Medium optimization for β-mannanase production from *Streptomyces sp.* RDA1496 using one factor at a time (OFAT) approach.

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Abstract: *Streptomyces sp.* RDA1496 strain was used for β-mannanase production to study optimization medium parameters. In this study various natural substrate and physical component were evaluate for enhancement of β-mannanase production. One factor at a time (OFAT) approach was applied for the purpose. Among the tested parameter, guar gum was found to be effective carbon source with the resulted activity of 106.33 IU/ml at the optimum concentration i.e, 8.0 g/L. Peptone found to be most effective as nitrogen source with the optimum concentration of 4.0 g/L with the highest activity of 130.17 IU/mL. Optimum condition for pH (8.0), temperature (30 $\pm$ 2), Medium Volume (100 mL), Inoculum size (3%), Incubation time (72 hours), Minerals (NaCl, K_2HPO_4, KH_2PO_4), Trace element ($CaCl_2$) were obtained for optimized condition. By using One Factor At a Time approach, the highest activity was obtained of 244.54 IU/mL.

Key words: Optimization, β-mannanase, *Streptomyces*, One factor at a time (OFAT).

I. INTRODUCTION

Hemicelluloses are structural polysaccharides of linear and branched chain in the cell walls of higher plants which are closely associated to the cellulose and lignin forming lignocelluloses biomass (Moreira and Filho, 2008). Mannan polysaccharide is the profound and most abundant part of polysaccharide found in softwood hemicellulose. Softwood mannan is consist of β-1,4-linked-Mannopyranose and D-glucopyranose found as a primary chain and partly substitutes by a-1-6-linked-D-galactosyl side chain. Mannan is found and can be obtained from seeds, coconut, palm kernel, coffee bean, tuber of konjac and copra (Lin and chain, 2004).

Enzymatic hydrolysis of mannan generates manno-oligosaccharide by the synergetic catalytic action of β-mannanase (EC.3.2.1.78), β-mannosidase (EC. 3.2.1.25) and β-glucosidase (EC.3.2.1.21) with the action on main chain of mannan polymer and with debranching enzyme such as Galactosidase (EC 3.2 1.22) and acetyl esterase (EC 3.1.1.6). (Singh et al., 2003; Petkowicz et al, 2007). The mannanase are endo-acting enzyme, breaking the internal glycosidic bond of backbone chain in mannan, releasing β-1-4-mannooligosaccharides (Dhawan and kaur, 2007; Chauhan et al., 2012; Chauhan et al, 2014b).

Production of β-mannanase have been reported from fungi, bacteria and yeast as well as seed of terrestrial plant (Kote et al, 2009; Meenakshi et al, 2010; Blibech et al, 2010; Chauhan et al, 2014b, Chauhan et al, 2014c). The production of β-mannanase from microbial source is more promising due to high production rate, low cost and in controlled condition (George et al, 2014a).

Medium composition has great importance mainly because its greatly influence the quality, production cost and production yield of any microbial product (Sondhi et al, 2014). Although medium component must be optimize at true concentration that supports and enhanced the production of biotechnological product. Optimization of medium composition for β-mannanase production was earlier reported (EI-Sharouny et al, 2015). The objective of current work is to optimize the selected medium component and to increase the β-mannanase production using submerge fermentation technique by One Factor At a Time (OFAT) method.

II. Materials and methods

2.1 Microorganism

Streptomyces sp. RDA 1496 isolated from natural soil sample, previously screened and confirms as β-mannanase production on agar plate assay (Abe et al, 1994). The strain performed for 16s rRNA technique and sequence was submitted to NCBI database with an accession no (KX656177). The actinomycetal strain maintained on Guar gum containing agar slant and sub-cultured at regular interval.

