

Application of Plackett-Burman Design for the Optimization of Xylanase Production by Alkalo-Tolerant *Streptomyces xantholiticus* K7

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Abstract

Xylanase has become an indispensable option for environmental industrial biotechnology applications. The increasing demand for its large-scale production is prompting the use of a statistical optimization approach. In this study, the production of xylanase by the alkalo-halotolerant *Streptomyces xantholiticus* K7, isolated from the soil of Djendli Sebkha (salt lake) in eastern Algeria, was investigated for the first time. The optimization of the enzymes production using six different agro-industrial residues: date molasses, date flour, wheat bran, wheat straw, barley bran, and barley straw as sole carbon source was realized. The Plackett-Burman design (N=20) demonstrated that the xylanase production was strongly affected by date molasses, date flour, wheat bran, wheat straw, barley bran, and barley straw. All the byproducts show a positive effect on production; besides, no additional chemicals were needed to induce the enzyme production. After 5 days of cultivation at 30°C under shacked fermentation, the yield of xylanase increased by about 387% compared to the Horikoshi liquid medium. Because the halotolerant *S. xantholiticus* has a basic requirement for xylanase production, it became a promising tool for the valorization of lignocellulosic by-products.

Keywords: xylanase; Plackett-Burman design; alkalo-tolerant; *Streptomyces xantholiticus*; agro-industrial residues.

Резюме

Ксиланазата се е превърнала в незаменима опция за приложения в индустриалната биотехнология, свързани с околната среда. Нарастващото търсене на нейното масово производство подтиква използването на статистически подходи за оптимизация. В това проучване за първи път беше изследвано производството на ксиланаза от алкало-халотолерантен щам *Streptomyces xantholiticus* K7, изолиран от почвата на Джендли Себка (солено езеро) в източен Алжир. Оптимизирано е производството на ензима, като са използвани шест различни агроиндустриални отпадъци - меласа и брашно от фурми, пшенични трици, пшенична слама, ечемични трици и ечемична слама като единствен източник на въглерод. Дизайнът на Plackett-Burman (N=20) показва, че производството на ксиланаза е силно повлияно от финиковата меласа, финиковото брашно, пшеничните трици, пшеничната слама, ечемичните трици и ечемичната слама. Всички странични продукти оказват положително въздействие върху производството, освен това не са необходими допълнителни химикали за стимулиране на производството на ензими. След 5-дневно култивиране при 30°C в условията на ферментация в сандъци добивът на ксиланаза се увеличава с около 387 % в сравнение с течната среда на Horikoshi. Тъй като халотолерантният *S. xantholiticus* има основни изисквания за производството на ксиланаза, той се превръща в обещаващ инструмент за валоризация на лигноцелулозни странични продукти.

Introduction

The hydrolysis of xylan, the second most abundant natural polysaccharide, is considered one of the major industrial applications of this biopolymer (Polizeli *et al.*, 2005; Abena and Simachew,

2024). The primary chain of xylan consists of β -xylopyranose residues linked by β -1,4-glycosidic bonds, and its complete hydrolysis requires the synergistic action of multiple enzymes, among which

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endo-1,4- β -D-xylanase (EC 3.2.1.8) plays a pivotal role in xylan depolymerization (Qeshmi *et al.*, 2020). The diversity of chemical structures of xy-lans derived from the cell tissues of wood, cereals, and various plant materials has resulted in a wide range of xylanases with diverse hydrolytic activities, physicochemical properties, and structural features. Moreover, xylan compounds are commonly employed to induce xylanase production (Haltrich *et al.*, 1996; Jin and Yoon, 2016; Walia *et al.*, 2017) by microorganisms, using either solid-state or submerged fermentation to achieve enhanced yields (Sinjaroonsak *et al.*, 2020; Intasit *et al.*, 2022).

