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Full Length Research Paper

Selection and production of insoluble xylan hydrolyzing enzyme by newly isolated *Thermomyces lanuginosus*

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Forty-two strains of *Thermomyces lanuginosus* isolated from various sources in Thailand were divide into 4 groups based on the soluble xylan hydrolyzing (SXH) and insoluble xylan hydrolyzing (IXH) enzyme activities in the supernatant obtained from 5-day culture at 50°C in the liquid medium using corncob as substrate. The strains which exhibited high IXH enzyme activity, showed high binding ability to insoluble xylan. Ten strains of *T. lanuginosus* produced high activity towards insoluble xylan. Among these, *T. lanuginosus* THKU-56 could produce considerable activity of insoluble xylan hydrolyzing enzyme with the highest stability that remained 28.2 and 58.9% of the original activity after treating at pH 3.5 and 37°C and at pH 6.0 and 70°C for 1 h, respectively. The optimized concentration of corncob, yeast extract, KH_2PO_4 and Tween80 were determined in shaking condition for the production of insoluble xylan hydrolyzing enzyme by *T. lanuginosus* THKU56 using a central composite design. Unlike other components, only corncob and yeast extract were found to affect the xylanase production. The optimal concentration of the active nutrients for xylanase production were 41 gl^{-1} of corncobs and 24 gl^{-1} of yeast extract, which gave a predicted and experimental yield of 526.7 and 541 Uml^{-1} at 5th day of culture, respectively.

Key words: *Thermomyces lanuginosus*, insoluble xylan hydrolyzing enzyme, xylan-binding ability, central composite design.

INTRODUCTION

Endo 1,4-β- D-xylan xylanohydrolase (EC 3.2.1.8), a glycosyl hydrolase, is generally secreted by micro-organisms grown on plant biomass, for hydrolyzing xylan in plant cell walls. These enzymes are important in various industrial processes such as food, feedstuffs and biobleaching process (Beg et al., 2001; Butt et al., 2008, Jiang et al., 2005; Kumar et al., 2009). Thermophilic fungus, *Thermomyces lanuginosus*, a potent strain to produce cellulase-free β-xylanase has been reported by several researchers (Gomes et al., 1993a; Hoq and Deckwer, 1995; Purkarthofer et al., 1993; Singh et al., 2003). Most reports focus on the enzyme production based on their hydrolysis ability to soluble xylan. However, in nature, the xylan molecules in the lignocellulosic materials are mainly in the insoluble form. The use of xylanase will be more feasible if the enzyme could degrade the insoluble xylan portion. Thermostable xylanase produced from *T. lanuginosus* belong to the family 11 of glycosyl hydrolase which have no binding site to insoluble substrates (Irwin et al., 1994; Nath and Rao, 2001). However, strains of *T. lanuginosus* which are able to hydrolyze insoluble xylan were selected from the collected strains previously isolated in Thailand (Khucharoenphaisan and Kitpreechavanich, 2004). This is the first report on the production of xylanase that are able to hydrolyze insoluble xylan from *T. lanuginosus*. To increase xylanase production, factors affecting the insoluble xylan hydrolyzing enzyme production by the *T.*

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Abbreviations: SXH, Soluble xylan hydrolyzing; **IXH,** insoluble xylan hydrolyzing; **CCD,** central composite design.

lanuginosus THKU-56 were optimized using response surface methodology.

MATERIALS AND METHODS

Microorganisms and cultivation

A total of 42 *T. lanuginosus* strains, isolated from soil and decayed plant materials and collected from various ecological systems at different geographical regions of Thailand were used in this study (Khucharoenphaisan and Kitpreechavanich, 2004). The *T. lanuginosus* strains were pre-cultured in 10% wheat bran agar for 10 days. One milliliter of spore suspension (10^5 to 10^6) in 1% Tween 80 solution of each strain was inoculated into 50 ml of the medium consisting of 15 gl $^{-1}$ corncobs, 15 gl $^{-1}$ yeast extract, 5 gl $^{-1}$ KH_2PO_4 and 0.3 mlL^{-1} Tween 80 in 250 ml cotton plug Erlenmeyer flasks and incubated at 50°C for 5 days on 120 rpm reciprocal shaker. The clear supernatants obtained from filtration of culture broth through filter paper (Whatman No.1) were used to assay the enzyme activities towards soluble and insoluble xylan. All experiments were done in duplicate.

