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Production, optimization growth conditions and properties of the xylanase from *Aspergillus carneus* M34

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Abstract

Growth conditions, including incubation times, temperature, agitation rate and initial pH of medium, that affect xylanase production by *Aspergillus carneus* M34 were studied sequentially use the classical "change-one-factor-at-a-time" method. Our results showed that there was a similar trend between cellular xylanase activity and extracellular xylanase activity. The optimal conditions for xylanase production, different from their cell growth, were on the third day, 30 °C, 100 rpm and pH 4, respectively, in this test. Response surface methodology (RSM) was further introduced to optimize the cultivation conditions and to evaluate the significance of these factors. The optimal cultivation conditions predicted from canonical analysis of this model were achieved by incubation at 35.08 °C with an agitation rate of 111.9 rpm and an initial pH of 5.16. In addition, temperature was the most critical factor for xylanase production by *A. carneus* M34. Xylanase activity of 22.2 U/mL was verified using the predicted optimal conditions and confirmed the fitness and applicability of the model. The optimal temperature and pH of the crude xylanase activity was observed at 60 °C and acidic pH, respectively. Sustained xylanase activity in the crude extract was also detected over a broad range of pH from 3 to 10. Considering its higher specificity toward agricultural wastes, especially corn cob and coba husk, this strain can be used to develop low-cost media for the mass-production of xylanase.

Keywords: *Aspergillus carneus* M34; Xylanase; Response surface methodology (RSM); Agricultural wastes

1. Introduction

Hemicellulose is an important biomass reservoir in the plant cell wall [1]. Agricultural wastes that contain hemicellulose were globally generated. With reference to biomass regeneration, hemicellulose degradation has been intensively studied in the last decade. Xylan, the principle component of hemicellulose, is comprised of β-1,4-linked D-xylopyranose residues with different substitute groups in the side chain. Xylanases (E.C.3, 2, 1, 8) that cleavage the backbone and initiate the depolymerization of xylan have received the most attention, mainly because of their potential use in pulp bleaching, clarifying fruit juices and wines, improving the nutritional value of animal feedstuff, and preparing xylooligosaccharides [2,3]. However, particular requirements must be satisfied in various fields of application; for example, cellulase-free, thermal- and alkaline-tolerant xylanase is required for pulp bleaching [4]. Hence, unique xylanase-producing strains must be screened continually for adaptations that make them useful in various fields.

Many microorganisms including bacteria, yeast, actinomycetes and filamentous fungi produce xylanase [5]. Filamentous fungi such as *Aspergillus* spp. and *Trichoderma* spp. are of particular interest, because they can excrete higher levels of xylanase than yeast and bacteria [6]. A novel strain of *Aspergillus carneus* M34, which was identified by the Bioresources Collection and Research Center (BCRC), is a phytase- and xylanase-producing strain, which was isolated by our lab [7]. *A. carneus* has been reported to be capable of secreting lipase that has industrially important characteristics, such as pH and thermal tolerance and stability, and excellent chemo- and regiospecificity in aqueous and non-aqueous media [8], raising the possibility that *A. carneus* M34 may be a potential source of xylanase.

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This study was aimed at evaluating the growth conditions that affect the production of xylanase by this novel strain and optimizing their cultivation conditions for xylanase production. It has been reported that xylan and xylan-containing residues can serve as inducers for promoting xylanase production [9]. In addition, several factors including pH, temperature, dissolved oxygen concentration, and shear stress can influence the production of fungal xylanases [10]. Thus, we used xylan-containing medium to evaluate the growth factors that mediate xylanase production by *A. carneus* M34. Furthermore, the response surface methodology (RSM) was adopted to analyze the significance of these factors and to optimize the growth conditions. The properties of the crude xylanase were also studied to identify its potential applications.