Xylanases and their producer microorganisms are currently applied in waste management for the generation of renewable fuels and chemicals. Furthermore, in the food, agro-fiber, pulp, and paper industries, these enzymes contribute to reducing ecological impact (Rojas-Pérez *et al.*, 2022). The oligosaccharides generated by xylanase activity are incorporated as functional food additives or as alternative sweeteners with beneficial properties. Several clinical trials in the pharmaceutical field have demonstrated that the consumption of xylo-oligosaccharides provides various health benefits, including a reduction in blood sugar and lipid levels, improved laxation, and positive effects on the immune response. Xylo-oligosaccharides are also widely recognized and consumed as prebiotics (Ibrahim, 2018; Abena and Simachew, 2024).

To satisfy industrial demands, more importance was given to the enzyme's stability in various processing conditions, including pH, temperature, and inhibitory ions, as well as its capacity to hydrolyze both soluble and insoluble xylan. Although numerous wild-type xylanases exhibit desirable properties such as thermostability and pH tolerance, and high catalytic activity, no single enzyme is sufficient to fulfill all the requirements of the food and feed industries.

Moreover, industrial applications requiring cost-effective enzymes, enhancing expression levels and efficient secretion of xylanases is essential for process sustainability; consequently, genetic engineering and recombinant DNA technology have a significant impact on the large-scale production of xylanases in both homologous or heterologous protein expression hosts (Chandel and Da Silva, 2013).

Actinobacteria are widely recognized for their role in nutrient recycling through the degradation of chitin, cellulose, starch, lipids, and complex carbohydrates, converting them into simple sugars via the secretion of diverse hydrolytic enzymes in

the rhizosphere (Vurukonda *et al.*, 2018). Recent studies highlight actinobacterial strains as promising xylanase producers, exhibiting broad pH and temperature stability profiles (Thomas *et al.*, 2025).

Nevertheless, many of these research studies employed commercial xylan for xylanase production (Liu *et al.*, 2020). Consequently, the applications of xylanase are limited due to the high cost of xylan, which increases the overall price of enzyme production. This challenge can be partially addressed by using low-cost nutrient sources as a suitable alternative in fermentation processes (Walia *et al.*, 2017). Among these inexpensive resources, agro-industrial by-products are favored because of their lower cost and greater availability. Accordingly, diverse by-product types, such as wheat bran, rice bran, rice straw, sugarcane bagasse, sawdust, and wheat husk, have been explored as carbon and nitrogen sources for microbial fermentation, thereby promoting xylanase biosynthesis in cost-effective bioprocesses (Oskay, 2025).

Optimizing the medium by modifying one independent variable while keeping the other factors constant has notable disadvantages: it is time-consuming and fails to identify the most influential factors (Liu and Tzeng, 1998). In contrast, statistical experimental designs, such as Plackett-Burman and response surface methods (RSM), successfully overcome the limitations by providing a more reliable evaluation of variable interaction and statistical significance. Among them, the Plackett-Burman design (Plackett and Burman, 1946) is a robust statistical tool that has been extensively employed to identify the most effective medium components for optimizing fermentation.

In line with the above, this research focused on optimizing and identifying the most significant factors influencing xylanase production through the Plackett-Burman design, using isolated actinomycetes for the conversion of agricultural waste into a valuable industrial enzyme.

Materials and Methods

Screening of xylanase production (Qualitative test)

Thirty actinomycetal strains of the genus *Streptomyces* sp. and *Nocardopsis* sp., isolated from three different sites in the Northeast of Algeria, Ezzemoul sebkha (35°53'14" N; 06°30'20" E), Djendli sebkha (35°43'15" N; 06°32'23" E), and Tinsilt chott (35°53'00" N; 6°29'00" E) - as previously reported in Smati and Kitouni (2019). The isolates were screened for their abilities to produce xylanase, by growing them on modified Nan-

mori medium (g/L): beechwood 10; Yeast extract 1; KH_2PO_4 4; NaCl 2; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 1; MnSO_4 0.05; $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ 0.05; CaCl_2 2; NH_4Cl 2; agar 15; pH 7) at 30°C for 7 days. The cultures were covered with a 1% (w/v) Congo Red solution for 10 minutes, and the elimination of excess Congo red was done by several washes with a 1M NaCl solution for 10 min (Nanmori *et al.*, 1990). The xylanase-producing strains were detected by the yellow spots around them against the red background and were selected as positive results (Ninawe *et al.*, 2008).

