that remained liquefied were indicative of slow gelatin hydrolysis.

2.4.6. Hydrolysis of starch

Starch hydrolysis was studied by sub-culturing the strains on starch agar medium and incubated at 28^0C. Observation was made after 7 days. The development of clear zone around the culture streaks, when the plates were flooded with Lugol's iodine solution should be recorded as the hydrolysis of starch. The medium is composed of ISP2 Media supplemented with 1% starch, which served as the polysaccharide substrate.

2.4.7. Coagulation of milk

Milk coagulation was studied with skimmed milk (Hi media). The skimmed milk tubes were inoculated and incubated at 37°C. The extent of coagulation was recorded on the 7th and 10th days of incubation. The importance of milk as a culture medium for the study of bacteria has long been recognized by workers in bacteriology. Although, milk is a very complex medium, it is more or less standard in composition and the reactions produced upon it by microorganisms are so characteristic, that it has found general acceptance. The plates were incubated for the 7 to 10 days after the inoculation.

2.4.8. Ability to grow in different pH

pH is defined as logarithm to the base 10 of the inverse of the hydrogen ion concentration (or preferably H^+ ion activity). It is also the negative logarithm to the base 10 of H^+ ion activity. This test was carried out on ISP2media. pH was adjusted to different ranges of 5, 6, 7, 8, 9 and 10. Duplicate slants were prepared for each strain of each range. After the incubation of 10-12 days readings were taken for each strain.

2.5. Screening for antimicrobial activity of isolated actinomycetes

Actinomycetes isolates were inoculated in the Starch casein broth and incubated at 28 ± 2º C for about 10-15 days. After incubation, dense colonies were observed on the broth and few milliliter of culture was taken and centrifuged at 7000 rpm, 4ºC. The cell free filtrate was removed and was used for both antibacterial and antifungal activity.

2.5.1. Antibacterial activity

20 ml of Mueller-Hinton agar was poured on petriplates and overnight of bacterial pathogens broth culture was swabbed on the surface of the agar media and further well was prepared by using well cutter. To each well, 200 µl of crude extract from actinomycetes was added and incubated at 28º C for 72hrs kept in a thermostat incubator. After incubation, the zone of inhibition around the well was calculated and observed as zone of inhibition in mm diameter by comparing them with positive and negative control.

2.5.2. Antifungal activity

20ml of Potato dextrose agar was poured on petriplates and overnight of fungal pathogens broth culture was swabbed on the surface of the agar media and further well was prepared by using well cutter. To each well, 200µl of crude extract from actinomycetes was added and incubated at 28º C for 48 hrs kept in a thermostat incubator. After incubation, the zone of inhibition around the well was calculated and observed as zone of inhibition in mm diameter by comparing them with positive and negative control.

2.6. alpha-glucosidase inhibition activity of actinomycetes isolates

The well grown slant cultures of actinomycetes isolates were inoculated into 50 ml medium in 250ml Erlenmeyer flasks containing the fermentation medium-3 with 25 percent of sea water and 75 percent of distilled water, the pH of the medium was adjusted to 7.2. The production cultures were incubated for 7 days at 28^oC, under constant agitation. A small aliquot (1.5 ml) of culture broth was sampled, after which the supernatants were prepared *via* centrifugation. The α-glucosidase inhibitory activity was then evaluated, using a slightly modified version of the ρ-nitrophenyl- α-D-glucoside (PNPG) method. α-glucosidase

© 2010, IJPBA. All Rights Reserved.

inhibition activity was assayed in 425 µl of reaction mixture, consisting of 100 µl of 0.1 M phosphate buffer (pH 7.2), 200 µl of culture broth, 25 µl of α-glucosidase (2 unit /ml) and 100 µl of PNPG (2 mM/ml) at 28°C for 10 min. The reaction was halted via the addition of 100 µl of 0.5 M Na_2CO_3, and the amount of ρ-nitrophenol (PNP) formed was determined by absorbance at 405 nm (ε=18,000M-1). As a control, culture broth was replaced by 0.1 M phosphate buffer (pH 7.2). Based on the results of antagonistic activity and α-glucosidase inhibition activity the strains were selected for animal study.

