Chapter III

Isolation and screening of glucose isomerase producing 
Streptomyces isolates

3.1. Introduction

Glucose isomerase (GI) (D-Xylose ketol isomerase, EC: 5.3.1.5) plays an essential role in the metabolism of sugars in microorganisms. GI catalyzes the forward and reverse isomerization of aldose (glucose) and ketose (fructose) sugars for industrial merits. The isomerization of D-glucose to D-fructose has a commercial importance in production of High Fructose Corn Syrup (Ben Hlima et al., 2012). Fructose is a GRAS (generally regarded as safe) sweetener used dietary supplement in pharmaceutical and food ingredients (Rocha et al., 2006). In many countries, consumer food products typically use HFCS as a sweetener (Abdel-rassol et al., 2012). Due to its industrial and commercial significance, the catalytic and physicochemical properties of GI have been comprehensively studied in various microorganisms. GI is commonly distributed in prokaryotes, in yeasts and fungus such as Candida utilis, Candida boidinii and Aspergillus oryzae (Bhosale et al., 1996). For example, Streptomyces rubiginosus, Streptomyces wedmorensis, Streptomyces phaeochromogens, Streptomyces murinus, Actinoplanes missouriensis and Bacillus coagulans are being used for commercial production of GI. Streptomyces from mangrove are widely recognized for their ability to produce industrially important enzymes. This made it as an important genus for scientific exploration of many useful products (Wate et al., 2001; Berdy, 2005; Chater et al., 2010). Moreover, Streptomyces genome is well regarded and found to be an important reservoir of natural products for many industrial applications (Cao et al., 2015).

Therefore, we have made an effort to collect samples from less explored mangrove regions of Tamilnadu. In this chapter, isolation of Streptomyces and its distribution in mangrove soil were studied. Potent GI producing isolates were identified based on morphological, physiological, biochemical and molecular characteristics and selected one of the efficient GI producing isolate S. lividans RSU26.
3.2. Materials and Methods

3.2.1. Sample collection

Soil samples were collected from four different sites of the Muthupet mangrove forest (Lat.10° 20’N & Long.79° 35’E) in Tamilnadu during May, 2012 situated in the south east coast of India (Fig 3.1). At each site, three samples were collected at a depth of 10-15 cm from the surface of the soil. The collected soil samples were placed in sterilized polythene bags and kept in dark for transport to laboratory. The physico-chemical parameters, such as temperature, pH and salinity profile of the soil sample were determined using Delux water and soil analysis kit (Model 191E).

3.2.2. Selective isolation of Streptomyces

Isolation of Streptomyces was performed by serial dilution plate technique using Starch casein agar (Kuster and Williams, 1964) medium. The medium was prepared in 50% sea water and sterilized at 121 °C in 15 lbs pressure for 15 min. The medium was supplemented with griseofulvin and streptomycin at 2 µg/ml to prevent other bacterial and fungal contamination. The medium was poured onto sterile petriplates. The collected soil samples were diluted up to 10⁻⁶. About 0.1 ml of the diluted sample was spread onto the agar plates. The inoculated plates were incubated at 28 ± 2 °C for seven to ten days. After incubation, the colonies were observed on the plates as colored, dried, rough, with irregular and regular margins. The isolated colonies were preserved on starch casein agar plates at 4 °C until further use.

3.2.3. Primary screening

3.2.3.1 Determination of glucose isomerase activity

The isolated 56 morphologically distinct Streptomyces-like colonies were screened for GI activity. Test isolates were grown on peptone-yeast agar containing glucose at 0.1 M used as carbon source at 28 °C for 96 h. After incubation, the plates were treated with the reaction mixture, containing 0.1% 2, 3, 5-triphenyltetrazolium chloride solution in 1 M NaOH at 55 °C for 10 min in dark. Development of pinkish red colored zone indicates glucose/xylose isomerase positive (Sapunova et al., 2004).
3.2.4. Characterization of marine isolates

3.2.4.1. Morphological, physiological and biochemical characteristics

The selected potential isolates were studied for morphological, physiological and biochemical characteristics according to the method previously described (Shirling and Gottlieb, 1966). All morphological characters were observed on Starch casein agar and were used for classification and identification as follows: colony appearance, aerial mass color and substrate mycelium, spore chain morphology, shape, texture and reverse side color of colonies according to Bergey’s manual of systematic bacteriology (Locci, 1989). Microscopic characterization was followed by cover slip culture technique (Kawato and Shinolue, 1959). The mycelium structure and spore arrangement was observed through high power oil immersion (100 X) objective by light microscope (Olympus, CH20i). The morphology of spore and spore bearing hyphae characteristics were identified using scanning electron microscope (Tescan, Vega3 LMU).

Physiological properties of Streptomyces isolates were experimented using eight different isolation media (International Streptomyces Project, ISP1-ISP7) and Starch casein agar. Colony growth was observed in 5-20 days of incubation. During the incubation period, the growth of the isolates was monitored and selected one of the best media for Streptomyces isolation and purification. The growth of the isolates was determined with different range of pH (6.0-8.5) and temperature (20-45 °C) on SCA media and tested for optimum pH and temperature.

The Streptomyces isolates utilize different carbon sources such as glucose, sucrose, fructose, xylose and maltose at 1% (w/v) and nitrogen sources such as, tryptone, casein and leucine at 1% (w/v). The isolates were inoculated onto SCA agar medium with different concentration of NaCl (2, 4, 6, and 8%, w/v), and the plates were incubated at 28 °C for 5-7 days. After incubation the salt tolerance was detected by growth appearance. To determine antibiotic resistance of the isolates streptomycin (5 μg ml⁻¹), ampicillin (2 μg ml⁻¹), kanamycin (2 μg ml⁻¹) and tetracycline (2 μg ml⁻¹) (Himedia, Mumbai, India) were added to SCA agar medium, and the growth was recorded according to the method of Shirling and Gottlieb (1966).
Biochemical characterization of isolates was carried out as described by Williams et al. (1983). To determine starch hydrolysis activity, the isolates were inoculated on starch agar plates and incubated at 28 °C for 5 days (Hankin and Anagnostakis, 1975). After incubation, iodine solution was flooded onto the plate and observed the development of clear zone around the colonies indicating the positive starch hydrolysis. To determine proteolytic activity, the isolates were inoculated onto plates containing SCA medium with two substrates like casein and gelatin separately, and incubated at 28 °C for 5-7 days. After incubation, the development of clear zone around the colonies, indicating the potential hydrolysis of proteins was recorded according to the method of Smibert and Krieg (1994). The isolates were screened for the production of various enzyme activities such as catalase, urease and nitrate reduction as described previously by Kampfer et al. (1991).