Screening of xylanase production (Quantitative test)

The selected twenty xylanase-positive strains were further tested for xylanase production by growing in Horikoshi liquid medium (g/L): wheat bran 20; Peptone 5; Yeast extract 5; KH_2PO_4 1; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ 0.1; pH 8.5) (Ninawe and Kuhad, 2005). One percent of each spore suspension of $\text{OD}_{600}=0.60$ is inoculated into production medium (50 mL) contained in Erlenmeyer flasks (100 mL) after autoclaved at 121°C for 20 min. The flasks were incubated in the rotary incubator shaker (180 rpm) at 30°C for 72 h. The cultures were stopped, centrifuged at 10000 rpm for 20 min at 4°C . The filtered supernatant was used as a source of enzymes.



Fig. 1. *Streptomyces xantholiticus* K7 on ISP2 media

Assay of xylanolytic activity

Xylanolytic activity was assayed using 1% (w/v) in sodium phosphate buffer (50Mm at pH 7) 4-O-Methyl-D-glucurono-D-xylan (SIGMA) as a substrate. The estimation of the reducing sugars released was determined by the dinitrosalicylic acid method (Miller, 1959) with xylose (SIGMA) used as a standard and expressed in terms of IU/mL. An international unit of enzyme activity (xylanase) was defined as the amount of enzyme required to release one μmol of reducing sugar/mL/min (xylose) under assay conditions.

Statistical optimization of xylanase production using Plackett-Burman design (PBD)

Plackett-Burman design (PBD) was applied to screen the medium and physical components for their influence on xylanase production by *S. xantholiticus* K7 (Fig. 1). By utilizing PBD, 19 independent and 5 dummies, they were assayed by representing them at two levels, low (-1) and high (+1), in 20 trials. Table 1 illustrates the levels of each factor and significance used in the experimental design.

Table 1. Variables and their levels screened in Plackett-Burman Design for the optimization of xylanase production conditions by *S. xantholiticus* K7

Variables	Units	Experimental values	
		(-1)	(+1)
A: wheat bran	g/L	0	20
B: wheat straw	g/L	0	20
C: dummy	-	-	-
D: barley bran	g/L	0	20
E: barley straw	g/L	0	20
F: dummy	-	-	-
G: date flour	g/L	0	20
H: date molasses	g/L	0	20
I: temperature	$^\circ\text{C}$	30	45
J: yeast extract	g/L	1	10
K: peptone	g/L	1	10
L: dummy	-	-	-
M: $(\text{NH}_4)_2\text{SO}_4$	g/L	0.5	5
N: $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	g/L	0.1	1
O: dummy	-	-	-
P: pH	-	05	09
Q: saline solution	mL/L	0	1%
R: dummy	-	-	-
S: $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	g/L	0.1	1

The flasks were incubated for 5 days in a rotary incubator at 100 rpm. Table 2 represents the design matrix.

Statistical analysis was conducted to provide a more robust and useful experimental design, using the STATISTICA software package. The BPD based on the first-order polynomial model:

$$Y = \beta_0 + \sum \beta_i X_i \quad (1)$$

Where Y is the response (enzyme), β_0 is the model intercept, β_i is the linear coefficient, and X_i is the level of the independent variable. This model did not describe the interaction among the factors. It was used to select and evaluate the important factors that affected the response. The magnitude of

Table 2. Plackett-Burman experimental design matrix for the evaluation of factors affecting xylanase production by *S. xantholiticus* K7

