этапов, таких как выращивание, подготовка, ферментация, выдержка, скручивание и уход. Среди них ферментация является основным звеном, влияющим на улучшение качества сигарного табака. Поэтому повышение качества и эффективности ферментации все чаще становится предметом исследований в области производства сигарного табака. Многочисленные исследования показали, что добавление экзогенных добавок играет важную роль в стимулировании ферментации листьев сигарного табака, что может эффективно улучшить качество и удобство использования листьев сигарного табака. В этой статье рассматривается ход исследований влияния добавления различных типов экзогенных добавок на ферментацию сигар, а также рассматривается направление исследований и важность экзогенных добавок.

Ключевые слова: *сигары, ферментация, экзогенные добавки, эффекты, качество табака.*

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SCREENING OF XYLANASE PRODUCING STRAINS AND ITS APPLICATION IN FLUE-CURED TOBACCO LEAF FERMENTATION

Abstract: *In this experimental study, a strain producing xylanase extracted from tobacco leaf was developed. Through the basic strain screening method and xylanase activity determination, strain M416 with the highest enzyme activity was preliminarily identified and M416 was A. niger, the enzyme has biological safety and can be used in the fermentation process of flue-cured tobacco. The fermentation conditions were optimized by shaking the flask culture, in which the best formulation of xylanase production from A. niger and the optimal culture temperature, pH, inoculum amount and culture time were obtained. Under the optimal fermentation and aroma production conditions, the strain are evenly sprayed to the surface of tobacco leaf to*

determine the changes of the composition and content of aroma substances in the fermentation process, and evaluate the influence of xylanase producing niger on the aroma composition in the process of tobacco leaf fermentation, which shows that the strain can effectively increase the content of aroma substances.

Key words: *xylanase, screening, identification, optimization, aroma substances, fermentation and aroma production/*

Introduction

Technical enzymes can be used as purified enzymes, partially purified enzymes or whole cells containing functional catalytic activities and are often obtained from a natural source or by recombinant expression in bacteria or yeast. There is also increasing interest in the use of plants as host expression systems for technical enzymes because their production can be easily scaled up with low production costs [1].

Xylan is a multimeric five-carbon sugar that is an important component of plant hemicellulose. It accounts for one-half of the total plant carbohydrates and is the second most abundant renewable resource in nature after cellulose. It is found in the cell walls of plants and in almost all parts of the plant [2]. Xylanase is a generic term for a class of enzymes that degrade xylan into oligosaccharides or xylose. The complete enzymatic degradation of a xylan molecule can be accomplished by endogenous xylanases (endo-beta-1,4-D-xylanase, EC 3.2.1.8) and xylosidases (beta-D-xylosidase, EC 3.2.1.37). Among them, xylanase can degrade xylan into oligo-xylan and xylan monosaccharides, which has a broad application prospect in feed, pulp and paper, food and pharmaceutical industries [3]. Many different technologies have been developed to produce xylanases in plants using stable transformation methods [4].

The study of xylanase transgenic plants has only begun in recent years Herbers et al. first expressed xylanase proteins derived from Clostridium thermocellum and Ruminococcus flavefaciens in tobacco, and the two xylanases were both expressed and showed activity [5-8]. Subsequently, xylanases from different sources were expressed and showed enzymatic activity in oil bodies of oilseed rape, barley seeds and rice, respectively [9-12]. The transgenic plants obtained in these studies were able to grow and develop normally. But the recombinant enzyme activity obtained in these transgenic plants was low.

In the process of tobacco fermentation, xylanase is the main component, which is involved in the sugar metabolism and amino acid synthesis of tobacco, and plays a key role in the colour, aroma, taste and freshness of tobacco. Xylanase causes the release of sugars from tobacco leaves, and as a result, the smoke becomes mellow and long, and the taste of tobacco is better [13]. Therefore, isolation of enzyme strains and qualitative fermentation of xylanase play an important role in the allocation of high quality tobacco. In the present study, xylanase-producing strains were isolated by targeted screening from tobacco fields and strain banks and applied in the fermentation of roasted tobacco.

Methodology

The test method is appropriately modified with reference to the experimental programme of GB/T 7714-2015[14], which is operated as follows:

Experimental material

Microbiological samples for isolation and Medicines

The soil was taken from a tobacco field at a depth of 10-15cm, dried at room temperature and sieved through a 60-mesh sieve.

NaOH, HCl, H_2SO_4, 3,5-dinitrosalicylic acid (DNS), phenol, Na_2SO_3, potassium sodium tartrate, dextrose, peptone, KH_2PO_4, $MgSO_4$, xylose, oat xylan (Sigma Company), all reagents were analytically pure.

Culture medium and Rescreen strain

Enrichment medium: xylan 1%, $(NH_4)_2SO_4$ 2.0%, KH_2PO_4 0.2%, pH natural.

Plate isolation medium: add 2% agar to the enrichment medium to form plate isolation medium.