2.2 Initial medium composition

All of the optimization parameters were carried out by using 250 ml of Erlenmeyer flask. During the study media sterilization was achieved by autoclaving at 15 lbs with 121°C for 15 min. Medium composition was re-modified on each step of optimization parameter based on obtained results. The Initial medium composition was formulated as (g/L): Guar gum 10.0, NaCl 1.0, K_2HPO_4 1.0, KH_2PO_4 1.0 dissolved in 100 mL of distilled water. Based on the results obtained during the secondary screening, the incubation time was taken for 120 hrs at 30 ± 2 °C with static condition for β-Mannanase production, and was considered as un-optimized condition (74.83 IU/mL) (Bhaturiwala and Modi, 2016).

2.3 Enzyme extraction and activity assay

The fermented broth medium was taken for centrifugation at 4000 rpm for 15 minutes at 4°C. The cell-free supernatant containing the crude enzyme was used for activity assay. β-Mannanase activity was assayed in the reaction mixture consisting of 0.5 ml of 50mM potassium phosphate buffer (pH 7.0) containing 1% Locust Bean Gum (LBG) (Hi-media Mumbai-INDIA) with 0.5 ml of the supernatant enzyme at 45°C for 30 min, a modified method of (El-Naggar et al, 2006). Amount of reducing sugar released was determined by the dinitrosalicylic acid reagent (DNS) (Miller, 1959). One unit of β-Mannanase activity was defined as an amount of enzyme producing 1 micromole of mannose per minute under the experimental conditions.

2.4 Optimization of Inoculum size

Different inoculum concentrations v/v (1.0 %, 3.0 %, 5.0 %, 7.0 % and 9.0 %) were evaluated for β-Mannanase production. The 24 hrs old seed culture, incubated in nutrient broth medium was used as inoculum culture. For the preparation of inoculum the optical density was set at 1.0 using sterilized distilled water (O.D. 660nm). The crude enzyme activity was determined at 120 hrs of incubation period.

2.5 Optimization of Carbon and Nitrogen source

For the optimization of carbon source various substrates namely, Locust Bean Gum, Guar gum, Wheat flour, Corn flour, Pineapple, Potato peel, Soyabean, Copra meal were evaluated for their effect on β-mannanase production. The concentration of 10.0 g/L was set for the selected carbon source. Subsequently, the effective carbon sources were evaluated for the optimal concentration.

Medium component (as Nitrogen source) namely, Yeast extract, Beef extract, Peptone, Ammonium chloride, Ammonium sulphate, Urea, Casein, KNO_3 were selected as nitrogen source for enhancement of β-mannanase production. The concentration of nitrogen source was selected 5.0 g/L to evaluate their effect on β-mannanase production followed by effective nitrogen source was evaluated for the optimal concentration.

2.6 Optimization of pH

The pH range was selected from 3.0 to 10.0 for the study. The effect of various pH were determined by the obtained crude enzyme activity. The crude enzymatic activity was measured at 120 hrs of the incubation period.

2.7 Optimization of Temperature

Temperature is the important factor for catalytic activity of the extracellular enzyme. Temperature ranging from 20 °C to 50 °C (at the interval of 5 °C) was taken for the evaluation for the optimal point. The effect of temperature on enzyme production was determined by the crude enzyme activity.

2.8 Optimization of Incubation time

The incubation period was studied up to 144 hrs for β-Mannanase production. The time scale study was performed to determine optimum time for maximum production of β-Mannanase after optimization of carbon and nitrogen source.

2.9 Optimization of Supplemental carbon source

The monosaccharides (Glucose, Galactose, Fructose, Arabinose and xylose) and disaccharides (Sucrose and Lactose) were selected as supplemental carbon source. The 2.0 g/L of each supplemental carbon source was added in to fermentation medium. The crude enzyme activity was measured and results were recorded.

2.10 Optimization of Salts

In this study, NaCl and potassium phosphate salts (KH_2PO_4, K_2HPO_4) and sodium phosphate salts (NaH_2PO_4, Na_2HPO_4) were evaluated for their effect on β-Mannanase production. The effect of selected salts was investigated for their individual and combinational effect. The concentration of 1.5 % (w/v) was taken for NaCl and sodium and phosphate salts were taken at the concentration of 1.0 % (w/v).