3.2.4.2. Molecular characterization

Totally, eight GI producing *Streptomyces* isolates were screened for molecular identification. The genomic DNA was isolated as described by Sambrook and Russell (2001). Polymerase Chain Reaction (PCR) reaction was carried out with Taq DNA polymerase using 27F-forward primer (5'-AGA GTT TGA TCG TGG CTC AG-3') and the 1492R-reverse primer (3'-GGT TAC CTT GTT ACG ACT T-5'). The conditions for thermal cycling were as follows: initial denaturation of the target DNA at 95 °C for 10 min, followed by 30 cycle of amplification with the condition of denaturation at 95 °C for 2 min, primer annealing at 58 °C for 1 min and the primer extension at 72 °C for 2 min. At the end of the cycle the reaction mixture was heated at 72 °C for 10 min and cooled to 4 °C. Amplified DNA was visualized at 100 V and 400 mA for 5 min on an agarose gel 1% (w/v) using 1xTAE buffer, 0.1 µl ethidium bromide solution. The DNA concentration was determined as described by Sambrook and Russell (2001). The amplified PCR products were purified with a QIAquick® PCR purification kit (Qiagen Ltd., Crawley, UK).

3.2.4.3. 16S rRNA gene sequencing and phylogenetic analysis

The 16S ribosomal RNA gene sequencing was done at Solgent Co. Ltd (Seoul, South Korea). The sequence similarity search was performed using Gen Bank BLASTN 2.2.28+ (National Center for Biotechnology Information). DNA sequence

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**Isolation and screening of glucose isomerase producing *Streptomyces* isolates**
contig alignment was computed through greedy algorithm (Zhang et al., 2000). Neighbour-joining phylogenetic tree (Saitou and Nei, 1987) based on analysis of nearly complete 1408-1445 nucleotides 16S rRNA gene sequences. The evolutionary distances were computed using the maximum composite Likelihood method (Tamura et al., 2004) Sequences of closely related taxa were retrieved and aligned using the CLUSTAL_W program (Thompson et al., 1997) and the alignment was corrected manually, phylogenetic tree was constructed using the neighbor joining DNA distance algorithm with the MEGA 6.0.2 (Molecular Evolutionary Genetic Analysis (http://www. megasoftware.net) version (Tamura et al., 2013).

3.2.5. Secondary screening

Totally eight potent GI producing isolates were selected from the primary screening such as RSU-1, 14, 15, 21, 23, 26, 30 and 51 and used for secondary screening such as chromatography separation and quantitative estimation of GI.

3.2.5.1. Determination of Biomass

The eight Streptomyces species were cultured in peptone yeast extract broth containing 50% sea water (pH 7.0) described by Uyar and Baysal, (2004). The cells were grown aerobically in 250 ml flask containing 100 ml medium at 28 °C with continuous shaking in an Orbital shaker (250 rpm). Streptomyces growth was monitored by measuring culture turbidity at OD $\text{nm}$ and estimated the biomass content. During stationary growth phase, the biomass was collected by centrifugation at $15,000 \times g$ for 10 min at 4 °C. The cell suspension was washed twice with distilled water and dried in hot air oven at 80 °C overnight. The cell dry weight was calculated per liter of culture broth $\geq 0.8$ OD $\text{nm}$ corresponds to g/L of cell dry biomass.

3.2.5.2. Crude cell free enzyme preparation

After shake flask fermentation, the biomass was harvested by centrifugation at $15,000 \times g$ 10 min at 4 °C. The cells were suspended in 50 ml distilled water and disrupted using ultrasonic disintegrator (Ultrasonic Homogenizer-Biologics, Inc-3000). The content was centrifuged at $12,000 \times g$ for 10 min at 4 °C. The supernatant containing cell- free extract was collected and used for soluble fraction of crude enzyme (Park and Toma, 1975). The extraction of insoluble protein, pellet aggregate

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containing cell debris was prepared 4M Urea solution used for solubilization (Sambrook et al., 1989). The soluble and insoluble fraction was used for determination of enzyme assay.

3.2.5.3. Chromatographic separation of glucose isomerase

Production of GI was confirmed by thin layer chromatography (TLC). Isomerization of glucose to fructose was detected as follows: the reaction mixture was prepared as described by Takasaki (1966) with few modifications. To prepare the mixture 0.8 ml 0.1 M D-glucose, 1.0 ml of potassium phosphate buffer (50 mM) pH 7.5, 0.2 ml of 10 mM MgSO₄·7H₂O, 0.2 ml 1 mM CoCl₂·6H₂O and 0.2 ml of the cell free intracellular enzyme solution were mixed and the final volume was brought up to 3 ml with deionized water and incubated 30 min at 55 °C. The isomerization of glucose → fructose was detected by TLC (Silica gel G, Merck) using a solvent system of ethyl acetate, ethanol, and water (4:5:1, v/v/v). The sugars were identified by spraying the reagent containing 9 ml 2% resorcinol and 1 ml of Orthophosphoric acid, on TLC plates. The plates were air-dried and observed the development of red spot, indicating the presence of fructose (Al-Tai et al., 1987).

3.2.6. Glucose isomerase Assay

3.2.6.1. Isomerization of D-fructose to D-glucose

GI activity was detected by fructose to glucose isomerization using a method described by Miller, 1959 with few modification. To prepare the reaction mixture 0.8 ml of 0.1 M D-fructose, 1.0 ml of 50 mM potassium phosphate buffer (pH 7.5), 0.2 ml of 10 mM MgSO₄·7H₂O, 0.2 ml of 1 mM CoCl₂·6H₂O and 0.2 ml of the intracellular (soluble and insoluble) crude enzyme extracts were mixed and the final volume was brought up to 3 ml with deionized water and incubated for 30 min at 40 °C. The reaction was stopped by adding 2 ml of Dinitrosalicylic acid (DNS) reagent. The development of red color indicates the isomerization of glucose. The reaction mixture was cooled in an ice water bath for 10-15 min and then centrifuged 5000 × g for 5 min at 4 °C. The quantity of glucose was measured at 540 nm using UV-Vis Spectrophotometer (Techcomp, 2310), with a blank sample as a reference. Standard calibration curve was constructed using D-glucose (Fig 3.9a). One unit of enzyme
activity was defined as the amount of GI needed to produce 1 µmol of D-glucose per min under the assay conditions.

