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# Characterization of Mannanase Isolated from Corncob Waste Bacteria

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Mannanase bacteria has been isolated from some sources such as oil waste, activated sludge, decayed konjac, soil and sagoo hump, which mannanase enzyme has been isolated from bacteria, fungi and some fruits. This study focused on the characterization of mannanase bacteria isolated from corncobs waste. Nine isolates bacteria were successfully screened, which 2 isolates showed the greatest mannolitic index *i.e.*, IB.1.6 and IB.1.10. The character of both isolates were Gram-negative and rod cells. Characteristics physiology of IB.1.6 and IB.1.10 isolates showed positive result on the fermentation of mannose, rhamnose, lactose and glucose, as well as utilization of citrate and catalase. Mannanase from IB.1.6 and IB.1.10 isolates were stable at wide temperature range 30-90 °C and pH range 5-8, which temperature and pH optimum of 60 and 8 °C, respectively. Type of metal ion ($Co^{2+}$ and $Cu^{2+}$) and surfactants (Tween 20 and Tween 80) affected the activity of 2 enzymes.

**Keywords: Mannanase, Corncob, Waste, Bacteria.**

## INTRODUCTION

Cellulose and hemicellulose are organic wastes that commonly found in nature. These wastes are potential carbon sources for microbial decomposers growth. Types of microbial decomposers that found in wastes vary widely depending on the substrates. One of the microbial decomposers is mannanase producing microbes, which can hydrolyze mannan substrates. The one of abundant mannan substrate is corncob that can be found in all corners of the market in Indonesia. Corncob contains cellulose, hemicelluloses and lignin approxymately 32.3-45.6, 39.8 and 6.7-13.9 %, respectively [1,2]. Most of the corncob has been used as fodder and bioenergy feedstock [3,4].

Corncob as source of mannanase microbes is largely based on hemicellulose content. Endo-β-1,4-mannanase (EC 3.2.1.78) is type of hydrolytic enzymes that can be expressed by various microorganisms such as bacteria and fungi. Mannanase catalyzes the random hydrolysis of β-1,4-mannosidic linkages in the backbone of β-mannan, glucomannan and galactomannan [5]. Mannan is a linear polysaccharide consisting of 1,4-β-D-mannosidic linkages or combination of glucose and mannose with α-1,6-linked galactosyl side groups [6]. Mannan is the major constituent of lignocellulose and hemicellulose in plant seeds, soft-woods and beans and is widely used as an energy source, animal feed or industrial feedstock [7-9].

Mannanase microbes have been isolated from various sources and types of wastes such as oil waste, activated sludge, decayed konjac and soil [8,10-12]. Several types of bacteria have been reported that have the ability of mannanase expression *i.e.*, *Bacillus* sp. N16-5 [13], *Pediococcus acidilactici* (M17) [14], *Bacillus subtilis* WD23 [15], *Bacillus circulans* NT 6.7 [16], *Pholiota adiposa* [17] and *Aspergilus* species [5,9] and *Bacillus pumilus* (M27) [18]. Mannanase has been applied to the detergent industry, pharmaceutical, paper and pulp, animal feeds, instant coffee and manno-oligosaccharides production [9,19,20], oil and gas [21] and slime control agent [22].

Application of mannanase is very broad, encouraged many researchers to explore various sources of microbes that can produce mannanase. Mannanase microbes and their enzymes expressed different characteristics. Even, the exploration of enzyme carried out at extreme locations like crater volcano, with the aim of finding microbes producing thermophilic enzymes. The exploration of mannanase microbes may also based on the availability of substrate, as had been reported by Wahyuni and Khaeruni [23] that isolated of mannanase microbes of sago hump waste. This research reported characteristics of mannanase that have been isolated from corncob waste bacteria.

## EXPERIMENTAL

All the materials used in this work were of analytical grade.Yeast extract, tryptone, $MgSO_4 \cdot K_2HPO_4$, $(NH_4)_2SO_4$, NaCl and locust bean gum were purchased from Sigma-Aldrich (Singapore). $CaCl_2$, $MgCl_2$, $ZnCl_2$, $CoCl_2$, $NiCl_2$, $FeCl_2$, $CuSO_4$, $CO(NH_2)_2$, 3,5-dinitrosalicylic acid (DNS), Tween 20 and Tween 80 were purchased from Merck (Germany). Corncob waste was taken from Mandonga market, Kendari, Southeast Sulawesi, Indonesia.

