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Effect of Inhibitors on partially purified alpha-amylase produced by *Brevibacillus borstelensis* R1

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ABSTRACT

The α-amylase produced by *Brevibacillus borstelensis* R1 has a number of applications in many fields such as bakery industry, food preparations, automation dishwashing, ethyl alcohol dual fermentation, sago and rice industrial effluent treatment, sewage water treatment, fodder production, laundry and textile industries. The effect of inhibitors ($AgNO_3$, $HgCl_2$, EDTA, $CuSO_4$, L-Glutamic acid and $ZnCl_2$) on partially purified α-amylase activity from *Brevibacillus borstelensis* R1 was studied. All the inhibitors inhibited the activity when compared with the control. The percentage of α-amylase activity retained after treating with 0.5M of L-Glutamic acid, $HgCl_2$, $AgNO_3$, $ZnCl_2$, $CuSO_4$ and EDTA was 31.59%, 27.79%, 24.58%, 23.69%, 20.19% and 19.03% respectively.

Keywords: Alpha-amylase, inhibitors, *Brevibacillus borstelensis* R1

1. INTRODUCTION

Alpha-amylases belong to family 13 (GH-13) of the glycoside hydrolyses group of enzymes (Bordbar et al., 2005). Numerous marine microorganisms secrete enzymes which can provide new insights and understanding of enzymes. Marine microorganisms have been attracting more attention as source for novel enzymes, because the microbial enzymes are relatively more stable and active than the enzymes derived from other sources (Bull et al., 2000; Kin, 2006). Higher metal ion concentrations often inhibit microbial growth and enzyme production. Adequate

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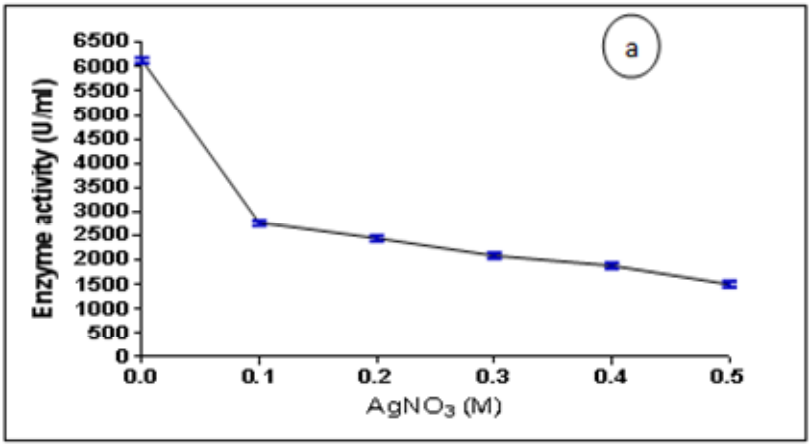

Figure 1

Effect of $AgNO_3$ on partially purified α-amylase activity; * P<0.0421 Values differ significantly at p<0.05 with fairly high degree of negative correlation. Y bars indicate the standard deviation of mean value

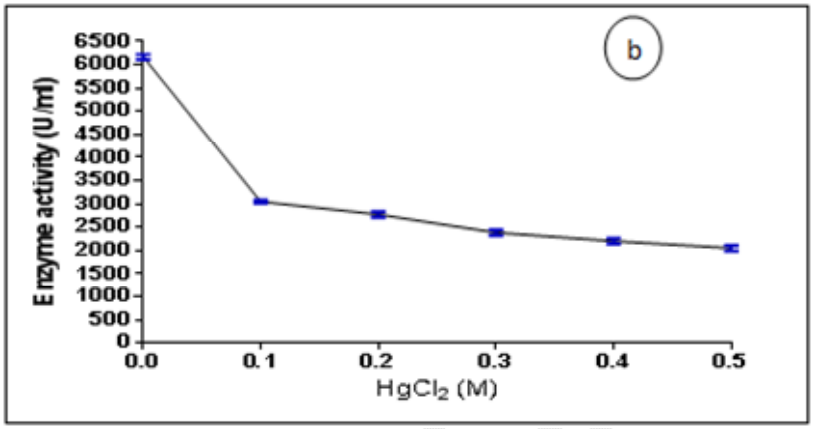

Figure 2

Effect of $HgCl_2$ on partially purified α-amylase activity; * P<0.0490 Values differ significantly at p<0.05 with fairly high degree of negative correlation. Y bars indicate the standard deviation of mean value