2. Materials and methods

2.1. Microorganism and culture media

A. carneus M34 was grown on potato dextrose (PD) agar. After 7–10 days of cultivation, the brown spores were scraped off and placed into sterilized water that contained 0.01% Triton X-100. Aliquots (1 mL) of a suspension containing 1×10^6 spores/mL were individually added to a 250 mL Erlenmeyer flask with a baffle that contained 50 mL of culture medium. The medium contained oat-spelt xylan, 5.0 g/L; peptone, 3.0 g/L; KH_2PO_4, 2.0 g/L; $CaCl_2$, 0.3 g/L; Tween 80, 2.0 g/L; $MgSO_4{\cdot}7H_2O$, 0.3 g/L; $FeSO_4{\cdot}7H_2O$, 0.005 g/L; $ZnSO_4{\cdot}7H_2O$, 0.0014 g/L; $MnSO_4{\cdot}H_2O$, 0.0016 g/L and $CoCl_2$, 0.002 g/L. The medium was sterilized at 121 °C, 15 lb/in^2 for 20 min; and the pH of the medium was then adjusted to 6.0 prior to inoculation with the organism [11].

2.2. Experimental design

The classical "change-one-factor-at-a-time" approach was employed to evaluate the effects of growth conditions, including incubation time, temperature, agitation rate and initial pH of the medium, on cell growth and xylanase production of *A. carneus* M34. The initial culture conditions were set at 30 °C, and pH 6 with an agitation rate of 130 rpm as recommended by Mandel and Reese [11]. The optimum conditions found to produce the maximum concentration of xylanase, as established by the preceding test, were then utilized in the following test. In the time-course study, cultures incubated under the conditions described above were harvested at 1, 3, 5, 7 and 9 days. Organisms grown in media with an initial pH of 6 were cultured at 20, 30, 40, 50 or 60 °C at an agitation rate of 130 rpm for 3 days to evaluate the effect of incubation temperature on xylanase production. In the agitation rate study, media with an initial pH of 6 were inoculated and cultivated at 30 °C for 3 days using agitation rates of 70, 100, 130, 160 or 190 rpm. To ascertain the influence of pH, the initial pH was adjusted to 2, 4, 6, 8 or 10 by the addition of 1 M NaOH or 2 M HCl to the sterile medium prior to inoculation. Following inoculation, cultures were incubated at 30 °C and 100 rpm for 3 days. Each experiment was performed three times.

Table 1
Values for the independent variables used in the three-level design with actual factor levels that correspond to coded factor levels

Independent variables X_i	Levels[a]		
	−1	0	1
X_1 agitation rate (rpm)	70	100	130
X_2 temperature (°C)	20	30	40
X_3 initial pH of medium	2	4	6

[a] Code level based on preliminary investigations.

2.3. Response surface methodology (RSM)

The optimal conditions were determined and the interaction of the environmental variables evaluated using a three-level design model in an RSM study. Three independent variables (agitation rate, temperature and initial pH) were selected. The boundary limits of each variable were determined from the preliminary results of the aforementioned single factor tests. Table 1 presents the actual levels, corresponding to the codes, of the process variables. Fifteen sets of treatment combinations were employed (Table 2). Data from the 15 sets were analyzed using SAS software (Version 8.0; SAS Institute, Cary, NC) to yield regression equations and regression coefficients (R^2). The significance of the regression coefficients was tested by a *t*-test. A second-order polynomial regression model was generated from the data according to the following equation:

$$Y = a_0 + a_1x_1 + a_2x_2 + a_3x_3 + a_{11}x_1^2 + a_{22}x_2^2 + a_{33}x_3^2 + a_{12}x_1x_2 + a_{13}x_1x_3 + a_{23}x_2x_3$$

where Y = predicted response (xylanase production, U/mL), x_i = variables, and a_i = model coefficient parameters.

2.4. Sample preparation

Xylanase was harvested by centrifuging each of the cultures at 10,000 × *g* for 20 min at 4 °C. The supernatant referred to as the extracellular protein faction was subjected to xylanase activity assay. The pellet was then washed three times in 0.2 M citrate acetate buffer (pH 4.8) and centrifuged at 10,000 × *g* for 20 min. The final pellet was homogenized with liquid nitrogen and sea sand using a mortar and pestle. Cell debris was redissolved in the same buffer and centrifuged at 10,000 × *g* for 20 min at 4 °C. The volume of the supernatant referred to as the cellular protein fraction was then adjusted to 10 mL and subjected to xylanase activity assay and protein assay as described subsequently.