**Isolation of bacteria:** Ten grams of corncob wastes were dissolved in 100 mL of sterile distilled water and incubated for 1 h using incubator shaker. Ten percent (v/v) solution of samples were suspended in sterile distilled water and made serial dilutions of $10^{-1}$, $10^{-2}$, $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, $10^{-7}$, $10^{-8}$, $10^{-9}$ and inoculated into Tryptic soy agar (TSA) media containing 1 % fungicide. Inoculum was incubated at 27-30 °C for 2 d. Bacteria colonies that growth from dilution of $10^{-2}$, $10^{-4}$, $10^{-6}$ and $10^{-8}$ were cultured in Tryptic soy agar media repeatedly to obtain pure cultures. Isolated bacteria was stored at 20 °C in 15 % glycerol.

**Screening of isolates:** Screening of mannolitic bacteria was carried out by growing isolates on mannan-containing media with composition of 0.2 % yeast extract, 0.2 % tryptone, 0.02 % $MgSO_4$, 0.14 % $K_2HPO_4$, 0.1 % $(NH_4)_2SO_4$, 20 % bacto agar and 0.3 % locust bean gum. One loop of isolated bacteria was suspended on 1 mL sterile distilled water and inoculated on the selection media that incubated at 37 °C. Mannolytic activity was determined by clear zone based on staining media using 0.1 % congo red. Isolates that had the greatest mannolytic index were used for the production and characterization of mannanase.

**Morphological and biochemical determination:** Morphological characterization of isolates was refered to the method contained on Bergey's Manual of Determinative Bacteriology [25], which includes the observation of microscopic and biochemical tests. Biochemical tests included Gram staining, citrate utilization, catalase and carbohydrate fermentations (mannose, rhamnose, lactose,and glucose).

**Production of β-mannanase enzyme:** Production of β-mannanase enzyme was refered to the method that had been reported by Wahyuni and Khaeruni [23]. Enzyme production was carried out by inoculating of 10 % starter into 250 mL production media (0.35 % yeast extract, 0.35 % tryptone, $MgSO_4$ 0.035 %, 0.245 % $K_2HPO_4$, 0.175 % $(NH_4)_2SO_4$, 0.2 % NaCl and 0.65 % locust bean gum) (w/v). The fermentation process was carried out using batch bioreactor system at room temperature. The optimum enzyme production period was measured by the growth of cell bacteria, concentration of enzyme and quantities of hydrolysis production. Mannanase crude extract was obtained by centrifugation at 15.000 rpm for 15 min.

**Activity of β-mannanase enzyme:** Mannanase activity was determined by reacting of 0.5 % solution of mannan (locust bean gum) and 50 mM citrate buffer, pH 6 (900 mL substrate locust bean gum and 100 mL of enzyme). The mixture was incubated at 80 °C for 30 min. The reaction was stopped by addition of 1 mL of 3,5-dinitrosalicylic acid to determine the reducing sugar [24] and heated for 15 min. The enzyme activity was determined by spectrometry at wavelength of 575 nm. One unit of enzyme activity was defined as the amount of enzyme that produce 1 mol mannose per min.

**Determination of stability and optimum temperature:** One milliliter of crude extract of mannase was treated with 1 mL locust bean gum substrate and incubated for 30 min at 30, 40, 50, 60, 70, 80 and 90 °C. Incubation was stopped by addition of 1 mL of 3,5-dinitrosalicylic acid and heated water for 15 min to calculate reducing sugar. The enzyme activity was determined by spectrophotometer at 575 nm. Thermal stability of the enzyme was determined by incubating the enzymes for 60 min at various temperatures in 50 mM citrate and phosphate buffers. Then, the residual enzyme activity was measured using the standard assay.

**Determination of optimum substrate concentration:** The optimum substrate concentration was determined by variation of substrate concentrations containing of 0.5-6 % locust bean gum with interval of 0.5 %. One milliliter of substrate was treated with 1 mL enzyme. 1 mL citrate buffer pH 6.0 and incubated for 60 min at the optimum temperature. The reaction was stopped by addition of 1 mL 3,5-dinitrosalicylic acid reagent and heated for 15 min. Determination of activity by measuring the absorbance using spectrophotometer at wavelength of 575 nm.

**Effects of metal ions and chemical reagents:** The effects of various additives such as metal ions, detergents and denaturants on the activity of IB.1.6 and IB.1.10 were examined. The effect of metal ions on enzyme activity was measured by reacting of 5 mM metal ion ($CaCl_2$, $MgCl_2$, $ZnCl_2$, $CoCl_2$, $NiCl_2$, $FeCl_2$ and $CuSO_4$), 1 mL of enzyme, 1 mL mannan substrate 0.5 % and 1 mL citrate buffer pH 6. The mixture was incubated for 60 min and stopped by addition of 1 mL of 3,5-dinitrosalicylic acid reagent and heated for 15 min. The detergents that used to test the stability of the enzyme were sodium dodecyl sulfate (SDS: 0.5 % and 1.0 %), Tween 80 (0.5 % and 1.0 %) and Tween 20 (0.5 % and 1.0 %). Denaturant agents used were NaCl (0.5 % and 1 %) and urea (0.5 % and 1 %). Measurement of residual enzyme activity was carried out by following the above procedure.

