## Original Research Article

# Screening Of Industrially Important Enzymes Of Potential Marine Actinobacteria Of The Neil Island, The Andamans, India

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**Abstract:** Marine actinobacteria are efficient producers of important secondary metabolites that show a range of biological activities and capable of catalyzing various biochemical reactions with novel enzymes. With this in mind, present study was carried out to screen the industrially important enzymes [L-asparaginase, Cellulase, Deoxyribonuclease (DNase) and Chitinase] of the marine actinobacteria, isolated from Neil island, the Andamans. Eight morphologically distinct actinobacterial strains (AUANI-1 to AUANI-8) were subjected to enzyme screening for L-asparaginase, cellulase, DNase and chitinase activity, using spot inoculation assay, with different enzymatic media. Among them, strain AUANI-1 showed higher L-asparaginase enzyme production with 12 mm of clear zone, strain AUANI-5 showed higher cellulase enzyme production with 18 mm of clear zone, strain AUANI-7 showed higher DNase enzyme production with 12 mm of clear zone and strain AUANI-8 showed higher chitinase enzyme production with 17 mm of clear zone. Based on the enzyme production performance, these four potential strains were selected for conventional identification. Results were: AUANI-1 – *Streptomyces nodosus,* AUANI-5- *S. craterifer,* AUANI-7-*S. moderatus* and AUANI-8-*S. aureofasciculus.* Thus, the present study concludes that the sediment samples of the Neil island, the Andamans contain a good member of culturable strains of *Streptomyces*. These strains are capable of producing different enzymes *viz.* L-asparaginase, cellulase, DNase and chitinase. These potential strains can be further evaluated for commercial scale production of enzymes that can be employed in varied biotechnological and industrial applications.

**Key words**: Actinobacteria, cellulase, chitinase, DNase, L-asparaginase, Neil island

## Introduction

Oceans are the home to huge microbial populations (Stach *et al.,* 2005; Sogin *et al.,* 2006) and microbes live in every corner of the ocean and their habitats are diverse; they are distributed in open waters, sediments, associated with many organisms, estuaries, hydrothermal vents etc. (Cevera *et al.,* 2005). They are always involved in the important processes of the sea in promoting organic material transformation and mineralization in the sediments and overlying waters (Das *et al.,* 2007). Among the different marine microbes, actinobacteria are ubiquitous in nature (Sethubathi *et al.,* 2013) and play important ecological roles and substantially impact the cycling of complex carbon substrates in the benthic and other ocean habitats (Mincer *et al.,* 2002).

Biological and chemical diversity of the marine environment has been the source of unique chemical compounds with the potential for development as pharmaceuticals, cosmetics, nutritional supplements, molecular probes, enzymes, fine chemicals and agrochemicals (Ireland

*et al.,* 1993). Especially, marine actinobacteria are efficient producers of innovative secondary metabolites that show a range of biological activities including antibacterial, antifungal, anticancer, antioxidant and insecticidal substances as well as enzyme inhibitors and enzymes (Solanki *et al.,* 2008). They are capable of catalyzing various biochemical reactions with novel enzymes (Sivakumar *et al.,* 2007), because they are metabolically active in the marine environment, producing various compounds that are not observed in terrestrial strains (Jensen *et al.,* 1991). Considering these, the present study was conducted to screen the industrially important enzymes (L-asparaginase, Cellulase, Deoxyribonuclease and Chitinase) produced by the marine actinobacteria of the Neil island, the Andamans and identify them with conventional methods.