Run	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	Xylanase activity U/mL
1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	6.615
2	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	18.495
3	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	1.415
4	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	2.615
5	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	39.11
6	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	27.485
7	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	16.935
8	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	33.9
9	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	1	32.43
10	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	-1	9.86
11	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	1	20.805
12	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	-1	36.82
13	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	-1	30.65
14	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	-1	27.235
15	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	-1	27.78
16	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	1	8.175
17	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	1	20.725
18	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	-1	15.94
19	-1	1	1	-1	-1	-1	-1	1	-1	1	-1	1	1	1	1	-1	-1	1	1	25.91
20	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	00.00

the coefficient of a positive or negative signified the corresponding impact on the title. The value of the coefficient approaches zero, signifying a low or no effect. The p -value was the probability that indicated the magnitude of a contrast coefficient that resulted from the random variability of the design process. A low p -value indicated a significant effect. The significance of each variable was established by employing the F value. The mean xylanase activity was registered as t -value, p -values, proportion of variance R^2 determined that the model was significant at $p < 0, 05$ and ANOVA.

Results and Discussion

The global xylanase market is set to develop in the coming years due to the continuous and increased requirement of xylanase in various industries. At the same time, the management of agro-industrial by-products is becoming a vital objective to minimize the potential environmental impacts of these lignocellulosic by-products and to valorize them into value-added products.

Screening and Selection of xylanase-producing Actinomycetes

Of thirty alkalo-tolerant strains screened for their xylanolytic activity, twenty isolates showed

clear zones around the colonies after staining with Congo red (Fig. 2), confirming their ability to produce xylanase.

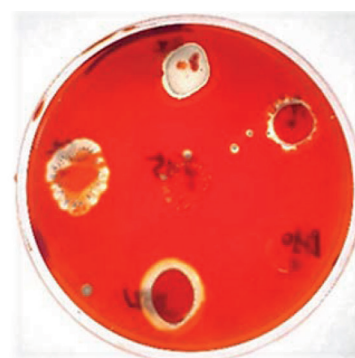


Fig. 2. Zones of clearance on modified Nammori medium (1%), beechwood by different species of actinobacteria

On further analysis, xylanase was produced from a lignocellulosic substrate, namely wheat bran, by these strains. It was found that the strains shown in Figure 3 reached the highest xylanase production levels of 10.1 IU/mL and 9.97 IU/mL, respectively, by the isolates K7 and J30.

Statistical optimization of media using Plackett-Burman design

The most commonly used statistical experimental designs are the Plackett-Burman design

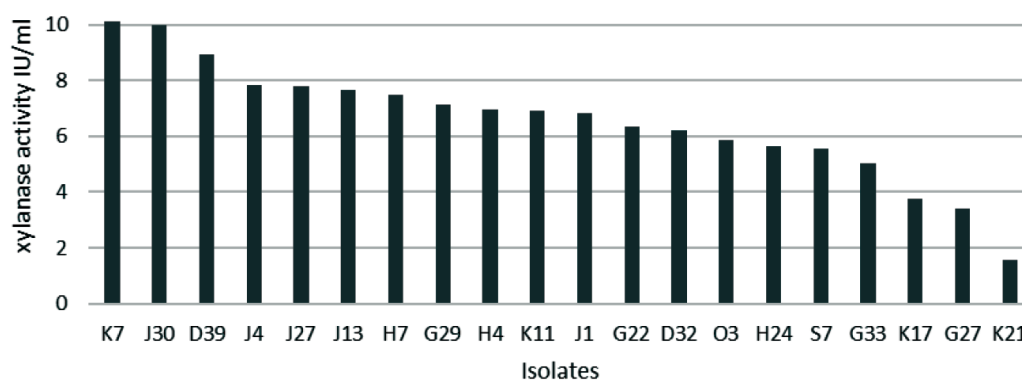


Fig. 3. Screening of different actinomycetes on Horikoshi medium for xylanase production

and the central composite design (Chaiyaso *et al.*, 2011). The main advantages of using these models are their simplicity and the capacity to evaluate a wide range of parameters on the relative effectiveness of a production process. Recent studies confirm their relevance: for instance, Plackett and Burmann combined with response surface methodology was successfully applied to optimize fermentation media and growth conditions of *B. velezensis* BHZ-29 (Shi *et al.*, 2024). Similarly, optimization of culture conditions for xylanase production by *Streptomyces* species remains an active research area, with recent work demonstrating improved yields using olive pomace as substrate and response surface methodology (Medouni-Haroune *et al.*, 2023). Op-

timization of culture conditions of xylanase production by estuarine *Streptomyces* sp. strain ER1 under submerged fermentation was reported.