2.5. Enzyme activity and protein assay

Xylanase activity was determined by measuring the concentration of reducing sugars released after incubation of 0.5 mL of authentic dilute enzyme solution with 0.5 mL 1% Birchwood xylan (in 0.2 M citrate acetate buffer, pH 4.8) at 40 °C for 30 min. Reducing sugars were determined using 3,5-dinitrosalicylic acid (DNS) as detailed elsewhere [12]. One unit (U) of enzyme activ-

Table 2
Experimental design and results of the three-level design for xylanase production

Run	X_1	X_2	X_3	Xylanase activity (U/mL)[a]
1	1	0	1	12.60 ± 0.44
2	1	1	0	15.21 ± 0.28
3	1	−1	0	0.60 ± 0.03
4	1	0	−1	11.10 ± 0.96
5	0	1	1	20.51 ± 0.41
6	0	−1	1	2.70 ± 0.34
7	0	1	−1	0.33 ± 0.01
8	0	−1	−1	0.03 ± 0.00
9	−1	0	1	10.12 ± 0.29
10	−1	1	0	7.66 ± 0.49
11	−1	−1	0	6.89 ± 0.31
12	−1	0	−1	8.44 ± 0.38
13	0	0	0	18.76 ± 0.04
14	0	0	0	18.66 ± 0.42
15	0	0	0	18.53 ± 0.08

[a] Each datum represents the mean ± S.D. from three independent experiments.

ity was defined as the production of 1 μmole of reducing sugar per minute under the assay conditions.

Protein concentration was determined using a BCA protein assay kit (Sigma Chemical Co., St. Louis, MO) with bovine serum albumin (BSA) as the standard. The assay was performed according to the manufacturer's instructions. Since xylan was insoluble in the medium, the amount of protein in the cellular fraction served as the indicator of cell growth.

2.6. Characterization of crude xylanase

The optimal temperature for crude xylanase activity was determined by performing the standard assay in 0.2 M citrate acetate buffer (pH 4.8) at 10 °C intervals from 10 to 100 °C. The optimal pH was evaluated by assaying the activity at 40 °C with 1% substrate that was dissolved in 20 mM glycine–HCl (pH 3), citric acid–trisodium phosphate (pH 4–5), phosphate (pH 6–8), glycine-NaOH (pH 9–10) and disodium phosphate (pH 11–12) buffers [13].

In a thermal inactivation test, the crude xylanase solution was incubated at 50, 60 and 70 °C and withdrawn at different times. After cooling, the residual enzyme activity was determined as described above.

In a pH stability test, the crude xylanase solution was placed in the aforementioned pH variant buffers and incubated at room temperature for 24 h. The residual enzyme activity was assayed as described above.

2.7. Substrate specificity to agricultural wastes

Twelve types of agricultural waste which are common in Taiwan – rice straw, rice bran, wheat bran, corn cob, coba husk, sugarcane bagasse, boiled tea leaves, peanut shell, peanut film, coconut shell, orange peel and banana peel – were collected from a local farm and a traditional market. Hemicellulose was prepared according to the method of Chen and Anderson [14]. One per cent of commercial xylan (Birchwood xylan, Beechwood xylan and Oat-spelt xylan purchased from Sigma Chemical Co., St. Louis, MO) and various prepared hemicelluloses served as substrates for the enzyme assays under the standard assay conditions.

3. Results

3.1. Xylanase production

When *A. carneus* M34 was grown for 1–9 days at 30 °C, 130 rpm in 50 mL of a xylan-containing medium at an initial pH of 6, the extracellular xylanase activity reached a maximum on day 3. As cultivation continued, total cellular protein (indicative of cell growth) reached a maximum on day 5. Cellular xylanase activity followed a similar trend to that observed for extracellular xylanase activity. The related xylanase-specific activity of extracellular xylanase rapidly increased between days 1 and 3, and reached a plateau on day 5. Since xylanase is the key enzyme in the degradation of xylan (carbon source), a process necessary for cell survival, the trend in xylanase production reveals that its production by *A. carneus* M34 increased in the early logarithmic phase of cell growth (Fig. 1A).