Slant medium: PDA medium.

Enzyme production medium: corn kernel meal 5.0%, glucose 0.5%, ($(NH_4)_2SO_4$ 0.1%, KH_2PO_4 0.5%, $MgSO_4$ 0.1%, Tween 80 0.01%, pH 6.5.

Complex sieving medium: corn kernel meal 5%, glucose 0.5%, $(NH_4)_2SO_4$ 0.1%, KH_2PO_4 0.5%,

$MgSO_4$ 0.1%, Tween 80 0.01%, pH 6.5.

Nine strains with higher hyaline rings were identified from the initial screening and numbered M401, M402, M407, M409, M410, M412, M416, M421, and M425.

Experimental Methods

Preliminary screening of strains

(1) Enrichment culture: the enrichment medium is divided into test tubes, and after sterilisation, each soil sample is separately connected to the enrichment culture solution test tube, each test tube is about 0.1 g of soil samples, and is incubated at 30℃ for 3~4d.

(2) Transparent circle method of primary screening: the enrichment of soil samples diluted to a certain concentration of liquid were inoculated into the separation of the plate, and placed in 30℃ culture for 3~4 d, to observe the colonies and colonies around the emergence of transparent hydrolysis circle and regular measurement. The diameter of transparent circle/colony diameter is HC value.

(3) Strain purification: use the line method and dilution plate method to isolate and purify the above strains that produce hyaline circle, pick a single colony into the slant, 30℃ culture for 3~4 d, save for later.

1.2.2 Re-screening of xylanase-producing strains

(1) Cultivation conditions of fermentation strains in shaking flasks: culture temperature 30℃, rotational speed 150r/min, culture cycle 72h.

(2) Determination of xylanase enzyme activity:

Definition of xylanase enzyme activity: under the condition of 50℃ and pH4.8, the amount of enzyme required to decompose the substrate xylan to produce the amount of reducing sugar equivalent to 1 μmol of xylose per minute is defined as a unit of enzyme activity, which is expressed as U.

Reagents: DNS reagent, pH4.8 acetic acid-sodium acetate buffer solution and 1% xylan solution were prepared with reference to GB/T23874-2009 standard.

Determination of xylanase activity Drawing xylose standard curve Take 8~10mL corked graduated test tubes, add xylose concentration of 0.00, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, 0.70 xylose standard solution 1.00mL, add 0.5M acetic acid-sodium acetate buffer (pH4.8) 1.00mL. add 3mL DNS reagent, mix thoroughly, and set in a room, and then add 0.5M acetic acid-sodium acetate buffer solution (PH4.8). Add 3mLDNS reagent, mix thoroughly, boil in boiling water bath for 5min, then quickly cool it down to room temperature with cold water, and then volume it to 10mL, and measure the absorbance value at 540nm. The absorbance value was measured at 540nm. Taking the absorbance value as the horizontal coordinate and the xylose content as the vertical coordinate, the standard curve was plotted and the regression equation was fitted, and the linear regression equation was obtained as y=0.0465x+0.0092, which is shown in Figure 1, and the regression coefficient of the linear regression equation was 0.9995, which is above 0.9990, and it is in line with the requirement of the linear regression equation.

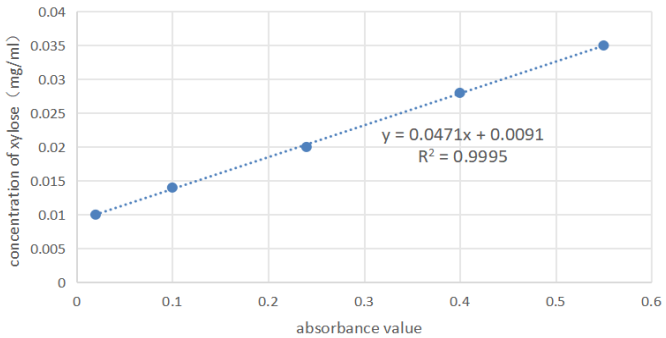

Figure 1 – Xylose standard curve

Determination of enzyme activity

Blank control: Take the sample in a centrifuge tube and centrifuge it at 5000r/min for 5min, take the supernatant and dilute it appropriately. Pipette 1.0mL of the diluted sample, add 3.0mL DNS reagent, mix thoroughly, then add 1% xylan substrate 1.0mL, react at 50℃ for 15min, put it into a

boiling water bath and boil for 5min, then cool it down to room temperature with cold water quickly, and then fix it to 10mL, and then measure the absorbance value at 540nm.

Measurement of samples: Take samples by centrifuge tube, centrifuge at 5000r/min for 5min, take the supernatant and dilute it appropriately, so that the absorbance value of the final measurement can be controlled between 0.2 and 0.3, if the absorbance value is out of the range, the dilution of the samples needs to be adjusted. Pipette 1.0mL of the diluted sample, add 1% xylan substrate 1.0mL, react at 50℃ for 15min, add 3.0mLDNS reagent, mix thoroughly, place in a boiling water bath and boil for 5min, quickly cool it to room temperature with cold water and then set it to 10mL, measure the absorbance value at 540nm, and then adjust the value to zero with the blank control.