concentrations of specific metal ions are very essential for microbial growth of *Bacillus subtilis* (Linden et al., 2003). Previous literature indicated that most amylase activities were inhibited in the presence of Ni^{2+}, Cd^{2+}, Cu^{2+}, Ag^{+}, Pb^{2+}, Fe^{2+} and Zn^{2+}. The α-amylases from *Bacillus spp.* were strongly inhibited by Ni^{2+}, Cd^{2+}, Zn^{2+} and Hg^{2+} (Cordeiro et al., 2002). Zinc chloride has inhibitory effect on the activity of enzyme in *Aspergillus flavus* (Abou zeid, 1997). The α-amylase from *Thermus sp.* was strongly inhibited by Cu^{2+} and Fe^{2+} (Shen et al., 1998) and the α-amylase from *Bacillus subtilis* was strongly inhibited by Zn^{2+}, Ag^{+}, Cu^{2+} and Fe^{2+} (Elif & Velittin, 2000). However, the activity of *Nocardiopsis sp.*7326 amylase was not affected by Zn^{2+}, Ni^{2+} and Fe^{2+} but activated by Cd^{2+} and Cu^{2+}. Alpha-amylase was strongly inhibited by Ag^{2+} in *Bacillus spp.* (Cordeiro et al., 2002; Gupta et al., 2003; Sun et al., 2010; Elif Demirkan & Demirkan, 2011) and α-amylase was inhibited by Hg^{2+} in *Bacillus spp.* (Pandey et al., 2000; Ramirez zavala et al., 2004; Sindhu 2005; Sun et al., 2010). The activity is inhibited in the presence of zinc ions in *Bacillus spp.* (Elif & Velittin 2000; Roheena abdullah, 2005), *Bacillus dipsosauri* (Deutch, 2002), *Bacillus stearothermophilus* (Chakraborty et al., 2000) and *Bacillus pumilus* (Ming et al., 1992). Ethylenediamine tetraacetic acid exhibited no effect on amylase activity in *Bacillus spp.* (Burhan et al., 2003) and *Bifidobacterium adolescentis* (Lee et al., 1997), but stimulated the activity of amylase in *Bacillus spp.* (Pandey et al., 2000; Gupta et al., 2003; Sindhu, 2005; Sun et al., 2010). Stimulatory effect of Cu^{2+} was reported by Krishnan & Chandra (1983).

2. MATERIALS AND METHODS

Maltose produced by the hydrolytic activity of α-amylase on α-1, 4 linkages present in starch, reduce 3, 5 dinitro salicylate to an orange red colored 5-nitro 3-amino salicylate which can be measured at 520nm. The starch substrate [0.5ml of 0.5% in 0.1M phosphate buffer (pH 6.8)] was mixed with 1% (0.2ml) NaCl in a test tube and pre incubated at 37^0C for 10 minutes. The supernatant collected from the centrifugation of the production media was used as enzyme source, 0.5ml of this was added to the reaction mixture. The reaction was terminated by the addition of 1.0 ml of 3, 5-dinitrosalicylic acid reagent [1.0 gm DNS in 0.8% NaOH, 60% Na K tartrate]. After incubation at 37^0C for 15 minutes. The contents were mixed well and kept in boiling water bath for 10 minutes. Then they were cooled and diluted with 10 ml of distilled H_2O. The color developed was read at 520nm. One unit of enzyme activity is defined as the amount of enzyme that releases 1.0 mmol of reducing sugar (maltose) per minute under the assay conditions (Miller, 1959).

The inhibitors were procured from Merk, Germany, (AR) grade ($HgCl_2$), Qualigen reagents, India, (AR) grade ($AgNO_3$, EDTA, L-Glutamic acid, $ZnCl_2$ and $CuSO_4$). The influence of inhibitors (0.1-0.5M) silver nitrate, mercury (II) chloride, ethylenediamine tetraacetic acid, copper sulphate, L-glutamic acid and zinc chloride on α-amylase activity was studied. The effect of and inhibitors on α-amylase activity was determined by DNS method (0.5% phosphate buffered starch as substrate) using standard conditions with partially purified enzyme. The control in all tests was assayed without any influencing agents respectively.