2.11 Optimization of Trace elements and Surfactant

The effect of various metal ions (as a trace element) was evaluated for the β-Mannanase production. Different metal ions namely, $CuSO_4$, $MgSO_4$, $FeSO_4$, $ZnSO_4$, NH_4Cl, $MnCl_2$, $CaCl_2$, $MnSO_4$ were used in the process. Effect of surfactants in the production of β-mannanase by *Streptomyces sp.* RDA1496 was determined for Sodium dodecyl sulfate (SDS), Tween 80 and Tween 40.

2.12 Optimization of Medium volume

The optimization of medium volume was carried out using the volume size ranging from 50 mL to 250 mL. The flasks were incubated for 96 hrs at 30 ± 2 ºC and enzyme assay was performed to determine the optimum value for medium volume.

2.13 Time Course study

At the completion of nutritional and physiological parameter studies for the optimization, the time course study was repeated to re-evaluate the optimum time for the maximum production of β-Mannanase. The incubation time was evaluated up to 144 hrs and crude enzymatic activity was measured at every 24 hrs of incubation period.

III. RESULTS AND DISCUSSION

Optimization of parameters

For the increase in β-mannanase production by *Streptomyces sp.* RDA 1496, optimization was carried out for physiochemical parameter by one variable at a time using submerged fermentation process.

3.1 Effect of inoculum size

The highest activity was observed at 3.0 % of inoculum size with activity of 80.61 IU/mL , further activity was decreased with increase inconcentration of inoculum size. At 1.0 % inoculum size activity was found to be 70.43 IU/mL as shown in Fig 3.1. Earlier inoculum size reported as a significant factor in production of β-mannanase and found to have optimum concentration of 5.5 % (v/v) using *Aspergillus terreus* SUK-1 (Rashid et al., 2011).

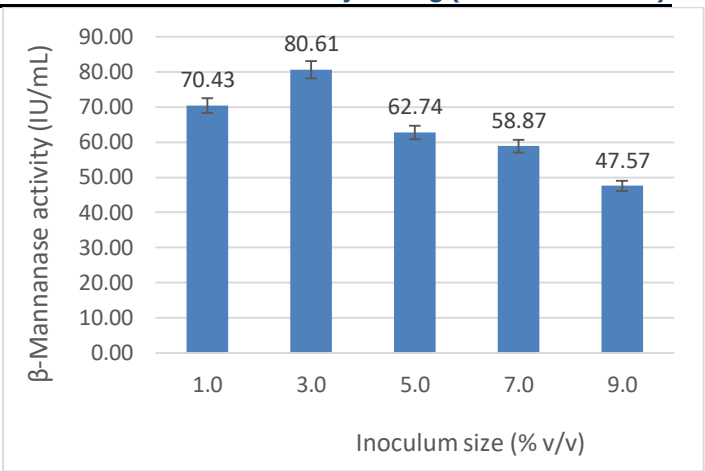

Fig 3.1 Effect of Inoculum size on β-Mannanase production by *Streptomyces sp.* RDA1496.

3.2 Effect of various natural carbon sources

The effect of various natural carbon sources on β-mannanase production is shown in Fig 3.2. Guar gum and Locust bean gum were found to be most effective with an activity of 80.51 IU/mL and 76.38 IU/mL respectively.

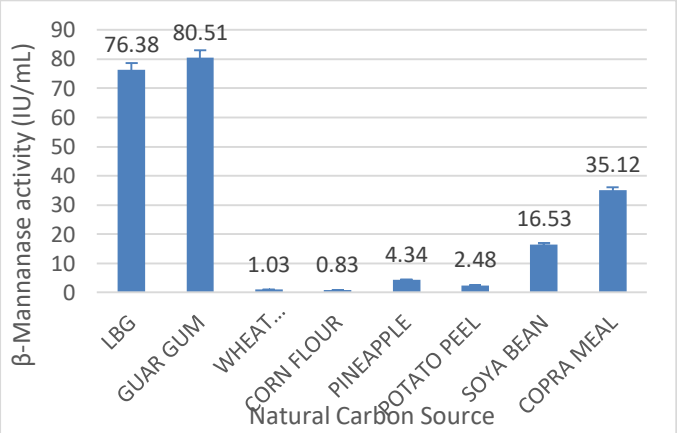

Fig 3.2 Effect of various natural carbon sources on β-Mannanase production by *Streptomyces sp.* RDA1496.