The xylanase activity was then calculated by substituting the absorbance value of the sample into the following equation (1)：

$$\text{Xylanase activity} = W \times D \times 1000 / 150.13 \times 15 \times 1.0 \quad (1)$$

In the formula:

W (mg): the amount of xylose produced by enzymatic reaction, obtained from the standard curve y=ax+b;

D: dilution times;

1000: conversion factor from mg to μg;

150.13: molecular weight of xylose;

15: enzymatic reaction time (min);

1.0: sample volume of the reaction system (mL).

Identification of strains

Observation of colony morphology and microscopic morphology: Inoculate the test strain into liquid medium, incubate at 30℃ for 48h, dip the inoculating needle into a small portion of the bacterial liquid, and then connect it to the centre of the PDA plate, and inoculate a total of 4 plates, 2 plates were used to observe the colony morphology, and 2 plates were used to observe the micro morphology of the hyphae and spores by using sterilized tweezers inserted obliquely into 4 pieces of sterilized coverslips about 2cm away from the point of inoculation. The other 2 plates were inserted diagonally into 4 pieces of sterilised coverslips each with sterilised forceps about 2 cm away from the inoculation point. The plates were placed in the incubator at a constant temperature of 30℃, and the growth of the colonies was observed regularly, and the microscopic observation of conidiophores, conidia and mycelium was carried out in time.

Identification methods: Morphological identification refer to Wei Jingchao «Fungal Identification Manual».

Optimisation of the fermentation conditions

The enzyme activity was used as the index to optimize the medium and culture conditions by single-factor tests on medium formulation, incubation temperature, initial pH, water content, inoculum amount and fermentation time. Each experiment was done 3 times in parallel and 2 times in repetition. The fermentation broth was roughly filtered with a filter cloth, and then centrifuged at 10000 r/min for 5 min to test the enzyme activity.

Strain in the fermentation process of roasted tobacco

After the screened strains were cultured under the optimal fermentation conditions, the fermentation broth was uniformly sprayed onto the surface of the tobacco leaves, and the unsprayed place was used as the control, and the tobacco leaves were put into the biofermentation cabinet for fermentation, and the samples were sampled every 1 d for measurement.

Results of research

Preliminary screening of strains

Some of the results of the initial screening are shown in Table 1. Figure 2 shows the hyaline circle diagram of the M416 strain that produced the largest hyaline circle after 21 strains were cultured.After the experimental culture, the size of the obvious transparent hydrolysis circle appeared on 21 petri dishes was recorded and compared, and the strains that produced relatively larger transparent hydrolysis circle indicated that they were more effective in hydrolysis of xylan.

Therefore, a total of 9 HC strains, M402, M406, M407, M409, M410, M412, M416, M418, and M421, were obtained for further re-screening.

Table 1 – The results of initial screening of xylanase-producing bacterial strains

Strain	HC value	Strain	HC value	Strain	HC value
M401	1,59	M408	1,37	M415	1,35
M402	1,68	M409	1,95	M416	1,96
M403	1,32	M410	1,88	M417	1,45
M404	1,55	M411	1,45	M418	1,61
M405	1,47	M412	1,90	M419	1,43
M406	1,64	M413	1,29	M420	1,20
M407	1,99	M414	1,49	M421	1,85

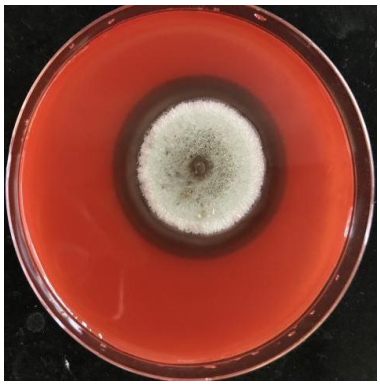

Figure 2 – M416 Transparency Ring Picture

Re-screening of xylanase-producing strains

The nine strains with higher enzyme activity were cultured in shake flasks, The absorbance values of the samples were determined separately after incubation and the corresponding enzyme viability values were calculated,and the results are shown in Table 2. From Table 1 and Table 2, the transparent circles of strains M402 and M406 were 1.68 and 1.64, and the enzyme activity of strains M402 and M406 was measured to be 72.36 U/mL and 52.53 U/mL, indicating that the size of the transparent circle was positively correlated with that of the enzyme activity, and so this method can be used to screen the hydrolysable xylanase-producing strains.

The results showed that M416 had the highest xylanase enzyme activity.