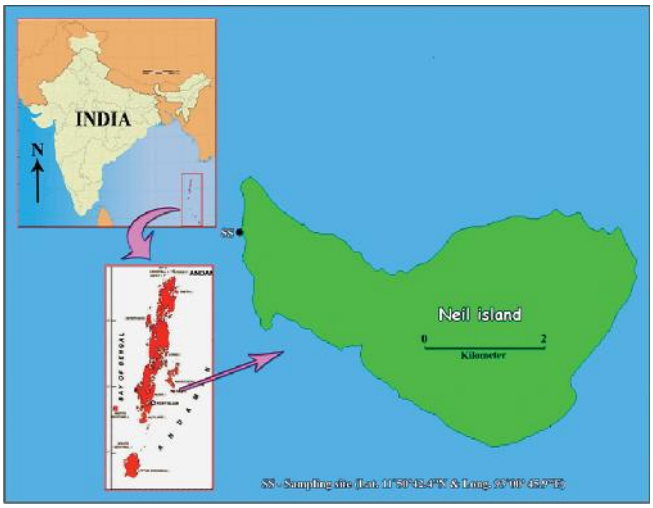


**Fig.**1. Map showing sampling station in the Neil island.

## Materials and methods

### Collection of samples

Sediment samples were collected from the Neil island (Fig.1), at a depth of 25 cm, using a sterile spatula, during November, 2012 for actinobacteriological analysis. The samples were placed in sterile polythene covers and brought to the field laboratory immediately and after arrival, necessary dilutions were made to carry out further analysis.

### Isolation of actinobacteria

Isolation of actinobacterial strains was carried out using Kuster's agar medium (Glycerol -10 g, Casein - 3 g, $KNO_3$ - 2 g, NaCl -2 g, $K_2HPO_4$ - 2 g, $MgSO_4 \cdot 7H_2O$ - 0.05 g, $CaCO_3$ - 0.02g, $FeSO_4 \cdot 7H_2O$ -0.01 g, Agar - 15 g, 50% Sea water -1000 ml, pH 7±0.1). Petriplates containing the autoclaved Kuster's agar medium were prepared aseptically. To minimize the fungal and bacterial contamination, the agar medium was supplemented with cycloheximide (10 µg/ml) and nalidixic acid (10 µg/ml) respectively (Lee *et al.,* 2014). One gram of pretreated sediment samples were serially diluted using sterile seawater and 0.1 ml of serially diluted samples were added to the petriplates containing Kuster's agar medium (Kuster and Williams, 1964) and spread using a 'L' shaped glass spreader. The plates were incubated at 37°C for seven days in an inverted position. Colonies of actinobacteria that appeared on the petriplates were counted from the 5$^{th}$ day onwards upto 28$^{th}$ day. After the incubation period, morphologically distinct colonies were picked up from the petriplates and restreaked in appropriate media and pure cultures were obtained and maintained at 4°C for further studies.

### Enzyme screening

Morphologically distinct actinobacterial strains were subjected to screening for L-asparaginase, cellulase, DNase and chitinase activity. Productions of extracellular enzymes by these strains were studied, using different enzymatic agar media and they are as follows.

For screening L-asparaginase, each actinobacterial strain was individually inoculated on glycerol asparagines (GA) agar (Pridham and Lyons, 1961), incorporated with pH indicator; pH was adjusted to 7.0 and incubated at 37 °C for seven days. Colonies with pink zones were considered as L-asparaginase producing active strains. Two control plates were also prepared using glycerol asparagine agar; one was without dye while the other was without asparagine.

For screening cellulase, carboxy methyl cellulose (CMC) agar medium was autoclaved and dispensed into petridishes and allowed to solidify. A loopful of culture was streaked on the medium and incubated at 37°C for 5-7 days. After the growth for 5-7 days, the plates were flooded with iodine chemical solution (0.1 ml HCl + 5 ml of 1% iodine in 2% KI). Formation of clear zone around the colony against

reddish-brown background indicated the cellulolytic activity of the strain (Radhakrishnan *et al.,* 2007).

DNase test (DNaseT) agar medium was autoclaved and dispensed into petriplates and allowed to solidify. A loopful of culture was streaked on the medium and incubated at 37°C for 5-7 days. After the growth for 5-7 days (DNase test agar containing toludine blue and methyl green was employed for this purpose), decolorization of blue and green colors was noted (Schreier, 1969, Smith *et al.,* 1969).

For screening chitinase, colloidal chitin (CC) agar medium was autoclaved and dispensed into petriplates and allowed to solidify. Colloidal chitin was prepared from the chitin (Hi Media) by the modified method of Hsu and Lockwood (Hsu and Lockwood, 1975). A loopful of culture was streaked on the medium and incubated at 37°C for 5-7 days. After the growth for 5-7 days, colonies showing clear zones on a creamish background were considered as chitinase producing.

Ratio of the clear zone diameter to colony diameter was measured in order to select the highest enzyme activity producing strains.