Guar gum and Locust Bean Gum contain the main chain of mannose and earlier reported as significant factor in the production of β-Mannanase by *Aspergillus terreus* (Soni et al, 2016), *Aspergillus fumigatus* IMI 385708 (Puchart et al., 2004), *Bacillus halodurans* PPKS-2 (Vijayalaxmi et al, 2013).

The optimum concentration of Guar gum for the production of β-Mannanase is reflected in Fig 3.3. The concentration at 8.0 g/L was found to be most effective with the activity of 106.33 IU/mL.

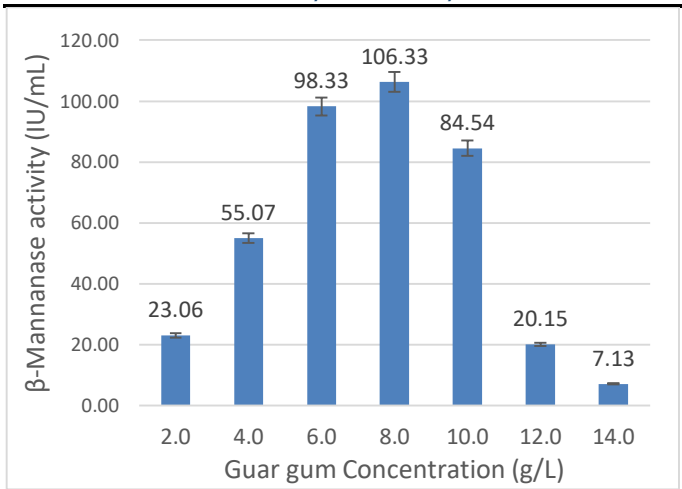

Fig 3.3 Effect of Guar gum concentration on β-Mannanase production by *Streptomyces sp.* RDA1496.

3.3 Effect of nitrogen source and optimum concentration

Various nitrogen sources were evaluated for the production of β-Mannanase production. Peptone was found to be most effective with an activity of 123.86 IU/mL. Yeast extract was found to have activity 109.06 IU/mL and other nitrogen sources were found to be least effective i.e, Beef extract, $(NH_4)_2SO_4$, urea, casein, $NaNO_2$ and NH_4Cl as shown in Fig 3.4.

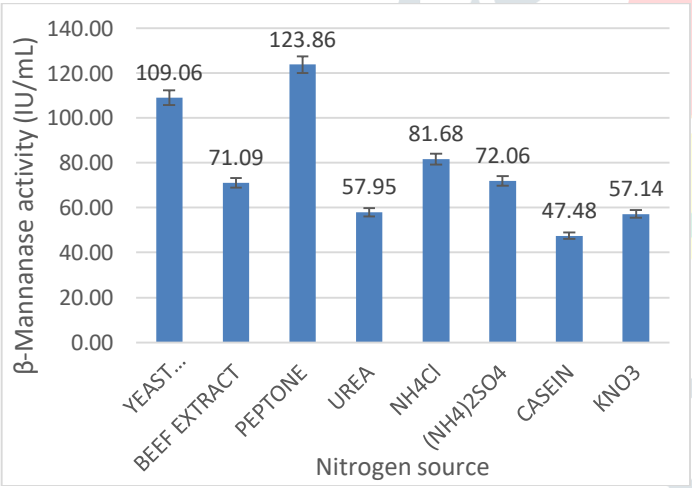

Fig 3.4 Effect of Nitrogen source on β-Mannanase production by *Streptomyces sp.* RDA1496.

The optimum concentration for peptone was evaluated for the range of 2.0 g/L to 10.0 g/L and shown in Fig 3.5. The optimum concentration was observed at 4.0 g/L with an activity of 130.17 IU/mL.