Determination of insoluble xylan binding ability

Crude enzyme produced by the selected isolates of *T. lanuginosus* was diluted to attain the xylanase activity of 50 units/ml in 0.1 M phosphate buffer (pH 6.0). A 5 ml of the diluted crude enzyme was added to 5 g of each substrate: insoluble oat spelt xylan, cellulose powder and raw cassava starch and incubated at 4°C for 30 min. After centrifugation, the insoluble xylan hydrolyzing (IXH) enzyme activity of the supernatant was determined and compared with the original activity. The binding ability is defined according to the equation:

% Binding = [(O-R)/O] x 100

Where, R indicates the residual IXH enzyme activity in the supernatant and O indicates that of the original crude enzyme solution activity.

Effect of pH and temperature on stability and activity

Crude enzymes produced from the 10 selected strains based on either high IXH and soluble xylan hydrolyzing (SXH) activities were determined for their stability at pH 3.5 and 37°C which is the comparable condition of swine maw and at 70°C with pH 6.0 for thermo stability. Each crude enzyme obtained was kept at each condition for 1 h. The residual IXA activities were determined.

The effect of pH and temperature on the enzyme activity and stability of crude enzyme produced by *T. lanuginosus* THKU 56 were investigated. To study the effect of pH on activity, the reaction mixture of 0.5 ml of enzyme and 0.5 ml of 1% insoluble oat spelt xylan in 50 mM buffers with various pH values (acetate buffer, pH 3 to 6; phosphate buffer, pH 6 to 9; glycine-NaOH buffer, 9.5 to 11) were incubated at 50°C for 10 min. For pH stability, the IXH activity was determined after keeping the crude enzyme in 50 mM of various buffers at pH 3 to 11 at 4°C for 24 h. To study the effect of temperature activity, the reaction mixture of 0.5 ml of enzyme and 0.5 ml of 1% insoluble oat spelt xylan in 50 mM sodium phosphate buffer (pH 6.0) was incubated at various temperatures (50 to 90°C) for 10 min. For thermostability, the IXH activity was determined after keeping the crude enzyme solution in 50 mM sodium phosphate buffer (pH 6.0) at various temperatures (50 to 90°C) for 30 min.

Medium optimization for insoluble xylan hydrolyzing enzyme production by *T. lanuginosus* THKU-56

Factors affecting insoluble xylan hydrolyzing xylanase production were investigated using central composite design (CCD), with k = 4, which was used to generate 26 treatment combinations, with corncob (X_1), yeast extract (X_2), KH_2PO_4 (X_3) and Tween 80 (X_4) as independent variables. Three levels of each variable were chosen, their upper and lower limits were set to be in the range described in the literature. The response value (Y) in each trial is the average of duplicates. Statistic program (SPSS) was used for regression and graphical analysis of the data. This design is represented by a second-order polynomial regression model to generate contour plot. The variance explained by the model is given by multiple coefficient of determination, R^2.

Xylanase activity assay

A 100 ml of 1% suspension of oat spelt xylan (Sigma Co, USA) in 0.1 M phosphate buffer (pH 6.0) was boiled for 10 min. The clear supernatant of soluble xylan was used as substrate for soluble xylan hydrolyzing enzyme activity. Insoluble xylan was prepared according to the method described by Ghangas et al. (1989). A suspension of 5% of oat spelt xylan was thoroughly mixed in 2 M NaOH at room temperature for 1 h. The insoluble portion was collected by centrifugation, suspended in distilled water and adjusted to pH 7.0. Then it was filtered and washed several times with distilled water. The insoluble portion, dried in hot air oven at 70°C for overnight, was collected and kept at room temperature. A suspension of 1% insoluble xylan was used as a substrate for insoluble xylan hydrolyzing enzyme activity. The activity of β-xylanase was assayed at pH 6.0 and 50°C for 10 min by determining the reducing sugars released with 3,5-dinitrosalicylic acid reagent (Miller, 1959). One unit of xylanase activity was defined as the amount of enzyme releasing 1 µmole equivalents of D-xylose per min.

