Optimization of Nutrition Constituents for Xylanase Activity by *Rhizopus stolonifer* Under Solid-State Fermentation on Corncob

Zhicai Zhang,[a,c,] *, Jiashao Li,[a] Fan Feng,[b] Dan Liu,[a] Qiaoxia Pang,[a] Ming Li,[a] and Keping Chen [b]

This study aims to optimize the medium for xylanase activity by a newly isolated strain of *Rhizopus stolonifer* JS-1008 (*R. stolonifer* JS-1008) under solid-state fermentation (SSF) on corncob. Four quantitative variables impacting the enzyme activity were selected through one-factor-at-a-time design. They were nitrogen source, initial moisture content (IMC), inorganic salt, and surface active agent. In addition, the interaction among these factors was further investigated by response surface methodology (RSM). Statistical analysis revealed that among these factors, IMC and urea significantly affected the xylanase activity. Our data indicate that the optimal medium contains (g/g dry corncob): urea, 0.15; $ZnSO_4$, 0.022; Tween-80, 0.08; IMC, 3. Under the optimal condition, the xylanase activity reached its maximum, 13.90 U/g dry substrate (DS), on the 10^{th} day of fermentation. This work provides a new potential strain to synthesize xylanase for biofuel production.

Keywords: Xylanase; Rhizopus stolonifer; Solid-state fermentation; Corncob

Contact information: a: School of Food and Biological Engineering, Jiangsu University, Zhenjiang 212013, P. R. China; b: Institute of Life Sciences, Jiangsu University, Zhenjiang 212013, P. R. China; c: Beijing Green Technology and Natural Biotechnology Co. Ltd., Beijing 102300, P. R. China;
** Corresponding author: zhangzhicai2001@yahoo.com.cn*

INTRODUCTION

Xylan is the main hemicellulosic polysaccharide of plant cell walls and is composed of a backbone chain of beta-1,4-linked xylosyl residues. Hemicelluloses comprise up to 20-35% dry weight of wood and agricultural wastes (Heck *et al.* 2005). Xylan protects cellulose against microorganisms. Xylanase, including endoxylanase, xylobiohydrolase, and xylotriohydrolase, is a class of enzymes that degrade the linear polysaccharide beta-1,4-xylan into xylose (Kabel *et al*. 2007; Komiyama *et al*. 2009; Oliveira *et al.* 2010). It has been extensively used in chlorine-free bleaching of wood pulp prior to the papermaking process, as food additives to poultry, in wheat flour to improve the dough handling and quality of baked products, in the extraction process of coffee, plant oils, and starch (Tan *et al.* 1987; Wong *et al*. 1988; Wong and Saddler 1992; Subramaniyan and Prema 2002) to improve the nutritional properties of agricultural silage and grain feed, and in combination with pectinase and cellulase to clarify the fruit juices and to degum the plant fiber sources such as flax, hemp, jute, and ramie. Although xylanase has many applications, studies on optimization of nutrition constitutes of medium to increase the xylanases activity, especially under SSF, remain scarce (Yang *et al.* 2006).

Fermentation for the production of active enzymes can be conducted in either liquid medium, known as submerged fermentation (SmF), or a solid medium, *i.e.* SSF. The latter is defined as the process of growing microorganisms in solid substrate without free water (Rahardjo *et al*. 2006). SSF has been particularly advantageous for the growth of filamentous fungi by simulating the natural habitat of these microorganisms. This benefit is extended to the activity of enzymes and has provided higher productivity compared to SmF (Acuna-Arguelles *et al*. 1995; Jain 1995; Aguilar *et al.* 2001; Ashok *et al.* 2001; Souza *et al*. 2003; Viniegra-González *et al*. 2003; Saqib *et al*. 2010; Colla *et al*. 2010). In addition, SSF is highlighted by being less susceptible to substrate inhibition (Acuna-Arguelles *et al*. 1995), having greater stability to changes in temperature and pH (Alazard and Raimbault 1981; Deschamps and Huet 1985), utilizing lesser energy (no vigorous agitations), being eco-friendly (low waste water output), producing high concentration of metabolites at low cost, and being technically easier to manipulate (Peixoto-Nogueira *et al*. 2009). However, approximately 90% of all industrial enzymes are produced by SmF, not SSF (Hölker *et al.* 2004). To date, only a few papers reported the production of xylanase by SSF using different microorganisms, such as *Aspergillus niger*, *Fusarium oxysporum*, *Thermoascus aurantiacus*, and *Trichoderma harzianum* (Hölker *et al*. 2004; Valls and Roncero 2009; Panagiotou *et al.* 2003; Santos *et al.* 2003; Rezende *et al*. 2003), and to our knowledge, the potential of SSF for the production of xylanases by *R. stolonifer* have not been evaluated thoroughly (Bailey *et al*. 1992).

In this work, a novel prime strain *R. stolonifer* Js-1008 was separated from Yudai river in Jiangsu University (Zhenjiang, China) and well identified and characterized by morphological and molecular biological methods. The fermentation of this strain for enzymatic activity was also investigated. To the best of our knowledge, this report is the first study to use *R. stolonifer* in xylanase production.