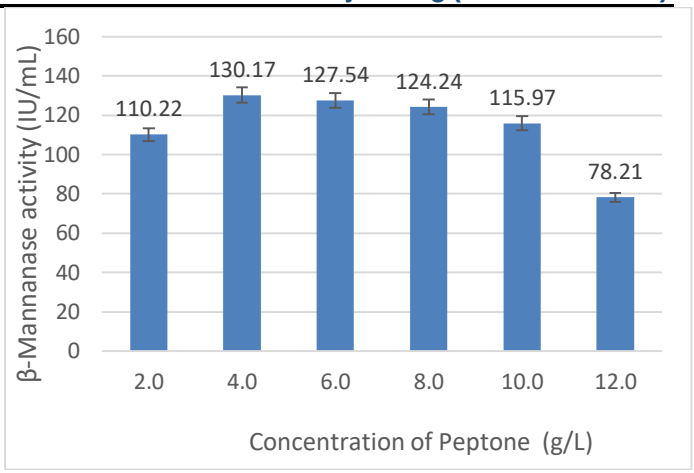

Fig 3.5 Effect of Peptone concentration on β-Mannanase production by *Streptomyces sp.* RDA1496.

β-Mannanase producers among the tested nitrogen source was reported by Chantron et al., (2013). In another study of optimization, ammonium nitrate was found to be significant as nitrogen source among the tested substrates (Yatmaz et al, 2016; Mabrouk and El-Ahwany, 2008) and ammonium sulphate 0.06 % (w/v) (Zakaria et al, 1998).

3.4 Optimization of pH

The production of β-Mannanase was favoured the slightly alkaline nature of the medium as shown in Fig 3.6. The pH 8.0 was reported to be most effective with the activity of 144.81 IU/mL. The acidic nature of the medium was shown to have an inhibitory effect on the enzyme production with the activity of 10.98 IU/mL.

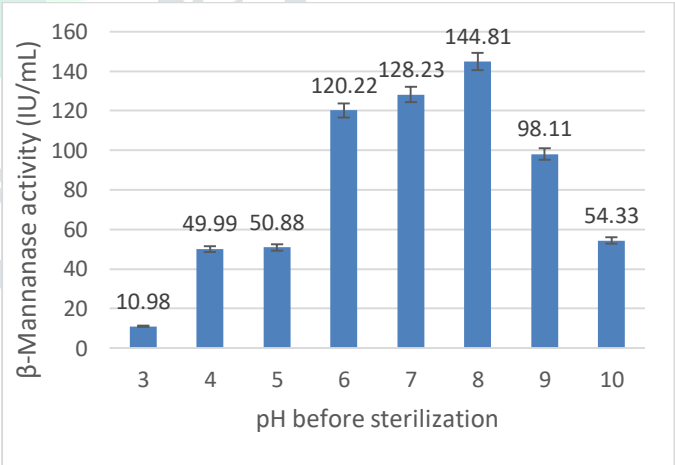

Fig 3.6 Effect of initial pH on β-Mannanase production by *Streptomyces sp.* RDA1496.

Zurmiati and colleagues studied the optimum conditions for β-Mannanase production by *Bacillus amyloliquefaciens* and reported optimum pH was 6.5 (Zurmiati et al, 2017). Similarly, pH 10.0 was found significant for β-Mannanase production by alkliphilic *Bacillus sp.* N16-5 (Lin et al, 2007).

3.5 Optimization of temperature

The production of β-mannanase was carried out at temperature ranging 20-50˚C at the interval of

every 5˚C (Fig 3.7). From the Results, the optimum temperature for β-Mannanase production was achieved at 30˚C with an activity 148.44 IU/mL. The optimum temperature of 30 °C was similar to earlier report for β-Mannanase production by *Streptomyces sp*. AZA12 (Zine and Peshwe, 2018).