All the experiments were conducted in triplicate. The results were given as mean value ± standard deviation. The conditions were analyzed to determine the significant difference between the variables by one way ANOVA, two way ANOVA and correlation analysis by using the scientific graph pad (Prism 6.1 version software). Analysis of variance (ANOVA) refers to the examination of differences among the sample means. It is used to examine the significance of

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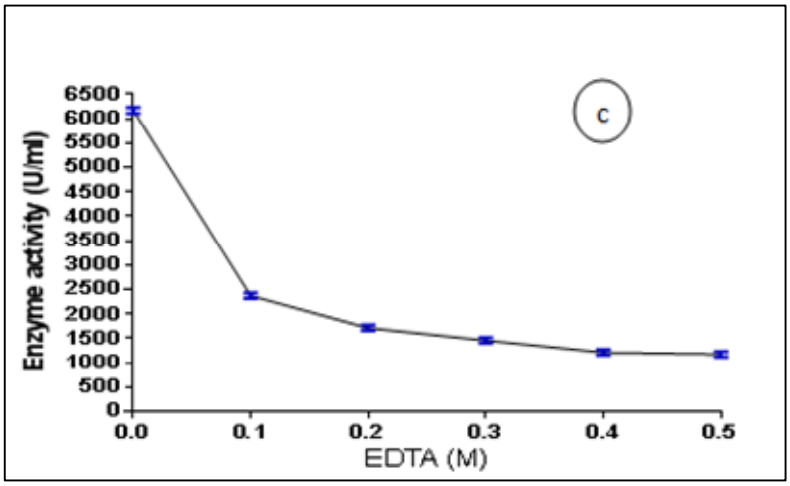

Figure 3

Effect of EDTA on partially purified α-amylase activity; * P<0.0466 Values differ significantly at p<0.05 with fairly high degree of negative correlation. Y bars indicate the standard deviation of mean value

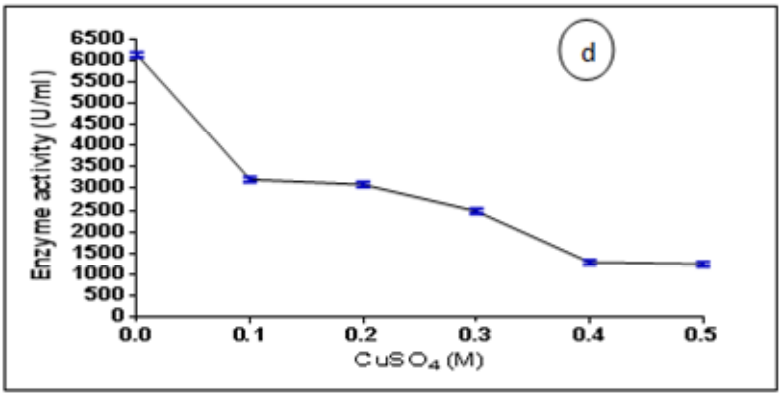

Figure 4

Effect of $CuSO_4$ on partially purified α-amylase activity; * *P<0.0097 Values differ significantly at p<0.05 with very high degree of negative correlation. Y bars indicate the standard deviation of mean value

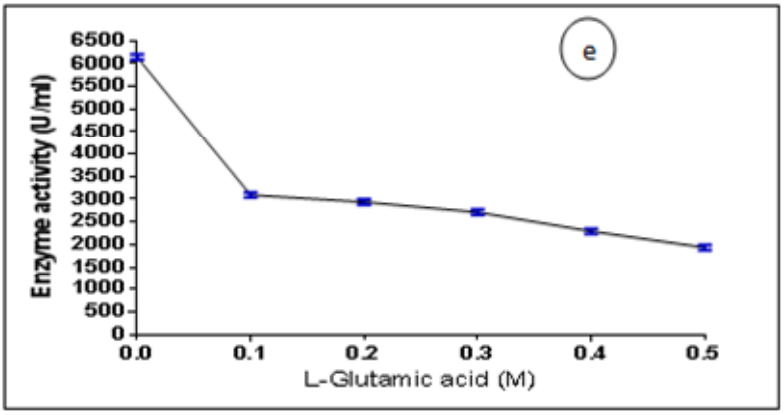

Figure 5

Effect of L-Glutamic acid on partially purified α-amylase activity; * P<0.0373 Values differ significantly at p<0.05 with fairly high degree of negative correlation. Y bars indicate the standard deviation of mean value

the difference amongst more than two sample means at the same time. Correlation analysis is concerned with measuring the strength or degree of relationship between variables. The measure of correlation is called the correlation co-efficient. The correlation measures the closeness of the relationship between variables.