EXPERIMENTAL

The Isolation and Identification of *R. stolonifer* JS-1008

The fungus was isolated from sludge in the Yudai river of Jiangsu University (Zhenjiang, China). After sampling, the sludge was diluted 10-fold with aseptic water, and the isolates were cultured by streaking onto a potato dextrose agar (PDA) plate, which was then incubated at 28 °C for 48 h. The representative strains of all colony types were isolated by sub-culturing onto PDA plate at the same temperature to form a single colony. The isolated typical strains were inoculated onto PDA slants, incubated at 28 °C for 72 h, and maintained in refrigerator at 4 °C.

After the strain was cultured on PDA plate at 32 °C for 4 to 6 days, the configuration and color were observed. Morphological characters including hyphae, spores, sporangium, *etc.*, were also recorded under a microscope.

A total of 1×10^6 spores from the strain slant were aseptically taken and inoculated into a 250 mL Erlenmeyer flask containing 100 mL of potato dextrose (PD) medium and cultured at 28 °C, 150 rpm for 4 days. The mycelial sphere was transferred into a mortar and ground into extractum by adding liquid nitrogen. Genomic DNA was then extracted using a Genomic DNA Extraction Kit (TaKaRa, China) according to the manufacturer's instructions. The primers for amplification of the 26s- rDNA regions were as follows: forward, 5'-GAGCGGATAACAATTTCACACGG-3' and reverse, 5-CGCCAGGGTTTTCCCAGTCACGAC-3'. PCR amplifications were performed

according to the manual of the Fungi Identification PCR kit (TaKaRa, China). Gel electrophoresis was then used to detect the amplified PCR product from *R. stolonifer* JS-1008 and viewed under ultraviolet light to detect the presence and size of the amplified DNA product. The band was excised from the gel and extracted for DNA with the Gel Extraction Kit (TaKaRa, China). The purified products were ligated into PMD18-T vector and transformed into *E. coli* DH5αMCR competent cells according to the manufacturer's instructions (TaKaRa, China) in order to facilitate the sequencing. Positive colonies were identified by blue/white screening on LB agar. The plasmid was extracted with Plasmid Extraction Kit (TaKaRa, China) and sequenced by Shanghai Bioasia Bio-technology Co, Ltd. (Shanghai, China). The sequences were analyzed using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/).

Medium and SSF

In addition to corncob, the substrate of SSF, different IMC, nitrogen sources, inorganic salts, and surface-active agents were studied. According to the experimental design, different concentrations of medium components were tested to obtain the optimal conditions. All fermentation media were sub-packaged into a stainless steel dish (15 cm in diameter) at 60 g per dish after being autoclaved at 121 °C for 60 min.

Total 1×10^6 spores from the strain slants were inoculated aseptically into a 250 mL Erlenmeyer flask containing 15 g bran and 20 mL H_2O (sterilized at 121 °C for 60 min) and incubated at 28 °C for 6 days to use as solid seed of following SSF.

Six grams of the aforementioned solid seed were then inoculated into the stainless tray containing 60 g fermentation medium. All materials were mixed thoroughly. The dishes were sealed with preservation film to maintain the humidity. All the SSFs were conducted at 28 °C for 10 days under static condition.

Experimental Design

Once the effective nutrient factors were identified by a "one-factor-at-a-time" set of tests, RSM was used to determine the optimal concentration of these parameters for xylanase activity by *R. stolonifer* JS-1008 under SSF. The Central Composite Design (CCD) with four factors at three levels was applied to optimize the IMC (A), urea (B), ZnSO4 (C), and Tween-80 (D) for xylanase activity. The response value (Y) in each run is the average of duplicates. The experimental design protocol for RSM was developed using Design-Expert Software (version 5.0.9; Stat-Ease Corporation, USA). The RSM experiment was conducted for 8 days at 32 °C, pH 7.5, and 1×10^6 spores/100 mL medium of inoculums size under static conditions.

Enzyme Extraction

After the fermentation, 5 g of the solid medium was transferred to a 250 mL Erlenmeyer flask, added with 100 mL distilled water, and stirred at 150 rpm for 60 min at 28 °C. The recovered enzyme extracts were stored in small volumes in 1.5 mL tubes at −18 °C for further analysis.

Assay of Enzyme Activity

Xylanase activity was measured based on the Bailey methodology (Bailey *et al*. 1992). Briefly, the appropriately diluted enzyme extract was incubated at 50 °C for 60 min with 1% beechwood xylan (Sigma, USA) in 0.05 mol/L acetate buffer (pH 4.5).

Xylose equivalents released from beechwood xylan (substrate) was measured using the dinitrosalicylic acid method (Miller 1959). One unit of xylanase corresponded to 1 μmol of xylose released per minute at pH 4.5 and 50 °C (Yang *et al.* 2006).

Assay of Moisture Content

Five g of solid medium or fermented product was dried to constant weight at 105 °C, and the dry weight was recorded. The moisture content was calculated according to Equation 1,

$$H = \frac{W_1 - W_2}{W_2} \quad (1)$$

where H is the moisture content of solid medium or fermented product (%), and W_1 and W_2 represent the weight of the solid medium or fermented product and the dry weight, respectively.

Data Analysis

The results were expressed as the mean of triplicates. The analysis of variance (ANOVA) was applied to distinguish the significance of parameters. The variables and their levels for the central composite experimental design was showed in Table 1. The optimal nutrition requirement was defined as achieving the highest xylanase activity in minimized culture time.

