Salt lakes and multi pond solar salterns are well known hyper saline habitats having high salt concentration and alkaline pH (Zafirilla et al., 2010). They possess enormous potential in terms of microbial diversity that can be exploited for many industrial applications. One such hypersaline habitat is Sambhar salt lake which is the largest inland saline lake of India that produces salt for human consumption and industrial needs. In the present study Sambhar Lake water was used to assess actinomycetes population and their bioactive potential. The study was divided into three different seasons during which water samples were collected and analyzed for their pH and salinity content. The results revealed that Sambhar Lake water has an alkaline pH that ranges from 9.0-12.5 and high salinity that ranges from 3-35.7%. Low salinity values were recorded in years of rainy season and in reservoir samples of winter and summer season. As the water evaporates from ponds salinity increases and leads to salt crystallization. Salt productions occur maximally during summers and at this time salinity of ponds were high. The alkaline pH and high salt present in water samples confirms Sambhar lake to be a hypersaline zone. Many previous studies also reported the same nature of Sambhar Salt lake (Sinha and Raymahashay, 2004; Jose and Jebakumar, 2013). Similar values were also reported for El-Djerid salt lake in southern Tunisia (Hedi et al., 2009) which is also athalassohaline in nature just as Sambhar Salt Lake. In contrast, athalassic lake Dead Sea, Israel was reported to be slightly acidic in nature. Waters of thalassohaline lakes as Great Salt Lake in Utah, USA are also slightly acidic to neutral in nature (Oren, 1993). Wadi Natrun and Mgadi lakes in Kenya are also examples of highly alkaline environments (Post, 1977). All these salt lakes are reported to be rich in NaCl concentration.

Actinomycetes from such halophilic habitats are composed of heterogeneous physiological group that belongs to different genera. They are identified by a polyphasic approach which is a combination of different phenotypic, chemotaxonomic and molecular test. In this study a total of 16 actinomycetes isolates (SSL 1-SSL 16) were recovered from solar salt pans of Sambhar Salt Lake, India. Other hypersaline environments studied for the presence of biotechnologically important actinomycetes producing enzymes or secondary metabolites are crystallizer ponds of solar salterns from Tuticorin in Bay of Bengal (Jose and Jebakumar, 2012) and saline desert of kutch, India (Thumar et al., 2010).
pH of the water samples from which actinomycetes were isolated was alkaline indicating the alkali tolerant to alkalophilic nature of isolates. Subramani and Mathivanan in 2009, isolated actinomycetes from Bay of Bengal and reported that majority of the isolates were found to grow between pH 8.1 and 8.5. In contrast, *Streptomyces* have also been reported from acidic soils with pH less than 5.0 (Lee and Hwang, 2002). It can be said that acidophilic or alkalophilic nature of any microorganism depends on pH of its habitat. The actinomycetes isolates of this study can therefore grow very well at pH 7.0 and above due to the alkaline nature of Sambhar Lake.

The seven isolated actinomycetes were categorized as moderate halophiles (optimum growth at 8 or 12% NaCl) and nine as halotolerant that grew well in absence of salt and in presence of wide range of salinity also. Similar behaviour of halotolerant and halophilic actinomycetes has been reported from other salt lake habitats (Oren, 2002; Cai *et al*., 2009, Jose and Jebakumar, 2013). In order to prevent osmotic lysis of cells in presence of salt most of the halophiles and halotolerant microorganisms accumulate compatible solutes in high concentrations within the cytoplasm (Galinski, 1993). This is achieved by biosynthesis, de novo or from storage material or can be taken up from medium. *Nocardiopsis halophila* isolated from a saline environment uses a hydroxy derivative of ectoine and β-glutamate as compatible solutes (Das Sarma and Arora, 2001).