Table 2 – Enzyme vigour of rescreened strains

Strain number	Enzyme viability（U/mL）
M402	72,36
M406	52,53
M407	37,83
M409	50,32
M410	62,30
M412	45,77
M416	92,15
M418	85,37
M421	27,08

Identification of strains

Colony Characterisation

Figure 3 shows the growth status of the strains after 40h of fermentation in shake flasks. Colonies were cultured on PDA medium plate at 30℃ for 4 d, with a diameter of 4-5 cm. the texture was thick velvety to flocculent, dark brown to black, the back of the colony was light yellowish brown, and the colony was in the shape of a straw hat, with radial extensions to the edge of the colony, see Figure 4.

Figure 3 – Growth status of strains in shake flask fermentation for 40h

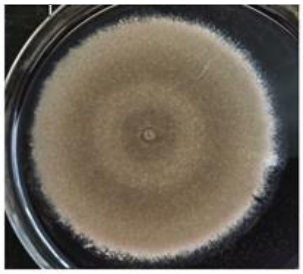

Figure 4 – M416 Colony Picture

Individual morphological characteristics

Mycelium is transparent, colourless and septate, part of it extends into the medium, part of it is aerial mycelium, mycelial cells are multinucleate; part of the mycelial cells form thick-walled, expanded foot cells, which extend vertically upwards in the middle of the foot cells to form a conidial peduncle, see Figure 5.

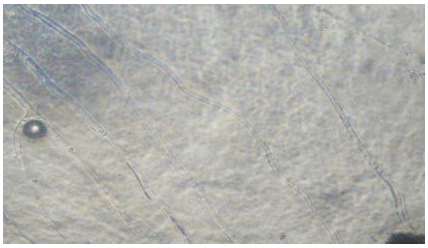

Figure 5 – M416 mycelium morphology

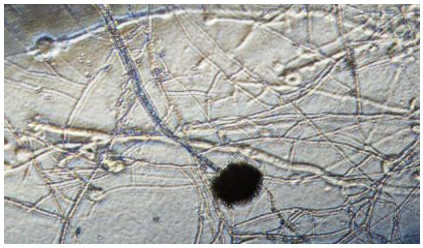

Figure 6 – Morphology of M416 conidial heads

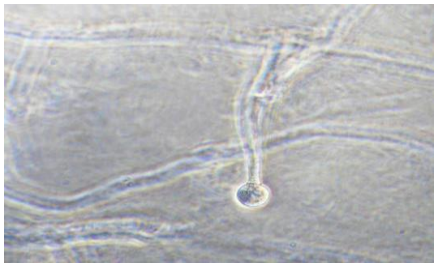

Figure 7 – Morphology of the M416 top capsule

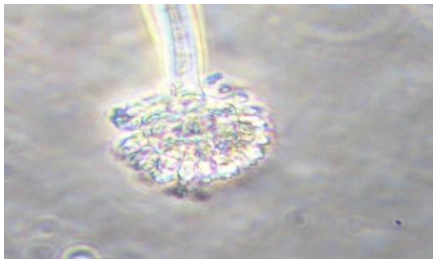

Figure 8 – M416 double-stacked peduncle

Conidiophore: diameter 10-20μm, length 1000-3000μm, wall thick and smooth, colourless. Conidiophores were spherical, radial, generally 300-800 μm in diameter, see Figure 6; acrospores were subglobose or flask-shaped, 30-60 μm in diameter; conidia were spherical to subglobose, black, 5-6 μm in diameter, see Figure 7 and Figure 8. The strain was identified as Aspergillus niger in the subphylum Deuteromycotina, class Hyphomycetes, class Hyphomycetales, family Moniliales, genus Aspergillus.

Strain safety description:

According to GB2760-2014 National Standard for Food Safety – Standard for the Use of Food Additives, Schedule C.3 "List of Enzyme Preparations for Food and their Sources", the requirements of strains for the production of enzyme preparations used for food contain about 20 species such as Bacillus subtilis, Aspergillus niger, Aspergillus oryzae, yeasts and actinomycetes. M416 was identified as Aspergillus niger and can be used for enzyme production for food.

Optimisation of the fermentation conditions

Effect of carbon and nitrogen sources and inorganic salts on enzyme activity

The different media were configured according to the formulation in Table 3 below, the optimum formulation for xylanase production by Aspergillus niger was obtained by comparing the effects of different carbon and nitrogen sources and inorganic salts on enzyme activity. The results showed that corn kernel and bran were the best carbon sources to promote xylanase production by Aspergillus niger strains. The inorganic salt $(NH_4)_2SO_4$ favoured the enzyme production by the strain, and its effect was better than that of the organic nitrogen source soya bean cake flour. The highest

enzyme activity was achieved when the ratio of corn kernel to bran was 8:2 and $(NH_4)_2SO_4$ was added at 1%, and the enzyme activity reached 220.5 U/mL.

