Glucose isomerase (GI), an enzyme with deserved high potential in the world market. GI plays a major role in high Fructose Corn Syrup Production (HFCS). HFCS is used as a sweetener in food and pharmaceutical industries. *Streptomyces* are well-known producers of various industrially valuable enzymes, including Glucose isomerase. Currently, recombinant strains have been available for the production of various enzymes, but it has limitation in the large scale production. Therefore, identifying effective *streptomyces* strains have emerged. The current study, the novel *S. lividans* RSU26 was isolated from a marine source and optimized its potential to produce glucose isomerase at different physical and chemical conditions. The optimum pH and temperature for GI and biomass production were 7.5 and 35°C, respectively at 96 h. Characterization study revealed that the approximate molar mass of GI was 43 kDa for monomeric and 170 kDa for tetrameric forms. Kinetic behavior exhibits $K_m$ and $V_{max}$ values for the conversion of fructose to glucose conversion were 48.8 mM and 2.54 U mg$^{-1}$ at 50°C and glucose to fructose were 29.4 mM and 2.38 U mg$^{-1}$ at 65°C protein, respectively. Therefore, the present study suggested that the wild-type *S. lividans* RSU26 has strong potential to produce glucose isomerase for various industrial applications.

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1. Introduction

$D$-Glucose/xylose isomerase (EC: 5.3.1.5) is an essential enzyme in various sectors such as food, fuel and medical field. GI involves isomerization of $D$-glucose into $D$-fructose (EFSA Panel on Food Contact Materials et al., 2019; Jin et al., 2017; Al-Dhabi et al., 2016). Particularly, the conversion of $D$-glucose into $D$-fructose has high commercial values for the production of High Fructose Corn Syrup (HFCS). The more attention in the reversible isomerization of glucose (G) into fructose (F) in the presence of GI has high profitable strategies in the HFCS production (Xu et al., 2014). The recent marketplace value of GI is approximately one billion US dollar (Vandamme et al., 2006; Al-Dhabi et al., 2019; Al-Dhabi et al., 2020). Generally, GI present in the various prokaryotes and fungi. Especially, *Streptomyces* are largely involved in GI production (Al-Dhabi et al., 2019c; Al-Dhabi et al., 2019d; Al-Dhabi et al., 2019e; Al-Dhabi et al., 2019f). The most commercial GI producers are *streptomyces* and *Bacillus* species (Al-Dhabi et al., 2019g). Recent findings of GI from various sources are widely studied and detected a few technical difficulties in the isomerization process. The wild-type GI producers exhibited very low specific activity, therмо-stability and high substrate specificity than for recombinant strains. Temperatures at 55–65°C and pH at 6.5–7.5 are the most suitable conditions for the fructose production industries (Rozanov et al., 2009). Therefore, we planned to identify novel GI producing *Streptomyces* from marine sources and optimize its potential to produce GI at various physical and chemical conditions and kinetic behavior also studied.
2. Materials and methods

2.1. Isolation and identification of Streptomyces

The soil samples were collected from different regions of Muthupet mangrove forest, Thanjavur, Tamil Nadu, India. The Streptomyces isolation was carried out by soil dilution plate techniques (Kuester and Williams, 1964; Al-Dhabi et al., 2018a, 2018b). GI production by isolated strains was screened (Sapunova et al., 2006). Based on the preliminary screening, a single strain was selected for further investigation. Physiochemical (Supplementary Tables 1 and 2) and 16srRNA sequences revealed that this strain belonged to the *S. lividans* and the sequence was deposited at NCBI gene bank (GenBank ID: KP698743.1).

2.2. Quantification of GI

The fresh *S. lividans* was cultured in peptone yeast extract broth with 50% of seawater (Uyar and Baysal, 2004). After fermentation, the biomass and supernatant were separated. The biomass was disrupted by ultrasonication and supernatant was separated by centrifugation at 12,000 rpm for 15 min (4 °C). The enzyme assay was determined towards d-fructose as a substrate for d-glucose production at OD 540 nm (Miller, 1959). Further, the interconversion reaction of d-glucose as a substrate for d-fructose production was read at OD 480 nm (Takasaki, 1966).

2.3. Scale-up process

*S. lividans* RSU26 with essential parameters were optimized by flask-scale fermentation. Initial GI optimizations were determined specific duration at 24–120 h. To standardize the optimum pH (pH 6.0–9.0) and temperature (20–50 °C); and impact of different carbon, nitrogen, metal ions also studied.