3. RESULTS

Partial purification of α-amylase carried out by using ammonium sulfate precipitation and gel filtration. The effect of inhibitors on partially purified enzyme activity is shown in figures 1-6. The control was the activity of the partially purified enzyme without inhibitor under standard conditions. All the inhibitors decreased the enzyme activity when compared with the control (Figures 1-6). The percent of α-amylase activity retained after treating with 0.5M of L-Glutamic acid, $HgCl_2$, $AgNO_3$, $ZnCl_2$, $CuSO_4$ and EDTA was 31.59%, 27.79%, 24.58%, 23.69%, 20.19% and 19.03% respectively.

4. DISCUSSION

The effect of inhibitors ($AgNO_3$, $HgCl_2$, EDTA, $CuSO_4$, L-Glutamic acid and $ZnCl_2$) on partially purified α-amylase activity from *Brevibacillus borstelensis* R1 was studied. All the inhibitors inhibited the activity when compared with the control. The % of amylase activity retained after treating with 0.5M of $AgNO_3$, $HgCl_2$, EDTA, $CuSO_4$, L-Glutamic acid and $ZnCl_2$ was 24.58%, 27.79%, 19.03%, 20.19%, 31.59% and 23.69% respectively. The α-amylase activity was strongly inhibited by EDTA in the present study. Similar observation was reported in *Bacillus sps.* by Pandey et al. (2000), Gupta et al. (2003), Sindhu (2005) and Sun et al. (2010).

It was reported that the α-amylase activity of *Bacillus spp.* was inhibited in the presence of zinc ions (Brown et al., 1990; Chunzhi et al., 1992; Abou Zeid, 1997; Lin et al., 1998; Elif & Velittin 2000; Cordeiro et al., 2002; Roheena abdullah, 2005). Amylase from *Bacillus dipsosauri* strain DD1 (Deutch, 2002), *Bacillus stearothermophilus* (Chakraborty et al., 2000), *Bifidobacterium adolescentis* (Lee et al., 1997), *Bacillus pumilus* (Ming et al., 1992), *Bacillus coagulans* (Hawary, 1991), *Bacillus licheniformis* A-4041 (Yuguo et al., 1994) and *Bacillus firmus* (Igarashi et al., 1998) was also reported to be inhibited by zinc ions. On the contrary, Zinc chloride reported to increase the activity of amylase in *Bacillus spp.* (Gupta et al., 2003; Sindhu, 2005; Sun et al., 2010). The stimulatory effect of Cu^{2+} was reported in *Bacillus licheniformis* CUMC-305 (Krishnan & Chandra, 1983).

5. CONCLUSION

The effect of inhibitors ($AgNO_3$, $HgCl_2$, EDTA, $CuSO_4$, L-Glutamic acid and $ZnCl_2$) on partially purified α-amylase activity from *Brevibacillus borstelensis* R1 was studied. All the inhibitors inhibited the activity when compared with the control. The percentage of α-amylase activity

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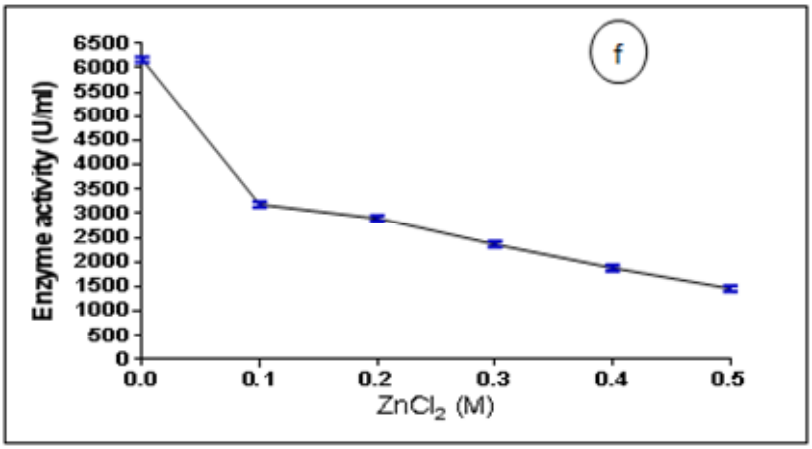

