Electronic Journal of Biotechnology 78 (2025) 76–85

Contents lists available at ScienceDirect

Electronic Journal of Biotechnology

journal homepage: www.elsevier.com/locate/ejbt

Research article

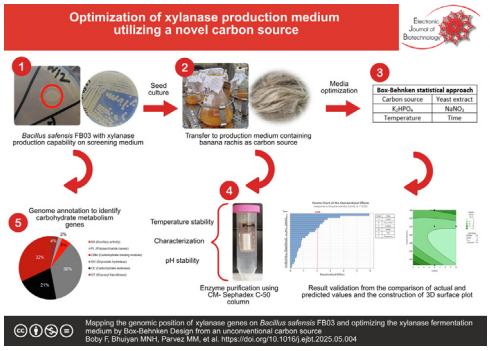
Mapping the genomic position of xylanase genes on *Bacillus safensis* FB03 and optimizing the xylanase fermentation medium by Box-Behnken Design from an unconventional carbon source ☆

Farhana Boby [a,*], Md Nurul Huda Bhuiyan [a], Md Mashud Parvez [a], Md Jahidul Islam [b], Ifrat Jannati [b]

[a] BCSIR Dhaka Laboratories, Bangladesh Council of Scientific and Industrial Research, Dhaka 1205, Bangladesh
[b] BCSIR Rajshahi Laboratories, Bangladesh Council of Scientific and Industrial Research, Rajshahi 6206, Bangladesh

GRAPHICAL ABSTRACT

Mapping the genomic position of xylanase genes on *Bacillus safensis* FB03 and optimizing the xylanase fermentation medium by Box-Behnken Design from an unconventional carbon source.



ARTICLE INFO

Article history:
Received 24 December 2024
Accepted 12 May 2025
Available online 31 July 2025

Keywords:
Bacillus safensis
Banana rachis
Box-Behnken Design (BBD)
Genome annotation
Industrially important enzymes
Lignocellulosic biomass
Media optimization
Peel of banana tree
Xylanase fermentation
Xylanase genes

ABSTRACT

Background: The ability of *Bacillus safensis* to synthesize xylanase and other industrially important enzymes utilizing lignocellulosic biomass makes it advantageous for a variety of biotechnology applications. Thus, the current investigation aimed to optimize conditions and medium components for maximizing xylanase production by a newly isolated *Bacillus safensis* strain using banana rachis (peel of banana tree) as a novel source of carbon.
Result: Upon employing Box-Behnken Design (BBD) statistical approach, the highest enzyme activity was obtained 25.24 U/ml at 2 g/L banana rachis, 1 g/L yeast extract, 1 g/L K_2HPO_4, 5 g/L $NaNO_3$, 35°C and 72 h of incubation time. The purified enzyme showed 10 times higher enzyme activity (143.6 U/ml) with 2.3 mg/ml protein concentration. The enzyme was found to maintain stability up to 60°C in a wide range of pH (6 to 10). Analysis of whole genome sequencing data revealed the presence of xylanase production and xylan metabolic genes (xynA, xynB, xylP, xylT) on *Bacillus safensis* FB03. Also, from genome annotation, different carbohydrate metabolic genes such as glycoside hydrolases (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), auxiliary activities (AAs), and carbohydrate binding modules (CBMs) were identified.

☆ Audio abstract available in Supplementary material.
Peer review under responsibility of Pontificia Universidad Católica de Valparaíso.
* Corresponding author.
E-mail address: farhana_boby@bcsir.gov.bd (F. Boby).

https://doi.org/10.1016/j.ejbt.2025.05.004
0717-3458/

Conclusions: In accordance with our research, banana rachis can be considered as a major medium component to develop an economical fermentation process for the production of xylanase by *Bacillus safensis* FB03. Additionally, identification of the genomic location of xyl genes provides valuable insight towards genetic engineering for the development of a more potent industrial strain.

How to cite: Boby F, Huda Bhuiyan MN, Parvez MM, et al. Mapping the genomic position of xylanase genes on *Bacillus safensis* FB03 and optimizing the xylanase fermentation medium by Box-Behnken Design from an unconventional carbon source. Electron J Biotechnol 2025;78. https://doi.org/10.1016/j.ejbt.2025.05.004.

1. Introduction

In recent days, exploration of novel biological sources for manufacturing industrial enzymes has become necessary due to increased industrial demand, harmful effects of synthetic chemicals, and other concerning issues. Xylanases are one of the key enzymes utilized in food, feed, agro-fiber, and paper and pulp processing industries to convert lignocellulosic biomass into value-added products [1]. Along with its bulk industrial applications, xylanase and their producers are being exploited in pollution control and biofuel generation [2]. Chemically, the enzyme is involved in the depolymerization of xylan to transform it into simple monosaccharide and xylo-oligosaccharides [3]. Since xylanase does not produce any harmful compounds during the enzymatic reaction, it is the greatest option for hydrolyzing xylan when compared to other alternative procedures [4,5].