3.2.6.2. Isomerization of D-glucose to D-fructose

The production of GI was detected by glucose to fructose isomerization using a method described by Takasaki, 1966 with few modifications. To prepare the mixture 0.8 ml of 0.1 M D-glucose, 1.0 ml of 50 mM potassium phosphate buffer (pH 7.5), 0.2 ml of 10 mM MgSO₄·7H₂O, 0.2 ml of 1 mM CoCl₂·6H₂O and 0.2 ml of the intracellular (soluble and insoluble) crude enzyme extracts were mixed and the final volume was brought up to 3 ml with deionized water and incubated 30 min at 55 °C. The reaction was stopped by adding 2 ml of HCl-Resorcinol reagents. The development of red color indicates the positive for fructose isomerization. The mixture was cooled in an ice water bath for 10-15 min and then centrifuged at 5000 × g for 5 min at 4 °C. The quantity of fructose was measured at 485 nm using using UV-Vis Spectrophotometer (Techcomp, 2310), with a blank sample as a reference. Standard calibration curve was constructed using D-fructose (Fig 3.9b). One unit of GI activity was defined as the amount of enzyme needed to produce 1 µmol of D-fructose per min under the assay conditions.

3.2.6.3. Estimation of total protein

The total protein content of the cell free extract was determined by the method described (Bradford, 1976). Briefly, 50 µl of cell-free enzyme solution was added into the tubes and mixed with 2.5 ml of Bradford reagent to each well, then incubated at room temperature for at least 5 min. The protein activity was estimated at 590 nm using UV-Vis Spectrophotometer (Techcomp, 2310) with a blank sample as a reference. Standard calibration curve was constructed using the absorbance of bovine serum albumin (Fig 3.9c).

3.3. Results

3.3.1. Soil analysis and isolation

The soil physico-chemical characteristics were studied and summarized in Table 3.1. The temperature was in the range of 24-27 °C, while the pH of sample 7.71 ± 0.43. The salinity recorded at the time of sample collection was in the range of 23.
25 g/L. Totally, 151 strains were isolated from mangrove samples collected from four different sites. Most of the strains appeared like Streptomyces. The distribution of isolates was represented in percentage (%) site I (16%); site II (20%); site III (54%) and site IV (10%).

3.3.2. Primary screening of glucose isomerase producing isolates

The primary screenings of GI are given in Fig 3.2. Among 151 isolates, 56 were appearing morphologically distinct and selected for GI primary screening. The total of 56 isolates, 22 exhibited positive for GI activity and 8 isolates exhibited better GI activities. The potent isolates were exhibited various diameters zone, ranging from 6-20 mm and individually expressed, RSU1 (4 mm), RSU14 (3 mm), RSU15 (4 mm), RSU21 (11 mm), RSU23 (15 mm), RSU26 (20 mm), RSU30 (12 mm) and RSU51 (15 mm) respectively. RSU26 had high GI activity than that of other seven isolates.

3.3.3. Characterization of marine isolates

3.3.3.1. Morphological, physiological and biochemical characteristics

The morphological characteristics of the isolates are given in Table 3.2. The cultural characterization of representative isolates was observed visually with the following appearance: grey, yellow, red, golden yellow, sandal and cream with white color on starch casein agar plate and produced different reverse color pigmentation (Fig 3.3). The isolates showed well developed aerial and substrate mycelia with sporulation on starch casein agar media. The isolates emerged different shapes like round, tiny ring, multi ring and button shape colonies, and the texture appeared powdery nature and have smooth spores (except) RSU15, which was produced rough. The growth diameter of the colonies was in the range of 3-8 mm except RSU21 and RSU26 maximum of 7 and 8 mm. In addition, the isolates had an earthy odor (presumably geosmin). As for the oxygen relationship, isolates was noted that all are aerobics.

The light microscopic and scanning electron microscopic (SEM) characteristics are given in Table 3.2. Spore chains arrangements of Streptomyces appeared like, flexible-rectiflexible (RF), one loops-rectiaculapierti (RA), spiraspirales (S), and flexibilis with spirals (FS), etc., (Fig 3.4). SEN
biverticillium, retinaculus - apertum like multi loops with filamentous, etc., (Fig 3.5). SEM characteristics, provides suitable information not only on spore surface characteristics, but also on spore arrangement and the presence of sheath surrounding sporophores.

The physiological characteristics of the isolates are given in Table 3.3. To determine the effect of media components on growth of isolates, the GI producing isolates were grown onto different ISP-1 to ISP-7 (International Streptomyces Project media) and SCA media for characterize their growth. The isolates exhibited poor growth onto ISP-1 media; the growth was developed in ≥15 days of incubation. The isolates RSU-1, 14 and 26 were grown ≥15 days on ISP-1 and ISP-4 media. The eight isolates was exhibited good growth onto ISP-3 and ISP5, except RSU51 and 21, in which growth appeared after 15 days. The ISP-1 and ISP-2 media have no significant effects. ISP-6 and ISP-7 media considered good for the isolates growth but few isolates did not show any effect on these media. All isolates showed well sporulation with excellent growth was observed only in starch casein agar (SCA) medium during 5-7 days of incubation. The optimized SCA media nutrient sources exhibited the same perspective growth to conventional SCA media.

The influence of pH and temperature on growth of isolates were studied. The eight isolates showed optimum growth at pH 7.2 ± 0.1 and temperature 28 ± 0.2 °C, the maximum growth was observed from the range of 25-35 °C. The isolates utilized glucose, starch, fructose and xylose; nitrogen sources, tryptone, casein and leucine. Significant growth was observed on starch as carbon source and casein as nitrogen source. The effect of different NaCl concentration was observed from the range of 2-8% and the optimum at 2% NaCl and all are able to tolerate 6% NaCl. The growth was inhibited 8% NaCl concentration. The isolates were exhibited capable of grown onto various antibiotics; especially RSU26 highly resistant to all antibiotics (Table 3.3).