A. carneus M34 was able to grow in temperatures ranging from 20 to 60 °C under agitation conditions of 130 rpm, initial pH 6 and a culture period of 3 days. The optimal growth and xylanase-producing temperatures were both 30 °C, indicating that *A. carneus* M34 is a mesophylic strain. However, higher xylanase specific activity but lower extracellular xylanase activity was observed at 40 °C but not at 30 °C (Fig. 1B).

Agitation can increase the levels of dissolved oxygen in the medium leading to good mass and heat transfer, all of which enhance the rate of cell growth [15]. The formation of mycelia pellets was observed and their diameters became gradually reduced, until a suspension was formed with increasing agitation speed. *Aspergillus carneus* M34 growth was enhanced as the agitation rate was increased to 130 rpm under cultivation conditions of 30 °C and an initial pH of 6 for a period of 3 days. However, the optimal agitation rate for extracellular xylanase activity was 100 rpm rather than 130 rpm and the amount of extracellular

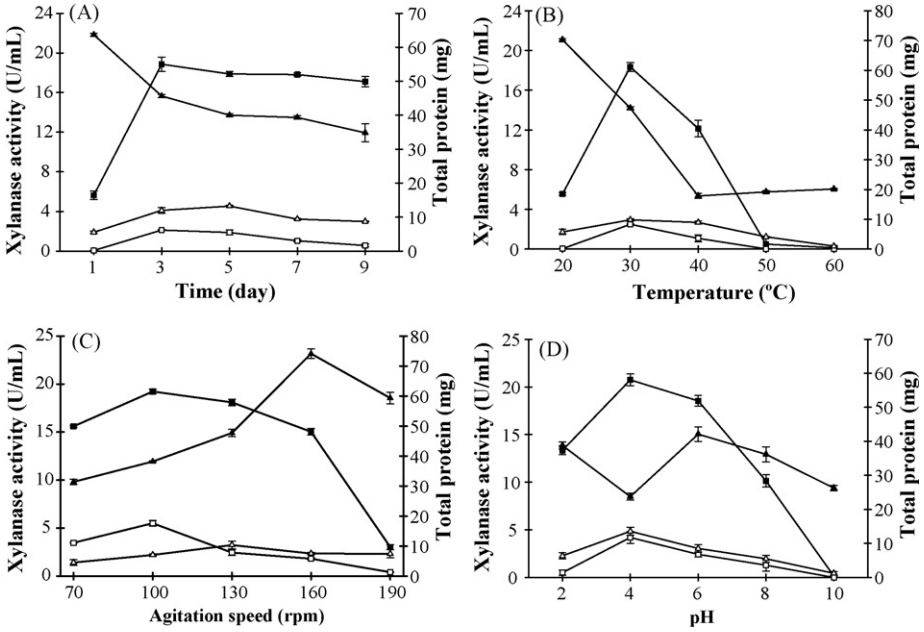

Fig. 1. Growth conditions affecting the xylanase production of *A. carneus* M34. Time course study (A), temperature study (B), agitation rate study (C) and pH study (D). (■) Extracellular xylanase activity; (□) cellular xylanase activity; (▲) extracellular total protein; (△) total cellular protein. All culture conditions used are described in Section 2.2.

protein apparently increased with the agitation speed (Fig. 1C).

The pH test was conducted at 30 °C and 100 rpm during 3 days of cultivation. Xylanase production by *A. carneus* M34 was favored by acidic conditions, but markedly declined when the initial pH of the growth medium exceeded 6. Both xylanase production and cell growth were optimal at pH 4 (Fig. 1D).

Based on the above results, all of the aforementioned factors may be important in influencing the xylanase production of *A. carneus* M34. To analyze the significance of these multiple factors in xylanase production and to optimize the cultivation conditions, response surface methodology (RSM) was employed.