### Identification of actinobacteria

Characterization and subsequent identification of the strains to the genus level were made based on the criteria of Cummins and Harris (1956), Shirling and Gottlieb (1966), Lechevalier and Lechevalier (1970) and Nonomura (1974).

Along with the cultural characteristics, melanoid pigments, reverse side pigments, soluble pigments, spore chain morphology and assimilation of carbon sources were studied, using the standard methods recommended by the International *Streptomyces* Project (Shirling and Gottlieb, 1966).

## Results

### Isolation of actinobacteria

Actinobacterial colonies were isolated from the sediment samples of the Neil Island using Kuster's agar medium (Fig. 2) and the actinobacterial population density was 12 x$10^3$ CFU $g^{-1}$. A total of eight morphologically distinct actinobacterial strains were selected. These strains were labeled as AUANI-1, AUANI-2, AUANI-3, AUANI-4, AUANI-5, AUANI-6, AUANI-7 and AUANI-8.

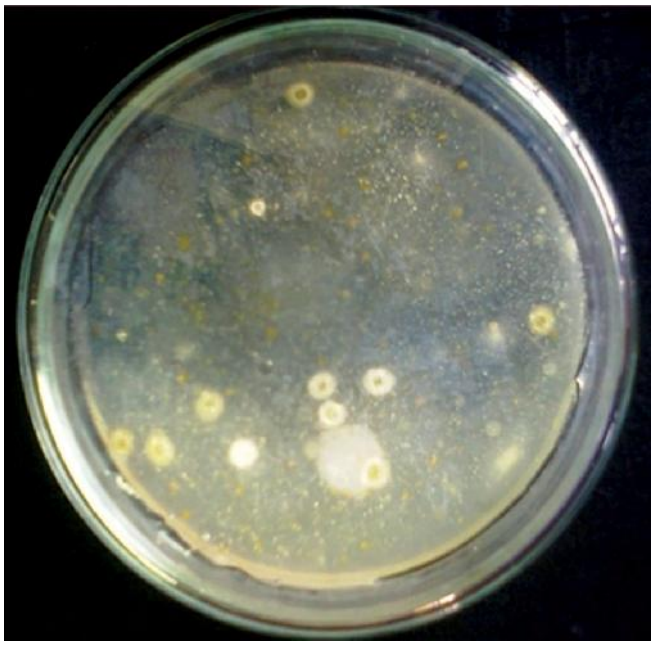

**Fig.**2. Actinobacterial colonies in Kuster's agar medium, isolated from the sediment samples of the Neil island.

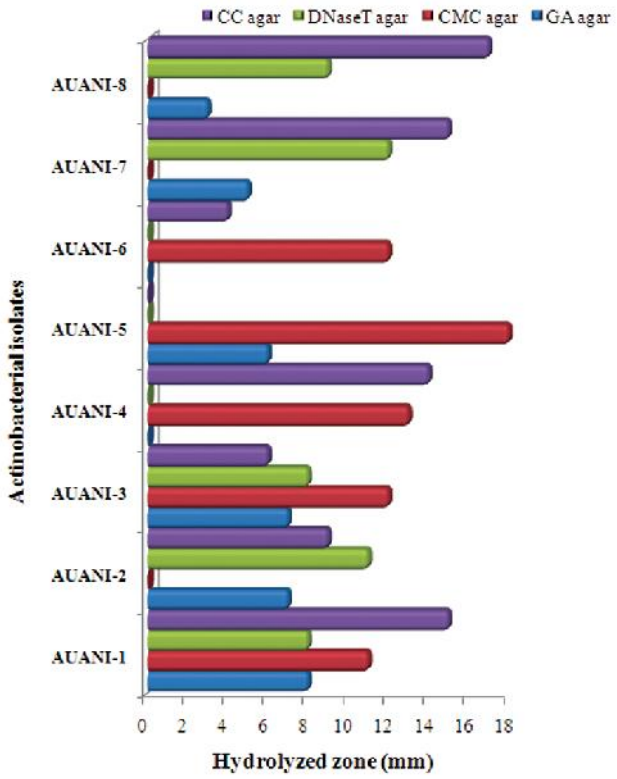


**Fig.** 3. Screening of actinobacterial strains for enzyme production in different enzymatic agar media.

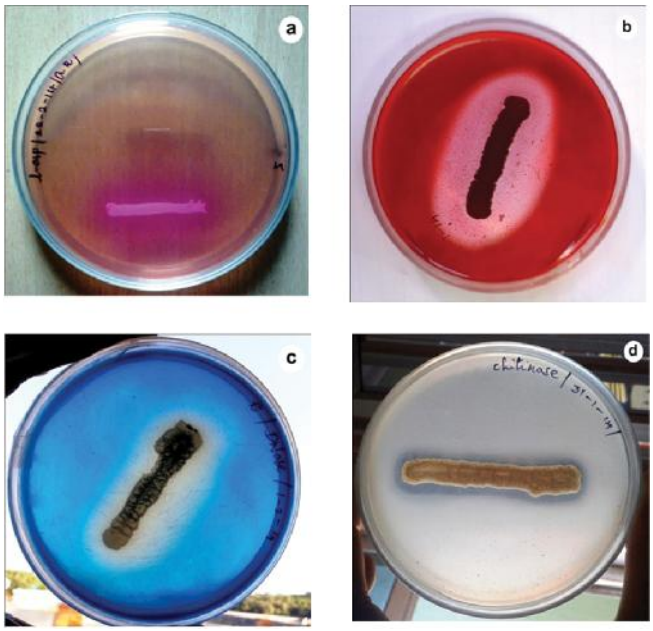


**Fig.** 4. a. L-asparaginase degradation activity of the strain AUANI-1, in Glycerol asparagine agar medium, b. Cellulose degradation activity of the strain AUANI-5, in Carboxy methyl cellulose agar medium, c. DNase degradation activity of the strain AUANI-7, in DNase test agar medium and d. Chitinase degradation activity of the strain AUANI-8, in Colloidal chitin agar medium.

## Enzyme screening

All the eight morphologically distinct strains isolated in the present study, were subjected to different enzyme screening assays *viz.* L-asparaginase, cellulase, DNase and chitinase using different enzymatic agar media and the results are depicted in Fig. 3.