Biochemical characteristics of the isolates are given in Table 3.4. All isolates are aerobic, G +ve, mesophilic, non-motile and non - endospore forming characteristics. The effect of carbohydrate fermentation, RSU23, 26 and 51 utilized glucose and sucrose as sole carbon source. All isolates positive for citrate, except
RSU15 and 21. The effect of starch and protein hydrolysis (casein and gelatin), all isolates exhibited positive. Starch and protein hydrolysis the diameter was determined to be in the range of 8-21 mm and 4-20 mm respectively. All isolates exhibited positive for urease utilization. RSU 1, 21, 23 and 26 were exhibited positive for catalase and nitrate, except RSU-14, 15, 30 and 51. Based on the above all biochemical characteristics these marine isolates were appeared similar to the genus *Streptomyces*.

### 3.3.3.2. Molecular characterization

The 16S rRNA genes of the eight representative isolates were sequenced. The obtained sequences were evaluated with BLASTN 2.2.28+ search, the available averaging 1408-1445 nucleotides of the sequences was compared with the other nucleotides of *Streptomyces* strains from genbank. The sequence reports revealed the strains homologous with related member of *Streptomyces* species: RSU1 (*S. albidoflavus*), RSU14 (*S. albus*), RSU15 (*S. fradiae*), RSU21 (*S. sampsonii*), RSU23 (*S. carpathicus*), RSU26 (*S. lividans*), RSU30 (*S. gresius*) and RSU51 (*S. krainskii*) ~99% similarity. The GI producing strains had a significant identity with various *Streptomyces* species. The phylogenetic tree was constructed on the basis of 16S rRNA gene sequences of the strains to illustrate the evolutionary relationship between the strains and distinct branch with most closely related *Streptomyces* species (Fig 3.6). Scale bar, 0.2 indicates the identities of substitutions per nucleotide position.

### 3.3.3.3. Nucleotide sequence accession numbers

The 16S rRNA sequences of GI producing *Streptomyces* species have been deposited in DDBJ/EMBL/Gen Bank under the accession no. (KP698738-KP698745) showed in Table 3.5. Based on the molecular characterization, the isolated *Streptomyces* was classified into species level.

### 3.3.4. Secondary screening of glucose isomerase producing isolates

#### 3.3.4.1. Biomass determination

The time course profile of eight *Streptomyces* species was monitored as optical density vs. incubation time (Fig 3.7). The maximum biomass production were
recorded only in *S. lividans* RSU26, it has reached initial stationary phase at 96 h and the maximum cell dry weight was 0.64 g L\(^{-1}\). The *S. albus*, *S. sapmsonii* and *S. krainskii* have reached the maximum cell dry weight to be in the range of 0.54-0.58 g L\(^{-1}\), respectively in 120 h.

3.3.4.2. Chromatographic separation

Thin layer chromatographic separations of GI are given in Fig 3.8. All *Streptomyces* species were expressed GI with different sizes and intensities of their spots. *S. lividans* RSU26 was expressed high intensity of red color and relative Rf value 0.46 cm, the value was similar to standard fructose. Remaining species showed low intensity and the Rf value were calculated to be in the range of ≥ 0.5-0.78 cm.

3.3.4.3. Activity of glucose isomerase

The efficiency of glucose isomerase producing *Streptomyces* species are given in Table 3.6. The total of eight isolates synthesizing soluble cell free extract had GI activity but the insoluble pellet extracts does not exhibited GI. Taking this observation, the isolates were harboring only soluble form of GI. The eight isolates was exhibited GI activity to be in the range of 0.19-0.42 U mg\(^{-1}\) protein during fructose → glucose isomerization at 40 °C (pH 7.5) and 0.17 to 0.40 U mg\(^{-1}\) of protein during glucose → fructose isomerization at 55 °C (pH 7.5). The GI specific activity highly exhibited in *S. lividans* RSU26, 0.42 and 0.40 U mg\(^{-1}\) protein, for its fructose → glucose and glucose → fructose conversion rate than compared to other seven *Streptomyces* isolates.

3.4. Discussion

This work was aimed to isolate potent GI harboring *Streptomyces* from marine environment. The determination of the primary ecological factors include pH, temperature and salinity of soil sample were recorded to their effects on the distribution of *Streptomyces* flora in mangroves. The population of *Streptomyces* was highly depending with physico-chemical properties of the sample. This is agreement with previous findings demonstrated with that the most of actinobacteria, particularly *Streptomyces* favor neutral or slightly alkaline pH for their growth (Kathireshan et al.,
2005; Vijayakumar et al., 2007; Das et al., 2008; Ravikumar et al., 2011; Thirumurugan and Vijayakumar, 2015).

The *Streptomyces* isolates count was observed least amount in all sampling sites except chief corer area (site III). It has unique environmental conditions, the soil pH 7.5 ± 0.9, temperature 26 ± 0.1 and salinity 25 g L⁻¹. This might be credited to this was noticeably described with high percentage of *Streptomyces* and adapts efficient intracellular GI producing isolates recovered from this site. The result described a diverse *Streptomyces* found in the mangrove sediment. While, there is no significance differences were observed between samples collected from different depths. The present work clearly revealed the *Streptomyces* are leading genus in mangrove ecosystem because similar predominance of *Streptomyces* was also reported in marine sediments (Hans-peter et al., 2005; Xu et al., 2009; Dhanasekaran et al., 2009; Sathya et al., 2010; Sivakumar et al., 2011; Ray et al., 2014; Chakraborty et al., 2015).

GI producing isolates were successfully screened from mangrove soil. Initial screening of GI, eight isolates exhibited better activity. Primary isolation of GI producing microorganism is usually carried out on solid media; the related approach was stated for screening by Sanchez and Smiley (1975). In view of screening, number of qualitative and quantitative determination of glucose (xylose) isomerase was reported (Chen and Anderson, 1979; Schenk and Bisswanger, 1998). Recently, GI screened from various *Streptomyces* species has been documented (Sapunova et al., 2004; Srivastava et al., 2010; Lawal et al., 2012).

The isolated colonies were showing typical morphology of *Streptomyces* were described earlier. All isolates appeared similar to the genus *Streptomyces* as reported by several workers (Sivakumar et al., 2005; Kampfer et al., 2008; Thirumalairaj et al., 2015). In accordance with the characteristics of *Streptomyces* aerial and substrate mycelium established in the Bergey’s manual of determinative bacteriology (Buchanan and Gibbons, 1974). The microscopical characterization was provided the key support for the identification of isolates. The technique of examining the shape and spore chains characteristics under the light microscopy and scanning electron
microscopy were previously described (Nonomura, 1974; Locci, 1989; Mitra et al., 2011; Whitman et al., 2012) and very well associated with the present findings.