RESULTS AND DISCUSSION

Soluble and insoluble xylan hydrolyzing activities produced by *T. lanuginosus* strains

The supernatants obtained from 42 different stains of *T. lanuginosus* grown on corncob liquid culture at 50°C for 5 days were determined for SXH and IXH activities. It was found that these isolates produced xylanases with different levels on SXH and IXH activities. *T. lanuginosus* THKU-86 produced the highest SXH activity at 737 U ml^{-1}, whereas *T. lanuginosus* THKU-4 exhibited the highest IXH activity at 335 U ml^{-1}. It was found that only 10 strains of *T. lanuginosus* were able to produce xylanase with high extent both on SXH and IXH activities. When comparing SXH activity with other strains of *T. lanuginosus* cultivated under similar conditions, it was found that strains RT9 (Hoq and Deckwer, 1995), DSM 5826 (Purkarthofer et al., 1993), ATCC22083, ATCC-58160, ATCC34626 (Singh et al., 2000b) and IOC-4145 (Damaso et al., 2000) produced 425, 1600, 455, 377, 1170 and 516 U ml^{-1}, respectively.

T. lanuginosus strains could be divided into 4 groups (A, B, C and D) based on SXH and IXH activities as

Table 1. Classification of *T. lanuginosus* strains based on SXHA and IXHA in corncob liquid medium.

Strain	SXHA (U ml^{-1})	IXHA (U ml^{-1})	Group	Strain	SXHA (U ml^{-1})	IXHA (U ml^{-1})	Group
THKU-3	479	185	C	THKU-56	682	317	A
THKU-4	451	335	C	THKU-58	464	126	D
THKU-7	527	92	B	THKU-61	567	133	B
THKU-9	473	262	C	THKU-63	478	198	C
THKU-12	548	110	B	THKU-64	605	120	B
THKU-15	555	157	A	THKU-66	588	177	A
THKU-20	499	247	C	THKU-67	613	171	A
THKU-21	617	139	B	THKU-68	29	15	D
THKU-23	568	270	A	THKU-69	616	202	A
THKU-24	629	247	A	THKU-70	555	75	B
THKU-25	670	65	B	THKU-71	636	136	B
THKU-32	486	139	D	THKU-74	529	3	D
THKU-33	551	128	B	THKU-75	734	241	A
THKU-39	602	102	B	THKU-76	514	138	B
THKU-34	491	139	D	THKU-77	687	198	A
THKU-42	537	232	A	THKU-80	427	112	D
THKU-45	636	173	A	THKU-84	683	234	A
THKU-47	491	169	C	THKU-85	868	70	B
THKU-50	633	148	B	THKU-86	737	133	B
THKU-53	516	133	B	THKU-87	564	147	B
THKU-55	448	121	D	THKU-88	626	317	A

shown in Table 1. Group A showed high activities in both SXH (>500 U/ml) and IXH (>150 Uml^{-1}). Group B produced xylanase with high SXH xylanase activity (>500 U/ml) but low activity for IXH (<150 Uml^{-1}). Group C was high for IXH (>150 Uml^{-1}) but SXH activity was less than 500 Uml^{-1}, while Group D showed low activity of both SXH and IXH.

Insoluble xylan-binding ability

The strains of *T. lanuginosus* which had either high activity on IXH or SXH were determined for insoluble xylan binding ability. It was found that the culture supernatant from the strains which produced high activity of IXH showed higher binding ability to insoluble xylan than those from the strains which produced high activity of SXH as shown in Table 2. However, all culture supernatant slightly bound to cellulose and did not bind to cassava starch. The crude enzyme of *T. lanuginosus* THKU-4 and THKU-56 showed high binding to insoluble xylan at 25.4 and 23.1%, respectively. It was found that the binding ability to insoluble xylan showed tendency to correspond with IXA activity. The binding ability of enzymes to their insoluble substrates occurred by hydrophobic interaction between aromatic amino acids such as tyrosine and tryptophan, in polysaccharide-binding domain and sugar ring of substrates (Tormo et al., 1996; Ponyi et al., 2000). Xylanase of *T. lanuginosus* in family 11 had only one domain of enzyme without xylan-binding domain (Irwin et al., 1994; Nath and Rao, 2001). Thus, the result of binding ability may be caused by the hydrophobic interaction between insoluble xylan and active site of xylanase. In addition, IXH enzyme from *T. lanuginosus* THKU-56 exhibited the highest pH and thermostability as shown in Table 3. The residual activity remained 22.3 and 58.9%, of original activity after been kept in 50 mM citrate phosphate buffer (pH 3.5) at 37°C which is a comparable condition in swine maw and at 70°C in 50 mM phosphate buffer (pH 6.0), respectively. The strain of *T. lanuginosus* THKU-56, which produced the highest IXH enzyme activity with high stability at low pH and high temperature was selected as potent strain for IXH enzyme production for animal feed.