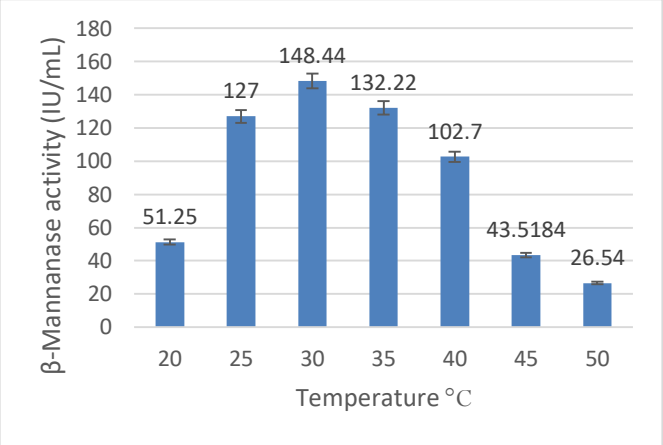

Fig 3.7 Effect of Temperature on β-Mannanase production by *Streptomyces sp.* RDA1496.

3.6 Optimization of incubation time

The maximum β-Mannanase activity was observed at 96 hrs of incubation time with an activity of 160.54 IU/mL, followed by 120 hrs activity of 144.11 IU/mL (Fig 3.8). The optimum incubation period was shifted to 96 hrs from the 120 hrs.

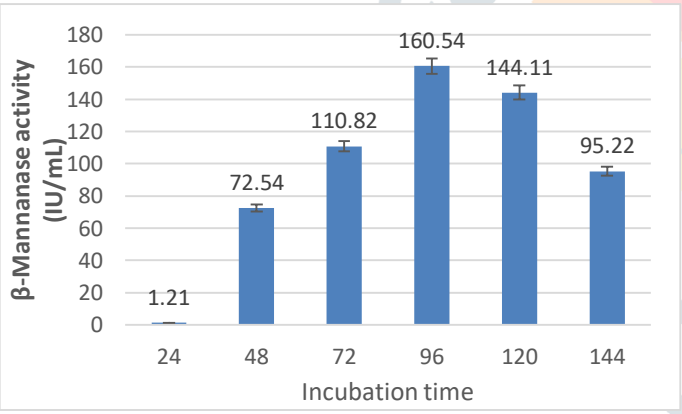

Fig 3.8 Effect of Incubation time on β-Mannanase production by *Streptomyces sp.* RDA1496.

The short incubation time can reduce the cost of overall process of biotechnological product (Mulay and Khale, 2011).

3.7 Optimization of supplemental carbon source

Among the tested supplemental carbon sources, fructose has highly enhanced the β-mannanase activity of 198.89 IU/mL followed by sucrose 188.72 IU/mL (Fig 3.9). Supplemental carbon source supports initial growth phase of microorganism. In similar study, carbon sources i.e. monosaccharides (glucose and maltose), oligosaccharides (sucrose, molasses and lactose) and polysaccharides (starch, molassis) were studied and molassis was found effective with an activity 411.09 U/g (Ab et al., 2009).

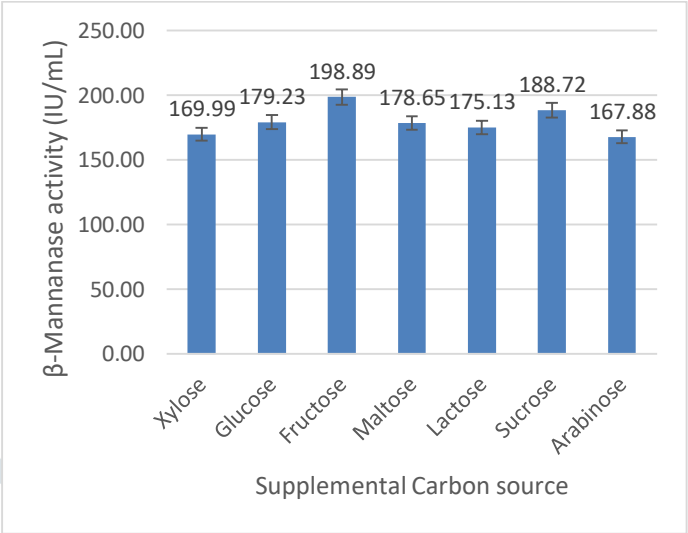

Fig 3.9 Effect of Supplemental carbon source on β-Mannanase production by *Streptomyces sp.* RDA1496.