Water samples having salinity more than 12 % and bitter samples also did not showed the presence of actinomycetes colonies. Salterns of Sambhar lake are connected to each other. The water sample from reservoir is transferred successively to connected ponds one after the other where it forms brine in presence of sunlight till the formation of NaCl crystals take place. The time of successive transfer of brine depends on intensity of sunlight and may vary from 10 days to a month. The salt crystallization is achieved when the density of brine increases to 29 °Be. Along with the transfer of this surface water, native actinomycete population is also transferred from ponds of low salinity to high salinity. It is suggested that some of these actinomycetes are not able to adapt and withstand salinities more than 12 % and hence their number decreases in salterns of high salinity. Moreover some of the
actinomycete population that is adapted to high salinity levels can be identified only by culture independent approaches. In the present study, actinomycetes were isolated using culture dependent methods and this could be responsible for their absence in highly saline water samples. Bittern is the rejected water left after removal of crystallized NaCl layer. It is rich in the presence of ions as sulphates, carbonates and bicarbonates along with remaining sodium and chloride. An excess of presence of these ions in bittern samples could be inhibitory for actinomycetes growth in culture medium and hence, techniques as metagenomics can be used for recovering uncultivable actinomycetes from such samples.

It was observed that actinomycetes were more abundant during winter season. The generic diversity was also maximum during winters than in rainy and summer season. Members of four genera (Streptomyces, Nocardiopsis, Actinobacterium, Saccharopolyspora) were isolated from sample water collected in winters whereas members of only two (Streptomyces, Nocardiopsis) and three genera (Streptomyces, Nocardiopsis and Microbispora) were isolated in rainy and summer season respectively. A similar observation was found in water samples of few lakes of China where more complex diversity of actinomycetes was present in dry season than in rainy season. They also reported that Streptomyces counts in rainy season were higher than dry season (Jiang and Xu, 1996). In the present investigation also more number of Streptomyces was found in rainy season than in any other season. It is suggested that during rainy season some of the Streptomyces would have been washed from the nearby vegetation and become a part of lake water. This belief is further confirmed by the observation that the isolates of rainy season can grow optimally either without salt or at low salt concentration (3 % NaCl) and their growth decreases above 5 % NaCl indicating their origin from terrestrial habitat lying nearby. However, the other isolates of the study showed rapid growth at high salt concentrations (5- 12 % NaCl).

Actinomycetes were isolated on AIA medium (supplemented with NaCl) as it promotes good growth and sporulation and hence it was chosen to study morphological features. The traditional criteria of identifying actinomycetes was
followed by Goodfellow and Cross in 1984 and includes characters as form and nature of AM and SM, spore chain morphology, pigments produced on ISP media and colony morphology on media plates. Colour of the AM is equally important for their identification (Pridham and Tresner, 1974). The International *Streptomyces* project of Shirling and Gottlieb, 1966 has still been recommended for observing AM colour in different media as important taxonomic feature which was followed in the present study also. It was found that on AIA medium 75% of the isolates had white coloured aerial spore mass and only one isolate SSL 13 forms light grey coloured aerial mycelium on maturity. 56.25% of the isolates produced white AM on ISP2 media and also showed the production of diffusible pigment on the same. On ISP6 medium most of the isolates produced white AM and 50% of them showed pigment production. On other ISP media also white colour was predominant in the isolates. The results were similar to the study of Barcina *et al.*, (1987), Patil *et al.*, (2001), Peela *et al.*, (2005) and Adinarayana *et al.*, (2006) who also reported isolates with white and gray coloured aerial mycelium.

Morphology of the spore bearing hyphae was studied by cover slip culture method and it was found that 50% of the isolates had rectiflexibles type of sporophore. Spiral spore chain was observed in only two isolates (SSL 3 and SSL 5) and Retinaculiaperti (SSL 4) and straight rectus (SSL 6) in only one isolate. Cover slip culture method used to identify spore chain morphology was also studied by Peela *et al.*, (2005) who reported the dominance of spiral spore chain actinomycetes in their study.