3.2. Response surface methodology (RSM)

RSM has been broadly applied in the search for the optimum conditions for multivariable systems and in the analysis of the interaction of variables [16,17]. The highest xylanase production by *A. carneus* M34 occurred on day 3 and the production rate exceeded that of most other xylanase-producing species [10]. Thus the benefit factor was fixed at day 3, and agitation rate (X_1), temperature (X_2) and pH (X_3) were chosen as variables in a three-level design assay (Table 1).

From the response surface regression (RSREG) data, a second-order polynomial equation was obtained:

$$Y\,(\mathrm{U/ml}) = 18.649 + 0.799X_1 + 4.187X_2 + 3.254X_3 - 3.192X_1^2 - 7.868X_2^2 - 4.891X_3^2 + 3.359X_1X_2 - 0.046X_1X_3 + 4.375X_2X_3$$

The determination coefficient ($R^2 = 0.93$) implies that the sample variation of 93% for xylanase production is attributable to the independent variables, and only about 7% of the total variation cannot be explained by the model. Significance of coefficients has been reported to be directly proportional to the *t*-value [18]. Positive coefficients for temperature (X_2) and initial pH (X_3) indicated a linear effect in the increase in xylanase production. The analysis of the quadratic effect indicated that all variables significantly contributed to this effect. With regard to the cross-product effects, the model clearly revealed that the most significant interaction was between temperature (X_2) and initial pH (X_3), and then agitation speed (X_1) and temperature (X_2) (Table 3). Results of the total analysis demonstrated that all the factors considered herein positively affected the production of xylanase by *A. carneus* M34, but the effect of temperature was the most predominant. The graphical representation provides a method for visualizing the relationship between the response and the interactions among test variables in order to determine the optimum conditions. The contour plot of Fig. 2A shows the effect of temperature and agitation speed on xylanase produc-

Table 3
Regression coefficients and *t*-values for full second-order polynomial equation for extracellular xylanase production

Parameters	Coefficient[a]	*t*-Value
Intercept	18.649	26.33
X_1	0.799	1.84
X_2	4.187	9.65[b]
X_3	3.254	7.50[b]
$X_1 \times X_1$	−3.192	−5.00[b]
$X_1 \times X_2$	3.359	5.64[b]
$X_2 \times X_2$	−7.868	−12.32[b]
$X_1 \times X_3$	−0.046	−0.07
$X_2 \times X_3$	4.375	7.13[b]
$X_3 \times X_3$	−4.891	−7.66[b]

[a] Coefficient of determination $R^2 = 0.93$.
[b] Means are significantly different at $\alpha < 0.05$.