## RESULTS AND DISCUSSION

**Screening for mannanase-producing bacteria:** Ten isolates of bacteria were isolated as source of mannanase enzyme. Isolates were screened from corncob wastes obtained from Mandonga market in Kendari, Southeast Sulawesi, Indonesia. Basic of isolate screening was the ability of isolate to form clear zone on agar media containing mannan (lacust bean gum). From the total of 10 isolated bacteria, one isolates did not show mannolitic activity. Nine isolates showed activity through measurements of mannolitic index ranged 0.67-2.5 cm (ratio of clearing zone to colony diameter). IB.1.6 and IB.1.10 isolates were isolates with the highest of mannolitic index approxymately 2.15 and 2.5, respectively (Table-1).

TABLE-1
MANNOLITIC INDEX OF MANNANASE BACTERIA ISOLATE

| No. | Isolate | Mannolytic index | No. | Isolate | Mannolytic index |
|---|---|---|---|---|---|
| 1 | IB. 1.1 | 0.80 | 6 | IB. 1.6 | 2.15 |
| 2 | IB. 1.2 | 1.81 | 7 | IB. 1.7 | 1.66 |
| 3 | IB. 1.3 | 0.67 | 8 | IB. 1.8 | 1.38 |
| 4 | IB. 1.4 | 1.63 | 9 | IB. 1.9 | 1.18 |
| 5 | IB. 1.5 | 1.33 | 10 | IB. 1.10 | 2.50 |

The ability of isolates to produce clear zone at screening media, showed that isolates could express mannanase extracellular enzymes.Visualization ofclear zone was using congo red of 0.1 %. Mannan polymers are not degraded form a complex mannan-congo red, thus the degradation area could be observed. The differences of clear zone size indicated that each isolates were expressing a different enzyme activity. The enzymatic activity of each microorganism is affected by microbes type. isolate source and substrate type. Wahyuni *et al.* [23] had reported that bacteria strain BLS.11-01 and BLS.11-02, isolated from hump sago waste, had different mannolitic index approximately 2.2 and 2.0, respectively. Besides, *Klebsiella oxytoca* and *Acinetobacter* sp., isolated from soil, had different mannolitic index about 1.3-3 mm [11].

Our results indicated that IB.1.6 and IB.1.10 isolates are potential isolates that can be used as mannanase enzyme source. For further purposes, both isolates were performed morphological and biochemical characterization based on Bergey's Manual of Systematic Bacteriology [25]. IB.1.6 and IB.1.10 isolates were Gram-negative bacteria through Gram stain using safranin and rod cells by microscopic observation (Fig. 1).

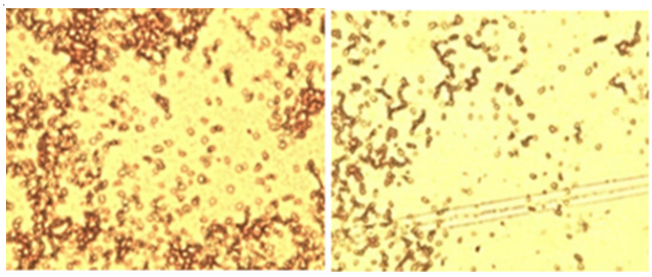


Fig. 1. Morphology of isolates bacteria cell (magnification of 1000 X)

Physiology characters of IB.1.6 and IB.1.10 isolates were identified by biochemical tests such as the use of citrate, the ability of carbohydrates fermentation, utilization of citrate and catalase test. IB.1.6 and IB.1.10 isolates showed positive results on the fermentation of mannose, rhamnosa, lactose, glucose, as well as utilization of citrate and catalase. The ability of isolates to give positive response on glucose and mannose indicated that both isolates could produce mannanase enzyme [26]. The same of physiology characters was also possessed by *Bacillus* sp. THCM3.1 [27] and *Bacillus* sp. IB-OR17 isolates [28].