Table 1. Experimental Design and Results of the Central Composite Design (CCD) (g/g corncob)

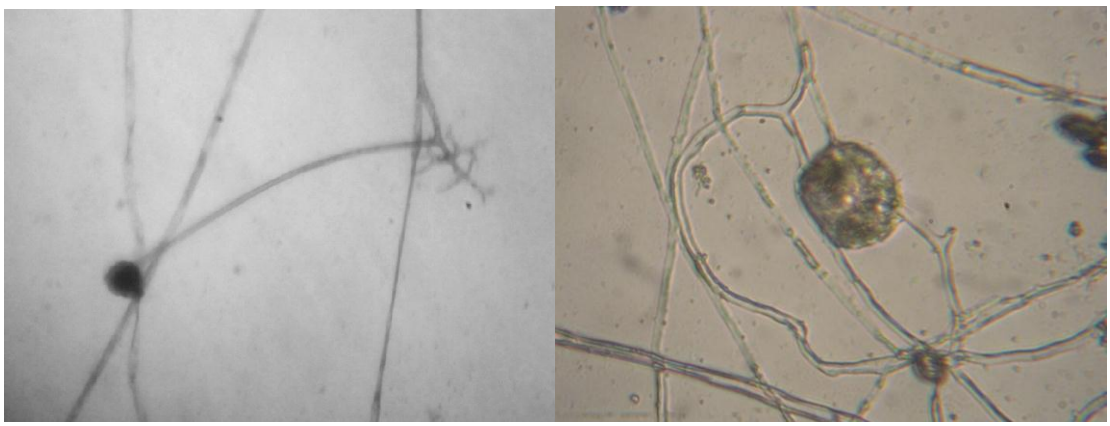
Dependent variables	Symbols	Real and code levels		
		-1	0	-1
IMC	A	2.0	2.5	3.0
Urea	B	0.15	0.2	0.25
Inorganic salt	C	0.01	0.02	0.03
SSA	D	0.08	0.09	0.1

RESULTS AND DISCUSSION

Identification of the Strain

The isolated strain JS-1008 was cultured on PDA at 30 °C; its color gradually changed from white (24 h) to gray (72 h) during the incubation process. The white branched hypha did not show through the diaphragm. Stolon hypha was seen on the surface of the medium and extended into the matrix to form a rhizoid (Fig. 1a), and produced one erect sporangiophore in the air (Fig. 1a), the top of which inflated to form a spore sac (Fig. 1a). The spore sac contained multi-core sporangium, and two hypha of the opposite sex formed zygospores (Fig. 1b). Based on these comparable characteristics, the isolated strain was identified preliminarily as *R. stolonifer*.

Approximately 1000 bp sized-fragment of the 26S rDNA gene of the strain was amplified and sequenced. The size of the fragment was confirmed by electrophoresis after PCR. The gene sequence of the strain is shown in Fig. 2. The result of the PCR is shown in Fig 3. It is consistent with the sequencing results.



a b

Fig. 1. Characteristic of morphology under the microscope. a: rhizoid and sporangiophore; b: zygospores

According to its 26S rDNA gene sequence, N-J method of Mega 5 software was applied to describe the molecular phylogenetic trees (Fig. 4). Compared with the BLASTN library, the homology between JS-1008 and *R. stolonifer* NRRL1477, *R. stolonifer* var. stolonifer strain BCRC 32002, *R. stolonifer* AFTOL-ID 632 was up to 99%. The homology between JS-1008 and *R. stolonifer* strain KACC 46105, *R. sexualis* var. *americanus* strain CBS 340.62, *R. sexualis* strain CBS 336.39 was 97%, and the value was 97% compared with *Syzygites megalocarpus* strain NRRL 54819. Phylogenetic trees indicated that the isolated strain was more primitive than *R. stolonifer* CBS340.62 and *Syzygites megalocarpus* NRRL 54819. Based on the above morphological characters and sequence comparison, the isolated strain was finally determined as *R. stolonifer* JS-1008.

GGGACTGGACCTGATGGTATCTCGAGTTCGTGTTCCAGGCGCATCCTCTGAGAGATTGAGCGAAAAT
AGGTTTAAACTTCAAGTCTTGTGACAATGCCACAGTCTGATCCGTAAATCCCAATAAAAGACTTTGC
CTCAAAAGAGAAGCATTCGCTGCGTTCCTCAATCCAATTCAAGGTATTTTCTAAAGGACTATAACAC
CAATAAATAAATATTGGCCACATTTCCAATAGTTTTTACACTCAAAAAAAATTGTCGTTGGCAGGCA
AATAGTCTGCAAGTGCATCCAGGCACGAAACCTAGATTGATTACAGACAAGCCAGTCTGGCTCCAAA
CGGTTCCCTTTTAACAATTTCACATACTGTTTAACTCTCTTTTCAAAGTTCTTTTCATCTTTCCCTC
ACGGTACTTGTTCGCTATCGGTTTCTCGCCAATATTTAGCTTTAGGTGAGATTTACCACCCAATTTA
GGCTGCATTCCCAAACAACCTGACTCTTTGAAAGTGTATCGCAAAAGACAAAATATCTCGAATCAAA
GACGGGATTCTCACCCTCTATGATGCCCTGTTCCAAAGGACTTATTAACTCGGATTGCCTGGAAAAC
ACTTCTACAGTTTACAATCCTGTTTAGCTAGGCCAAACAGGTTCCAACTTTGAGCTCTTTCCTCTTC
ACTCGCCGTTACTAGGGAAATCATTGTTATTTTCTTTTCCTCCGCTTATTGATATGCGTCGTGACTG
GGAAAACCCTGGCGAATCGTCCACTTGCCGTCATGCTTCTTTGGCACTGGCCGTCGTTTTACAACGT
CGTGACTGGGAAAACCCTGGCGTTACCCAACTTAATCGCCTTGCAGCACATCCCCCTTTCGCCAGCT
GGCGTAATAGCGAAGAGGCCCGCACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATG
GCGCCTGATGCGGTATTTTCTCCTTACGCATCTGTGCGGTATTTCACACCGCATATGGTGC