This work, suggested the starch casein agar (SCA) medium was suitable for selective isolation of *Streptomyces*. SCA medium highly suppressed bacterial and fungal contamination than for ISP1-7. The SCA medium was originally developed by Grein and Meyers (1958). Furthermore, recommended many workers (Kokare et al., 2003) also suggested SCA medium is more effective for the isolation of marine actinobacteria. All isolates exhibiting the physiological and biochemical characteristics are comparable with previous reports (Hakvag et al., 2008; Willey et al., 2010; Chacko Vijai Sharma and David 2012; Rajeswari et al., 2015). Number of scientific reports have predicted the similar characterization of *Streptomyces* (Kalyani et al., 2012; Sirisha et al., 2013; Cruz et al., 2015; Haque et al., 2015).

To make the taxonomic position of the isolated species, 16S rRNA gene as a phylogenetic marker, as it only provides an accurate and reliable classification to the genus and species level of *Streptomyces*. The *Streptomyces* isolates and their closest NCBI relatives based on almost complete 16S rRNA gene sequences similar with previous findings, *Streptomyces albus* subsp. *albus* NRRLB-1811(T), *S. fradiae*, *S. gresius*, and *S. carpaticus* species forms a cluster with present 16S rRNA gene sequences (Pathom–aree et al., 2006; Haritha et al., 2012; Labeda et al., 2013; Ranjith kumar et al., 2014; Ian et al., 2014). A vast array of *Streptomyces* species reported in German collection of microorganism and cell cultures listed approximately 650 species (Wilkins and Scholler, 2009). The utility of 16S rRNA gene sequence and confirm the molecular identity of *Streptomyces* species (Lanoot et al., 2004; Yan et al., 2006; Maleki et al., 2013; Seipke, 2015).

In order to increase the prospects to screen for the most suitable strain for high level of GI expression were determined by chromatography. Secondary screening of GI was performed by thin layer chromatographic separations; provide accurate results for GI. This method is less time consuming compare to other screening experiments. Only few reports are available for screening of GI by TLC. The result was appropriate and coincides with previous work of GI detection by chromatography (Takasaki, 1966; Al-Tai et al., 1987; Sathya and Ushadevi, 2014).
Quantitative screening of GI production, *S. lividans* RSU26 is highly significant with interpretation of other species. It is interestingly to note that *S. lividans* RSU26 has a linear function with 96 h of incubation and high cell density favor for GI production. This is very attractive character found only in *S. lividans* RSU26 and exhibited high cell dry weight and specific activity of 0.64 g L\(^{-1}\) and 0.42 U mg\(^{-1}\) protein. The previous reports achieved lower specific activity, *Bacillus thermoantarticus*, 0.18 U mg\(^{-1}\) protein (Lama et al., 2001), wild-type *E. coli*, 0.065 U mg\(^{-1}\) (Voronovsky et al., 2005); *Arthrobacter ureafaciens*, 0.06 U mg\(^{-1}\) (Sapunova et al., 2008).

The present study describes the isolated *Streptomyces* species have high biological activities. Our investigation suggested that the marine *Streptomyces* produce efficient source of GI. It has been suggested that number of GI pattern are available in *Streptomyces* species, but too little evidence is yet to prove this idea in marine environment. This work motivating the GI producing *Streptomyces* are found in mangroves as well as *S. lividans* RSU26 are considered as a robust glucose isomerase producer.

Hence, primary and secondary screening of GI producing capabilities of *S. lividans* RSU26, appeared certainly compete with other seven GI producers. The high catalytic activity of aldose and ketose interconversion was observed by RSU26 GI. Following further optimization and characterization, *S. lividans* RSU26 may become a good source for industrial production of glucose isomerase.
Table 3.1. Physico-chemical properties of mangrove soil collected from Muthupet coastal region.

<table>
<thead>
<tr>
<th>Site of sampling</th>
<th>Sample</th>
<th>Depth (cm)</th>
<th>Temperature (°C)</th>
<th>pH</th>
<th>Salinity (g/L)</th>
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<tbody>
<tr>
<td>I</td>
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<td>10-15 cm</td>
<td>25</td>
<td>7.36</td>
<td>23</td>
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<td></td>
<td>2</td>
<td>10-15 cm</td>
<td>24</td>
<td>7.28</td>
<td>25</td>
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<td></td>
<td>3</td>
<td>10-15 cm</td>
<td>25</td>
<td>7.35</td>
<td>24</td>
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<td></td>
<td>1</td>
<td>10-15 cm</td>
<td>26</td>
<td>7.62</td>
<td>24</td>
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<tr>
<td>II</td>
<td>2</td>
<td>10-15 cm</td>
<td>25</td>
<td>7.87</td>
<td>28</td>
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<td></td>
<td>3</td>
<td>10-15 cm</td>
<td>24</td>
<td>7.62</td>
<td>26</td>
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<td></td>
<td>1</td>
<td>10-15 cm</td>
<td>26</td>
<td>7.54</td>
<td>25</td>
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<tr>
<td>III*</td>
<td>2</td>
<td>10-15 cm</td>
<td>25</td>
<td>7.56</td>
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<td></td>
<td>3</td>
<td>10-15 cm</td>
<td>27</td>
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<td>25</td>
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<td>IV</td>
<td>1</td>
<td>10-15 cm</td>
<td>25</td>
<td>7.66</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10-15 cm</td>
<td>24</td>
<td>7.88</td>
<td>28</td>
</tr>
</tbody>
</table>

Sampling sites: I - Selli munai, II - Sedhukkuda, III - Chief corner, IV - Xeiver point.
Note: The soil physico-chemical properties were analyzed by soil analysis kit (Model 191).

*Sampling site III: Glucose isomerase producing *Streptomyces* isolates was found only this region. There is no drastic changes occurs between physico-chemical parameters of three core samples in this region, pH 7.5 ± 0.9, temperature 26 ± 0.1 and salinity 25 g L⁻¹ respectively.

The distribution of *Streptomyces* isolates was found high in chief corner (site III), the data represented as percentage of distribution, site III (54%); other three sampling site ≤ 20% of population recorded.