2.4. Purification of GI

Ammonium sulfate precipitation method was used to precipitate GI initially. Then the cell-free extract was dialyzed by 110 kDa membrane (HiMedia-Mumbai, India). Dialyzed fraction was then subjected to the purification of GI by gel filtration chromatography using a Sephacryl S-200 column (Chen and Anderson, 1979) (Sigma Aldrich- Mumbai, India). The protein content of samples was quantified (Bradford, 1976). Purified GI molecular mass was determined by SDS and native PAG gel electrophoresis (Laemmli, 1970).

2.5. Characterization of GI

The effects of pH on the activity of GI were determined and indicate pH range between 6 and 9 were considered as most suitable for the activity of GI. The different pH was prepared in acetate and phosphate buffers. The pH 6–6.5 was adjusted in acetate buffer; pH 7–7.5 in phosphate buffer; pH 8–9 in Tris buffer at 45 °C for Fig. 1. Effects of different physiochemical and substrates on GI productions: Influences of physiochemical effects (a-incubation periods; b-pH; c-temperatures) on GI production and different substrates carbon (d); Nitrogen (e); metals (f) on GI production. Carbon sources (1%, w/v of glucose, xylose, starch, fructose and maltose); nitrogen sources (1.2%, w/v of peptone, beef extract, gelatin, casein and tryptone); metal ions, MgCl$_2$ (5.0 mM), Mg$^{2+}$ (5.0 mM), Co$^{2+}$ (0.05 mM), Mg$^{2+}$Co$^{2+}$ (10.0 and 0.05 mM), Mg$^{2+}$Co$^{2+}$ (10.0 and 0.1 mM) (magnesium and cobalt ions were added in the form of sulfate and chloride salts).
glucose and 60 °C for fructose conversion. Changes in the GI activity by different temperatures (35–80 °C at pH 7.5) were determined. The isomerization reaction was performed at 35–65 °C glucose and 50–80 °C for fructose.

The pH stabilities of GI were ranged from 7 to 8. The enzyme pre-incubated with different pH in phosphate buffer (pH 7–7.5); and Tris buffer (pH 8) at a different phase of incubation time (0–30; 30–60; 60–90 min) at 4 °C. The thermal stabilities of GI were determined in the range between 45–75 °C. The purified GI was pre-incubated with a different phase of incubation time (0–10; 10–20; 20–30 min), isomerization at 50 °C and 60 °C. The effects of various metal ions were determined by adding different concentration Na+, Mg2+, Co2+, Mn2+, Fe2+, Ca2+, Ag+, Hg2+, Cu2+ and Zn2+ under the standard condition at 50 °C for glucose and 65 °C for fructose production (Wang et al., 2011). The kinetic characterizations of GI were determined with D-glucose and D-fructose as a substrate (10–1000 mM). The affinity of both substrates such as glucose and fructose were calculated during the isomerization process.

3. Results

3.1. Scale-up process

*S. lividans* RSU26 was produced significant amounts of glucose isomerase (GI) in crude form (0.42 U/mg), and the cell dry weight was 0.64 g/L. The optimum conditions such as incubation periods, pH and temperature for the GI production were 96 h, pH 7.5 and 35 °C, respectively. The cell dry weight was reached 1.05 g/L and specific activity 0.57 U/mg (G) and 0.55 U/mg (F) proteins. GI pro-

### Table 1

GI activity in different forms, extracted from *S. lividans* RSU26.

| Enzyme in different forms | Total Activity (µM) | Protein (mg/mL) | Specific Activity | Purified Yield (µM) | Fold (%) |
|--------------------------|---------------------|-----------------|------------------|--------------------|---------|
| CEE                      | 1.053 ± 0.002       | 1.064 ± 0.003   | 1.46 ± 0.004     | 0.718 ± 0.008      | 100     |
| ASP                      | 1.332 ± 0.004       | 1.397 ± 0.019   | 1.23 ± 0.002     | 1.080 ± 0.011      | 1.5     |
| EED                      | 1.503 ± 0.009       | 1.556 ± 0.004   | 0.99 ± 0.001     | 1.509 ± 0.028      | 2.1     |
| PES                      | 1.550 ± 0.003       | 1.685 ± 0.022   | 0.79 ± 0.014     | 1.943 ± 0.083      | 2.7     |

CEE—Crude enzyme extract; ASP—Ammonium Sulfate precipitation; EED—Enzyme extract after dialysis; PES—Purified enzyme by Sephacryl S-200.