Effect of pH and temperature on activity and stability of IXH enzyme produced by *T. lanuginosus* THKU-56

High stability at wide range of pH and high temperature is required for the enzyme in animal feed. The optimal pH and pH stability of crude enzyme from *T. lanuginosus* THKU-56 were at 7.0 and in the range of pH 6 to 9, respectively, as shown in Figure 1A. Maximal enzyme activity was found at 70°C. The thermostability of the enzyme showed that the enzyme retained its full activity

Table 2. Binding ability of *T. lanuginosus* xylanase on insoluble polysaccharides.

Strains	Activity (U ml^{-1})		Percentage binding		
	SXH	IXH	Insoluble xylan	Cellulose	Cassava starch
THKU-4	451	335	25.4	1.7	0
THKU-20	499	247	18.7	2.9	0
THKU-56	682	317	23.1	5.6	0
THKU-75	734	241	16.3	3.6	0
THKU-85	686	70	5.9	3.2	0
THKU-86	737	133	9.2	2.5	0

Table 3. pH stability and thermostability of insoluble xylan degrading enzyme from the selected *T. lanuginosus* strains.

Strains	Initial IXD activity (U/ml)	Residual activity (%)	
		37°C, pH 3.5, 60 min	70°C, pH 6.0, 60 min
THKU-4	335	19.2	33.4
THKU-9	262	11.5	24.2
THKU-20	247	8.7	38.5
THKU-23	270	13.7	28.1
THKU-24	247	7.9	27.9
THKU-42	232	16.7	34.7
THKU-56	317	22.3	58.9
THKU-75	241	20.9	50.2
THKU-84	234	18.3	27.4
THKU-88	317	9.8	22.3

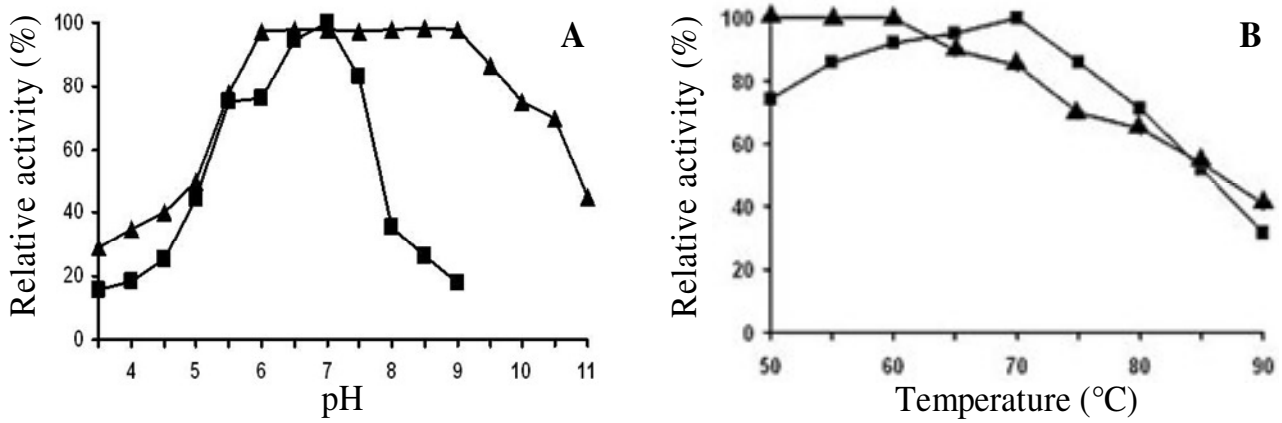

Figure 1. Effect of pH (A) and temperature (B) on enzyme activity (-■-) and stability (-▲-) of crude xylanase produced by *T. lanuginosus* THKU-56.

up to 60°C (Figure 1B). These properties of xylanase obtained from *T. lanuginosus* THKU-56 were in the range similar to the previous reports (Gomes et al., 1993b; Singh et al., 2000a; Singh et al., 2003; Jiang et al., 2005) indicating the potential application of this enzyme for animal feed. Therefore, *T. lanuginosus* THKU-56 was selected as potent strain for further study on the enzyme production.