After morphological characterization all the isolates were subjected to a variety of biochemical tests. Presence of catalase and oxidase enzyme was found in all isolated actinomycetes suggesting their aerobic nature. All the isolates except SSL 4, SSL 5 and SSL 11 were not able to degrade gelatin. The *Streptomyces* isolated from biotopes in Bulgaria were also found to be negative for gelatin liquefaction test (Naidenova and Vladimirova, 2002). However gelatin hydrolysis was found to be positive in *Streptomyces sannanensis* strain RJT-1 isolated from the alkaline soil sample of Saurashtra University Campus, Rajkot, India (Vasavada *et al.*, 2006). Only five isolates were found to degrade tyrosine, seven degraded
hypoxanthine and majority of them degraded xanthine and casein. But the extent of degradation of these compounds was different as inferred from their hydrolysis pattern on appropriate media plates. It was also found that they were able to utilize most of the carbon compounds. The difference in the biochemical nature of isolates is attributed to their different genetic makeup. All the isolates were Gram positive in nature.

The isolates identified up to genus level by morphological and biochemical test were found to be fitted to the description of *Streptomyces* (SSL 2, SSL 3, SSL 4, SSL 5, SSL 10 and SSL 15), *Nocardiopsis* (SSL 1, SSL 11 and SSL 14), *Pseudonocardia* (SSL 7), *Microbispora* (SSL 13) and *Saccharopolyspora* (SSL 16) genera as per Bergeys manual of determinative bacteriology. Three Non filamentous actinomycetes were proposed to be identified as *Georgenia* (SSL 8) and *Kokuria* (SSL 9 and SSL 12).

The results of these phenotypic characterization were supported by 16S rDNA sequencing in five of the isolates and they were confirmed to be members of genera *Streptomyces* (SSL 2, SSL 4 and SSL 10), *Nocardiopsis* (SSL 11 and SSL 14). In only one of the isolate SSL 3 the results of molecular identification were found contradictory to morphological studies. This isolate was found to have both AM and SM which is a typical feature of filamentous actinomycetes. SM was non fragmenting and sporophores bearing spiral spore chains were present on AM. These features in combination with biochemical tests confirm SSL 3 to be member of *Streptomyces* whereas 16S rDNA sequencing study showed 100 % homology to *Arthrobacter* sp. A5 (Accession number EU882856.1). The cells of *Arthrobacter* shows typical rod-coccus life cycle at maturity which was not observed in SSL 3. Even the phylogenetic analyses of SSL 3 also showed 99 % similarity to *Streptomyces* sp. LD48 (accession number AY641538.1) and hence it was believed to be member of *Streptomyces* genus only and thereby identified as *Streptomyces* sp. SSL 3. Isolate SSL 6 although morphologically resembled *Saccharopolyspora* but the phylogenetic analyses report it to be 99 % similar to *Actinobacterium* kmd_222 (Accession number EU723156.1). *Actinobacterium* belongs to a group of actinobacteria that is still not classified. It is rare genus and its description is not
found in Bergys manual of Determinative Bacteriology (Holt et al, 2000). Filamentous morphology and presence of AM and SM in SSL 6 confirms it to be actinomycetes and phylogenetic analyses and sequence homology identifies it to be similar to Actinobacterium. There is a possibility that this isolate might be a novel actinomycete but more studies are needed in order to confirm its novelty hence for the present study SSL 6 was considered to be Actinobacterium SSL 6 only.