Table 3 – Enzyme viability of different culture media

Composition of the culture medium	**Proportions**	**Enzyme viability（U/mL）**
Corn cobs: bran: soya bean cake flour	7:2:1	185,2
Corn cobs: bran: soya bean cake flour	2:6:2	106,4
Corn cobs: bran: soya bean cake flour	7:2:1	89,5
Corn kernels: maize flour，$(NH_4)_2SO_4$	7:2，1,0%	96,4
Bran: maize flour，KH_2PO_4	8:2，0,5%	101,8
Bran: soya bean cake flour	8:2	65,6
Corn cobs: Bean cake flour	8:2	79,8
Corn cobs: bran，$(NH_4)_2SO_4$	8:2，1%	220,5

Effect of the incubation temperature on the enzyme viability

The results in Table 4 showed that the enzyme viability was highest at the incubation temperature of 30℃ with consistent inoculum and culture conditions. In the culture process, it was found that 20℃, 25℃, 40℃, 40℃, 45℃ are not suitable for the growth of A. niger, small biomass, resulting in low enzyme vitality; 35℃, Aspergillus niger biomass, but not conducive to the secretion of enzyme production, resulting in low enzyme vitality.

Table 4 – Relationship between incubation temperature and enzyme production

Incubation temperature（℃）	Enzyme activity（U/mL）
20	65,1
25	48,5
30	208,7
35	153,2
40	67,9
45	51,3

Effect of the initial pH on the enzyme viability

The results in Table 5 showed that the pH had a significant effect on the enzyme production ability of A. niger, with the appropriate pH range of medium starting from 6 to 7 and the highest enzyme viability at pH of 6.5.

Table 5 – Effect of material initial PH on enzyme activity

pH	Enzyme activity（**U/mL**）
5,0	86,2
6,0	185,9
6,5	220,1
7,0	192,6
8,0	56,5
9,0	47,9

Effect of the inoculum size on the enzyme viability

Table 6 results indicate that the inoculum size affects the enzyme viability of xylanase production in A. niger. With the increase of the inoculum, the enzyme production capacity of A. niger tended to increase first and then weaken, with the highest enzyme viability at 5%, reaching 214.0U/mL. When the inoculum capacity is too small, the fermentation is slow; when the inoculum is too large, the rapid growth of bacteria leads to the lack of nutrients and oxygen, causing premature failure of bacteria, thus affecting the decline of enzyme production ability.

Table 6 – Effects of inoculum size on enzyme activity

Inoculum size （**%**）	Enzyme activity （**U/mL**）
1,0	139,8
3,0	156,3
5,0	214,0
7,0	122,6
9,0	109,5

Effect of incubation time on enzyme viability

The results are shown in Table 7. According to the table, after 36h of fermentation, the enzyme activity was 46.5U/mL, and then gradually increased, and at 72h, the highest enzyme activity reached 221.1U/mL. In the late stage of fermentation, the enzyme vitality is gradually reduced because the nutrients are gradually consumed. Therefore, the fermentation period was selected for 72h.

Table 7 – Effect of incubation time on enzyme activity

Incubation time （**h**）	Enzyme activity （**U/mL**）
36	46,5
48	68,3
60	126,9
72	221,1
84	174,6
96	142,7

Strain in the fermentation process of roasted tobacco

The Table 8 and 9 were obtained after analysis by high performance liquid chromatograph. There were different aroma substances under different aroma types, and the content of aroma substances between the experimental group and the control group varied from the first day to the seventh day of fermentation. In general, the total content of aroma substances in the experimental group was higher than that of the control group.