Fig. 2. The DNA sequence of the 26S rDNA gene of the new isolated strain

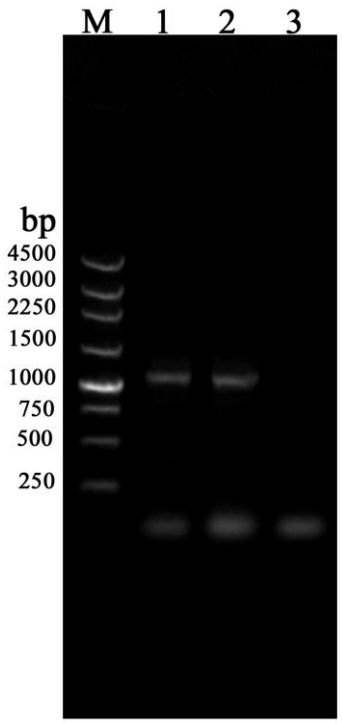


Fig. 3. Specific fragment on a 1.5% agarose gel electrophoresis M: DNA mark; 1, 2, and 3: samples

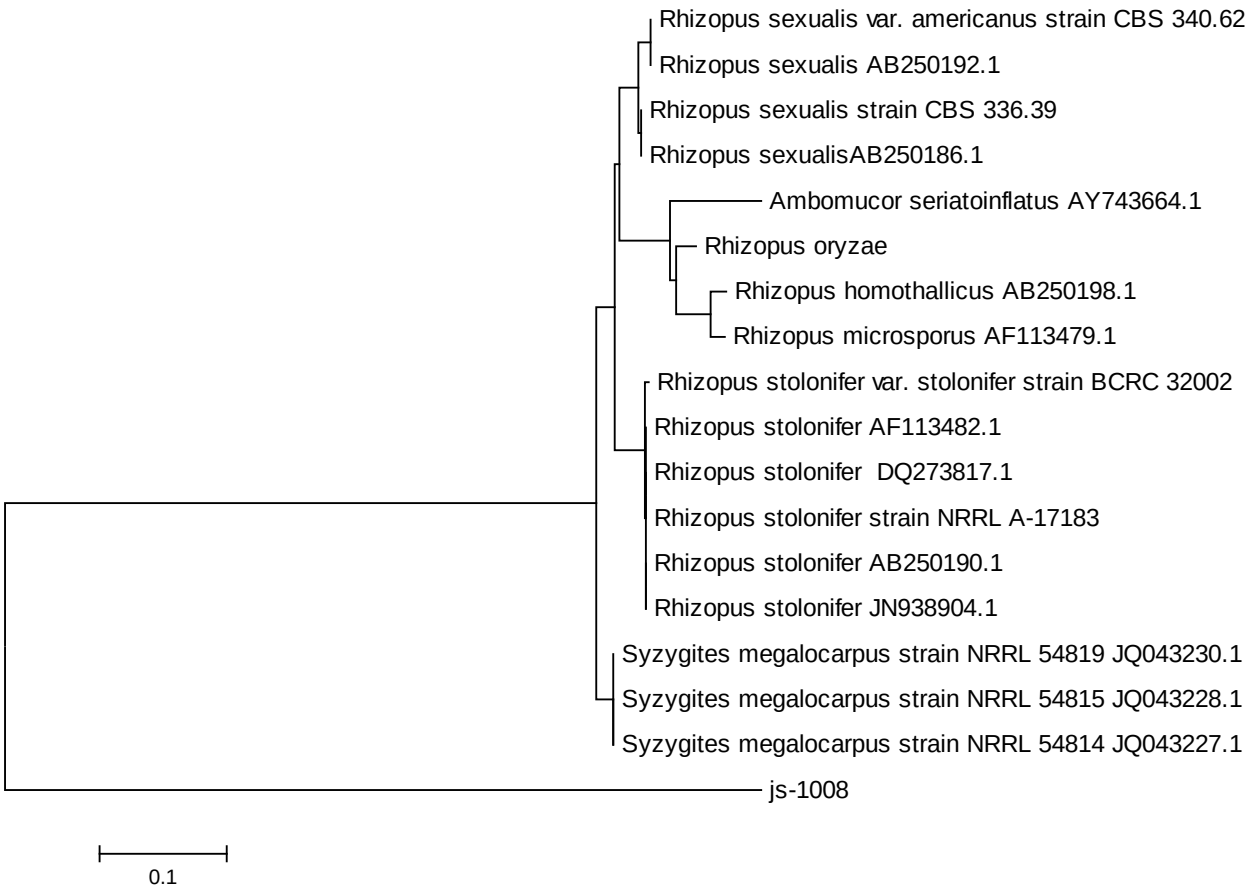


Fig. 4. The phylogenetic trees (N-J) of isolated strain and associated strains

The Effect of IMC

In the process of SSF, material exchange takes place between the gas-, liquid-, and solid-phase. If the IMC in medium is too high, then the material exchange cannot be conducted, and the energy also cannot be released. The SSF cannot be normally conducted. Conversely, if the IMC is too low, water is not able to dissolve enough material to meet the requirement of mycelia growth, and the SSF also cannot be performed. Therefore, the effect of different IMC on xylanase activity was first analyzed. The results (Fig. 5) indicate that the xylanase activity was the highest on the 6th day (6.63 U/g DS) when the IMC was 2.5 g/g corncob (Fig. 5). The enzyme activity decreased sharply when the IMC exceeded 2.5 g/g corncob.