Screening for L-asparaginase enzyme production was done for the actinobacterial strains, using the Glycerol asparagine agar medium. Strain AUANI-1 produced higher amount of L-asparaginase enzyme with 12 mm of clear zone (Fig. 4a) and lower amount (3 mm) in AUANI-8. Strains AUANI-4 and AUANI-6 did not produce L-asparaginase.

Screening for cellulase enzyme production was done for the eight isolated actinobacterial strains, using the Carboxy methyl cellulose agar medium. Higher cellulase enzyme production was found in the strain AUANI-5 with 18 mm of clear zone (Fig. 4b) and lower enzyme production (12 mm) was found in AUANI-3 and AUANI-6. Strains AUANI-2, AUANI-7 and AUANI-8 did not produce cellulase.

Screening for DNase enzyme production was done for the eight isolated actinobacterial strains, using the DNase test agar medium. Higher DNase enzyme production was found in the strain AUANI-7 with 12 mm of clear zone (Fig. 4c) and lower enzyme production (8 mm) was found in AUANI-1 and AUANI-3. Strains AUANI-4, AUANI-5 and AUANI-6 did not produce DNase enzyme.

Screening for chitinase enzyme production was also done for the eight isolated actinobacterial strains, using the Colloidal chitin agar medium. Higher chitinase enzyme production was found in the strain AUANI-8 with 17 mm of clear zone (Fig. 4d) and lower enzyme production (4 mm) was found in AUANI-6. Strain AUANI-5 did not produce chitinase enzyme.

**Table** 1. Cell wall analysis of the strains isolated from the Neil island.

| Strain No. | Cell wall amino acids | | | Whole cell sugar | Cell wall chemo type |
|---|---|---|---|---|---|
| | LL-DAP | Meso DAP | Glycine | | |
| AUANI-1 | + | - | + | * | I |
| AUANI-5 | + | - | + | * | I |
| AUANI-7 | + | - | + | * | I |
| AUANI-8 | + | - | + | * | I |

(+) Presence, (-) Absence, (*) No characteristic sugar pattern.