Plackett-Burman is a fractional factorial design used to identify the significant factors affecting a response with two-factor interactions. It was carried out by using 20 runs (Table 2). A, B, D, E, G, H, I, J, K, M, N, P, Q, and S represent the constituents of the medium, and C, F, L, O, and R represent the dummy variables.

A data set of 19 media parameters was examined for their impact on xylanase production by *S. xantholiticus* K7 (Table 1). The experimental design protocol and the results are shown in Table 2. The data indicated a wide variation in the xylanase

Table 3. Estimated regression coefficients for xylanase production

Variables	Coefficient	Effect	-t-value	-p-value
constant	20.145	20.145	26.22414	0.000002
A: wheat bran	2.4435	4.887	3.18087	0.024514 ^a
B: wheat straw	2.2515	4.503	2.93093	0.032597 ^a
C: Dummy	-	-	-	-
D: barley bran	2.081	4.162	2.70898	0.042328 ^a
E: barley straw	2.038	4.076	2.65301	0.045263 ^a
F: Dummy	-	-	-	-
G: date flour	3.0735	6.147	4.00099	0.010313 ^a
H: date molasses	10.0595	20.119	13.09515	0.000046 ^a
I: Temperature	0.1655	0.331	0.21544	0.837935
J: yeast extract	0.837	1.674	1.08958	0.325603
K: peptone	0.3155	0.631	0.41071	0.698295
L: Dummy	-	-	-	-
M: (NH ₄) ₂ SO ₄	-0.3185	-0.637	-0.41461	0.695613
N: CaCl ₂ ·2H ₂ O	0.825	1.65	1.07396	0.331913
O: Dummy	-	-	-	-
P: pH	-2.414	-4.828	-3.14247	0.025595 ^a
Q: Saline solution	0.123	0.246	0.16012	0.879056
R: Dummy	-	-	-	-
S: MgSO ₄ ·7H ₂ O	-0.0645	-0.129	-0.08396	0.936343

* Significant at 5% level (p < 0.05)

activity from 0.00 to 39.11IU/mL in the 20 trials. The variables with confidence levels more than 95% were considered as the significant parameters.

The analysis of the regression coefficients and the *t*-values of 14 factors (Table 3) was significantly affected by seven factors. These were: (H) date molasses percent with an effect of 10.0595 ($p < 0.001$), followed by (G) date flour 3.0735 ($p < 0.01$), (A) wheat bran 2.4435 ($p < 0.05$), (B) wheat straw 2.2515 ($p < 0.05$), (D) barley bran 2.081 ($p < 0.05$) and (E) barley straw 2.038 ($p < 0.05$), whereas (P) pH -2.414 ($p < 0.05$) had negative significant effect on the xylanase production.

However, in this study, the three main variables (peptone, $(\text{NH}_4)_2\text{SO}_4$ and MgSO_4) had no effect on xylanase production ($p > 0.05$). The highest activity was observed in run 5, where five of the six significant factors were present and at their highest levels, while runs 20 and 3 showed the lowest activities of 0.00 and 1.415 U/mL, respectively. This could be related to the absence of all the significant medium components in run 20.