3. RESULTS AND DISCUSSION

3.1. Isolation and enumeration of *Actinomycetes* sp:

Distribution and diversity 0f actinobacteria have been reported from marine habitats such marine sediments by Jensen *et al* [8]. The *Actinomycetes* sp. was isolated from the soil samples of two different sites of Mangroves in Pichavaram east costal region of Tamil Nadu. The sediment soil sample was inoculated by Serial dilution techniques and seven pure actinomycetes isolates were obtained by Spread plates and maximum population was noted in dense mangroves (18.25 cfu g^{-4}) (**Table 1**). The strains were identified on the basis of their physiological and biochemical characteristics. Spirals - S; Retinaculum - apertum- RA; Biverticillus – spira - BIV-S.

3.2. Physiological characteristics of the Actinomycetes isolates

The cultural and microscopic characterizations of actinomycetes were showed in (**Table 2**). Aerial mass colour of the substrate mycelium was determined by observing the plates after 7 to 10 days. It was done only after seeing the heavy spore mass surface. The common colours found in the strains were White (W), Yellow (Y), Grey (G) and dark ash. The aerial mass colour of the isolates AP1and AP8 showed white colour. The isolate AP3, AP4 and AP6 exhibited grey coloured aerial mass and the isolates AP2, AP5 and AM7 were Greyish white in colour [9] have also reported that white colour series of actinomycetes they were the dominant forms. Colour series were also recorded in soil, morphological observation of colonial characteristics such as amount and colour of vegetative growth, and the presence and colour of aerial mycelium and spores, and again the presence of diffusible pigments are recorded for each strain studied colonial growth on agar plate. The strains were divided into two groups according to their ability to Produce pigments on the reverse side of the colony, namely distinctive (+) and not distinctive or none (-). Reverse side pigments and Melanoid pigmentation was observed by the formation of greenish brown, brownish black or distinct brown pigment. The colours observed for not distinctive isolates were pale yellow, olive or yellowish brown colour marked as (-). Spore chain morphology was done by the Coverslip culture technique. The slides were examined under microscope of 40x. Spira was showed the strains namely AP1, AP4, AP5, AP7 and AP8. The strains AP2 have showed the Retinaculum-apertum and AP4 have showed the Biverticillus-spira- BIV-S. (10) Reported that the cultural characteristics (pigment production), morphological characteristics and antimicrobial activities of the different *Streptomyces* isolates are presented. Out of 64 *Streptomyces* isolates, 29 produced melanin, 18 showed distinctive reverse side pigment and 11 produced soluble pigments.

The ability of different actinomycetes strains utilizing various carbon compounds namely arabinose, sucrose, fructose, xylose, inositol, raffinose, mannitol, cellulose and rhamnose as source of energy was studied and the results were indicated in (**Table 3**). Sodium chloride tolerance test was very important to understand the native nature of the marine actinomycetes isolates and showed in (**Table 4**). The result was obtained after 7 to 15 days of incubation. Growth was compared with the control plate. The biochemical parameters *viz.*, cellulase activity, H_2S production, gelatinase activity, amylase activity and caseinase activity were shown in (**Table 5**).

The results obtained from the experiments done were matched with the keys given for 458 species of actinomycetes included in ISP (International Streptomyces Project) and the species identification was done, the results obtained are showed in (**Table 6**). The match was done on the basis of maximum percentage of resemblance of characteristics.

3.3. Antimicrobial Activity

3.3.1. Anti-bacterial activity of actinomycetes sp. isolated from

Antibacterial activity of *Actinomycetes* sp. isolated from Pichavaram mangrove was showed in (**Table 7**). Among the eight isolates, only AP8

© 2010, IJPBA. All Rights Reserved.

has showed the maximum zone of inhibition against all the pathogens. The isolate AP1 and AP5 have showed the zone of inhibition against *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus aureus* and did not showed the inhibition against *Bacillus cereus.* The isolates AP3 and AP7 showed the zone of inhibition against *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Bacillus cereus* and did not show any zone of inhibition against *Escherichia coli* and *Staphylococcus aureus.* The isolates AP4 showed the zone of inhibition against all pathogens except *Pseudomonas aeruginosa.* The isolates AP6 showed the zone of inhibition against *Escherichia coli, Bacillus cereus* and *Staphylococcus aureus* and did not show activity against *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*.