## Identification of actinobacteria

Based on the enzyme production performance, four potential strains were selected for conventional identification. They were AUANI-1 (L-asparaginase), AUANI-5 (cellulase), AUANI-7 (DNase) and AUANI-8 (chitinase). All these strains (AUANI-1, AUANI-5, AUANI-7 and AUANI-8) showed the presence of LL-DAP along with glycine of the peptidoglycan layer with no characteristic sugar pattern, indicating that these strains belong to the cell wall chemo type I (Table 1). The genera belonging to the cell wall type–I are *Streptomyces, Streptoverticillium, Chainia, Actinopycnidium, Actinosporangium, Elytrosporangium, Microellobosporia, Sporichthya* and *Intrasporangium* (Lechevalier and Lechevalier, 1970). It is important to note that the presence of spores in a long chain occurring on the aerial mycelium and branched nature of the substrate mycelium eliminate all the other genera having the cell wall type I except *Streptomyces* (Lechevalier

and Lechevalier, 1970). This clearly indicated that these four stains belong to the genus *Streptomyces.*

Cultural and morphological characters and their carbon source utilization were also analyzed to identify the isolates. Key characters of the potential strains were compared with those of the *Streptomyces* species given in the key of Nonomura (1974). Results of the identification of the four actinobacterial strains are as follows.

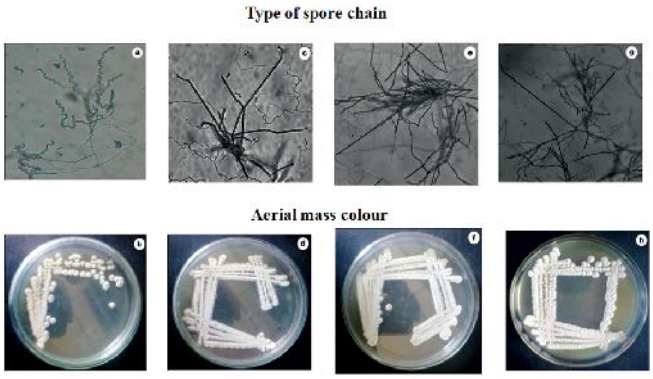


**Fig.** 5. Morphological characters of the strains, AUANI-1 (a,b), AUANI-5 (c,d), AUANI-7 (e,f) and AUANI-8 (g,h).

## Strain AUANI-1

Strain AUANI-1 is a mesophilic actinobacterium, which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into short, spiral spore chains (Fig. 5a). Gray coloured aerial spores were formed in ISP2 agar (Fig. 5b). Reverse side pigment and soluble pigments were produced on Peptone yeast extract iron agar. Melanin pigment was absent in ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* raffinose and rhamnose. There was weak growth in arabinose, xylose, inositol, mannitol, fructose and sucrose. AUANI-1 showed the presence of LL-DAP along with glycine of the peptidoglycan layer with no characteristic sugar pattern, indicating that this strain belongs to the cell wall chemo type I (Table 2).

Results of the cultural, cell wall chemotypical, morphological and physiological characters were compared between the strain AUANI-1 and its closest *S. nodosus*. The strain AUANI-1 showed variation in some characters when compared to those of the reference species *S. nodosus* i.e.

**Table.** 2. General characteristics of the strains AUANI-1, 5, 7 and 8 and their closest related *Streptomyces* species.

| Characters studied | AUANI-1 | *S. nodosus* | AUANI-5 | *S. craterifer* | AUANI-7 | *S. moderatus* | AUANI-8 | *S. aureofasciculus* |
|---|---|---|---|---|---|---|---|---|
| **I. Cell wall amino acids** | | | | | | | | |
| LL-DAP | + | + | + | + | + | + | + | + |
| Meso- DAP | - | - | - | - | - | - | - | - |
| Glycine | + | + | + | + | + | + | + | + |
| **II. Cell wall chemotype** | **I** | **I** | **I** | **I** | **I** | **I** | **I** | **I** |
| **III. Characters studied** | | | | | | | | |
| (as per Nonomura key) | | | | | | | | |
| Colour of aerial mycelium | Gray | Gray | White | Whitish gray | White | Whitish gray | Whitish yellow | White |
| Melanoid pigment | - | - | - | - | - | - | + | + |
| Reverse side pigment | + | - | - | - | + | - | + | + |
| Soluble pigment | + | + | - | - | - | - | - | - |
| Spore chain | S | S | RF | RF | RF | RF | RF | RF |
| **Carbon source assimilation** | | | | | | | | |
| Arabinose | ± | ± | + | + | ± | + | ± | + |
| Xylose | ± | ± | ± | + | + | + | + | + |
| Inositol | ± | ± | + | - | + | + | + | + |
| Mannitol | ± | ± | ± | ± | ± | + | ± | + |
| Fructose | ± | ± | ± | ± | + | + | + | + |
| Rhamnose | + | + | + | + | + | + | + | + |
| Sucrose | ± | ± | ± | ± | + | + | + | ± |
| Raffinose | + | - | ± | ± | + | + | + | + |