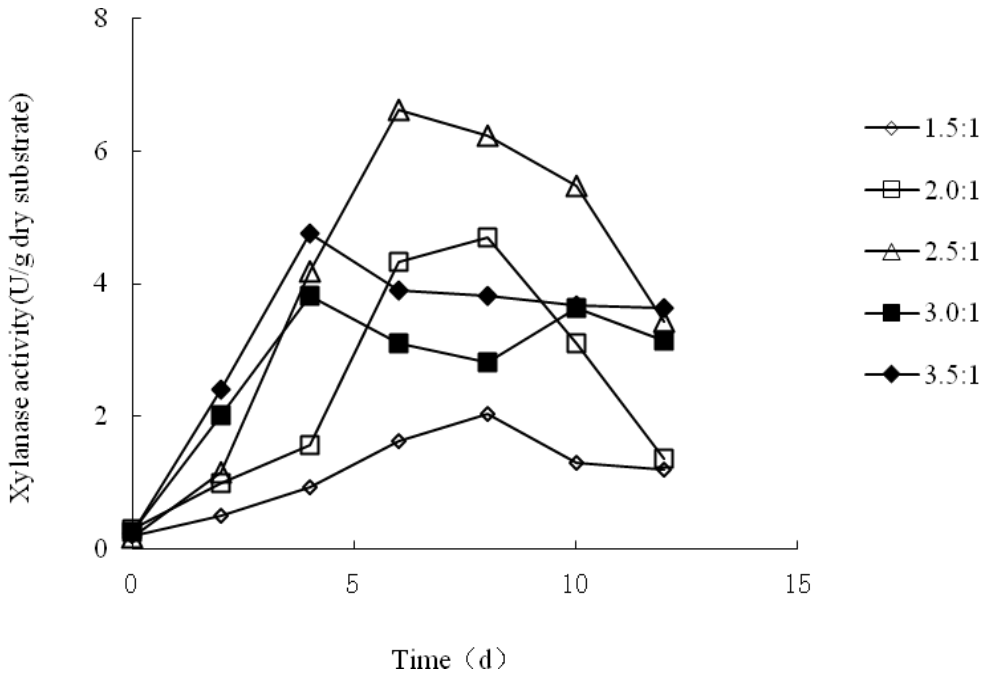


Fig. 5. The effect of different IMC on activity of xylanase. The unit of IMC is g/g corncob

The Effect of Nitrogen Sources

The nitrogen source was indispensable for mycelia growth and xylanase activity. To study the effect of nitrogen source on xylanase activity, the solid medium was supplied with five kinds of different nitrogen sources: $(NH_4)_2SO_4$, urea, beef extract, peptone, and yeast extract at the concentration of 0.2g/g dry corncob, respectively. The fermentation was conducted at 28 °C for 10 days. The maximum xylanase activity (9.30 U/g DS) was obtained on the 8th day with the medium supplied with 0.15 g urea /g dry corncob, and significantly higher than that of the control (without nitrogen sources). While the beef and yeast extracts failed to promote the xylanase activity, $(NH_4)_2SO_4$ and peptone turned to inhibit SSF instead of promoting it compared to the control (Fig. 6).

The Effect of Different Inorganic Salts

To study the effect of inorganic salt on xylanase activity, different inorganic salts were added at the concentration of 0.02 g/g corncob, respectively. All the inorganic salts displayed certain promotional effects on the activity of xylanase. The maximum activity (7.00 U/g DS) was obtained on the 8th day in the medium supplied with $ZnSO_4$ (Fig. 7).