Considering the results displayed in Tables 3 and 4 and after exclusion of insignificant model terms ($p > 0.05$), the reduced polynomial equation (1) may be written as follows for the xylanase production:

$$Y = 20.145 + 2.4435 A + 2.2515 B + 2.081 D + 2.038 E + 3.0735 G + 10.0595 H - 2.414 P \quad (2)$$

Where A = wheat bran, B = wheat straw, D = barley bran, E = barley straw, G = date flour, H = date molasses, P = pH

The success of the design for *S. xantholiticus* (K7) xylanase production was analyzed by ANOVA as shown in Table 4. The model was further validated by R^2 and adj R^2 which were 0.9701 and 0.92024, respectively, indicating that 97% of the variability in the output could be explained by the model.

In the search for low-cost, effective media to support the growth of *S. xantholiticus* and induce xylanase production, different sources of carbon were introduced, including wheat bran, wheat straw, barley bran, barley straw, date flour, and date molasses. Our data demonstrated that date molasses (date syrup) is a superior xylanase inducer, with a positive effect ($p < 0.001$).

Date syrup is a by-product of date processing, which is widely used in the food industry as a sweetener and food preservative. It is also used for the bioproduction of value-added products, such as citric acid (Mostafa and Alamri, 2012), lactic acid (Ahmad *et al.*, 2021), arachidonic acid (Asadi *et al.*, 2018), and biohydrogen (Kamyab *et al.*, 2019). There are a few reports of enzyme productions using date syrup, like invertase (Abdul *et al.*, 2017), alpha amylase (Acourene and Ammouche, 2012). More recent studies have emphasized the valorization of date syrup and its by-products for microbial

Table 4. Statistical analysis by Plackett-Burman Design for xylanase production

Source	Sum of squares	df	Mean square	F-value	p-value Prob>F	
Model	38533.656	14	2752.404	233.2115	0.05	
A	119.414	1	119.414	10.1180	0.024514	
B	101.385	1	101.385	8.5904	0.032597	
D	86.611	1	86.611	7.3386	0.042328	
E	83.069	1	83.069	7.0384	0.045263	
G	188.928	1	188.928	16.0079	0.010313	
H	2023.871	1	2023.871	171.4829	0.000046	
I	0.548	1	0.548	0.0464	0.837935	
J	14.011	1	14.011	1.1872	0.325603	
K	1.991	1	1.991	0.1687	0.698295	
M	2.029	1	2.029	0.1719	0.695613	
N	13.613	1	13.613	1.1534	0.331913	
P	116.548	1	116.548	9.8751	0.025595	
Q	0.303	1	0.303	0.0256	0.879056	
S	0.083	1	0.083	0.0070	0.936343	
Residual	59.011	5	11.802			
Cor total	2811.414	19				

$R^2 = 0.97901$; Adj. $R^2 = 0.92024$

Significant

fermentations and sustainable bioprocesses (Khorshidian *et al.*, 2022; Alqahtani, 2024).

Date flour is the second most effective factor ($p < 0.01$). Similar findings have been reported, *B. licheniformis* KIBGE-IB3 producing xylanase using date fruit wastes as substrate through solid state fermentation (Aslam *et al.*, 2020).

Approximately 30% of the date fruits harvested in Algeria are wasted during the post-harvest stages (Oladzad *et al.*, 2021). The second quality dates, harvested after the separation operation, are processed as raw products in the date transformation industry to produce the aforementioned value-added products (Sirisena *et al.*, 2015). Since dates contain a significant amount of microbial growth nutrients, they are considered a valuable substrate for the processes of fermentation (Matloob, 2014). Date syrup contains sugars such as glucose, fructose, sucrose, and various reducing sugars; minerals, which include selenium, copper, phosphorus, potassium, and magnesium; as well as vitamins (Al-Farsi and Lee, 2008), acting as a substrate that might simultaneously supply the bacteria with all necessary nutrients for both bacterial growth and enzymatic induction.

Converting date waste into value-added products is a sustainable approach that provides both environmental and economic advantages while minimizing the challenges of traditionally managing date waste (Oladzad *et al.*, 2021; Khorshidian *et al.*, 2022; Alqahtani, 2024). These results indicate that wheat bran, wheat straw, barley bran, and barley straw can induce considerable levels of xylanase from *S. Xantholiticus* (K7).