Positive (+); Negative (-); weakly utilized (±); Spirales (S); Rectiflexibiles (RF).

reverse side pigment and positive utilization of raffinose as carbon source. Except these, all the other characters were similar to those of *S. nodosus* (Table 2). Hence, the strain AUANI-1 was identified as a species close to *S. nodosus.*

### Strain AUANI-5

Strain AUANI-5 is a mesophilic actinobacterium, which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into short, rectiflexible spore chains (Fig. 5c). White coloured aerial spores were formed in ISP2 agar (Fig. 5d). Reverse side pigment and soluble pigments were not produced on Peptone yeast extract iron agar. Melanin pigment was also absent on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* arabinose, inositol and rhamnose. There was weak growth in xylose, mannitol, fructose, sucrose and raffinose. AUANI-5 showed the presence of LL-DAP along with glycine of the peptidoglycan layer with no characteristic sugar pattern, indicating that this strain belongs to the cell wall chemo type I (Table 2).

Results of the cultural, cell wall chemotypical, morphological and physiological characters were compared between the strain AUANI-5 and its closest species *S. craterifer*. AUANI-5 showed variation in some characters when compared to those of the reference species, *S. craterifer* i.e. aerial mycelial colour and positive utilization of inositol as carbon source. Except these, all the other characters were similar to those of *S. craterifer* (Table 2). Hence, the strain AUANI-5 was identified as a species close to *S. craterifer.*

### Strain AUANI-7

Strain AUANI-7 is a mesophilic actinobacterium, which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into short, rectiflexible spore chains (Fig. 5e). White coloured aerial spores were formed in ISP2 agar (Fig. 5f). Reverse side pigment was produced and soluble pigments were not produced on Peptone yeast extract iron agar. Melanin pigment was present on ISP7 agar. Culture grew well when it was supplemented with the carbon sources *viz.* xylose, inositol, fructose, rhamnose, sucrose and raffinose. Weak growth was seen in arabinose and mannitol. The strain AUANI-7 showed the presence of LL-DAP along with glycine of the peptidoglycan layer with no characteristic sugar pattern, indicating that this strain belongs to the cell wall chemo type I (Table 2).

Results of the cultural, cell wall chemotypical, morphological and physiological characters were compared between the strain AUANI-7 and its closest *S. moderatus*. AUANI-7 showed variation in only one character when compared to those of the reference species *S. moderatus* i.e. aerial mycelial colour. Except this, all the other characters were similar to those of *S. moderatus* (Table 2). Hence, the strain AUANI-7 was identified as a species close to *S. moderatus.*

### Strain AUANI-8

Strain AUANI-8 is a mesophilic actinobacterium, which forms an extensively branched substrate mycelium and aerial hyphae that differentiate into short, rectiflexible spore chains (Fig. 5g). Whitish yellow coloured aerial spores were formed in ISP2 agar (Fig. 5h). Reverse side pigment was produced and soluble pigments were not produced on Peptone yeast extract iron agar. Melanin pigment was present on ISP7 agar. Culture grew well when it was supplemented with the carbon sources, xylose, inositol, fructose, rhamnose, sucrose and raffinose. Weak growth was noticed in arabinose and mannitol. AUANI-8 showed the presence of LL-DAP along with glycine of the peptidoglycan layer with no characteristic sugar pattern, indicating that this strain belongs to the cell wall chemo type I (Table 2).

Results of the cultural, cell wall chemotypical, morphological and physiological characters were compared between the strain AUANI-8 and its closest *S. aureofasciculus*. AUANI-8 showed variation only in one character when compared to those of the reference species, *S. aureofasciculus* i.e. aerial mycelial colour. Except this, all the other characters were similar to those of *S. aureofasciculus* (Table 2). Hence, the strain AUANI-8 was identified as a species close to *S. aureofasciculus*.