3.8 Optimization of salts

Effect of various salts on β-Mannanase production is shown in Fig 3.10. The highest activity was observed with a combination of NaCl and K_2HPO_4 with an activity of 209.82 IU/mL, followed by the NaCl alone showing the activity of 195.99 IU/mL. Similarly, NaCl was also observed as important factor for β-Mannanase production by alkaliphilic strain Bacillus sp. N16-5, the optimum concentration of NaCl was 84.4 g/L (Lin et al, 2007). In this study, potassium salts were found to be more effective compare to sodium salts.

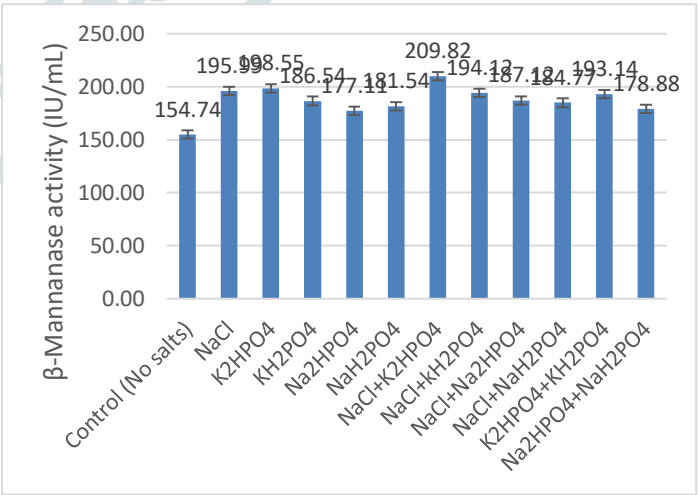

Fig 3.10 Effect of Salts on β-Mannanase production by Streptomyces sp. RDA1496.

3.9 Optimization of Trace elements and surfactant

In earlier report, Effect of Metal ions (i.e $FeSO_4$ and $MnSO_4$) on β-Mannanase production was found to enhance the β-Mannanase yield up to 193 U/g and conditions were optimized by response surface methodology (Rana et al, 2014). In our study,

CaCl2 was found highly effective trace element to increase activity of β-Mannanase (218.22 IU/mL). While, $MnSO_4$ and $CuSO_4$ inhibited the β-Mannanase production compare to last optimized condition. The surfactant was found to have reduce the β-Mannanase activity as shown in Fig. 3.11

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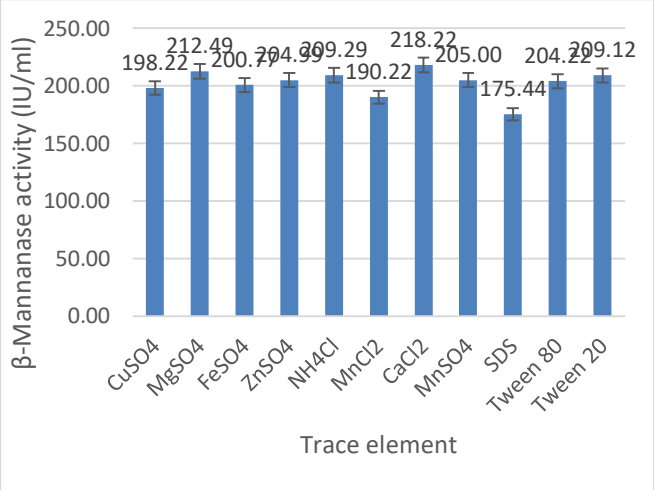

Fig 3.11 Effect of Trace elements and surfactant on β-Mannanase production by *Streptomyces sp.* RDA1496.