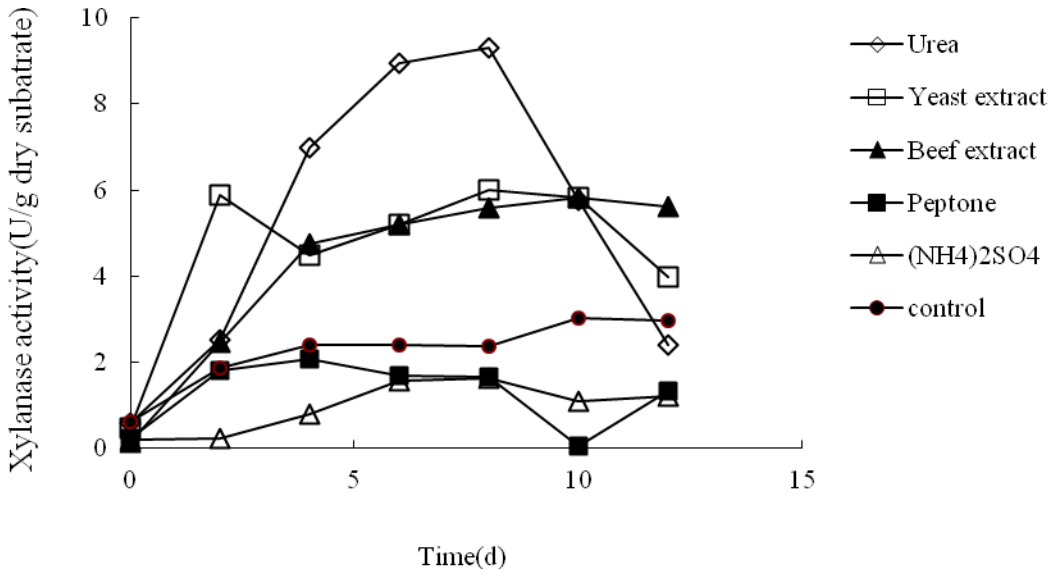


Fig. 6. Effect of different nitrogen source on xylanase activity

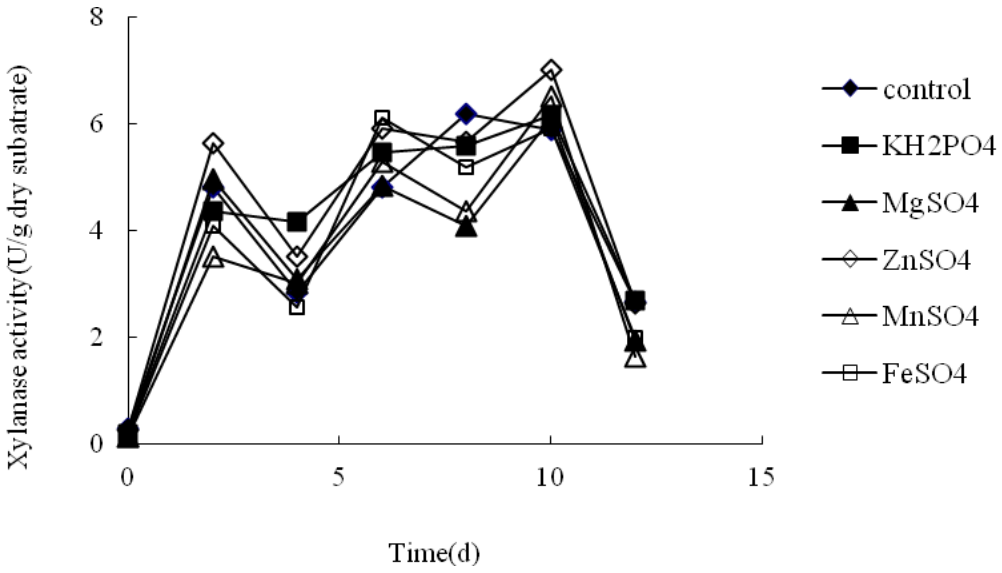


Fig. 7. Effect of different inorganic salts on xylanase activity

The Effect of Different Surface-Active Agents

Wu *et al* (2006) found that the surface-active agents could increase enzymatic activity. Therefore, the effect of surface-active agents on xylanase activity was further studied. Sodium dodecyl sulfate (SDS) and Tween-80 are two surface-active agents often used in experiments because of their low mammalian toxicity. Both surface-active agents were added at the concentration of 0.09 g/g corncob. Their effects on xylanase activity

were investigated in this study. Compared with the control (without addition of surface active agents), Tween-80 significantly increased the activity of xylanase with the maximum value of xylanase activity (6.98 U/g DS) observed on the 10th day (Fig. 8). Inversely, SDS exhibited inhibitory effect on the activity of xylanase.

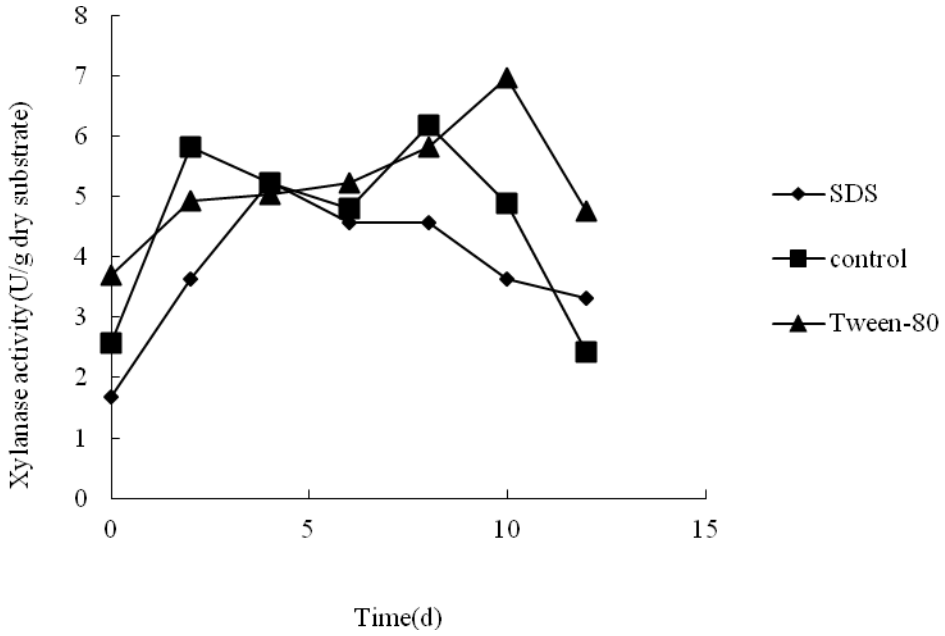


Fig. 8. Effect of different surface-active agents (SAA)