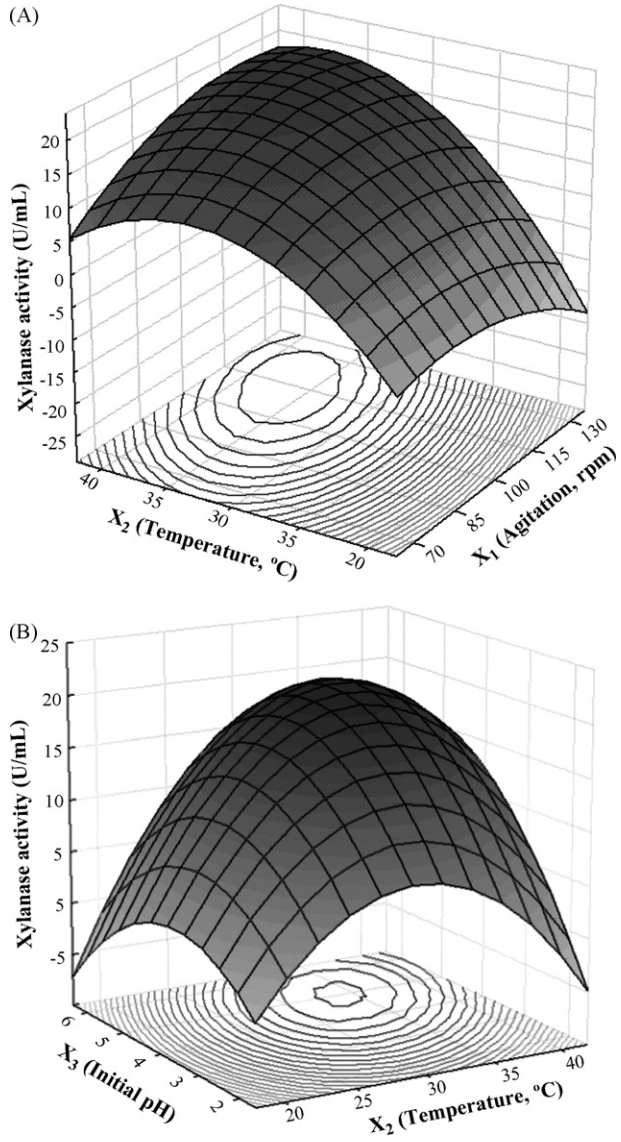

Fig. 2. Response surface and contour plot of the combined effects of (A) agitation rate and temperature and (B) temperature and initial pH on xylanase production by *Aspergillus carneus* M34.

tion at a pH of 6. It can be seen that when the temperature was below 30 °C, the effect of agitation speed on xylanase production was not significant. At higher temperatures (32–37 °C), the yield of xylanase increased gradually with the increase in agitation speed, but decreased at speeds beyond the optimal agitation range (100–125 rpm). The effect of temperature and initial pH on xylanase production at an agitation speed of 100 rpm is shown in Fig. 2B. Fig. 2B shows that increasing the initial pH and temperature within the range tested improved xylanase production and that maximum xylanase production is achieved at an initial pH in the range of 4.5–5.5 and a temperature in the range of 33–36 °C.

3.3. Characteristics of crude xylanase and substrate specificity

The optimal temperature for crude xylanase activity was 60 °C. This result agrees with the fact that the optimum temperature for the xylanase produced by most fungi is in the range of 40–60 °C [19]. Thermal inactivation yields half-life values (T_{50}) of 101.2, 5.7 and 1.5 min at 50, 60 and 70 °C, respectively (Fig. 3). The short T_{50} at the temperature of optimal xylanase activity (60 °C) can be attributed to the protection of insoluble substrate [20]. The optimal pH range for production of xylanases was acidic (Fig. 4), and therefore similar to the range observed for production of xylanases by *Aspergillus kawachii* and *Penicillium herque* [21,22]. The results are also in accord

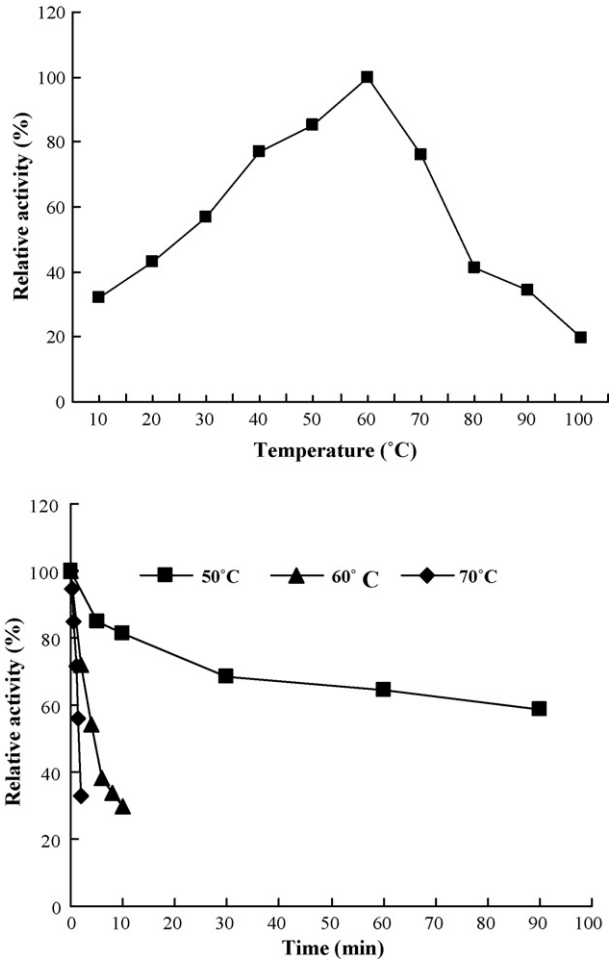

Fig. 3. Optimal temperature test (A) and thermal inactivation test (B) applied to extracellular crude xylanase produced by *A. carneus* M34.