When the phylogenetic trees are constructed, many times halophiles and non halophiles are present together and different genera and families have members with varied salt requirement and salt tolerance (Oren, 2008). Halophilic and halotolerant species have been reported for the genera Streptomyces, Pseudonocardia, Streptosporangium, Nocardiopsis, Salinispora and Streptomonspora (Hamedi et al., 2013). The results of the present study were in accordance to it as Streptomyces and Nocardiopsis were also found in waters of Sambhar lake and further it was also noticed that most of the actinomycetes isolated from the Sambhar lake water belongs to the genus Streptomyces (37.5 %) thus it was regarded as most dominant of all. Streptomyces is already reported as predominant genus in marine environment having largest number of species that differ from each other in their morphology and biochemical activities (Jensen et al., 1991; Dharmaraj, 2010) and this was found true in waters of non marine but halophilic Sambhar lake also. Many other studies on marine sediments also reported Streptomyces as the most dominant genera (Lakshmanaperumalsamy, 1978; Siva Kumar, 2001; Kokare et al., 2004a, 2004b; Hans-Peter et al., 2005). In contrast Micromonospora was reported as dominant genus in some of the other marine sediments also (Jensen et al., 1991; Mincer et al., 2002). Similar to the present findings, members of the genus Streptomyces and Saccharopolyspora have been isolated from marine sediment samples near islands of the Andaman Coast of the Bay of Bengal and there also Streptomyces was reported to be dominant (Peela et al., 2005). Soil samples from solar salterns along Tuticorin coastline in Bay of Bengal (Jose and Jebakumar, 2012) and marine sediment samples of Palk Strait region of Bay of Bengal, (Vijayakumar et al., 2007) India also revealed the presence of these filamentous actinomycetes. Nocardiopsis was the second most abundant actinomycete isolated from the Sambhar salterns of
varying salinity having 3 %, 8.2 % and 12 % NaCl. Similar to this, *Nocardiopsis* sp. was also reported from Kovalam, Puthalum and Thamaraikulam salt works, India (Jenifer *et al*., 2013). The presence of halo tolerant *Microbispora* sp. in waters of Sambhar Lake was supported by the studies reported in saline soil samples of Kuwait (Abbas, 2006) and in sediments of Yangzong lake in China (Jiang and Xu, 1996).

Sufficient studies were not available on actinomycetes population of Sambhar Lake water till 2012 but recently in 2013 Yadav *et al*., published reports on actinomycetes diversity from Sambhar lake water which was studied by both culture dependent and culture independent methods. Members of *Streptomyces, Actinopolyspora, Microbispora, Saccharopolyspora*, and *Actinoplanes* genera were isolated by culture dependent methods. Culture independent method was based on 16S rRNA sequencing studies of environmental DNA sample and it was reported that members of *Streptomyces, Micromospora, Streptosporangium, Thermomonospora* and *Dactylosporangium* were present in Sambhar Lake at different vertical stratification levels. They also reported the presence of *Streptomyces* as dominant genera. Since the present study was based on culture dependent approach hence some actinomycetes genera reported in their study were not recovered in the present investigation. However they also reported the presence of *Streptomyces, Microbispora* and *Saccharopolyspora* using culture dependent methods which was in accordance to present study. Not only water samples but soil sample of inland solar salterns of Sambhar Salt lake were also studied and presence of total 14 actinomycetes (*Streptomyces, Pseudonocardia* and *Actinoalloteichus*) were reported with *Streptomyces* as dominant genera (Jose and Jebakumar, 2013). The results were again similar to present study with reference to the isolation of *Streptomyces* and *Pseudonocardia*. Many other reports also describe the presence of *Pseudonocardia* from marine hypersaline environments as Bay of Bengal, India (Peela *et al*., 2005) and deep sea sediment sample in South China Sea (Tian *et al*., 2013).
It is emphasized that actinomycetes recovered in the present study represented only a fraction of total actinomycete population of Sambhar Lake because culture dependent method was used for isolation and the criterion used for recognition of actinomycetes colonies were selective. Chemical nature of cell wall is also an important criterion for identifying actinomycetes which was not a part of the present study. Moreover phylogenetic studies were also not conducted on nine of the isolates.