1 U/ mg protein.

Table 1: GI activity in different forms, extracted from *S. lividans* RSU26.

Fig. 2. Determination of purified GI activity and molar mass: Eluted fractions of purified GI protein (a) and its specific activity (b); SDS and Native Page profile of purified GI protein mass (c and d).
duction has strictly adhered with substrates used in the medium. The final step of the scale-up process of GI has reached maximum dry cell weight 3.0 g/L and specific activity 0.69 and 0.68 U/mg (t = 96 h) protein (Fig. 1a–f).

3.2. Purification of GI

The *S. lividans* RSU26 produced GI was purified successfully by the multistep process (Table 1). The initial precipitation and dialysis of GI protein were expressed to be 1.5–1.56 U/mg proteins. The purity was improved greater than two-fold and 67% of protein. The final step of gel filtration chromatography eluted fractions was measured at OD 280 nm. The GI was expressed total protein 54% yield, and purity increased higher than threefold (Fig. 2a and b). The SDS and Native PAGE analysis of purified GI molar mass were expressed to be 43 kDa (monomer) and 170 kDa (tetramer) (Fig. 2c and d).

3.3. Characteristics of GI

The optimum pH and temperature for the activity of purified GI were noted at 7.5 (98%), temperatures 50 °C for glucose and 65 °C for fructose (≥98%) yield (Fig. 3a and b). The pH stabilities of purified GI protein residual activity ranged from 98 to 92%. The GI protein half-life stability was maintained at 90 min. The stability was reduced by ≥20% when pH was increased (pH 8) during 30–90 min (Fig. 3c and d). Thermal stabilities of purified GI protein activities were exhibited at ranges between 40 and 50 °C at 10–30 min. The GI half-life was stabled until temperatures between 60 and

| Metals and other Compound | Fructose → glucose | Glucose → fructose |
|---------------------------|-------------------|-------------------|
| Relative activity (%)     | Relative activity (%) |
| Without metals            | 5.80 ± 0.546       | 5.70 ± 0.824      |
| Mg^{2+} (5.0 mM)           | 54.9 ± 0.376       | 51.2 ± 0.468      |
| Ca^{2+} (0.5 mM)           | 6.40 ± 4.584       | 7.20 ± 4.929      |
| Co^{2+} (0.1 mM)           | 5.00 ± 4.742       | 4.80 ± 4.755      |
| Co^{2+} (0.5 mM)           | 49.0 ± 3.257       | 47.2 ± 2.670      |
| Co^{2+} (1.0 mM)           | 61.6 ± 3.818       | 61.3 ± 3.902      |
| Mg^{2+} (5.0 mM) + Co^{2+} (0.1 mM) | 75.3 ± 1.355       | 78.8 ± 0.125      |
| Mg^{2+} (10.0 mM) + Co^{2+} (0.5 mM) | 90.0 ± 3.110       | 86.5 ± 0.785      |
| Mg^{2+} (10.0 mM) + Co^{2+} (1.0 mM) | 98.7 ± 0.947       | 96.3 ± 3.108      |
| MgCl2 (0.5 mM)             | 28.3 ± 0.310       | 18.1 ± 2.459      |
| MgSO4 (0.5 mM)             | 38.2 ± 0.100       | 41.3 ± 0.451      |

Fig. 3. Relative and residual activity of purified GI from *S. lividans* RSU26: Relative activity of pH (a) and Temperature (b); Residual activity of pH (c and d) and Temperature (e and f).
65 °C for 10 min. The purified GI activity was started to denature at higher than 65 °C (20–30 min), which denotes the GI relatively thermostable. The GI was completely denatured at 75–80 °C (Fig. 3e and f). The relative activity of *S. lividans* RSU26 GI was predicted in the presence of different metal ions added in the final isomerization. The combination of Mg$^{2+}$ and Co$^{2+}$ at the concentrations of 10.0 mM-Mg$^{2+}$ and 1.0 mM-Co$^{2+}$ respectively increased maximum relative activity (98%). The combination of Mg$^{2+}$ Co$^{2+}$ significantly enhanced the activity (Table 2).