Table 8 – Changes of neutral aromatic substances content in CK tobacco

Odorous constitue	Odorous constitue	Neutral fragrances content（μg/g）						
		1	2	3	4	5	6	7
Degradation products of plastid pigments	Geranylacetone	2,15	2,13	1,16	1,05	1,43	1,19	1,14
	4-(2,6,6-Trimethyl-1-cyclohexe	4,80	2,32	1,49	3,54	0,63	2,26	1,45
	(2R,6R)-6,10-dimethyl-2-prop-1-en-2-yl-spiro[4.5]dec-9-en-8-one	1,93	2,15	1,51	1,03	2,25	1,78	1,68
	Megastigmatrienone D	3,82	2,91	1,96	2,72	3,04	2,54	2,91
	Megastigmatrienone B	2,18	2,12	0,82	1,83	1,36	1,38	1,41
	Megastigmatrienone A	-	-	-	0,41	-	-	-
	Linalool	0,66	0,78	0,88	0,76	0,89	0,78	0,87
	Farnesyl acetone	9,02	8,52	5,48	4,20	7,29	7,82	5,89
	2,6,6-Trimethyl-2-hydroxycycl	0,93	1,09	0,70	0,59	0,86	0,62	1,00
	beta-Damascenone	12,05	9,65	7,74	8,10	8,46	7,69	7,69
	Total	37,54	31,68	21,76	24,21	26,21	26,06	24,03
Phenylalanine aromatic substances	Dibutyl phthalate	4,90	3,62	-	-	-	-	-
	1,4-Benzenedicarboxylicacid	1,89	7,50	3,09	1,09	1,44	1,97	2,30
	Phenylacetaldehyde	0,59	0,73	0,61	0,49	0,69	0,58	0,74
	Total	7,39	11,85	3,70	1,58	2,12	2,55	3,04
Cypress like degradation products	Solanone	47,90	39,25	28,55	29,32	30,78	27,61	29,20
	b-Cembrenediol	31,80	28,55	39,49	33,38	47,57	31,71	37,08
	Total	79,70	67,80	68,03	62,70	78,36	59,32	66,28
Maillard reaction products	Furfural	4,50	8,04	5,08	2,61	4,96	3,52	3,68
	γ-sitosterol	1,15	1,47	0,65	0,69	0,58	0,74	0,88
	Total	5,65	9,52	5,73	3,29	5,53	4,26	4,56
Other fragrance substances	Methyl hexadecanoate	1,58	1,40	1,84	0,84	1,64	2,12	2,62
	Palmitic acid	23,88	44,24	42,11	10,35	52,81	42,23	50,07
	Linolenic acid	8,62	8,87	8,86	8,80	8,44	9,94	10,15
	Geranylgeraniol	49,18	40,44	36,30	24,22	47,27	46,41	41,05
	Nootkatone	2,40	1,71	1,64	1,20	2,03	1,25	1,88
	Eicosane	0,94	1,09	0,78	0,51	1,61	0,81	5,50
	Thunbergol	2,00	2,88	2,37	1,74	1,73	3,45	2,05
	2-Methylicosane	2,36	6,29	3,08	0,97	4,56	3,59	2,51
	triacontanol	37,05	41,23	28,33	12,91	40,68	50,03	34,75
	Total	128,01	148,15	125,30	61,53	160,76	159,83	150,59
Neophytadiene		288,25	243,92	222,53	185,38	240,73	219,81	231,70
Total		546,54	512,91	447,05	338,71	513,72	471,83	480,20

Table 9 – Changes of neutral aromatic substances content in spraying xylanase tobacco

Odorous constitue	Odorous constitue	Neutral fragrances content（μg/g）						
		1	2	3	4	5	6	7
Degradation products of plastid pigments	Geranylacetone	3,37	2,35	1,83	2,26	1,61	1,51	1,32
	4-(2,6,6-Trimethyl-1-cyclohexe	1,55	2,74	2,31	3,85	2,74	2,95	2,92
	(2R,6R)-6,10-dimethyl-2-prop-1-en-2-yl-spiro[4.5]dec-9-en-8-one	3,52	3,01	2,53	2,69	2,58	2,50	2,19
	Megastigmatrienone D	5,12	4,54	4,65	5,75	5,18	4,55	3,66
	Megastigmatrienone B	2,89	2,17	2,21	3,00	2,41	2,20	1,50
	Megastigmatrienone A	1,12	-	0,64	0,71	0,79	0,68	0,66
	Linalool	1,40	1,22	1,40	1,49	1,19	1,13	1,02
	Farnesyl acetone	9,85	11,16	3,97	4,48	6,58	8,07	3,97
	2,6,6-Trimethyl-2-hydroxycycl	0,94	0,91	0,61	0,94	0,91	0,93	1,10
	beta-Damascenone	11,41	10,24	8,95	10,29	7,84	7,05	5,73
	Total	41,18	38,34	29,11	35,47	31,82	31,56	24,07
Phenylalanine aromatic substances	Dibutyl phthalate	3,72	-	-	0,83	-	-	-
	1,4-Benzenedicarboxylicacid	1,14	1,14	1,99	1,98	14,56	0,84	6,72
	Phenylacetaldehyde	-	-	0,64	-	-	0,73	0,80
	Total	4,86	1,14	2,62	2,81	14,56	1,57	7,52
Cypress like degradation products	Solanone	28,85	22,78	21,07	23,30	21,40	17,90	15,88
	b-Cembrenediol	39,84	42,76	37,88	46,65	57,01	41,70	43,39
	Total	68,69	65,54	58,95	69,95	78,41	59,61	59,27
Maillard reaction products	Furfural	5,30	5,63	4,78	3,72	4,34	4,78	4,58
	γ-sitosterol	1,32	0,69	0,55	0,98	0,93	1,29	0,95
	Total	6,62	6,32	5,32	4,70	5,27	6,07	5,53
Other fragrance substances	Methyl hexadecanoate	0,94	0,93	0,94	1,78	1,18	1,30	1,63
	Palmitic acid	39,89	38,04	32,63	42,17	42,88	37,98	39,15
	Linolenic acid	6,08	6,24	5,12	9,03	7,65	9,37	9,10
	Geranylgeraniol	42,93	37,46	32,69	44,16	18,01	32,13	33,06
	Nootkatone	2,93	2,48	2,79	3,01	3,29	1,65	2,56
	Eicosane	1,21	1,14	1,96	0,72	0,70	0,71	0,69
	Thunbergol	1,40	1,22	1,00	1,19	0,88	0,82	1,15
	2-Methylicosane	2,78	2,53	2,83	2,96	2,30	2,15	2,36
	triacontanol	23,20	17,71	12,55	17,77	11,27	17,90	9,79
	Total	121,36	107,76	92,52	122,79	88,16	104,01	99,50
Neophytadiene		353,95	305,10	263,00	309,48	279,48	225,74	220,21
Total		596,65	524,19	451,53	545,19	497,69	428,57	416,11