Medium optimization for insoluble xylan hydrolyzing enzyme production

The experimental design of CCD and the results of IXH

Table 4. Process variable used in CCD, showing the treatment combinations and the mean experimental response.

Treatment	Corncob (g l^{-1})	Yeast extract (g l^{-1})	KH_2PO_4 (g l^{-1})	Tween 80 (ml l^{-1})	IXH (U ml^{-1})
1	10	10	0	0	128
2	10	10	0	1	126
3	10	10	10	0	186
4	10	10	10	1	160
5	10	40	0	0	121
6	10	40	0	1	124
7	10	40	10	0	126
8	10	40	10	1	80
9	10	20	5	0.3	142
10	40	10	0	0	477
11	40	10	0	1	442
12	40	10	10	0	461
13	40	10	10	1	467
14	40	40	0	0	450
15	40	40	0	1	445
16	40	40	10	0	446
17	40	40	10	1	434
18	40	20	5	0.3	550
19	20	10	5	0.3	286
20	20	40	5	0.3	301
21	20	20	0	0.3	381
22	20	20	10	0.3	386
23	20	20	5	0	396
24	20	20	5	1	344
25	20	20	5	0.3	376
26	20	20	5	0.3	362
27	20	20	5	0.3	368

Table 5. Coefficient estimates by regression model for optimization.

Parameter	Coefficient	Computed t-value	P-value
X_1	28.281	8.690	0.000
X_2	14.778	4.557	0.000
X_3	0.160	0.509	0.617
X_4	-0.052	-1.701	0.104
X_1^2	-0.343	-5.405	0.000
X_2^2	-0.311	-4.899	0.000
X_3^2	0.024	0.741	0.467
X_4^2	-0.049	-1.525	0.142
X_1 X_2	0.038	0.447	0.660
X_1 X_3	0.004	0.116	0.908
X_1 X_4	-0.049	-1.414	0.172
X_2 X_3	-0.014	-0.387	0.703
X_3 X_4	-0.029	-0.925	0.365
R^2		0.979	0.365

Statistically significant at 95% of confidence level; X_1 = corncob (g.l^{-1}); X_2 = yeast extract (gl^{-1}); X_3 = KH_2PO_4 (g.l^{-1}); X_4 = Tween 80 (ml l^{-1}).

enzyme activity of *T. lanuginosus* THKU 56 are shown in Table 4. The treatment numbers 10 to 18 showed a high level of IXH enzyme activity (434 to 550 units/ml), of which treatment no. 18 gave the highest activity. The statistical significance regression model checked by F-test and the analysis of variance (ANOVA) demonstrated that the model was highly significant. The determination coefficient (R^2) implied that the sample variation of 97.9% for IXH enzyme production was attributed to the independent variables. This suggested a satisfactory representation of the process model. Also, there was a good correlation between the experimental and predicted values. The results (Table 5) also suggested that, among the independent variables, KH_2PO_4 and Tween 80 did not play significantly role in the enzyme formation, whereas X_1 (corncob) and X_2 (yeast extract) had a significant effect on production of IXH activity. The quadric term of these two variables also had a significant effect. However, no interactions between the two variables were found to contribute to the response at a significant level. Purkarthofer et al. (1993) also studied the effect of various organic nitrogen compounds at 30 g/l (yeast extract, casein peptone, meat extract, fish peptone, fish

peptone with 30% yeast blend, soya meal and cottonseed) on the formation of xylanase by *T. lanuginosus* DSM5826 under submerged growth conditions in shake flasks using basal medium together with corncobs or xylan (25 g/l). Yeast extract showed the best nitrogen source for xylanase production. However, Hog et al. (1994) demonstrated that a combination of yeast extract and $(NH_4)_2HPO_4$ supported the best biomass growth, extracellular protein and xylanase production by *T. lanuginosus* RT 9.