IB.1.6 and IB.1.10 isolates were selected to be used for the production of mannanase, which had activities of 0.209 and 0.304 U/mL, respectively. Mannanase activity of *Bacillus* sp. was lower than that of IB.1.6 and IB.1.10 isolates [29]. However, mannanase activities of BLS.11-01 and BLS.11-02 isolates from sago hump waste were higher than that of IB.1.6 and IB.1.10 isolates, which are about 0.485 and 0.700 U/mL, respectively. Alsarrani [30] reported that mannase activity of *Aspergilus niger*, *A. flavus* and *A. ochraceous* were 2.90, 2.54 and 2.16 U/mL, respectively. These results indicated that IB.1.6 and IB.1.10 isolates have potential as source of mannanase enzyme that can be used for the production of manno oligosaccharides by utilizing corncob waste or other agricultural wastes that containing hemicellulose.

**Optimum of substrate concentration:** Substrate that used in this study was locust bean gum (LBG). Mannanase extract of IB.1.6 and IB.1.10 isolates was used to determine locust bean gum catalytic activity at concentration of 0.5-6 %. The effect of substrate concentration on the activity of mannanase was shown in Fig. 2.

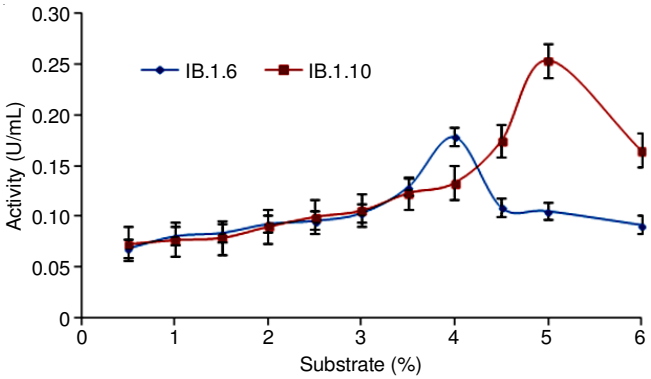


Fig. 2. Optimum of substrate concentration of mannanase

Fig. 2 showed that the catalytic activity of mannanase from IB.1.10 was higher than that of IB.1.6. Both enzymes showed different activity trends, which mannanase from IB.1.6 and IB.1.10 showed saturation of active site (ES) at subtrate concentrations of 4 % (0.178 U/mL) and 5 % (0.25 U/mL), respectively. Araujo and Ward [31] reported that β-mannanase of fungi had optimum substrate concentration at 3.5 % with the activity of 0.012 U/mL. Optimum of substrate concentration of β-mannanase from *Thermotoga neopalitana* and its activity were 5 % and 0.080 U/mL, respectively [32]. Each of enzyme from different sources showed the ability to hydrolyze substrate at various concentrations. The optimum of substrate concentration was important to know because it relates to the application of enzymes, for example in production of manno oligosaccharides.

**Effects of temperature and pH:** The effect of temperature and pH on the activity of mannanase from IB.1.6 and IB.1.10 isolates were tested at varying pH and temperature. The optimum activity of crude mannanase was determined at 30-90 °C and pH 3-11. The effect of temperature on the activity of mannanase is shown in Fig. 3.

The results showed that IB.1.6 (80 °C) was thermostable than IB.1.10 (60 °C), however, both isolates showed stability of wide temperature about 30-90 °C. Mannanase character varied widely depending on the type of microbes, isolate source and specification of substrate. Wahyuni and Khaeruni [23] reported that the optimum temperature and stability of mannanase of BLS.11-01 and BLS.11-02 isolated from sago hump waste were 70 and 60 °C, respectively. For the stability, mannanase from both corncob and sago hump wastes isolates were stable at wide temperature of 30-90 °C. Titapoka *et al.* [11] reported that the optimum temperature of mannanase from ST1-1 and CW2-3 isolates were 40 and 50 °C. In general, bacterial mannanases are optimally active at 50-70 °C. Besides, some reports about the optimum temperature of mannanase from native and recombinant sources had been reported by Mendoza *et al.* [33] (40-50 °C); Zakaria *et al.* [34] (40-50 °C); Zhang *et al.* [35] (40-50 °C); Zheng *et al.* [13] (70 °C); Nadaroglu *et al.* [14] (60 °C) and Katsimpouras *et al.* [36] (60 °C).