Discussion

In this study, the subjects of tobacco, Through a series of strain screening, purification, identification, A. niger with high xylanase production, And optimized the fermentation process of the shake flask, Several major factors, such as medium formulation, culture temperature, inoculum size, and fermentation time, Finally, the optimal fermentation conditions were obtained, According to the standard of this condition, the fermentation liquid was evenly sprayed onto the surface of the test tobacco leaves, And the first day to the seventh day of fermentation samples were sampled to determine the aroma substance content, Found that this A. niger species significantly increased the aroma material content of fermented tobacco leaves, It also further proves that xylanase plays a key role in the color, aroma and taste of tobacco leaves, While xylanase is in greater demand in industry, But the industrial yields are low, If this A. niger can be further used to explore the process conditions and optimization for the industrial production of xylanase, It can further increase the industrial production.

Conclusion

The primary and rescreening obtained the highest strain of the xylanase, and the dominant strain was A. niger. By comparing the effects of different carbon and nitrogen sources and inorganic salts on enzyme motility, the optimal formulation of A. niger was obtained. The results show that corn cob and bran are the best carbon sources to promote xylanase production in A. niger species. Inorganic salt (NH4) 2SO4 is conducive to bacterial enzyme production, its effect is better than

organic nitrogen source bean cake powder enzyme. The ratio of corn cob and bran was 8:2, and when (NH4) 2SO4 was added to 1%, reaching 220,5 U/mL.

The temperature test showed that the highest enzyme activity was observed at 30℃, which was the optimal culture temperature for A. niger. The pH value had a significant effect on the enzyme production ability of A. niger, with the initial pH range from 6 to 7, and the highest enzyme viability was obtained at pH 6.5. The inoculum size affected the activity of A. niger, and the capacity increased first and then decreased. When the inoculum was 5.0%, the enzyme viability was highest, reaching 214.0U/mL. With the extension of culture time, the enzyme viability changed significantly. After 36h fermentation, the enzyme activity was 46.5U/mL, gradually increased, 72h the highest enzyme activity, reached 221.1U/mL, after which the enzyme viability gradually decreased.

References

1. Sharma A.K., Sharma M.K. Plants as bioreactors: Recent developments and emerging opportunities / A.K. Sharma, M.K. Sharma // Biotechnol Adv. – 2009. – № 27(6). – P. 811-832. DOI: https://doi.org/10.1016/j.biotechadv.2009.06.004.
2. Prade R.A. Xylanases: from biology to biotechnology / R.A. Prade // Biotechnol Genet Eng Rev. – 1996. – № 13. – P. 101-131. DOI: https://doi.org/10.1080/02648725.1996.10647925.
3. Omogbenigun F.O. Dietary supplementation with multienzyme preparations improves nutrient utilization and growth performance in weaned pigs / F.O. Omogbenigun, C.M. Nyachoti, B.A. Slominski // J Anim Sci. – 2004. – № 82(4). – P. 1053-1061. DOI: https://doi.org/10.2527/2004.8241053x.
4. The expression of a xylanase targeted to ER-protein bodies provides a simple strategy to produce active insoluble enzyme polymers in tobacco plants / I. Llop-Tous et al // PLoS One. – 2011. – № 6(4). – P. 19474. DOI: https://doi.org/10.1371/journal.pone.0019474.
5. Herbers K. Thermostable Xylanase from Clostridium thermocellum Expressed at High Levels in the Apoplast of Transgenic Tobacco Has No Detrimental Effects and Is Easily Purified / K. Herbers, I. Wilke, U.A. Sonnewald // Bio/technology. – 1995. – № 13(1). – P. 63-66. DOI: https://doi.org/10.1038/nbt0195-63.
6. Production of Recombinant Proteins in Tobacco Guttation Fluid / S. Komarnytsky et al // Plant physiology. – 2000. – № 124(3). – P. 927-934. DOI: https://doi.org/10.1104/pp.124.3.927.
7. Production of recombinant proteins in plant root exudates / N.V. Borisjuk et al // Nature Biotechnology. – 1999. – № 17(5). – P. 466-469.
8. Herbers K. Apoplastic expression of the xylanase and β(1-3, 1-4) glucanase domains of the xyn D gene from Ruminococcus flavefaciens leads to functional polypeptides in transgenic tobacco plants / K. Herbers, H.J. Flint, U. Sonnewald // Molecular Breeding. – 1996. – № 2(1). – P. 81-87. DOI: https://doi.org/10.1007/BF00171354.
9. High expression of the xylanase B gene from Clostridium stercorarium in tobacco cells / J. Sun et al // Journal of Fermentation & Bioengineering. – 1997. – № 84(3). – P. 219-223. DOI: https://doi.org/10.1016/S0922-338X(97)82057-0.
10. Patent US6137032 United States. Xylanase obtained from an anaerobic fungus / K.J. Cheng, L.B. Selinger, J.H. Liu et al; Application filed by Agriculture and Agri Food Canada AAFC – US09/390,200 events; 07.09.99; 24.10.00. – 25 p.
11. Transgenic barley expressing a fungal xylanase gene in the endosperm of the developing grains / M. Patel et al // Molecular Breeding. – 2000. – № 6(1). – P. 113-124. DOI: https://doi.org/10.1023/A:1009640427515.
12. Molecular breeding of transgenic rice expressing a xylanase domain of the xynA gene from Clostridium thermocellum / T. Kimura et al // Applied Microbiology and Biotechnology. – 2003. – № 62(4). – P. 374-379. DOI: https://doi.org/10.1007/s00253-003-1301-z.
13. Isolation and identification of xylanase-producing strains during tobacco fermentation / FAN Yi-Wen et al // Modern Food Science and Technology. – 2013. – № 029(011). – P. 2658-2662.
14. GUO Qingji Screening of xylanase-producing bacteria, enzyme properties and construction and screening of macrogenomic library / GUO Qingji // Qingdao University of Science and Technology, 2008. DOI: https://doi.org/10.7666/d.y1399894.