Isolation and screening of glucose isomerase producing *Streptomyces* isolates
Table 3.2. Morphological characterization of glucose isomerase producing *Streptomyces* isolates on starch casein agar medium.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RSU1</th>
<th>RSU14</th>
<th>RSU15</th>
<th>RSU21</th>
<th>RSU23</th>
<th>RSU26</th>
<th>RSU30</th>
<th>RSU51</th>
</tr>
</thead>
<tbody>
<tr>
<td>Appearance</td>
<td>Sandal with white colonies</td>
<td>Dark yellow thick colonies</td>
<td>Light red color colonies</td>
<td>Cream with grey color</td>
<td>Brown color colonies</td>
<td>Yellow with white color colonies</td>
<td>Light white thick colonies</td>
<td>Yellow with white colonies</td>
</tr>
<tr>
<td>Aerial mycelium</td>
<td>Light grey</td>
<td>Ash color</td>
<td>Sandal color</td>
<td>Ash color</td>
<td>Violet with white</td>
<td>Light white color</td>
<td>White color</td>
<td>Light cream with grey</td>
</tr>
<tr>
<td>Substrate mycelium</td>
<td>Whitish grey Smooth</td>
<td>Sandal with white Smooth</td>
<td>Yellow color Rough</td>
<td>Blue color Smooth</td>
<td>Black color Smooth</td>
<td>Light pink Smooth</td>
<td>Black color Smooth</td>
<td>Green color Smooth</td>
</tr>
<tr>
<td>Spore surface</td>
<td>6 mm</td>
<td>3-5 mm</td>
<td>6 mm</td>
<td>3-7 mm</td>
<td>3 mm</td>
<td>8 mm</td>
<td>4 mm</td>
<td>3-6 mm</td>
</tr>
<tr>
<td>Reverse color</td>
<td>Dark brown</td>
<td>Light yellow</td>
<td>Red</td>
<td>Light Violet</td>
<td>Dark pink</td>
<td>Light Pink</td>
<td>Orange</td>
<td>Pink</td>
</tr>
<tr>
<td>Texture</td>
<td>Powdery</td>
<td>Powdery</td>
<td>Powdery</td>
<td>Powdery</td>
<td>Powdery</td>
<td>Powdery</td>
<td>Powdery</td>
<td>Cottony</td>
</tr>
<tr>
<td>Shape</td>
<td>Double ring</td>
<td>Round</td>
<td>Button</td>
<td>Button</td>
<td>Tiny round</td>
<td>Multi ring</td>
<td>Round</td>
<td>Ring</td>
</tr>
<tr>
<td>Odor</td>
<td>Geosmin</td>
<td>Geosmin</td>
<td>Geosmin</td>
<td>Geosmin</td>
<td>Geosmin</td>
<td>Geosmin</td>
<td>Geosmin</td>
<td>Geosmin</td>
</tr>
<tr>
<td>Oxygen relationship</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
<td>Aerobic</td>
</tr>
<tr>
<td>†Light microscope</td>
<td>RF</td>
<td>F</td>
<td>RA</td>
<td>F</td>
<td>F-SH</td>
<td>F-SL</td>
<td>F-RF</td>
<td>SF</td>
</tr>
<tr>
<td>‡Scanning electron</td>
<td>SF</td>
<td>RS-CF</td>
<td>S-LH</td>
<td>RF</td>
<td>BIV</td>
<td>F-RA</td>
<td>F</td>
<td>RF-S</td>
</tr>
<tr>
<td>microscope</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

†RF, Rectus with filamentous; F, Filamentous; RA, Retinaculum Apartum; F-SH, Filamentous with spiral hooks; F-SL, Filamentous with spiral loops; F-RF, Filamentous with recti flexibilis; SF, Spiral with filamentous.

‡ SF, Straight with flexibilis; RS-CF, Regular Smooth tiny and compact filamentous; S-LH, Spiral with open loops hooks extended; RF, Recti flexibilis spore chain with irregular filaments; BIV, Biverticillium (compound verticillus, long axis) F-RA, Filamentous with retinaculus - aperture like multi loops with hooks extended; F, Filamentous with multi young spores; RF-S, Recti flexibilis simple with compact spiral spores.

*Note:* The isolates were grown in sea water (50%, v/v) containing basal starch casein agar medium (Kuster and Williams, 1964).

*Streptomyces* characteristics reported in both, Bergey’s manual of systematic Bacteriology (Locci, 1989; Whitman et al., 2012), and International *Streptomyces* Project (Shirling and Gottlieb, 1966).

Microscopic specimens were prepared by coverslip culture technique; † Spore arrangement was observed by oil immersion objective (100 X) (Kawato and Shinolue, 1959).

‡‡ Spore arrangements of the isolates were observed slightly contradictory under light microscope and scanning electron microscope.

Isolation and screening of glucose isomerase producing *Streptomyces* isolates
Table 3.3. Physiological characteristics of glucose isomerase producing *Streptomyces* isolates.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>RSU1</th>
<th>RSU14</th>
<th>RSU15</th>
<th>RSU21</th>
<th>RSU23</th>
<th>RSU26</th>
<th>RSU30</th>
<th>RSU51</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Growth on different media</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yeast extract agar (ISP I)</td>
<td>Poor</td>
<td>Poor</td>
<td>Poor</td>
<td>Poor</td>
<td>Poor</td>
<td>Poor</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Yeast extract malt extract agar (ISP II)</td>
<td>Poor</td>
<td>Poor</td>
<td>Moderate</td>
<td>Good</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
<td>Good</td>
</tr>
<tr>
<td>Oat meal agar (ISP III)</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Inorganic salt agar (ISP IV)</td>
<td>Poor</td>
<td>Poor</td>
<td>Good</td>
<td>Moderate</td>
<td>Good</td>
<td>Poor</td>
<td>Moderate</td>
<td>Poor</td>
</tr>
<tr>
<td>Glycerol asparagine agar (ISP V)</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Poor</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td>Peptone yeast extract agar (ISP VI)</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Good</td>
<td>Poor</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Tyrosine agar (ISP VII)</td>
<td>Moderate</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Poor</td>
<td>Poor</td>
</tr>
<tr>
<td>Starch casein agar</td>
<td>Excellent</td>
<td>Excellent</td>
<td>Good</td>
<td>Good</td>
<td>Excellent</td>
<td>Good</td>
<td>Good</td>
<td>Good</td>
</tr>
<tr>
<td><strong>Optimum temperature (°C)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>28</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Optimum pH</strong></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>7.2</td>
<td>++</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td><strong>Carbon source (1%, w/v)</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Starch</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Fructose</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+</td>
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<tr>
<td><strong>Nitrogen source (1%, w/v)</strong></td>
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<td></td>
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<tr>
<td>Tryptone</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Casein</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Leucine</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td><strong>Effect of NaCl (w/v)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2%</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>4%</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>6%</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8%</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>Antibiotic resistant</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomycin (50 μg/100 ml)</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Kanamycin (20 μg/100 ml)</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Tetracycline (20 μg/100 ml)</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Ampicillin (20 μg/100 ml)</td>
<td>+</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

+, Moderate Growth; ++, Good growth; ++++, Excellent growth; ISP- International *Streptomyces* Project medium. The isolates grown in sea water (50%, v/v) containing media (Shirling and Gottlieb, 1966; Kuster and Williams, 1964). Excellent, growth observed in 5 days; Good, growth observed in 7 days; Moderate, growth observed in ≥ 15 days.