Analysis with RSM

Based on the "one-factor-at-a-time" design, IMC (A), urea (B), ZnSO4 (C), and Tween-80 (D) that exhibited high impact on xylanase activity were selected. Central Composite Design (CCD), which is one version of the RSM approach, has been demonstrated as an efficient method to optimize medium compositions (Sonia *et al.* 2005; Dobrev *et al.* 2007; Senthilkumar *et al*. 2005), and it was used to determine the optimum concentration of these four nutrients. The experimental recipes (horizontal lines as shown in Table 2) for different nutrient interactions (and their concentrations) were generated by Design-Expert software. The running results of these experiments of CCD are shown in Table 2. A polynomial mathematical model on the xylanase activity incorporating the different interactions of low and high concentrations of different nutrient factors was proposed in equation (2):

$$Y = 3.240 + 2.239A - 5.142B + 9.770C - 35.63D + 0.903AB + 2.761AC - 3.168AD - 6.256BC + 6.335BD + 41.967CD + 0.0315A^2 - 0.721B^2 - 97.27C^2 - 26.207D^2 \quad (2),$$

where Y represents xylanase activity (U/g DS).

The summary of the analysis of variance (ANOVA) representing the result of the quadratic response surface model for xylanase is shown in Table 3. The Model F-value of

9.54 implied the significance of the model; *i.e.*, there was only a 0.01% chance that a noise could occur with such high a "Model F-value." The values of "Prob>F" less than 0.0500 indicated that the model terms were significant. In this case, A, B, BD, C^2, and D^2 were significant model terms. According to equation (2), the predicted value of experimental recipes (horizontal lines as shown in Table 2) based on the interactions among different nutrient factors (their concentrations) was generated (the last column). F-value of "Lack of Fit" was 2.78, implying that the Lack of Fit was not significant relative to the pure error. Therefore, equation (2) fits with these results from running the experimental recipes of CCD (horizontal lines as shown in Table 2).

Table 2. The Variables and their Levels for the Central Composite Experimental Design

Experiment	Coded levels				Xylanase activity (U/g dry substrate)	
	A	B	C	D	Observed	Predicted
1[a]	2.50	2.00	0.20	0.90	11.75	11.89
2	2.50	1.00	0.20	0.90	12.15	12.49
3	2.50	2.00	0.20	1.10	9.99	9.74
4	3.00	1.50	0.15	1.00	11.63	11.32
5	1.50	2.00	0.20	0.90	9.44	10.02
6	2.50	2.00	0.10	0.90	10.27	10.61
7	2.00	2.50	0.25	0.80	9.33	8.80
8	3.00	2.50	0.15	1.00	11.06	11.39
9	2.00	1.50	0.15	1.00	10.18	10.32
10	2.00	2.50	0.25	1.00	9.13	9.77
11[a]	2.50	2.00	0.20	0.90	12.27	11.89
12	2.00	2.50	0.15	1.00	10.38	9.50
13	2.00	1.50	0.25	1.00	12.06	11.22
14[a]	2.50	2.00	0.20	0.90	12.10	11.89
15	2.50	2.00	0.30	0.90	11.26	11.22
16	2.00	1.50	0.25	0.80	11.30	11.51
17	3.00	2.50	0.15	0.80	11.90	11.90
18	2.00	2.50	0.15	0.80	9.13	9.36
19	2.50	2.00	0.20	0.70	11.23	11.16
20	3.00	1.50	0.25	1.00	12.17	12.49
21	3.00	2.50	0.25	0.80	11.19	11.60]
22	3.00	1.50	0.25	0.80	13.39	13.42
23	3.00	1.50	0.15	0.80	13.19	13.09
24[a]	2.50	2.00	0.20	0.90	11.73	11.89
25	2.50	3.00	0.20	0.90	9.88	9.85
26[a]	2.50	2.00	0.20	0.90	11.27	11.89
27	3.00	2.50	0.25	1.00	12.38	11.94
28	3.50	2.00	0.20	0.90	14.10	13.82
29[a]	2.50	2.00	0.20	0.90	12.21	11.89
30	2.00	1.50	0.15	0.80	11.87	11.46

[a] center points

Table 3. Results of the Regression Analysis of the Central Composite Rotatory Design (CCD)

Source	Mean square	F-value	Probability>F
Model	2.97	9.54	<0.0001
A	21.70	69.75	<0.0001
B	10.44	33.56	<0.0001
C	0.55	1.76	0.2046
D	0.96	3.08	0.0996
AB	0.81	2.62	0.1265
AC	0.076	0.24	0.6279
AD	0.40	1.29	0.2737
BC	0.39	1.26	0.2797
BD	1.61	5.16	0.0383
CD	0.70	2.26	0.1531
A2	0.0017	0.0055	0.9419
B2	0.89	2.86	0.1113
C2	1.62	5.21	0.0374
D2	2.18	7.01	0.0182
Lack of Fit	0.40	2.78	0.1357