Actinomycetes are studied most extensively for antibiotic productions but less focus is on enzyme production especially of those isolated from halophilic habitats (Chakraborty et al., 2009). In this study all the 16 actinomycete isolates were screened for the production of two extracellular enzymes i.e. amylase and cellulase by agar plate assay in a medium containing starch and CMC respectively. Eighty one percent of the isolates were found to be positive for both enzymes. The relative enzyme activity of isolates were compared and it was observed that the isolate SSL 6 identified as *Actinobacterium* sp. and the isolate SSL 14 identified as *Nocardiopsis* sp. showed the maximum amylase and cellulase activity respectively hence they were selected for further enzyme optimization studies in submerged fermentation method following the one factor at a time approach (OFAT). Similar enzyme optimization studies using OFAT approach were performed for cellulase production by *Streptomyces* sp. B-PNG23 (Azzeddine et al., 2013) and by *Fomitopsis* sp.RCK 2010 (Deswal et al., 2011).

Actinomycetes are slow growing bacteria. It has been reported that enzyme production by actinomycetes starts in early log phase, increases in late log phase and continues till the start of stationary phase after which it shows a decline (Chakraborty et al., 2011). The results of the present study were found to be in accordance with the above theory for both amylase and cellulase productions.

Maximum production of amylase by *Actinobacterium* SSL6 strain occurred when the production medium was incubated for a period of 96 h in its late log phase and at 120 h also amylase activity did not declined significantly. This was supported by post hoc LSD test that showed no significant difference in amylase activity at 96
and 120 h of incubation (Table 4.13.3), however after this time period amylase activity decreased (Table 4.7.1). This kind of growth associated amylase production was also observed in a marine *Streptomyces* isolate BTS 1001 that showed optimum amylase production at 96 h of incubation (Acharyabhatta *et al*., 2013) and in *Streptosporangium* (Stamford *et al*., 2002) where maximum production was noticed at 72 h of incubation (log phase). It is well known that amylase acts on starch and releases glucose as an end product. It can be therefore be suggested that the release of high levels of glucose during stationary phase and thereafter are responsible for the decrease in amylase production after 120 h of incubation. These results were similar to those reported for α amylase produced by *Halomonas meridiana* (Coronado *et al*., 2000) and halotolerant *Chromohalobacter* sp. TVSP 101 (Prakash *et al*., 2009).

CMCase production by *Nocardiopsis* SSL 14 strain was also highest at 96 h of incubation but a significant difference in cellulase activity between 96 and 120 h (p < 0.05) was observed. A novel actinomycete *Nocardiopsis* sp. KNU was found to produce highest amount of cellulase in late log phase that continued up to stationary phase (Saratale and Oh, 2011). Non growth associated CMCase production was observed in *S. drozdowiczii* where highest activity was present at the start of stationary phase (Lima *et al*., 2005). In another study on *Streptomyces* sp. maximum enzyme production was observed after 5 days of incubation (Alani *et al*., 2008). It has been already reported in the literature that end product act as inhibitors to hydrolysis of cellulose (Van Dycke, 1972; Howell and Mangat, 1978) and therefore are responsible for decline in activity after few days. Another possible explanation of decline in CMCase activity after few days could be attributed to the depletion of nutrients in the medium that adversely affected the enzyme production (Nochure *et al*., 1993).

In both the cases enzyme activity declined after 120 h. These results suggested that enzyme production was associated with growth in both the isolates.

Many literature suggest 40-55 °C as an optimum temperature for amylase production especially in thermostable actinomycetes (Kar and Ray, 2008; Chakraborty
Discussion

et al., 2009). *Streptomyces gulbargensis* sp DAS 131 was found to produce maximum amylase at 45 °C (Dastager et al., 2009). But the results of amylase production by SSL 6 were found to be an exception as it produced maximum enzyme at 30 °C only. Similar results were found in a study on fungi, *P. fellutanum* where highest amylase production was also noticed at 30 °C (Kathiresan and Manivannan, 2006). Aiba et al., (1983) suggested that genes responsible for starch degrading enzymes are inactivated at higher temperatures. This provides the most possible explanation of sudden decrease in enzyme activity at temperatures above 50 °C as noticed in this study.