## Discussion

Kuster's agar supports isolation of various types of actinobacteria especially from the mangrove sediments (Sivakumar, 2001). Similarly, Sahu *et al.* (2005) isolated higher number of actinobacteria using this medium from the Vellar estuary. Raghavendrudu and Kondalarao (2007) studied the distribution of actinobacteria in the Gaderu mangroves of the Gautami- Godavari estuarine system, east coast of India, using five different agar media for isolation; among them, Kuster's agar was found to be suitable for the isolation of the genus *Streptomyces.* Baskaran *et al.* (2011) also reported that the Kuster's agar medium supports the growth of the marine actinobacterial population. Further, Sethubathi *et al.* (2013) found that the Kuster's agar medium yielded higher counts of actinobacterial colonies and Mohseni *et al.* (2013) isolated 44 actinobacterial strains from the sediments of the Caspian Sea using the Kuster's agar. Gobalakrishnan (2013) has also isolated more actinobacterial colonies from the Havelock island of the Andamans, using Kuster's agar. Therefore, Kuster's agar was chosen to isolate the actinobacteria from the sediments of the Neil island, in the present study.

Enzymes are the ideal catalysts used in the food industries, owing to their specificity, mild reaction condition and non-toxicity. Therefore, these have attracted the attention of the researchers all over the world due to their wide range of physiological, analytical and industrial applications, because of their broad biochemical diversity, feasibility of mass culture and ease of genetic manipulation (Nalini Singh, 2012). Enzymes of the marine microbes have unique protein molecules. Properties like higher salt tolerance, thermo stability and barophilisity of the marine microbes prompted the scientists to consider these kinds of enzymes for commercial purpose (Sivakumar *et al.,* 2007) and the reason to prefer them is their biodegradable, non-toxic nature and they can be administered at the local site quite easily (Wakil and Adelegan, 2015). So, the eight morphologically distinct strains isolated in the present study, were subjected to different enzyme screening assays *viz.* L-asparaginase, cellulase, DNase and chitinase using different enzymatic agar media.

L-asparaginase is present in many animal tissues, bacteria, plants and in the serum of certain rodents, but not in man and is produced by a large number of microorganisms (Mukherjee *et al.,* 2000). L-asparaginase has been investigated recently in higher plants on account of its key role in the nitrogen nutrition. Though, L-asparaginase is an excellent nitrogen source for the growth of eukaryotic microalgae, it is a nutritional requirement for all normal cells. It is a potent antitumor enzyme that catalyzes the hydrolysis of L-asparagine to L-aspartic acid and ammonium ion. So, this enzyme has been widely exploited in the treatment of certain kinds of cancers, especially acute lymphoblastic leukaemia (Mohamed *et al.*, 2015).

Actinobacteria are the potential sources for the production of L-asparaginase. Particularly, *Mycobacterium tuberculosis, Streptomyces griseus, S. karnatakensis, S. venezuelae, S. longsporusflavus* F- 15*, S. phaeochromogenes* FS-39 and *Nocardia asteroides* have proved this (Kavitha and Vijayalakshmi, 2012). Due to the therapeutic potential of the enzyme, L-asparaginase, screening of microbial sources for its activity has been greatly intensified (Clavell *et al.,* 1986; Mercado and Arenas, 1999). In the present study, among the eight strains, strain AUANI-1 produced higher amount of L-asparaginase enzyme with 12 mm of clear zone. Similarly, Sivasankar *et al.* (2013) have reported L-asparaginase activity of the actinobacteria isolated from the Bay of Bengal, India, in addition to Dhevangi and Poorani (2006), Sahu *et al.* (2007a,b) and Khamna *et al.* (2009). It is worth mentioning here that Jha *et al.* (2012) have presented a detailed review on the microbial L-asparaginases.