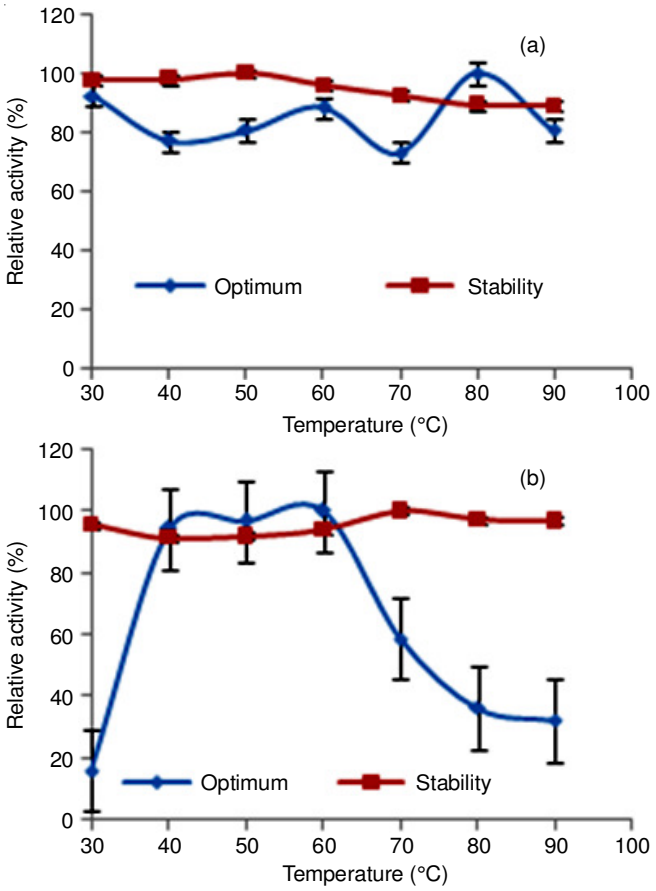


Fig. 3. Effect of temperature on mannanase activity: (a) IB.1.6 (b) IB.1.10 isolates

pH is chemical parameters that affect enzyme activity directly. Each of enzyme showed different activity in the pH range of acids and bases. The enzyme activity at optimum pH showed optimum ionization in active site of enzyme.The strength of enzyme maintains the conformation influenced by enzymes stabilizing forces consisting of hydrogen and hydrophobic bonding, ionic interactions and disulfide bridges [37]. Mannanase from IB.1.6 and IB.1.10 isolates with optimum activity at neutral pH was shown in Fig. 4.

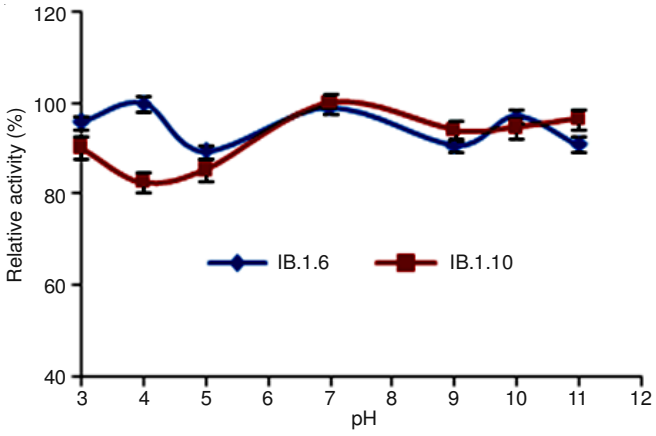


Fig. 4. Effect of pH on mannanase activity of IB.1.6 and IB.1.10 isolates

Catalytic activity of mannanase from both isolates (Fig. 4) showed the stability in broad pH. Generally, crude extracts and pure mannanase had optimum activity at pH 5-8. Wahyuni and Khaeruni [23] reported that the crude extracts of mannanase from BLS.11-01 and BLS.11-02 isolates had optimum activity at pH 7 and 6, respectively. Li *et al.* [15] and Yu *et al.* [5] reported recombinant mannanase had optimum activity at pH 7. Titapoka *et al.* [11] also reported mannanase from ST1-1 and CW2-3 isolates had optimum activity at pH 6 and 7, respectively. The optimum activity of mannanase from *Bacillus circulans* NT 6.7. was at pH 6 [16]. pH stability of enzyme reflects the tolerance to acidic and alkaline conditions. The tolerance of enzyme to acidic and alkaline conditions showed that an enzyme has very wide application.

**Effect of metal ions and chemical reagents on mannase activity:** Some enzymes need cations for their activity, as well as mannanase enzyme which needs cationic compounds such as $CaCl_2$, $ZnCl_2$, $CoCl_2$, $NiCl_2$, $FeCl_2$ and $CuSO_4$. The effect of metal cations on enzyme activity was shown in Fig. 5.