Figure 6

Effect of $ZnCl_2$ on partially purified α-amylase activity; * P<0.0174 Values differ significantly at p<0.05 with fairly high degree of negative correlation. Y bars indicate the standard deviation of mean value

retained after treating with 0.5M of L-Glutamic acid, $HgCl_2$, $AgNO_3$, $ZnCl_2$, $CuSO_4$ and EDTA was 31.59%, 27.79%, 24.58%, 23.69%, 20.19% and 19.03% respectively.

SUMMARY OF RESEARCH

1. The effect of inhibitors ($AgNO_3$, $HgCl_2$, EDTA, $CuSO_4$, L-Glutamic acid and $ZnCl_2$) on partially purified α-amylase activity from *Brevibacillus borstelensis* R1 was studied.
2. All the inhibitors inhibited the activity when compared with the control (6000U/ml).
3. The percentage of α-amylase activity retained after treating with 0.5M of L-Glutamic acid was 31.59%.
4. The percentage of α-amylase activity retained after treating with 0.5M of EDTA was 19.03%.

FUTURE ISSUES

The Effect of Inhibitors on partially purified alpha-amylase produced by *Brevibacillus borostelensis* R1 was studied. There is a need to study about the reagents that neutralize the effect inhibitors.

DISCLOSURE STATEMENT

There is no special financial support for this research work from the funding agency.

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REFERENCES

1. Abou Zeid AM. Production, purification and characterization of an extracellular alpha-amylase enzyme isolated from *Aspergillus flavus*. *Microbios*. 1997, 89(358), 55-66 [PMID: 9218355]
2. Bordbar AK, Omidiyan K, Hosseinzadeh R. Study on interaction of α-amylase from *Bacillus subtilis* with cetyl trimethylammonium bromide. *Colloids Surf. B: Biointerfaces,* 2005, 40(1), 67–71 [PMID: 15620842]
3. Brown SH, Costantino HR, Kelly RM. Characterization of amylolytic enzyme activities associated with the hyperthermophilic archaebacterium *Pyrococcus furiosus*. *Appl. and Envir. Microb.* 1990, 56(7), 1985-1991 [PMID: 16348235]
4. Bull AT, Ward AC, Goodfellow M. Search and discovery strategies for biotechnology: The paradigm shift. *Microbiol. Mol. Biol. Rev.* 2000, 64(3), 573–606 [PMID: 10974127]
5. Burhan A, Nisa U, Gökhan C, Ömer C, Ashabil A, Osman G. Enzymatic properties of a novel thermostable, thermophilic, alkaline and chelator resistant amylase from an alkaliphilic *Bacillus* sp. Isolate ANT-6. *Proc. Biochem.* 2003, 38(10), 1397-1403
6. Chakraborty K, Bhattacharyya BK, Sen SK. Purification and characterization of a thermostable alpha-amylase from *Bacillus stearothermophilus*. *Folia Microbiol.* 2000, 45(3), 207-210 [PMID: 11271801]
7. Chunzhi QZ, Jin F, Hua W, Ziaqiang L, Cheng Y. Purification and characterization of a thermo stable alpha- amylase from *Bacillus spp.*-JF strain. *J. Gen. Appl. Microbiol.* 1992, 38, 293-302
8. Cordeiro CAM, Martins MLL, Luciano AB. Production and properties of α-amylase from thermophilic *Bacillus sp. Braz. J. Microbiol.* 2002, 33, 57- 61
9. Deutch CE. Characterization of a salt-tolerant extracellular α-amylase from *Bacillus dipsosauri*. *Lett Appl. Microbiol.* 2002, 35(1), 78-84 [PMID: 12081555]
10. Elif Demirkan, Demirkane. Production, purification and characterization of α-amylase by *Bacillus subtilis* and its mutant derivates 705. *Turk J Biol.* 2011, 35, 705-712