Isolation and screening of glucose isomerase producing *Streptomyces* isolates
Table 3.4. Biochemical characteristics of glucose isomerase producing *Streptomyces* isolates.

<table>
<thead>
<tr>
<th>Experiments</th>
<th>RSU1</th>
<th>RSU14</th>
<th>RSU15</th>
<th>RSU21</th>
<th>RSU23</th>
<th>RSU26</th>
<th>RSU30</th>
<th>RSU51</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Staining ‡</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gram’s staining</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Endospore</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td><strong>CHO fermentation ∗</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td><strong>Starch hydrolysis</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>++</td>
</tr>
<tr>
<td>Protein hydrolysis *</td>
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<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Gelatin</td>
<td>+</td>
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<td>+++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Casein</td>
<td>+</td>
<td>+</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
<td>+++</td>
<td>+</td>
</tr>
<tr>
<td><strong>Enzyme activity †</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Citrate</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Urease</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Nitrate reduction</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

–, Negative; +, positive.
+++, Good positive (8-17 mm diameter zone); +++, strongly positive (18-21 mm diameter zone).
†† Based on characteristics reported in International *Streptomyces* Project (Shirling and Gottlieb, 1966).
* Comparative protein hydrolysis followed by the method of Smibert and Krieg (1994).
† Starch hydrolysis by Hankin and Anagnostakis (1975).

Table 3.5. 16S rRNA gene sequences of glucose isomerase producing *Streptomyces* sp.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Nucleotide contig</th>
<th>Percentage (%)</th>
<th>E value</th>
<th>Accession. No.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. albidoflavus</em> strain RSU1</td>
<td>1437</td>
<td>99</td>
<td>0.0</td>
<td>&gt;gb [KP698738.1]</td>
</tr>
<tr>
<td><em>S. albus</em> strain RSU14</td>
<td>1445</td>
<td>99</td>
<td>0.0</td>
<td>&gt;gb [KP698739.1]</td>
</tr>
<tr>
<td><em>S. fradiae</em> strain RSU15</td>
<td>1438</td>
<td>99</td>
<td>0.0</td>
<td>&gt;gb [KP698740.1]</td>
</tr>
<tr>
<td><em>S. sampsoni</em> strain RSU21</td>
<td>1443</td>
<td>99</td>
<td>0.0</td>
<td>&gt;gb [KP698741.1]</td>
</tr>
<tr>
<td><em>S. carpaticus</em> strain RSU23</td>
<td>1408</td>
<td>99</td>
<td>0.0</td>
<td>&gt;gb [KP698742.1]</td>
</tr>
<tr>
<td><em>S. lividans</em> strain RSU26</td>
<td>1438</td>
<td>99</td>
<td>0.0</td>
<td>&gt;gb [KP698743.1]</td>
</tr>
<tr>
<td><em>S. gersius</em> strain RSU30</td>
<td>1438</td>
<td>99</td>
<td>0.0</td>
<td>&gt;gb [KP698744.1]</td>
</tr>
<tr>
<td><em>S. krainskii</em> strain RSU51</td>
<td>1437</td>
<td>99</td>
<td>0.0</td>
<td>&gt;gb [KP698745.1]</td>
</tr>
</tbody>
</table>

Sequences are accessible in NCBI/DDBJ/EMBL/Gen Bank data base.
Sequence alignment retrieved from BLASTN 2.2.28+ and nucleotide contig sequence aligned greedy algorithm followed by Zhang et al. (2000).
Table 3.6. Efficiency of glucose isomerase producing *Streptomyces* isolates.

<table>
<thead>
<tr>
<th>Sources</th>
<th>Cell Biomass (cdw, g/L)*</th>
<th>Glucose isomerase activity (U/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fructose → glucose</td>
</tr>
<tr>
<td><em>S. albidosflavus</em> strain RSU1</td>
<td>0.40 ± 0.002</td>
<td>0.24 ± 0.006</td>
</tr>
<tr>
<td><em>S. albus</em> strain RSU14</td>
<td>0.58 ± 0.002</td>
<td>0.25 ± 0.004</td>
</tr>
<tr>
<td><em>S. fradias</em> strain RSU15</td>
<td>0.43 ± 0.006</td>
<td>0.35 ± 0.001</td>
</tr>
<tr>
<td><em>S. sampsonii</em> strain RSU21</td>
<td>0.54 ± 0.001</td>
<td>0.36 ± 0.002</td>
</tr>
<tr>
<td><em>S. carpaticus</em> strain RSU23</td>
<td>0.43 ± 0.006</td>
<td>0.19 ± 0.001</td>
</tr>
<tr>
<td><em>S. lividans</em> strain RSU26†</td>
<td>0.64 ± 0.004</td>
<td>0.42 ± 0.003</td>
</tr>
<tr>
<td><em>S. gresius</em> strain RSU30</td>
<td>0.43 ± 0.008</td>
<td>0.31 ± 0.007</td>
</tr>
<tr>
<td><em>S. krainskii</em> strain RSU51</td>
<td>0.54 ± 0.001</td>
<td>0.38 ± 0.003</td>
</tr>
</tbody>
</table>

*Streptomyces* isolates were grown in peptone yeast extract broth (pH 7.0) at 28 °C for 96 h (Uyar and Baysal, 2004).

Cell free enzyme preparation described by Park and Toma, 1975; GI activity was determined by two catalytic isomerization reactions.

Fructose to glucose isomerization was followed by the method (Miller, 1959) with slight modification and glucose to fructose isomerization was performed by Takasaki (1966).

*cdw, cell dry weight.