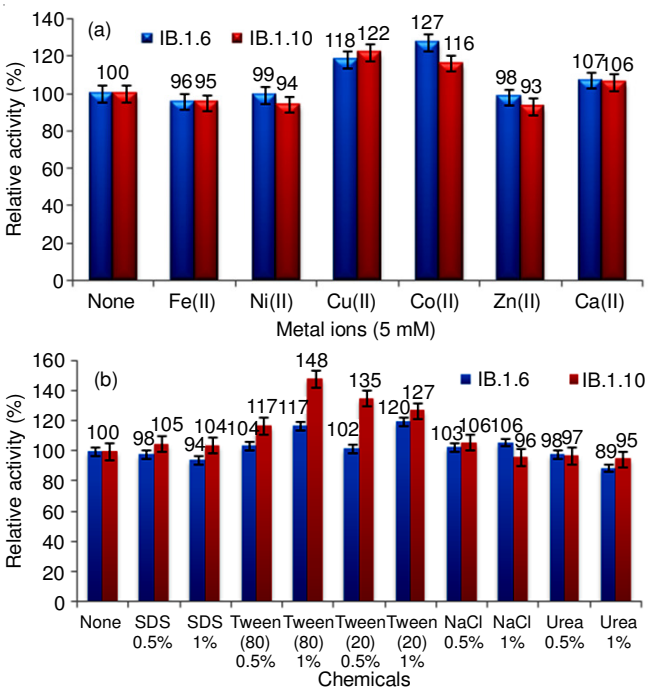


Fig. 5. Effect of metal ions (a) and chemical reagents (b) on mannanase activity

Mannanase from IB.1.6 and IB.1.10 isolates showed similar activities characters. Copper(II) and coablt(II) were metal ions that enhances the mannanase activity from both isolates. While Fe(II), Ni(II), Zn(II) and Ca(II) ions showed similar activity. Similar results were reported by Wahyuni and Khaeruni [23] that Cu(II) and Co(II) ions were cofactors of mannanase from bacteria isolated sago hump waste. Yoo *et al.* [38]; Li *et al.* [15] and Pangsri *et al.* [16] reported that Co(II) wasan activator of mannanase from *Streptomyces tendae* and *Bacillus subtilis* WD23. Besides, Co(II) ion might be associated with active A.Pase. which was essential for activity and maintaining the conformational stability of the enzyme [39]. Wang *et al.* [40] reported that Mg(II) ion was a cofactor of endo-β-mannanase AtMan5-2 from *Arabidopsis thaliana*. Besides, mannanase activity of *P. acidilactici* (M17) was influenced by $Mn^{2+}$ and $Zn^{2+}$ ions [14]. The mechanism of metal ions in inhibiting or

enhancing enzyme activity is unknown. It suggested that metal ions interact with the local structures of enzymes that can stabilize or destabilize the enzyme. Some enzymes need metal ions with varying concentrations to increase their activities [41], which it might have direct influence on the electric charge on the surface of enzyme [42].

Various chemical reagents were also investigated on their effects on the activity of mannanase from IB.1.6 and IB.1.10 isolates (Fig. 5b). Tween 20 and Tween 80 were type of reagents which can increase the activity of mannanase from both isolates. Mannanase of IB.1.10 showed the greatest activity. sodium chloride, urea and sodium dodecyl sulfate did not affect the activity of mannanase from both isolates significantly. Mannanase activity of IB.1.10 was strongly influenced by the chemical reagent of Tween 80 (1 %). Tween 80 (1 %) could enhance the catalytic activity about 48 %. In contrast to Tween 80, increasing concentrations of Tween 20 (1 %) could decrease mannanase activity of 7 %. Mannanase of IB.1.6 was very different in the presence of Tween 20 and 80, which increased the activity of 17 and 20 %, respectively. Generally, Tween is a type of reagent that has function as cofactor in IB.1.10 and IB.1.6 mannanase.The effects of various chemical reagents were often studied related to enzyme applications in the future. Wang *et al*. [43] and Lu *et al*. [44] reported the use of β-mercaptoethanol detergent increased the mannanase activity of *Xanthomonas campestris*, *Bacillus* sp. JB-99 and *Thielavia arenaria* XZ7 by nutralizing the oxidation effects of the S-S linkage between cysteine residues. Urea and SDS were type of chemical reagents that did not effectively used on both concentrations of (0.5 and 1 %) for both isolates.

## Conclusion

In this study, the mannanase enzymes produced by IB.1.6 and IB.1.10 isolates, which isolated from corncobs waste were characterized. The enzymes produced from both isolates showed thermophilic properties and stable in broad pH range. Copper(II), cobalt(II), Tween 20 and Tween 80 are type of metal ions and chemical that can enhanced catalytic activity of mannanase from both isolates. However, Ni(II), Zn(II), Fe(II), urea, SDS and NaCl were metal ions and chemical that is not effectively used in applications of mannanase from both isolates.