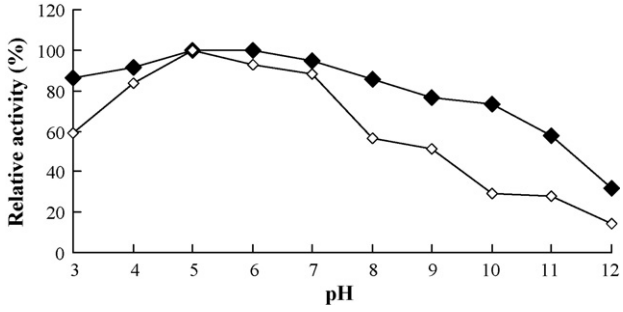

Fig. 4. pH stability (♦) and optimal pH (◊) of extracellular crude xylanase produced by *A. carneus* M34. Buffers of various pH values were used as described in Section 2.

Table 4
Substrate specificity of crude enzyme for various agricultural wastes

Samples	Hemicellulose contents (%)	Reducing sugar produced (mg/min × mg protein)
Beechwood xylan		2.40 ± 0.07
Birchwood xylan		2.15 ± 0.12
Oat-spelt xylan		1.71 ± 0.05
Rice straw	17.30 ± 0.71	2.08 ± 0.05
Rice bran	7.55 ± 0.49	0.17 ± 0.01
Wheat bran	9.60 ± 1.98	1.41 ± 0.04
Corn cob	16.20 ± 0.57	2.60 ± 0.07
Coba husk	21.97 ± 2.42	2.77 ± 0.04
Sugarcane bagasse	16.35 ± 0.49	2.02 ± 0.04
Boiled tea leaves	12.55 ± 0.42	0.18 ± 0.01
Peanut shell	5.10 ± 0.28	1.43 ± 0.05
Peanut film	20.05 ± 0.78	0.25 ± 0.05
Coconut shell	8.25 ± 0.35	0.23 ± 0.03
Orange peel	6.95 ± 0.35	0.38 ± 0.02
Banana peel	16.40 ± 0.42	0.25 ± 0.02

Each datum represents the mean ± S.D. from three independent experiments.

with those indicating that most xylanases of fungal origin are active under acidic conditions [19]. However, the pH tolerance of crude xylanase from *A. carneus* M34 was stable in the range of 3–10 (>70% relative activity). This result substantiates the finding that most crude xylanases of fungal origin are stable over a broad pH range [19]. We suggest that this observation is attributable to other extracellular proteins which are associated with xylanase and which protect it from denaturation by the effects of pH. This characteristic makes this enzyme potentially useful in a broad range of industrial applications. The hemicellulose content of the various agricultural wastes used varied from 5 to ~22%. Substrate specificity tests revealed that this xylanase showed greater specificity for hemicelluloses of coba husk and corn cob than for those of commercial xylan (Table 4).

4. Discussion

This work is the first to investigate the production of xylanase by *Aspergillus carneus*. The xylanase that is produced by this strain is similar to most xylanases of fungal origin; these enzymes are the major extracellular product of these organisms. Extracellular xylanase activity was found to be more than five times higher than cellular xylanase activity at every stage. The activity of cellular xylanase follows a trend similar to that observed for extracellular xylanase, suggesting that xylanase is secreted immediately following its production. Both xylanase production and cell growth were optimized at 30 °C. However, related xylanase-specific activity at 40 °C exceeded that at 30 °C. Thermostable *Aspergillus* FP-470 cultured at 37 and 45 °C also exhibited higher specific activity at higher temperatures [23]. The higher specific activity at higher temperature may involve cellular adjustments to mechanisms such as the membrane lipid composition, protein synthesis and secretory machinery which occur under thermal stress, as suggested by Rizzatti et al. [24]. In the agitation speed study, a dramatic increase in extracellular protein was observed along with a corresponding decline in xylanase activity and cellular growth as the agitation rate increased above 130 rpm, perhaps because the higher sheering force causes the cells to lyse and release proteases which inhibit xylanase production. Relative xylanase specific activities at agitation speeds of 70 and 100 rpm were observed to be higher than those observed at 130 rpm which produces optimum growth, revealing that the prevalence of xylanase in the extracellular proteins increased as the agitation speed decreased. Xylanase is an inducible enzyme and we suggest that it may be involved in the accessibility of the inducer for enzyme induction. However, this possibility should be further examined.