**Assay conditions:** 50 mM potassium phosphate buffer (pH 7.5); 0.1 M, D-glucose and 0.1 M, D-fructose individually; 10 mM, MgSO₄; 1 mM, CoCl₂; and an appropriate amount of intracellular enzyme. The temperature was set at 40 °C for fructose → glucose and 55 °C for glucose → fructose conversion. All experiments were performed in triplicates and the mean values along with standard error mean (n=3) are presented.

† *S. lividans* RSU26 exhibited biomass cell dry weight maximum of 0.64 g L⁻¹ and specific activity 0.4 and 0.40 U mg⁻¹ protein, for its fructose → glucose and glucose → fructose conversion respectively.

Isolation and screening of glucose isomerase producing *Streptomyces* isolates
Fig 3.1. Study area of Muthupet mangroves, Tamilnadu, India: a) Indian continental slope of Bay of Bengal; b) Satellite map of Muthupet mangrove forest; Site of Sampling: I- Selli munai, II- Sedhikkuda, III- Chief Corner, and IV- Xevier point. At each site, three core soil samples were collected at a depth of 10-15 cm from the surface of the soil.
Fig 3.2. Screening of glucose (xylose) isomerase producing *Streptomyces* isolates; the isolates were grown on peptone yeast agar containing 0.1 M, D-glucose. After incubation, treated with the reaction mixture 0.1% 2, 3, 5-triphenyltetrazolium in 1M NaOH at 55 °C for 10 min, the development of pink color zone was observed around the colonies (Sapunova et al., 2004). RSU- Isolate code: Measurement of pink color zone diameters, RSU1 (4 mm), RSU14 (3 mm), RSU15 (4 mm), RSU21 (11 mm), RSU23 (15 mm), *RSU26 (20 mm), RSU30 (12 mm) and RSU51 (15 mm). * indicates robust producer of glucose isomerase.
Fig 3.3. Colony morphology of *Streptomyces* isolates on starch casein agar plates: Representative isolates of glucose isomerase producers associated with mangrove soil; RSU- Isolate code; RSU1- *S. albidoflavus*; RSU14- *S. albus*; RSU15- *S. fradiae*; RSU21- *S. sampsonii*; RSU23- *S. carpaticus*; RSU26- *S. lividans*; RSU30- *S. gresius*; RSU51- *S. krainskii*. 
Fig 3.4. Light microscopic image of *Streptomyces* isolates: Experiments were performed by coverslip culture technique (Kawato and Shinolue, 1959). RSU1- *S. albidosflavus*, RSU14- *S. albus*; RSU15- *S. fradiae*; RSU21- *S. sampsonii*; RSU23- *S. carpaticus*; RSU26- *S. lividans*; RSU30- *S. gresius*; RSU51- *S. krainskii*. All *Streptomyces* isolates were observed under 100-X oil immersion objective.
Fig 3.5. Scanning electron microscopic image of *Streptomyces* isolates: Experiments were performed by coverslip culture technique (Kawato and Shinolue, 1959). Isolates: RSU1- *S. albidoflavus*, RSU14- *S. albus*; RSU15- *S. fradiae*; RSU21- *S. sampsonii*. For SEM, this specimen accelerate HV-high Voltage and calibrates image from 1.0kX-800X magnification and the image pattern covers 20-50 µm. Scale bar (µm) and WD, working distance (mm) are shown on each photomicrograph.
Isolates: RSU23- *S. carpaticus*; RSU26- *S. lividans*; RSU30- *S. gregius*; RSU51- *S. krainskii*. Branching filaments with various structure of aerial and substrate mycelium characteristics share common features of related *Streptomyces*, and spores are clearly evident for genus level determination. Scale bar (μm) and WD, working distance (mm) are shown on each photomicrograph.
Fig 3.6. Neighbour-joining phylogenetic tree of 16S rRNA gene sequences: The analysis (Saitou and Nei, 1987) based on nearly complete 1408-1445 nt sequences. The optimal tree with the sum of branch length=23.19187154 is shown. The evolutionary distances were computed using the maximum composite Likelihood method (Tamura et al., 2004) and are the units of the number of base substitutions per site. The analysis involved 70 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were removed. There were a total of 8 positions in the final dataset-Evolutionary analyses were conducted in MEGA.
Fig 3.7. Time course profile of glucose isomerase producing *Streptomyces* isolates: The growth phase was determined with peptone yeast extract broth. The growth rate was observed regularly at 24 h time intervals and the concentration was measured at OD 600 nm during 24-168 h. The right portion of the curve was observed only in RSU26, and high level of growth exhibited during 96 h than other isolates. The RSU26 growth was exhibited, OD 600 nm 0.821 (t = 96 h) but the other isolates reached the OD 0.78 - 0.8 (t = 168 h). Each isolates growth were performed in triplicates and the mean values along with standard error mean (n=3) are presented.

Fig 3.8. Thin layer chromatography separation of fructose by crude glucose isomerase: Isomerization of glucose to fructose was detected by TLC (Silica gel G, Merck). A total of 8 *Streptomyces* isolates crude cell free extract were used for the conversion of glucose → fructose. To prepare the standard assay: 0.8 ml 0.1 M, D-glucose; 1.0 ml of potassium phosphate buffer (50 mM) pH 7.5; 0.2 ml of 10 mM, MgSO4·7H2O; 0.2 ml of 1 mM, CoCl2·6H2O and 0.2 ml of the cell free intracellular enzyme solution, the reaction was carried out 55 °C for 30 min. Ratio of solvent system-Ethyl acetate: Ethanol: Water (4:5:1, v/v/v). Spraying reagents 9 ml of 2% resorcinol and 1 ml of Orthophosphoric acid was used to determine fructose by the method (Al-Tai et al., 1987). The development of red color band was indicating fructose production due to glucose isomerase. RSU26 was exhibited desirable Rf value 0.46 cm and high intensity of red color, for its significant isomerization of glucose → fructose conversion. SF - Standard Fructose, RSU- isolate code of glucose isomerase producing *Streptomyces*. 
Fig 3.9. Standard graph of substrates and protein: a) glucose standard graph was plotted in the range of 10-100 mM concentration (OD 540 nm); b) fructose standard graph was plotted in the range of 10-100 mM concentration (OD 485 nm); c) Protein (Bovine Serum Albumin) standard graph was plotted in the range of 10-100 µg concentration (OD 590 nm). All standard were performed in triplicates and the mean values along with standard error mean (n=3) are presented.