A considerable amount of work published on different *Streptomyces* sp. also report 45 °C as an optimum temperature for CMCase activity (McCarthy, 1987). In a recent study on St-1 (*S. griseorubens*) which was isolated from Indian soil maximum cellulase activity was found at 45 °C (Prasad et al., 2013). Contradictory to the described pattern for other actinomycetes, the present findings of study indicated that optimum enzyme production occurs at 30 °C by *Nocardiopsis* SSL 14.

Goodfellow and Williams in 1983 suggested that most of the actinomycetes behave as mesophiles and have an optimum growth temperature ranging from 30-37 °C. In the present study the effect of temperature on the enzyme productions was found to be related to growth temperature being highest at 30 °C.

Among the physical factors, pH of the production media is important as it can induce morphological changes in microorganisms and alter enzyme production also. Stability of the product in the medium is also affected by change of pH (Gupta et al., 2003). pH of the growth medium also effects transport of various enzymes and chemical products across cell membrane thereby affecting enzymatic reactions (Kapoor et al., 2008; Liang et al., 2009). The results also suggested this as both the enzyme productions are affected by change of pH of the medium.

*Actinobacterium* SSL6 showed amylase production in broad pH range of 7.0-9.0 with optimum at pH 8.0. *Streptosporangium* also produced highest amount of glucoamylase at pH 8.0 (Stamford et al., 2002). Maximum amylase production at alkaline pH (9.0) was also reported in *Streptomyces* sp D1 (Chakraborty et al., 2009)
and *Chromohalobacter* sp. TVSP 101 (Prakash et al., 2009). Amylase production by SSL 6 was found to be less at pH values below 6.0 similar to those reported for *Halobacillus* sp. strain MA-2 (Amoozegar et al., 2003).

In actinomycetes, most of the studies are available on *Streptomyces* species that reported maximum CMCase production over a broad pH range from 5.5 in *S. lividans* (Theberge et al., 1992), to pH 6.0 in strain J2 (*Streptomyces* sp.) (Jaradat et al., 2008), pH 7.0 in *S. griseorubens* (Prasad et al., 2013) and in *S. drozdowiczii* (Semedo et al., 2000). In a study on *Thermonospora curvata* also optimum cellulase production occurred at pH 8.0 (Stutzenberger, 1972). An alkaline novel *Streptomyces* species from east African soda lakes also showed an optimum enzyme production at pH 8.0 (Solingen et al., 2001). CMCase production by *Nocardiopsis* SSL 14 was found to be highest at pH 9.0, although no significant difference between pH 8.0 and 9.0 was observed (Table 4.15.4).

It is a well known fact that enzymes are not stable at very high or very low pH values as they get denatured and thus highly basic and highly acidic pH values adversely affects enzyme production by microorganisms (Haltrich et al., 1996). The results obtained here support this as the production of both amylase and cellulase was very low at low pH values. However it was also noticed that both the enzymes were produced at an alkaline pH which can be attributed to their origin and thereby adaptation in saline habitat of Sambhar lake.

Microorganisms thriving in saline habitats are capable of growing not only at alkaline pH but also at high salt concentrations. Both the isolates SSL 6 and SSL 14 were not found to be an exception to this.

Maximum amylase production by *Actinobacterium* SSL6 strain occurred when the medium was supplemented with 9 % NaCl (Table 4.10.1) which indicated mild halophilic nature of the isolate. The finding is similar to that reported in *Streptomyces* sp D1 (Chakraborty et al., 2009) and in *Streptomyces* BTSS 1001 which produced maximum amylase at 7 % NaCl (Acharyabhatta et al., 2013). However in the present study amylase production at all salt concentrations tested
was comparable and the result at most of them were not significant statistically (Table 4.16.3).

*Nocardiopsis* SSL 14 showed highest CMCase activity when the medium was supplemented with 12 % NaCl and in contrast to amylase production by SSL 6, significant difference was observed at all the salt concentrations tested (Table 4.16.4). Least cellulase activity was observed at low salt concentrations (0.5 and 1 % NaCl) and it gradually increased with the increase in amount of NaCl (Fig. 4.11.2). These results suggest the moderately halophilic nature of the isolate SSL 14.