3.10 Optimization of medium volume

Medium volume ranging from 50ml to 250 ml for β-Mannanase production. However, there was no larger difference was observed up to 200 mL medium volume and activity was reduced in 250 mL of medium volume (Fig 3.12)

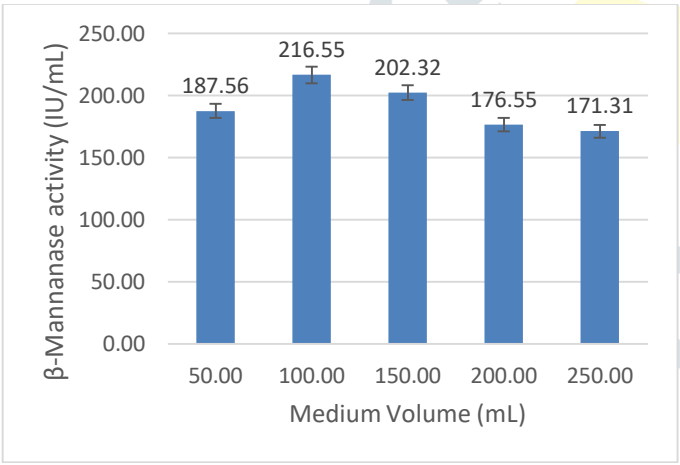

Fig 3.12 Effect of Medium Volume on β-Mannanase production by *Streptomyces sp.* RDA1496.

3.11 Time course study for β-Mannanase production

The study of optimization of incubation period for the β-Mannanase was performed at the end of OFAT optimization and is shown in Fig 3.13. The incubation period is an important factor to reduce cost of overall process. The 72 hrs of incubation period was found to optimum for the maximum enzyme production (244.54 IU/mL). Prior to optimization, the optimum time was 120 hrs and reduced to 72 hrs mainly due to the optimization of various factors.

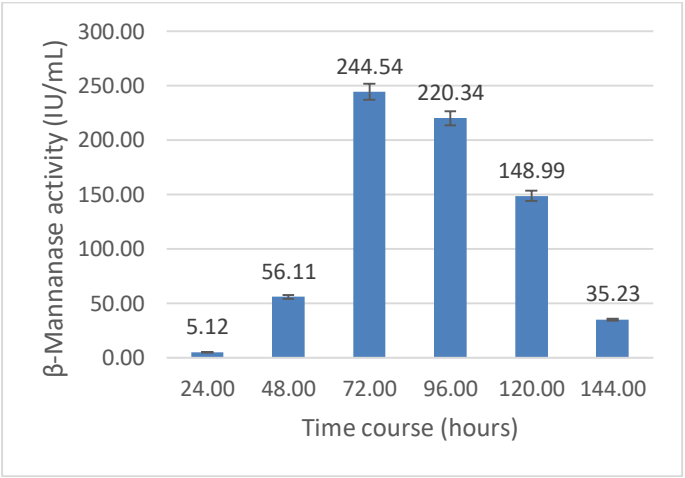

Fig 3.13 Effect of Time course study of β-Mannanase production by *Streptomyces sp.* RDA1496.

The One Factor At a Time (OFAT) approach was proved to be effective strategy for the β-Mannanase production. The final crude enzyme activity was 244.54 IU/mL, which is 1.7 fold increase of initial activity. The optimized medium components and conditions are as follow : guar gum 8.0 g/L, peptone 3.0 g/L, fructose 2.0 g/L, NaCl 1.5 g/L, K_2HPO_4 1.0 g/L, KH_2PO_4 1.0 g/L, $CaCl_2$ 0.5 g/L, inoculum size 3.0 %, medium volume 100 mL, initial pH 8.0, temperature 30°C with static condition and Incubation time 72 hrs.

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IV. CONCLUSIONS

The result highlights *Streptomyces sp.* RDA 1496 is potential to produce β-mannanase and in future may be useful for β-mannanase production at industrial level by scaling up the fermentation process. β-mannanase production achieve with incorporation of β-mannan chain as a sole source of carbon i.e., Guar gum and peptone was found to be most effective nitrogen source. Supplemental carbon source support the growth of *Streptomyces sp.* RDA 1496 in the medium and eventually increase the β-mannanase activity. NaCl and potassium phosphate salts found to be effective. Optimization by the One Factor At a Time (OFAT) method increase the activity by 3.26 fold increase in β-mannanase compare to un-optimize medium condition.

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