3.3.2. Antifungal Activity

Antifungal activity of *Actinomycetes* sp. isolated from Pichavaram mangroves and the results were showed in (**Table 9**). Among the eight isolates, only AP8 has showed the maximum zone of inhibition against all the pathogens. All isolates has showed zone of inhibition against *Aspergillus niger*. The Isolates AP1, AP2, AP4, AP5 and AP6 has showed zone of inhibition against *Aspergillus oryzae.* The isolates AP3, AP4 and AP5 has showed zone of inhibition against *Aspergillus terreus* and other isolates did not showed activity. All the isolates did not show the inhibition against *Penicillium citrinum.* Various reports suggested that the actinomycetes inhibiting the growth of antibiotic - resistant microorganisms produce more active antimicrobial substances than the conventional antibacterial sources. The selected 10 isolates showed good antibacterial activity. This characteristic features not remains with antibacterial alone and also added antifungal activity too. This wide spectrum activity nature was very common in marine actinomycetes [11].

3.3.3. Effect of the α-glucosidase inhibition activity of ethyl acetate extracts actinomycetes

α-glucosidase activity of *Actinomycetes* sp. isolated from mangrove sediments and results were showed in Table-9. Among all the isolates, AP8 has showed the maximum α-glucosidase inhibition activity. The isolates AP4, AP7, have showed least inhibition of α-glucosidase activity. Other strain did not show the α-glucosidase activity. Alpha-glucosidase inhibitors interfere with enzymatic action in the brush border of the small bowel, slowing the breakdown of polysaccharides and disaccharides to glucose and thereby delaying glucose absorption and decreasing postprandial plasma glucose levels. Due of this only the α-glucosidase inhibitors lowers the postprandial and not fasting plasma glucose levels [12]. Most α -glucosidase inhibitors of microbial origin have been isolated from terrestrial *Actinobacteria* and there are no reports on α -glucosidase inhibitor producing marine microbes except of [13] who reported the ability of the *Streptomyces* strains in production of inhibitors in presence of salt.

Table1: Enumeration of Actinomycetes

Isolates	Total actinomycetes Population ($x10^4$CFU/g) in Pichavaram
AP1	12.23
AP2	14.23
AP3	14.45
AP4	13.74
AP5	16.64
AP6	17.34
AP7	12.23
AP8	18.25

Table 2: Physiological characteristics of the Actinomycetes isolates

Isolates	Aerial mass colour	Melanoid pigmentation	Reverse side pigments	Spore chain morphology
AP1	W	_	_	S
AP2	Gy (W)	+	+	R
AP3	Gy	_	_	BIV-S
AP4	Gy	+	_	S
AP5	Gy (W)	+	_	S
AP6	Gy	+	+	RA
AP7	Gy (W)	+	+	S
AP8	W	-	+	S

Spirals- S; Retinaculum-apertum- RA; Biverticillus-spira- BIV-S

Table 3: Results of Assimilation of carbon sources in isolated *Actiomycetes* sp

S.No	Isolates	Xylose	Inositol	Sucrose	Raffinose	Fructose	Rhamnose	Mannitol
1	AP1	+	-	_	_	±	_	+
2	AP2	+	+	+	±	+	+	+
3	AP3	+	+	+	±	+	+	+
4	AP4	+	+	+	+	±	+	+
5	AP5	+	+	+	±	+	+	+
6	AP6	+	+	+	_	+	+	+
7	AP7	+	+	+	+	+	+	+
8	AP8	+	+	+	+	+	+	±

© 2010, IJPBA. All Rights Reserved.

Table 4: Results of Sodium chloride tolerance test at different concentrations

Isolates	5%	10%	15%	20%
AP1	+	-	-	-
AP2	+	±	-	-
AP3	+	±	-	-
AP4	+	-	-	-
AP5	+	-	-	-
AP6	+	±	-	-
AP7	+	-	-	-
AP8	+	±	-	-

Table 5: Biochemical activity of actinomycetes

Isolates	Cellulase activity	H_2S production	Gelatinase activity	Amylase activity	Caseinase activity
AP1	+	-	_	+++	+
AP2	+	+	+	+	+
AP3	+	+	+	-	-
AP4	+	+	+	+	±
AP5	+	+	+	+	+
AP6	+	+	+	+++	++
AP7	+	+	+	+++	++
AP8	+	+	+	+	++

Table 6: Results obtained from the keys given for 458 species of Actinomycetes included in ISP (International Streptomyces Project)

S. No	Isolates	Species Name
1	AP1	*Streptomyces almquisti*
2	AP2	*Streptomyces vastus*
3	AP3	*Streptomyces alni*
4	AP4	*Streptomyces luteogriseus*
5	AP5	*Actinomycetes longiporus*
6	AP6	*Actinomycetes malachitorectus*
7	AP7	*Streptomyces neyagawaensis*
8	AP8	*Streptomyces xantholiticus*

Table 7: Anti-bacterial activity of *Actinomycetes* sp. isolated from Pichavaram mangroves