### 3.4. Kinetic behavior

The GI reaction was performed in the presence of two different substrates, such as D-glucose and D-fructose at various concentrations individually (Table 3). The double reciprocal plots of the GI reaction rate were estimated against the two substrates. The $K_m$ (48.8 and 29.5 mM) and $V_{max}$ (2.5 and 2.3 U/mg protein) value were calculated. The $K_{cat}/K_m$ value was expressed as 0.9 fold for fructose production (Fig. 4a-d). The *S. lividans* RSU26 producing GI had high affinity with glucose than the fructose.

| Substrates | Products | $V_{max}$ (U/mg protein) | $K_m$ (mM) | $K_{cat}$ (s$^{-1}$) | $K_{cat}/K_m \times 10^3$(M$^{-1}$ s$^{-1}$) |
|------------|----------|--------------------------|------------|---------------------|------------------------------------------|
| D-Fructose | D-Glucose| 2.544 ± 0.10             | 48.85 ± 9.50| 12.72 ± 0.70        | 0.260 ± 0.07                             |
| D-Glucose | D-Fructose| 2.388 ± 0.09             | 29.43 ± 4.59| 11.94 ± 0.45        | 0.405 ± 0.09                             |

### 4. Discussion

The current study, we isolated novel *S. lividans* RSU26 from marine soil and scaled the GI productions. This study suggested that *S. lividans* RSU26 is the highly valuable strain for the production of GI. *S. lividans* RSU26 significantly produced the desired quantity of GI in the medium than the other isolates. The result was concurrent with previous reports (Lama et al., 2001; Arasu et al., 2013; Balachandran et al., 2015; Arasu et al., 2017). Also, the flask scale parameters such as incubation time (96 h), pH (7.5) and temperature (35 °C) report coincided with earlier findings of GI (Givry and Duchiro, 2008). Different source of carbon, nitrogen and minerals could influence GI production. The dependence of nutrient sources such as glucose, peptone and metal ions supplement could be improved activity of GI greater than 10%, our current result coincided with previous reports (Chanitnun and Pinphanichakarn, 2012; Kaneko et al., 2001). The overall fermentation of GI yield 0.69 U/mg proteins and the cell dry weight 3 g/L was noted. The scale-up process could increase the GI production up to >4 fold (Sapunova et al., 2006). Therefore, the production medium constituents with peptone, 12 g; Yeast extract, 5g; glucose 10 g, K$_2$HPO$_4$, 3 g; MgSO$_4$, 1 g; CoCl$_2$, 0.1 g was recommended for enhancing the production of GI yield by *S. lividans* RSU26. The result was slightly comparable activity with earlier fermentation process (Yassien, 2012).

*S. lividans* RSU26 produced GI expressed desired molar mass indicated that the protein bands in gel were denoted as 43 kDa and ~170 kDa. The similar molar mass was observed in previous reports (Akan, 2018; Joo et al., 2005). The relative pH for purified GI was noted at pH 7.5. The relative temperature for GI activity exhibited at 50 °C and 65 °C for both product conversions.
individually. The residual activity of GI noted at pH 7.5 for 90 min, and thermal stability retained, after incubation at 65 °C for 10 min. The GI active up to 60 °C, the result strongly associated with the previous report (Staudigl et al., 2014). The purified GI was active in tetrameric forms and required Mg²⁺, Mn²⁺ or Co²⁺ for catalytic activity and thermostability. While, Ca²⁺ as a solid competitive inhibitor and Cu²⁺, Zn²⁺, Hg²⁺ were inhibited GI activity. The S. lividans RSU26 was required only a trace amount of Co²⁺ for activity (Bhosale et al., 1996; Karaoğlu et al., 2013). The kinetic behavior of GI was tiny similar and dissimilar with previous work (Akan, 2018; Sripiraphund et al., 2000).

5. Conclusion

In this study, the wild-type S. lividans RSU26 was isolated and optimized its GI production. Furthermore, GI was purified by Sephacryl S-200 column and characterized its kinetic behavior. The strain was grown in limited nutrients with suitable physical and chemical conditions. The S. lividans produced GI without any raw materials and inducer (xylose). Hence, the production has been an economically feasible and inexpensive process. The kinetics of GI effectively indicated both conversions (glucose and fructose) have a reversible and irreversible mode. In conclusion, S. lividans RSU26 is highly recommended for GI production. Hence, the upcoming work bonds with the heterologous system will be used for GI production.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2019.12.024.

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