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11. Elif S, Velittin G. Increase of the α-amylase yield by some *Bacillus strains. Turk J Biol.* 2000, 24, 299–308
12. Gupta R, Gigras P, Mohapatra H, Goswami VK, Chauhan B. Microbial α-amylase: a biotechnological perspective. *Proc. Biochem.* 2003, 38, 1599–1616
13. Hawary FI. Purification and characterization of alpha amylase produced by thermophilic isolate of *Bacillus coagulans*. *Acta. Aliment*. 1991, 20, 85-95
14. Igarashi K, Hatada Y, Hagihara H, Saeki H, Takaiwa M, Uemura T Ara K, Olali K, Kawai S, Kobayashi T, Ito S. Enzymatic properties of a novel liquefying α-amylase from an alkaliphilic *Bacillus* isolate and entire nucleotide and amino acid sequences. *App. and Env. Microbio.* 1998, 64, 3282–3289
15. Kin SL. Discovery of novel metabolites from marine actinomycetes. *Curr. Opin. Microbiol.* 2006, 9(3), 245–251 [PMID: 16675289]
16. Krishnan T and Chandra AK. Purification and characterization of *α*-amylase from *Bacillus licheniformis* CUMC305. *Appl. and Env. Microbio.* 1983, 46(2), 430-437 [PMID: 16346366]
17. Lee SK, Kim YB, Ji GE. Purification of amylase secreted from Bifidobacterium adolescentis. *J Appl. Microbiol.* 1997, 83(3), 267-272 [PMID: 9351206]
18. Lin LL, Chyau CC, Hsu WH. Production and properties of a raw-starch-degrading amylase from thermofilic and alkaliphilic *Bacillus sp.* TS-23. *Biotech. Appl. Biochem.* 1998, 28(Pt 1), 61-68 [PMID: 9693090]
19. Linden A, Mayans O, Meyer-Klaucke W, Antranikian G, Wilmanns M. Differential regulation of a hyperthermophilic amylase with a novel (Ca, Zn) two-metal center by Zinc. *J. Biol. Chem.* 2003, 278(11), 9875- 9884 [PMID: 12482867]
20. Malhotra R, Noorwez SM, Satyanarayana T. Production and partial characterization of thermostable and calcium-independent α-amylase of an extreme thermophile *Bacillus thermooleovorans* NP54. *Lett. Appl. Microbiol*. 2000, 31(5), 378-384 [PMID: 11069641]
21. Miller GL. Use of Dinitro salicylic acid reagent for determination of reducing sugar. *Analy. Chem.* 1959, 31, 426-429
22. Ming M, Jiang R, Wan CH. Studies on the characteristics of alpha amylase produced by *Bacillus apiaries* 289(PBX97). *Acta. Microbial. Sin.* 1992, 30, 405-407
23. Pandey A, Nigam P, Soccol CR, Soccol VT, Singh D, Mohan R. Advances in microbial amylases. *Biotechnol. Appl. Biochem.* 2000, 31(Pt 2), 135-152 [PMID: 10744959]
24. Ramirez-Zavala B, Mercado-Flores Y, Hernadez-Rodriguez C, Villa-Tanaca L. Purification and characterization of lysine aminopeptidase from *Kluyveromyces marxiamus*. *FEMS Microbiol. Lett.* 2004, 235, 369-375
25. Roheena Abdullah. Studies on the production of alpha amylase by *Aspergillus oryzae* using submerged fermentation, institute of industrial biotechnology gc university lahore, Thesis, 2005, 20-91
26. Shen G, Saha B, Lee Y, Bhatnagar L, Zeikus J. Purification and properties of an extracellular α-amylase from *Thermus sp. Bot Bull Acad Sci.* 1998, 36, 195–200
27. Sindhu R. Isolation, purification and characterization of α-amylase from *Penicillium janthhinellum*. Ph.D. Thesis. School of Biosciences, Mahatma Gandhi University. 2005, 175
28. Sun H, Zhao P, Ge X, Xia Y, Hao Z, Liu J, Peng M. Recent advances in microbial raw starch degrading enzymes. *Appl. Biochem. Biotechnol.* 2010, 160(4), 988-1003 [PMID: 19277485]
29. Yuguo Z, Xiaoru C, Xiaoquin IL. Experimental study on the technology conditions of alpha amylase fermentation with *Bacillus* mutant Zhefiang. *Gangxxeynan. Xuababoo.* 1994, 2, 56-61

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