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## REFERENCES

1. T.T. Clark and E.C. Lathrop, USDA-ARS North Regional Research Lab., Peoria, IL, AIC-177 (1953).
2. K. Foley, Ohysical Properties, Chemical Properties and Uses of the Anderson's Corncob Products, The Andersons, Maumee, OH, USA (1978).
3. M. Chen, L. Xia and P. Xue, *Int. Biodeter. Biodegrad*, **59**, 85 (2007); https://doi.org/10.1016/j.ibiod.2006.07.011.
4. T. Ansah, A.A. Agbolosu, G.A. Teye, A. Akwasi and M.O. Agyeman, *Anim. Sci. Biotechnol.*, **45**, 7 (2012).
5. S. Yu, Z. Li, Y. Wang, W. Chen, L. Fu, W. Tang, C. Chen, Y. Liu, X. Zhang and L. Ma, *Biotechnol. Lett.*, **37**, 1853 (2015); https://doi.org/10.1007/s10529-015-1848-7.
6. M. Akita, N. Takeda, K. Hirasawa, H. Sakai, M. Kawamoto, M. Yamamoto, W.D. Grant, Y. Hatada, S. Ito and K. Horikoshi, *Acta Crystallogr. D Biol. Crystallogr.*, **60**, 1490 (2004); https://doi.org/10.1107/S0907444904014313.
7. S. Dhawan and J. Kaur, *Crit. Rev. Biotechnol.*, **27**, 197 (2007); https://doi.org/10.1080/07388550701775919.
8. E.L. Carr, P. Kampfer, B.K.C. Patel, V. Gurtler and R.J. Seviour, *Int. J. Syst. Evol. Microbiol.*, **53**, 953 (2003); https://doi.org/10.1099/ijs.0.02486-0.
9. W.H. Van Zyl, S.H. Rose, K. Trollope and J.F. Gorgens, *Process Biochem.*, **45**, 1203 (2010); https://doi.org/10.1016/j.procbio.2010.05.011.
10. Y. Oda, T. Komaki and K. Tonomura, *J. Ferment. Bioeng.*, **76**, 14 (1993); https://doi.org/10.1016/0922-338X(93)90045-A.
11. S. Titapoka, S. Keawsompong, D. Haltrich and S. Nitisinprasert, *World J. Microbiol. Biotechnol.*, **24**, 1425 (2008); https://doi.org/10.1007/s11274-007-9627-9.
12. H. Mou, F. Zhou, X. Jiang and Z. Liu, *J. Food Biochem.*, **35**, 1451 (2011); https://doi.org/10.1111/j.1745-4514.2010.00466.x.
13. H. Zheng, Z. Yu, X. Fu, S. Li, J. Xu, H. Song and Y. Ma, *J. Ind. Microbiol. Biotechnol.*, **43**, 977 (2016); https://doi.org/10.1007/s10295-016-1773-3.
14. H. Nadaroglu, G. Adiguzel, A. Adiguzel and Z. Sonmez, *Eur. Food Res. Technol.*, **243**, 193 (2016); https://doi.org/10.1007/s00217-016-2735-8.
15. H. Li, Z. Liu, C. Wang, S. Huang and M. Zhao, *Eur. Food Res. Technol.*, **240**, 671 (2015); https://doi.org/10.1007/s00217-014-2369-7.
16. P. Pangsri, Y. Piwpankaew, A. Ingkakul, S. Nitisinprasert and S. Keawsompong, *Springerplus*, **4**, 771 (2015); https://doi.org/10.1186/s40064-015-1565-7.
17. P. Ramachandran, Z. Zhao, R. Singh, S.S. Dhiman, J.H. Choi, D. Kim, J.R. Haw and J.K. Lee, *Bioprocess Biosyst. Eng.*, **37**, 1817 (2014); https://doi.org/10.1007/s00449-014-1156-y.
18. A. Adiguzel, H. Nadaroglu and G. Adiguzel, *J. Food Sci. Technol.*, **52**, 5292 (2015); https://doi.org/10.1007/s13197-014-1609-y.
19. W. Aehle and R.N. Perham, Enzymes in Industry: Production and Applications, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim, edn 2 (2004).
20. H. Lu, H. Luo, P. Shi, H. Huang, K. Meng, P. Yang and B. Yao, *Appl. Microbiol. Biotechnol.*, **98**, 2155 (2014); https://doi.org/10.1007/s00253-013-5112-6.
21. D.A. Comfort, S.R. Chhabra, S.B. Conners, C.-J. Chou, K.L. Epting, M.R. Johnson, K.L. Jones, A.C. Sehgal and R.M. Kelly, *Green Chem.*, **6**, 459 (2004); https://doi.org/10.1039/b406297c.
22. V. Pee, K.L. Ignatius, V. Speybroeck, M.P. Michel and V.P. Jozef, Use of Mannanases as a Slime Control Agents, US Patent 0871596 (2002).
23. S. Wahyuni and A. Khaeruni R., L. Lianto, S. Sidarmin, H. Holilah, W.P. Utomo and A. Asranudin *Waste Technol.*, **4**, 1 (2016); https://doi.org/10.12777/wastech.4.1.1-6.
24. G.L. Miller, *Anal. Chem.*, **31**, 426 (1959); https://doi.org/10.1021/ac60147a030.
25. J.G. Holt, N.R. Krieg, P.H.A. Sneath, J.T. Staley and S.T. Williams, Bergey's Manual of Determinative Bacteriology, Williams & Wilkins USA, edn 9 (1994).
26. A. Sachslehner, B. Nidetzky, K.D. Kulbe and D. Haltrich, *Appl. Environ. Microbiol.*, **64**, 594 (1998).
27. P. Kanjanavas, P. Khawsak, A. Pakpitcharoen, S. Areekit, T. Sriyaphai, K. Pothivejkul, S. Santiwatanakul, K. Matsui, T. Kajiwara and K. Chansiri, *Sci. Asia*, **35**, 17 (2009); https://doi.org/10.2306/scienceasia1513-1874.2009.35.017.
28. A.L. Melentiev, N.F. Galimzianova, E.A. Gilvanova, E.A. Shchelchkova, Y.L. Kuzmina, T.F. Boyko, N.G. Usanov and G.E. Aktuganov, *Adv. Microbiol.*, **4**, 455 (2014); https://doi.org/10.4236/aim.2014.48050.
29. J. Abe, M.Z. Hossain and S. Huzukuri, *J. Ferment. Bioeng.*, **78**, 259 (1994); https://doi.org/10.1016/0922-338X(94)90301-8.