The response surface methodology (RSM) provided a feasible tool for screening significant factors in, and optimal conditions for, xylanase production in this work. From the model used in this study it was determined that the temperature and initial pH of the medium were the most influential factors in xylanase production. Both of these factors also played significant roles in xylanase production by *Penicillium oxalicum* ZH-30 under submerged fermentation [25]. Temperature, examined by the RSM method as described here, was the factor that most strongly affected xylanase production. Moreover, the observation that the characteristics and chromatographic profiles of xylanases varied with the cultivation temperature has also been reported by other authors [26]. Thus, temperature can be a critical factor that affects xylanase production and also manipulates the expression of various biochemical properties of xylanases. The optimal production of xylanase is achieved by using temperatures in the range of 32–37 °C, agitation rates between 100 and 125 rpm, and a pH in the range of 4.5–5.5 as shown in Fig. 2. These observations were also verified by canonical analysis of response surface. Canonical analysis reveals that the optimum values for xylanase production were $X_1 = 0.3967$, $X_2 = 0.5085$ and $X_3 = 0.5582$. These correspond to an actual agitation rate of 111.9 rpm, a temperature of 35.08 °C, and an initial pH of 5.16, respectively. The maximum activity of xylanase predicted by this model is 20.78 U/mL. To verify the optimal conditions, an experiment was carried out at 35 °C, an initial pH of 5.2, and an agitation rate of 112 rpm with harvest of the product, xylanase, being carried out on day 3. The experimental results revealed a maximum yield of xylanase activity of 22.2 U/mL, superior to any optimum point in the single factor experiments, confirming that the model was in accord with the experimental results and demonstrating the applicability of this model. This result represents an improvement of about 20% over the single variable optimization of culture conditions. Comparison to xylanases from fungi produced in submerged culture with oat-spelt xylan as the carbon source, demonstrated that the enzyme activity found in this study was lower than that of *Thielavia terrestris* (36 U/mL) but higher than that of *Aspergillus nidulans* CECT 2544 (9.75 U/mL), *Aspergillus* sp. PK-7 (10.6 U/mL), *Crptococcus albidius* (4.13 U/mL), *Phanerochaete chrysosporium* (19.6 U/mL) and *Termitomyces clypeatus* (20 U/mL) [10]. This result shows that this strain is also an excellent xylanase producer.

Developing a low-cost method for xylanase production is valuable since xylanase has a wide range of potential uses as stated earlier. According to Saha [27], the conversion of hemicellulose to value-added useful products by enzymatic

and/or fermentation routes holds considerable promise for converting discarded and underutilized agricultural residues to useable products. From substrate specificity tests, this particular xylanase exhibited greater specificity for the agricultural waste produced from coba husk and corn cob than for that produced from commercial xylan. This fact suggests that *Asp. carneus* M34 can potentially be used for the mass-production of xylanase or the preparation of useful products, such as xylooligosaccharide, by using a low-cost substrate such as coba husk or corn cob.

5. Conclusions

This investigation has clearly established the effect of growth conditions on the production of xylanase by *Aspergillus carneus* M34. Following RSM optimization, an approximately 1.2-fold increase (22.2 U/mL) in the enzyme activity was obtained. Temperature was the most significant factor to have a major effect on the production of xylanase by this strain. Based on our results, it was found that crude xylanase had a higher pH tolerance over a broad range of pH values and showed specificity for various agricultural wastes, especially that resulting from the use of coba husk. Considering the optimum conditions for xylanase production and specific substrate of the xylanase produced, this strain of aspergillus has a potential role in the development of a bioprocess for the mass-production of xylanase using low-cost media.

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