		Zone of inhibition (mm)				
S.No	Isolates	*P.aeruginosa*	*E.coli*	*K.pneumoniae*	*B.cereus*	*S.aureus*
1	AP1	15	12	10	NZ	10
2	AP2	18	10	16	17	16
3	AP3	13	NZ	8	10	NZ
4	AP4	NZ	13	10	14	7
5	AP5	12	9	12	NZ	11
6	AP6	NZ	13	NZ	14	12
7	AP7	16	NZ	5	10	NZ
8	AP8	35	40	28	37	32

NZ – No zone of inhibition

Table 8: Antifungal activity of *Actinomycetes* sp. isolated from Pichavaram mangroves

S. No	Isolates	Zone of inhibition (mm)			
		A.niger	*A.oryzae*	*A.terreus*	*P.citrinum*
1	AP1	10	16	NZ	NZ
2	AP2	7	10	NZ	NZ
3	AP3	13	NZ	8	NZ
4	AP4	11	13	10	NZ
5	AP5	12	9	13	NZ
6	AP6	8	13	NZ	NZ
7	AP7	14	NZ	8	NZ
8	AP8	32	28	36	25

Table 9: Effect of the α-glucosidase inhibition activity of ethyl acetate extracts actinomycetes

Isolates	OD 405 (nm) / inhibition concentration of r-nitrophenol (µM)
Control	2.425
AP1	2.254
AP2	2.204
AP3	2.345
AP4	1.984
AP5	2.408
AP6	2.345
AP7	1.879
AP8	0.482

REFERENCES

1. XiaohongLiua., Fang Xu., Changlun Shao., Zhigang Shea., YongchengLin., and Wing Lai Chan., 2008. Bioactive metabolites from marine microorganisms. Atta-ur-Rahman(Ed.) *Studies in Natural Products Chemistry*, Vol. 35.
2. Busti, E., 2006. Antibiotic-producing ability by representatives of a newly discovered linage of actinomycetes. J. *Microbiology* 152, 675– 683
3. Baltz, R. H., 2006. Marcel Faber Roundtable, Is our antibiotic pipeline unproductive because of starvation, constitution or lack of inspiration? *Journal of Industrial Microbiology and Biotechnology* 33, 507-513.
4. Harvey, A., 2008. Strategies for discovering drugs from previously unexplored natural products. *DDT;*5: 294–300.
5. Watve, M.G., Tickoo, R., Jog, M.M and Bhole, B.D., 2001. Antibiotics are produced by the genus *Streptomyces*. *Arch. Microbiol*. 176, 386–390.
6. Jenkins, DJ., Taylor, RH., Goff, DV., Fielden, H., Misiewicz, JJ., Sarson, DL., Bloom, SR and Alberti, KG., 1981. Scope and Specificity of Acarbose in Slowing Carbohydrates absorption in Man. Diabetes, 30: 951- 954.
7. Hamato, N., Takano, R., Kamei-Hayashi, T., Imada, C and Hara, S., 1992. Leupeptins produced by the marine Alteromonas sp. B-10-31. *Biosci. Biotech. Biochem.*, 56: 1316-1318.
8. Jensen, P. R., Gontang, E., Mafnas, C., Mincer, T. J., and Fenical, W., 2005.Culturable marine Actinomycetes diversity from tropical Pacific Ocean sediments. *Applied and Environmental Microbiology* 7,1039- 1048.
9. Vanajakumar, Selvakumar.N and Natarajan.R,(1995). Antagonistic properties of actinomycetes isolated from mollusks of the porto Novo region, south india; 267-274.
10. SujathaPeela, VVSN.,BapirajuKurada., and RamanaTerli., 2005. Studies on antagonistic marine actinomycetes from the Bay of Bengal. *World Journal of Microbiology & Biotechnology* 21:583–585.
11. Ramesh, S., 2009. Marine actinomycetes diversity in Bay of Bengal, India: Isolation and characterization of bioactive compounds from *Streptomyces*

© 2010, IJPBA. All Rights Reserved.

fungicidicus MML 1614. Ph.D., thesis, University of Madras, Chennai, India.

12. Bell D,S., 2004. Type 2 diabetes mellitus: what is the optimal treatment regimen? Am. J. Med., 116: 23-29.
13. Imada, C., 2005. Enzymes inhibitors of marine microbial origin with pharmaceutical importance. *Mar. Biotechnol*., 6: 193-198.

© 2010, IJPBA. All Rights Reserved.