This kind of adaptation of proteins in a media having high salt concentrations is attributed to the presence of large number of acidic residues on protein surface supported by electrostatic interactions and an increased number of salt bridges (Danson and Hough, 1997).

In bacteria, most of the genes and operons that code for enzymes involved in regulation of transport and carbohydrate catabolism are expressed only when medium contains the corresponding carbohydrate and lacks the preferred carbon source. Such kind of regulatory mechanisms are called as induction and carbon catabolite repression (CCR) respectively (Stulke et al., 1998). A study conducted in *Streptomyces* suggests that in these filamentous bacteria many cis-acting sequences are involved in the CCR of many genes and operons (Virolle and Gagnat, 1994). Therefore it was assumed that when glucose is present in the medium, synthesis of negative transcriptional regulator occurs that in turn binds to cis-acting sequences thus causing repression of the corresponding operon or gene (Mellouli et al., 2002).

Various carbon sources were tested for the production of amylase by *Actinobacterium* SSL 6. Starch was found to be most effective carbon source giving highest amylase activity (6.597 ± 0.489 U/ml) followed by maltose. Least activity was shown in a medium containing dextrose (3.457 ± 0.378 U/ml). This confirms the inducible nature of amylase in presence of starch and the results are supported by the studies of Vijayalakshmi et al., 2012 who also reported starch as best source for amylase production. Narayana and Vijayalakshmi in 2008 also reported starch as
best carbon source for amylase production in *Streptomyces albidos flavus*. In a study on *Thermoactinomycetes vulgaris* also highest production was found when starch or maltose was used (Kuo and Hartman, 1966). Similar inductive amylase production was found in *Bacillus* sp. strain TSCVKK and *Bacillus* sp. 64 (Khire, 1994; Kiran and Chandra, 2008). However in a *Halobacillus* sp. strain MA-2 constitutive production of amylase was reported (Amoozegar et al., 2003). It has been suggested by Welker and Campbell (1963) that maltodextrins act as direct inducers of α amylase in *B. stearothermophilus*. They also proved that the inductive effect of starch and maltose on amylase production is due to the presence of maltodextrins which can be formed from starch during fermentation or are present as impurities in technical grade maltose.

Synthesis of cellulase enzyme is also subjected to induction and catabolite repression in both bacteria and fungi. Cellulose and its derivatives act as inducers and soluble sugars like glucose, maltose etc. act as repressors (Sternberg and Mandels, 1980; Stoppok et al., 1982). Consistent with these theories, in present study also maximum production of enzyme occurred in presence of CMC and decreased significantly in presence of glucose and maltose. Thus CMC was easily metabolized by SSL 14 and also acted as inducer for CMCase production. In a study on *Streptomyces* sp it was found that CMC (1 %) was better than glucose and other carbon sources for endoglucanase production (Janga and Chen, 2003). In a halophilic bacterium *Salinivibrio* sp NTU-05 also CMC was found as best carbon source for cellulase production (Wang et al., 2009).

Nitrogen plays an important role in the growth and development of the bacteria hence in this study different nitrogen sources were used for optimizing enzyme productions. Among the different nitrogen sources tested beef extract was found to give maximum amylase activity. A similar finding has also been observed in *Streptomyces erumpens* MTCC 7317 that showed optimum amylase production using beef extract and starch (Kar and Ray, 2008). In *Streptomyces gulbargensis* sp DAS 131 also peptone was found to give higher amylase production when supplemented in the medium (Dastager et al., 2009). In *Halobacterium salinarum* and *Bacillus thermooleovorans*, tryptone and peptone were found to good nitrogen
sources for amylase production (Patel and Jain, 1993; Malhotra et al., 2000). Similar to this in the present study also amylase production was found to be high when peptone was used in the production medium. Low amylase activity was observed when the production medium contained ammonium nitrate as a nitrogen source. In contrast to these findings, Michelena and Castillo (1984) have reported increased amylase activity on supplementation of medium with inorganic nitrogen salts in Aspergillus foetidus. In a study reported by Prakash et al., in 2009 inorganic nitrogen sources as urea and ammonium chloride did not support amylase production. The inhibitory effect of inorganic salts might be related to changes in pH resulting to decline in enzyme activity.