By applying multiple regression analysis on the experimental data, the following second order polynomial equation (Equation 1) was used to explain the enzyme production:

$$Y = -220.402 + 28.281X_1 + 14.778X_2 - 0.343X_1^2 - 0.31X_2^2 \quad (1)$$

Where, Y is the predicted enzyme activity, X_1 is the corncob concentration and X_2 is the yeast extract concentration.

The contour and three-dimensional plots of interactions among the variables showed an increase in IXH production as the concentration of corncob and yeast extract increased up to 41 and 24 gl^{-1}, respectively (Figure 2). The model was validated by repeating the experiment under the optimized condition. The result suggested that the maximum experimental response IXH enzyme production after 5 days culture at the temperature of 50°C was 541 Uml^{-1}, whereas the predicted value was 526.7 Uml^{-1}. Purkarthofer et al. (1993) employed a central composite design to optimize the fermentation medium with respect to medium components for xylnase production by the strain of *T. lanuginosu* DSM5826. The medium for the optimal production (31.2 gl^{-1}, corn cobs, 30.2 g l^{-1}yeast extract and 5.0 g $l^{-1}KH_2PO_4$), yielded 36,200 nkat ml^{-1} within 7 days of cultivation. Trace elements did not show important role in the enzyme production. On the other hand, Damaso et al. (2000) used 2^{4-1} fractional factorial design (FFD) with two values of each factor to attain a low cost medium for xylanase production by *T. lanuginosus* IOC-4145. The results indicated that corncob was shown to be the most important factor that affects the enzyme production. In addition, peptone and meat extract used in the media did not have any significant effect on xylanase production, while NaCl presents a moderate effect. Slight stimulation of xylanase production (5 to 15%) by *T. lanuginosus* IOC-4145 was achieved with NaCl and urea, both at 3 and 5 mM concentration, respectively. The results of 2^{4-1} FFD indicated that the medium having 30 and 10 gl^{-1} corn cobs and NaCl, respectively, had a high influence on xylanase production of 248 U/ml. In addition, Su et al. (2010) employed Plackett–Burman design to evaluate the effects of nine variables (powdered corncobs, soybean meal, Tween-80, $CaCl_2$, $MgSO_4 \cdot 7H_2O$, $FeSO_4$, KH_2PO_4, initial pH and inoculum culture volume) on the production of a thermostable xylanase from *T. lanuginosus* SDYKY-1. The results showed that corncobs, soybean meal and $FeSO_4$ significantly influenced the xylanase production. The concentrations of these three factors were therefore optimized using central composite design and RSM. Adjusting the concentration of corncobs to 38.7 gl^{-1}, soybean meal to 17.5 gl^{-1} and $FeSO_4$ to 0.26 gl^{-1}, favored maximum xylanase production. Xylanase activity of 3078 Uml^{-1} was obtained after optimization, which was a 144% increase that was obtained before optimization (1264 U ml^{-1}). Our findings are similar to the report of Su et al. (2010) that Tween-80 and KH_2PO_4 did not play significant role in the enzyme formation.

Conclusion

Selection of *T. lanuginosus* strains which produced high level of insoluble xylan hydrolyzing enzyme in the liquid medium using corncob as raw material was investigated from the forty-two strains isolated from different sources in Thailand. It was found that *T. lanuginosus* THKU-56 produced high IXH xylanase activity (317 U/ml) which was stable at low pH and high temperature, which is suitable for practical application in animal feed. In addition, all the collected strains of *T. lanuginosus* were divided into 4 groups based on their ability to produce soluble xylan hydrolyzing and insoluble xylan hydrolyzing enzyme activities. Strains exhibiting high insoluble xylan IXH activity also showed high binding ability on insoluble xylan. CCD was found to be a good tool for optimizing the production of enzyme. The second order polynomial regression model was in agreement with the experimental result of β–xylanase having R^2 of 0.979 ($P < 0.05$). The optimal concentration of the active nutrients for xylanase production was 41 gl^{-1} of corncobs and 24 gl^{-1} of yeast extract. The medium consisting of 41 gl^{-1} corncobs, 24 gl^{-1} yeast extract, 5 gl^{-1} KH_2PO_4 and 0.3 ml l^{-1} Tween 80 gave a predicted yield of 526.7 Uml^{-1} in 5 days culture. The xylanase activity obtained from the experiment was 541 Uml^{-1} which was close to the predicted value. This is the first report on the production of insoluble xylan degrading xylanase by *T. lanuginosus.*