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КСИЛАНАЗА ТҮЗЕТІН ШТАММДАРДЫҢ СКРИНИНГІ ЖӘНЕ ОНЫ МҰРЖАДА КЕПТІРІЛГЕН ТЕМЕКІ ЖАПЫРАҚТАРЫН АШЫТУ КЕЗІНДЕ ҚОЛДАНУ

Осы эксперименттік зерттеуде темекі жапырағынан оқшауланған ксиланаза шығаратын штамм шығарылды. Штаммдарды скринингтің және ксиланаза белсенділігін анықтаудың негізгі әдісімен ең жоғары ферментативті белсенділігі бар М416 штаммы алдын ала анықталды және М416 A. niger болды, фермент биологиялық қауіпсіздікке ие және оны мұржада кептірілген темекіні ашыту процесінде қолдануға болады. Ашыту шарттары колбадағы мәдениетті шайқау арқылы оңтайландырылды, нәтижесінде А-дан ксиланаза өндірісінің ең жақсы формуласы пайда болды. niger және оңтайлы өсіру температурасы, РН, егу мөлшері және өсіру уақыты алынды. Оңтайлы ашыту және хош иіс алу жағдайында штамм ашыту процесінде хош иісті заттардың құрамы мен құрамының өзгеруін анықтау және темекі жапырағын ашыту процесінде Нигер өндіретін ксиланазаның хош иісті құрамға әсерін бағалау үшін темекі жапырағының бетіне біркелкі шашыратылады, бұл штамм хош иісті заттардың құрамын тиімді арттыра алатынын көрсетеді.

Түйін сөздер: *ксиланаза, скрининг, сәйкестендіру, оңтайландыру, хош иісті заттар, ашыту және хош иісті өндіру.*

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СКРИНИНГ ШТАММОВ, ПРОДУЦИРУЮЩИХ КСИЛАНАЗУ, И ЕГО ПРИМЕНЕНИЕ ПРИ ФЕРМЕНТАЦИИ ВЫСУШЕННЫХ В ДЫМОХОДЕ ТАБАЧНЫХ ЛИСТЬЕВ

В ходе этого экспериментального исследования был выведен штамм, продуцирующий ксиланазу, выделенную из табачного листа. С помощью основного метода скрининга штаммов и определения активности ксиланазы был предварительно идентифицирован штамм М416 с наивысшей ферментативной активностью, и М416 был A. niger, фермент обладает биологической безопасностью и может быть использован в процессе ферментации табака, высушенного в дымоходе. Условия ферментации были оптимизированы путем встряхивания культуры в колбе, в результате чего была получена наилучшая рецептура производства ксиланазы из А. были получены niger и оптимальная температура культивирования, рН, количество инокулята и время культивирования. При оптимальных условиях ферментации и получения аромата штамм равномерно распыляют на поверхность табачного листа, чтобы определить изменения состава и содержания ароматических веществ в процессе ферментации и оценить влияние ксиланазы, продуцирующей нигер, на ароматическую композицию в процессе ферментации табачного листа, что показывает, что штамм может эффективно увеличивать содержание ароматических веществ.

Ключевые слова: *ксиланаза, скрининг, идентификация, оптимизация, ароматические вещества, ферментация и производство аромата.*

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