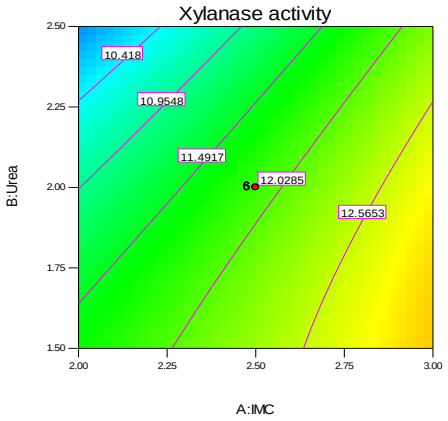


Fig. 9. Contour plot of combined effects of IMC and urea on the xylanase activity by *R. stolonifer* JS-1008

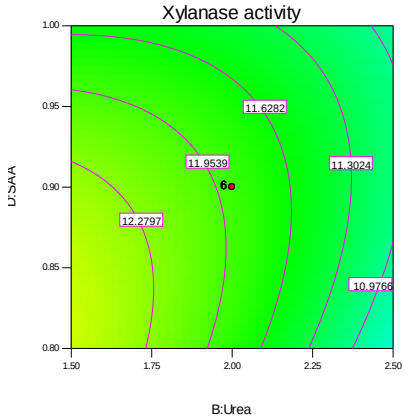


Fig. 10. Contour plot of the combined effects of urea and surface active agent (SAA) on the xylanase activity by *R. stolonifer* JS-1008

Contour plots of nutrient interactions were plotted based on the model equation (2) to investigate the interaction among variables and the optimal concentration of each factor and the effect of the interactions on xylanase activity by *R. stolonifer* JS-1008. From the contour plot, the following conclusions can be obtained: 1) the activity of xylanase gradually increased with the increase of IMC and/or urea, and the maximum xylanase was obtained at the IMC of 3 and/or the urea of 0.15 g/g dry corncob, respectively (Fig. 9), 2) the xylanase activity was higher when the concentration of urea and surface active agent was at a lower level (Fig. 10), 3) unlike urea and surface active agent, the effect of inorganic salt on xylanase activity was not significant (Fig. 11), 4) an initial increase with the increase of inorganic salt was shown before the decrease in the xylanase activity with a further increase in the concentration of inorganic salt, and 5) IMC and urea had a major role in the xylanase activity compared to $ZnSO_4$ and Tween-80, while $ZnSO_4$ and Tween-80 had no significant effect on the xylanase activity (Fig. 9).

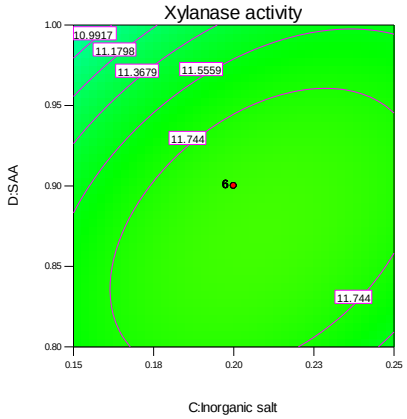


Fig. 11. Contour plot of the combined effects of inorganic salt and SAA on the xylanase activity by *R. stolonifer* JS-1008

Consistent with previous report that the optimal working conditions were defined as achieving the optimum xylanase activity in minimized cultivation time (Bocchini *et al.* 2002), the optimum concentration in the present study was determined as the following (g/g dry corncob): IMC, 3; urea, 0.15; ZnSO4, 0.022; and SAA, 0.08. Under such conditions, our model predicted a xylanase activity of 13.52 U/g DS.

Over the last few decades, although a number of studies regarding optimization of the production of xylanase have been reported, most of the information was focused on the shaking culture, with little about SSF. The SSF system using corncob as support is a feasible and economical method for the production of xylanase based on the fact that corncob is a cheap and abundant agro-waste. *R. stolonifer* is an unusual fungal strain that produces cellulase, xylanse, and hemicellulase, while xylanase has been shown to solubilize pulp actively and hence is worth applying in industry. In this study, we used RSM and CCD to optimize culture media to increase xylanase activity by *R. stolonifer* JS-1008 from an initial 3.75 U/g DS to a final 13.90 U/g DS, for four times; although it is still far lower than that reported (Kapilan and Arasaratnam 2011), *R. stolonifer* JS-1008 is more primitive and potently applicable. Therefore, more in-depth studies are needed in the future to improve the yield of xylanase.

CONCLUSIONS

1. The nutrition requirement for maximum xylanase activity by *R. stolonifer* JS-1008 contained (g/kg dry corncob): urea, 0.15; ZnSO4, 0.022; Tween-80, 0.08; and IMC, 3.
2. The maximum experimental xylanase activity of 13.90 U/g DS obtained under the optimal medium condition was slightly higher than the predicted 13.52 U/g DS on the 8th day of fermentation and far higher than the enzyme activity (3.75 U/g DS) obtained with the initial medium on the same day.

ACKNOWLEDGMENTS

This work was supported by a grant from the Department of Sequencing, Shanghai and grants from National Natural Science Foundation of China (No. 31101269), Anhui United Agricultural Science and Technology Programs in Changjiang Delta District (No. 10140702021), Natural Science Foundation of Jiangsu province (No. BK2011154), Nantong Social Development Fund (No. S2011003), and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

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Article submitted: July 27, 2012; Peer review completed: October 14, 2012; Revised version received: November 2, 2012; Accepted: February 18, 2013; Published: February 27, 2013.