Yeast extract was found to be best source for CMCase production by SSL 14 similar to that reported for Streptomyces sp. F2621 (Smruti et al., 1995) and Streptomyces drozdowiczii (Lima et al., 2005). The other complex organic nitrogen sources, beef extract and peptone also gave higher amount of enzyme production whereas inorganic sources as ammonium nitrate and peptone did not showed any significant difference. Similar finding have been also reported in Streptomyces sp. T3-1 by Jang and Chen in 2003 where addition of ammonium salt and peptone made no significant difference in production. Wang in 1982 reported that ammonium ions can be absorbed to the mycelium resulting in lowering of the pH thereby inhibiting cellulase production. This could be responsible for lowering of CMCase production in presence of ammonium salt.

A striking observation made on actinomycetes isolates was that not a single isolate showed inhibitory activity against the test bacterial strains. This finding was contradictory to the fact that most of the actinomycetes especially Streptomyces produces antimicrobial compounds. It is already reported that the actinomycetes present in the soil of salterns of not only Sambhar lake but other coastal areas also are antagonistic in nature (Jose and Jebakumar, 2012; Jose and Jebakumar, 2013) but the SSL isolates of present study behaved in an opposite manner. Production of any antibiotic in excess is controlled by the genetic make up and is affected by nutritional and environmental factors (Vasavada et al., 2006). Many times antibiotic producing genes are activated only under specific conditions as in presence of other
competing microbes. In the present study isolates were studied in isolation in laboratory conditions and their antagonistic action was also studied only against a limited number of bacterial strains. They might have required specific signals as from environment (stress) or from surrounding competitors or symbionts to produce antibiotics and in this context ecology of the original habitat plays a very important role (Zhu et al., 2014). Thereby it is suggested that the isolates either does not produce antibiotics or they require specific activation signals and clues to produce them. If the first case is true they can be considered as an exception rather than a contradiction but in depth studies are required to prove or disprove any of the above case. However the presence of antagonistic actinomycetes in soil samples of Sambhar lake reported by Jose and Jebakumar, 2013 can be explained as their isolates were different from presently studied isolates both at phenetic and genetic levels and hence cannot be compared.

Actinomycetes adapted to saline and alkaline environments are of general scientific concern as they can form halophilic/halotolerant or aklaophilic/alkalotolerant enzymes. Amylase of halophilic origin can be used to treat saline waste waters containing starch residues. Halotolerant cellulase is being used for treatment of agricultural waste and bioremediation of cellulosic materials and production of bioenergy (Margesin and Schinner, 2001). Halophilic cellulase are now being employed for hydrolysis of cellulosic biomass in presence of ionic liquids (ILs) which is an ecofriendly pre treatment method for liberation of cellulose from complex lignocellulosic materials (Gunny and Arbain, 2013; Gunny et al., 2014). Hence, the cellulase produced by Nocardiopsis SSL14 under high salt concentrations (12 % NaCl) and alkaline pH is therefore of biotechnological interest as it can be used for such industrial processes.

The findings of the present investigation thus project the isolates of Sambhar salt lake for their biotechnological application in different areas. The production of enzymes by SSL6 and SSL14 at alkaline pH and high salt concentrations is advantageous and makes them suitable for further studies where they can be purified and characterized for their activity and stability at extreme conditions of pH, temperature, salt and metal ions. These purified enzyme preparations which are
expected to work optimally at high pH and salt can be then used for variety of industrial applications as detergent formulations, food industries, waste treatment for bio-based sustainable product formations and biofuel production where higher salt concentrations and pH inhibit enzymatic conversions.