Wheat bran is an inexpensive by-product that contains a high amount of xylan. It is one of the most popular components of complex media for xylanase production by *Streptomyces*, such as *S. thermocarboxydus* subspecies MW8, *S. cuspidosporus*, and *Streptomyces* sp. P12-137 (Coman and Bahrim, 2011), *Streptomyces* sp. QG-11-3 (Beg *et al.*, 2000) and *S. thermocarboxydus* (Tran *et al.*, 2021).

Similarly, wheat straw can induce considerable levels of xylanase from *S. xantholiticus* K7. This is supported by literature, which promotes the production of xylanase by an array of strains, such as *Streptomyces* sp. strain MS-S2 (Danzo *et al.*, 2022), *Cellulomonas* sp. CX4, *Cellulomonas* sp. CX5, *Bacillus* sp. CX6, *Bacillus* sp. CX10, *P. illinoisensis* CX11, *P. sp.* CX14, *B. cereus* CX15, and *Bacillus* sp. CX16, *P. barcinonensis* CX17, and *Cellulomonas* sp. CX20 (Ahmed *et al.*, 2018).

As a rule of thumb, the incorporation of zero-cost substrates (e.g., lignocellulose by-products) in the production media will definitely decrease the expenditure of the bioprocess.

Similar findings were documented by Abd El Nasser and Foda (1995), who showed that a variety of agricultural by-products, such as wheat bran, wheat straw, and corn cobs, enhanced the levels of xylanase activities relative to xylanase produced on pure xylan by *S. griseoflavus* and *S. violarius*.

Results indicate that barley bran also has a potential xylanase inducer with the other substrates used in this study. Bran fraction is rich in fibers (e.g., cellulose, hemicellulose, and lignin), which have an impact on microbial fermentation and enzyme production (xylanase, amylase, cellulase) (Katina *et al.*, 2007; Messia *et al.*, 2016). These findings align with recent studies on the valorization of lignocellulosic wastes for sustainable enzyme production, where agricultural residues have been shown to facilitate high enzymatic titers (El-Gendi *et al.*, 2023).

Intestinal isolate *S. marcescens* HK2 produced higher xylanase activity when grown in media containing barley straw as the sole carbon source (Sharma *et al.*, 2021). Our results agree with the previous finding that barley straw may serve as a suitable alternative to costly xylan in production media.

The tested ranges (5-9) of the initial medium pH result in a significant negative effect on xylanase production by *S. xantholiticus*. However, if the primary effect value of the tested variable is positive, then its impact on the response variables is more significant at a higher level; when the value is negative, its influence is more important at a lower level (Maan *et al.*, 2016). pH controls xylanase production by affecting the enzymatic system (Subramaniyan and Prema, 2002; Maan *et al.*, 2016).

While *S. olivaceus* (MSU3) was maximum at pH 7 (Rosmine *et al.*, 2019), and another *Streptomyces* sp. The initial optimal pH of the media was 7 to 8 (Bajaj and Singh, 2010).

Conclusion

The xylanase used in this study has unique characteristics, providing a useful strain on an industrial scale. In the present work, we demonstrated the optimization of *S. xantholiticus* K7 culture conditions and medium composition using a factorial experiment design, resulting in a significantly increased xylanase production yield. This method has proven to be a successful and valuable strat-

egy for screening, optimization, and modeling of the fermentation process. Xylanase production through submerged fermentation on agro-residues, including date molasses, date flour, wheat bran, wheat straw, barley bran, and barley straw, constitutes an alternative lignocellulosic source for enzyme production, bio-technological applications, and the most effective xylanase production inducer. The impact of different processing parameters on enzyme yield and production was shown to be significantly influenced by date molasses, date flour, wheat bran, wheat straw, bran, and barley straw. The six influential variables selected by the Plackett-Burman design were identified as the primary significant factors in xylanase production by *S. xantholicus*.

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