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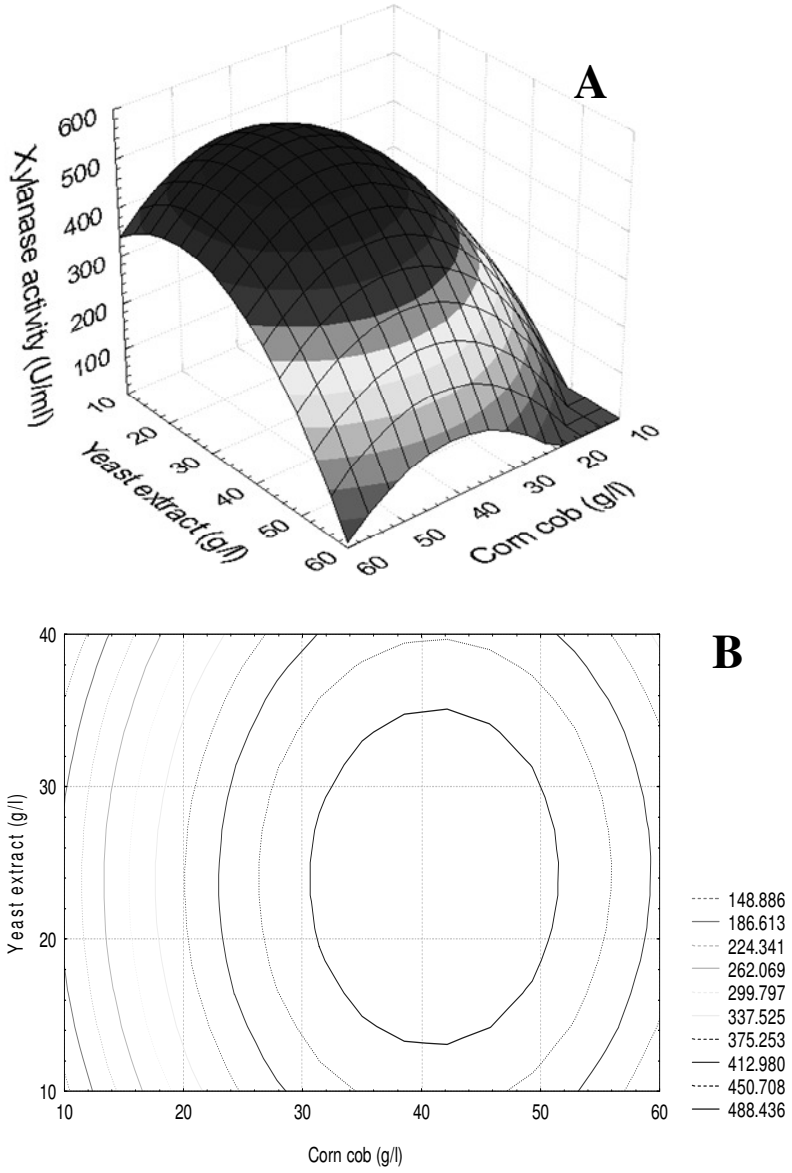

Figure 2. Response surface analysis (A) and contour plot (B) of factors affecting IXH activity by *T. lanuginosus* THKU 56.

REFERENCES

Beg OK, Kapoor M, Mahajan L, Hoondai GS (2001). Microbial xylanases and their industrial applications: a review. Appl. Microbiol. Biotechnol. 56: 326-338.

Butt MS, Tahir-Nadeem M, Ahmad Z, Sultan M (2008). Xylanases and Their Applications in Baking Industry. Food Technol. Biotechnol. 46(1): 22-31.

Damaso MCT, Andrade CMMC, Pereira Jr. N (2000). Production and properties of the cellulase-free xylanase from *Thermomyces lanuginosus* IOC-4145. Braz. J. Microbiol.33: 333-338.