30. A.Q. Alsarrani, *J Taibah Univ. Sci.*, **5**, 1 (2011).
31. P. Araujo and O.P. Ward, *J. Ind. Microbiol.*, **6**, 171 (1990); https://doi.org/10.1007/BF01577692.
32. G.D. Duffaud, C.M. McCutchen, P. Leduc, K.N. Parker and R.M. Kelly, *Appl. Environ. Microbiol.*, **63**, 169 (1997).
33. N.S. Mendoza, M. Arai, T. Kawaguchi, T. Yoshida and L.M. Joson, *World J. Microbiol. Biotechnol.*, **10**, 551 (1994); https://doi.org/10.1007/BF00367665.
34. M.M. Zakaria, S. Yamamoto and T. Yagi, *FEMS Microbiol. Lett.*, **158**, 25 (1998).
35. J. Zhang, Z. He and K. Hu, *Biotechnol. Lett.*, **22**, 1375 (2000); https://doi.org/10.1023/A:1005644414762.
36. C. Katsimpouras, M. Dimarogona, P. Petropoulos, P. Christakopoulos and E. Topakas, *Appl. Microbiol. Biotechnol.*, **100**, 8385 (2016); https://doi.org/10.1007/s00253-016-7609-2.
37. C. Edwards, Thermophiles: Microbiology of Extreme Environments, Alden Press, Oxford, pp. 1-32 (1990).
38. H.Y. Yoo, G.C. Pradeep, S.W. Kim, D.H. Park, Y.H. Choi, J.W. Suh and J.C. Yoo, *Biotechnol. Bioprocess Eng.*, **20**, 453 (2015); https://doi.org/10.1007/s12257-014-0885-8.
39. D.B. Spencer, C.P. Chen and F.M. Hulett, *J. Bacteriol.*, **145**, 926 (1981).
40. Y. Wang, S. Azhar, R. Gandini, C. Divne, I. Ezcurra and H. Aspeborg, *Plant Sci.*, **241**, 151 (2015); https://doi.org/10.1016/j.plantsci.2015.10.002.
41. Y.M. Hsiao, Y.F. Liu, M.C. Fang and Y.H. Tseng, *J. Agric. Food Chem.*, **58**, 1653 (2010); https://doi.org/10.1021/jf903637s.
42. H. Zhao, *J. Mol. Catal., B Enzym.*, **37**, 16 (2005); https://doi.org/10.1016/j.molcatb.2005.08.007.
43. C. Wang, J. Zhang, Y. Wang, C. Niu, R. Ma, Y. Wang, Y. Bai, H. Luo and B. Yao, *Food Chem.*, **197**, 474 (2016); https://doi.org/10.1016/j.foodchem.2015.10.115.
44. H. Lu, H. Zhang, P. Shi, H. Luo, Y. Wang, P. Yang and B. Yao, *Appl. Microbiol. Biotechnol.*, **97**, 8121 (2013); https://doi.org/10.1007/s00253-012-4656-1.