Actinobacteria as cellulase producers have enhanced the research interest due to their potential application (Arunachalam *et al.,* 2010). A wide variety of bacteria are known for their production of hydrolytic enzymes including cellulases and *Streptomyces* has been reported as the best (Chellapandi and Jani, 2008). In the present study, cellulase activity was screened in eight isolates and among them, AUANI-5 exhibited higher cellulase activity with 18 mm of clear zone and this strain was identified as *Streptomyces.*

Murugan *et al.* (2007) isolated 35 actinobacterial strains from the Vellar estuary, southeast coast of India and examined their cellulase production. Among them, CL-30 (*S. actuosus*) showed higher cellulase activity (45.5¼g glucose/ml/h). Sirisha *et al.* (2013) identified bioactive compounds from the marine actinobacteria, isolated from the sediments of the Bay of Bengal and 24% of the strains exhibited cellulase activity. Meena *et al.* (2013) isolated 26 actinobacterial strains from the Andaman and Nicobar islands and among them, two *Streptomyces* strains (NIOT-VKKMA02 and NIOT-VKKMA26) showed excellent cellulase activity. Gobalakrishnan (2013) identified cellulase producing actinobacterial stains from the Havelock island of the Andamans and *Actinoalloteichus* sp. MHA15 was the promising candidate. Recently, Pradhan *et al.* (2015) have found that *Streptomyces* sp. AC-I, *Actinomycetes* sp.AC-II, *Actinomycetes* sp.AC-III and *Nocardia* sp. AC-IV are the potential sources for microbial cellulases.

Deoxyribonucleases are the enzymes which can break phosphodiester linkages of deoxyribonucleic acid (DNA). Though DNases are the part of every cell, there are very few microorganisms which produce DNases extracellularly (Sheikh and Hosseini, 2014). By using nucleases in different ways, it has become easier to recombine DNA, remove harmful genes and replace single gene on DNA strand; applications include gene therapy for genetic diseases, genetic engineering etc. DNases are also used in chemotherapeutic and industrial fields (Kamble *et al.,* 2011). In the present study, among the eight strains, higher DNase enzyme production was found in the strain AUANI-7 with 12 mm of clear zone. Recently, Narasaiah *et al.* (2015) have reported *S. albus* CN-4 (isolated from laterite soil sample) as an ample producer of DNase enzyme. Pradhan *et al.* (2015) have found that *Actinomycetes* sp. AC-III and *Nocardia* sp. AC-IV isolated from the Chilika Lake, Odisha are good sources of DNase.

Chitinase is important due to its wide range of biotechnological applications, especially in agriculture bio-control of phytopathogenic fungi and harmful insects. Approximately 75% of the total weight of shellfish such as shrimps, crabs and krills is considered as waste, of which, chitin comprises 20-58% of the dry weight of the said waste, which can be used as a substrate for microbial chitinase production.

Among the microbes, approximately 90-99% of the chitinolytic populations are actinomycetes (Mukherjee and Sen, 2004). Sowmya *et al.* (2012) reported that chitinase of *Streptomyces* is active over a wide range of operating and environmental conditions and hence it is one of the best organisms to study the production as well biochemical aspects of chitinase. Recently, Pradhan *et al.* (2015) have reported that *Nocardia* sp. AC-IV isolated from the Chilika Lake, Odisha is also a source of chitinase enzyme. Corroborating these findings, present study has revealed higher amount of chitinase production in the strain AUANI-8 with 17mm of clear zone.

So far, many species of actinobacteria belonging to 28 genera have been recorded from the marine environment. Among them, *Streptomyces* is dominant (Karthik *et al.,* 2010), as found in the present study, where all the four strains identified belong to *Streptomyces*. Success of the *Streptomyces* in its establishment could be attributed to its ability to produce an array of catabolic enzymes that degrade biopolymers and also a mixture of antimicrobial compounds, depending on their substrates. Further, of the scores of microorganisms, *Streptomyces* spices have been found to be the most prolific producers of a variety of clinically important biochemicals (Tarkka *et al.,* 2008). In this regard, strains AUANI-1, AUANI-5, AUANI-7 and AUANI-8, identified as *Streptomyces,* merit further indepth studies for their biopotentials.

Present investigation concludes that the sediment samples of the Neil island, the Andamans, contain potential actinobacterial strains of *Streptomyces*, producing different enzymes *viz.* L-asparaginase, cellulase, dioxyribonuclease and chitinase which are of biotechnological and industrial importance. Hence, these strains can be further evaluated and studied in detail for commercial scale production of useful enzymes.

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