Ghangas GS, Hu YJ, Wilson OB (1989). Cloning of a *Thermomonospora fusca* xylanase gene and its expression in *Escherichia coli* and *Streptomyces lividans*. J. Bacteriol. 171: 2963-2969.

Gomes J, Gomes I, Kreiner W, Esterbauer H, Sinner M, Steiner W (1993a). Production of high level of cellulase-free and thermostable xylanase by a wild strain of *Thermomyces lanuginosus* using beechwood xylan. J. Biotechnol. 30: 283-297.

Gomes J, Purkarthofer H, Hayn M, Kapplmüller J, Sinner M, Steiner W (1993b). Production of a high level of cellulase-free xylanase by the thermophilic fungus *Thermomyces lanuginosus* in laboratory and pilot scales using lignocellulosic materials. Appl. Microbiol. Biotechnol. 39: 700-707.

Hoq MM, Deckwer WD (1995). Cellulase-free xylanase by thermophilic fungi: A comparison of xylanase production by two *Thermomyces lanuginosus* strains. Appl. Microbiol. Biotechnol. 43: 604-609.

Irwin D, Jung ED, Wilson DB (1994). Characterization and sequence of *Thermomonospora fusca* xylanase. Appl. Environ. Microbiol. 60: 763-770.

Jiang ZQ, Yang SQ, Tan SS, Li L, Li XT (2005). Characterization of a xylanase from the newly isolated thermophilic *Thermomyces lanuginosus* CAU44 and its application in bread making. Lett. Appl. Microbiol. 41: 69-76.

Khucharoenphaisan K, Kitpreechavanich V (2004) High thermostable xylanase producing *Thermomyces lanuginosus* isolated from Thailand. In: Proceeding of the 1st KMITL International Conference on Integration of Science & Technology for Sustainable Development, Bangkok, Thailand, pp. 447-451.

Kumar KS, Manimaran A, Permaul K, Singh S (2009). Production of β-xylanase by a *Thermomyces lanuginosus* MC 134 mutant on corn cobs and its application in biobleaching of bagasse pulp. J. Biosci. Bioeng. 107: 494-498.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31: 426-428.

Nath D, Rao M (2001). pH dependent conformational and structural changes of xylanase from an alkalophilic thermophilic *Bacillus* sp. (NCIM 59). Enzyme Microb. Technol. 28(4): 397-403.

Ponyi T, Szabo L, Nagy T, Orosz L, Simson PJ, Williamson MP, Gilbert HJ. (2000). Trp22 Trp24 and Tyr8 play a pivotal role in the binding of family 10 cellulose-binding module from *Pseudomonas* xylanase A to insoluble ligands. Biochem. 39: 985-991.

Purkarthofer H, Sinner M, Steiner W (1993). Cellulase-free xylanase from *Thermomyces lanuginosus*: Optimization of production in submerged and solid state culture. Enzyme Microb.Technol. 15: 677-682.

Singh S, Madlala AM, Prior BA (2003). *Thermomyces lanuginosus*: properties of strains and their hemicellulases. FEMS Microbiol. Rev. 765: 1-14.

Singh S, Pillay B, Dilsook V, Prior BA (2000a). Production and properties of hemicellulases by a *Thermomyces lanuginosus* strain. J. Appl. Microbiol. 88: 975-982.

Singh S, Reddy P, Haarhoff J, Biely P, Janse B, Pillay B, Pillay D, Prior BA (2000b). Relatedness of *Thermomices lanuginosus* strains producing a thermostable xylanase. J. Biotechnol. 8: 119-128.

Su Y, Zhang X, Hou Z, Zhu X, Guo X, Ling P (2010). Improvement of xylanase production by thermophilic fungus *Thermomyces lanuginosus* SDYKY-1 using response surface methodology. New Biotechnol. doi:10.1016/j.nbt.2010.06.002.

Tormo J, Lamed R, Chirino AJ, Morag E, Bayer AE, Shoham Y, Steitz TA (1996). Crystal structure of bacterial family-III cellulose-binding domain: a general mechanism for attachment to cellulose. EMBO. J. 15: 5739-5751.