Industrial-scale xylanase is synthesized mostly by microorganisms like bacteria, fungi and actinomycetes [6]. There are types of bacteria commonly used in industrial fermentation as they mostly synthetize extracellular enzymes aiding ease of downstream processing [7,8]. However, the prohibitive cost of growth and production media is one obstacle to the commercial use of microorganisms in industrial biosynthesis processes. Over the last few years, lignocellulosic biomass has drawn a lot of research interest as a way to succeed with this biotechnological strategy of low-cost enzyme production [9]. Additionally, bacterial enzyme biosynthesis utilizing a wide range of inexpensive substrates including waste materials, agricultural residues, and lignocellulosic biomass as a carbon source is considered an eco-friendly and sustainable technique. Along with other bacterial species, some researchers have reported *B. safensis,* a bacterium from the *Bacillus pumilus* group, as a potent producer of xylanase [10,11]. However, variation of enzyme production among different species has necessitated the urge of screening out the most potent producer strain to support industrial purposes [7]. *Bacillus safensis* is a well-described species with the efficiency of producing industrially important enzymes like protease [12], lipase [13], chitinase [14], amylase [15], inulinase [16], beta-galactosidase [17], xylanase [18], keratinase [19] and others. Apart from this, it serves as a plant growth promoter in halophilic environments under high salinity stress [20,21], probiotic [22], biocontrol and bioremediation organisms [23,24]. Its iniquitousness and non-pathogenicity towards humans have made it a safe industrial microorganism to produce industrial enzymes and industrially applicable secondary metabolites.

Therefore, to ensure a sustainable eco-friendly economy, along with sorting out potential xylanase-producing *B. safensis*, optimization of media components for maximizing production is a must. Due to excessive cost, it is impractical to apply pure xylan as a substrate of enzyme synthesis. Though a number of agricultural residues like rice husk, coconut husk, corn stock, corncob, wheat bran, rice bran have already been utilized as cheap carbon and nitrogen sources [25], banana rachis (peel of banana tree) is still an unexplored item that deserves special attention as every year tons of them are wasted worldwide.

In consideration with these facts, the present study aims to report the xylanase synthesis potential of locally isolated *Bacillus safensis* FB03 utilizing banana rachis as a novel growth substrate. Our objective is to optimize the cultivation condition using Response Surface Methodology (RSM) combined with Box-Behnken statistical Design (BBD) followed by enzyme purification, biochemical characterization, and detecting the precise genomic location of xylanase genes.

2. Materials and methods

B. safensis FB03 was isolated and identified from the rhizosphere soil of the Bahrind region of Bangladesh. The bacterium was identified, sequenced, and the sequence data were deposited on NCBI gene bank (https://www.ncbi.nlm.nih.gov/bioproject/?term=PRJNA1161822) with accession number JBHILD000000000.

2.1. Screening of xylanase-producing isolate

100 µl of soil sample diluted into normal saline was spread over the surface of a nutrient agar plate and incubated at 37°C for 24 h. To verify purity, the obtained discrete colonies were sub-cultured for several times. The potency of these purified isolates as xylanase producers were evaluated on Minimal Salt Media (MSM) plate (contained g/L $NaNO_3$, 5; KH_2PO_4, 1; K_2HPO_4, 2, $MgSO_4{\cdot}7H_2O$, 0.5; KCl, 0.1; $CaCl_2$, 0.01; $FeSO_4{\cdot}7H_2O$, 0.02; agar, 17, distilled H_2O, 1 L; pH = 7) supplemented with 0.5% beech wood xylan [26]. Further, bacteria were allowed to grow and produce an enzyme on 50 ml MSM containing 2% banana rachis in place of xylan as a carbon source. After incubating at 37°C for 48 h under 120 rpm shaking condition, the supernatant was separated by centrifugation to measure enzyme activity.

2.2. Quantitative enzyme assay and protein concentration

Enzyme activity was measured by following the methodology described previously [27]. It was extracted through centrifugation of the culture at 6000 rpm for 20 min. 1% (wt/v) xylan prepared in 100 mM sodium phosphate buffer (pH 7.0) was used as substrate, and the liberated xylose was measured by 3′2,5 di-nitro salicylic acid (DNS) utilizing a xylose standard curve. Herein, "the amount of enzyme required to release 1 µmol of xylose per minute under standard assay conditions was defined as "one unit of *endo*-1, 4-β-xylanase". The total protein content was estimated by the Bradford protein estimation method using Bovine serum albumin as standard [28]. The further work was conducted with the isolate having the highest enzyme activity.

2.3. Optimization of production parameters: The Box-Behnken Design (BBD)

To ensure the maximum productivity, the fermentation media were optimized by the Box-Behnken statistical method using minitab statistical software. Here, six factors were considered as critical for basal medium composition (Table 1), and 54 runs were executed where each factor had three levels (−1, 0, +1). In BBD, the maximum xylanase production condition was determined by comparing the experimental results obtained at 0-point value with that found at −1 or +1 values. Following the rule R = n + 1, 54 experiments were generated where R denotes the number of runs, n stands for the number of critical factors. The Box-Behnken experimental design was achieved based on the following first-order model:

$$Y = \beta 0 + \sum BiXi$$

where Y represents the response (xylanase activity U/mL), β0 is the model intercept, Bi is the linear coefficient, Xi is the level of independent variable, and k is the number of involved variables.

2.4. Scaled-up production in the optimized medium

To scale up xylanase production, the optical parameters from the statistical design experiments were employed. A 5% bacterial culture was added to each Erlenmeyer flask containing 400 ml of the medium, and the flasks were then incubated under shaking at the ideal conditions. Following incubation, the cell-free supernatant was recovered and enzyme activity was measured as described previously.

2.5. Purification

The resultant supernatant was then purified by chromatography on CM-Sephadex C-50 column (20 cm × 2.0 cm), pre-equilibrated with 50 mM sodium phosphate buffer (pH 6.0) at a flow rate of 30 ml/h. using a continuous gradient of 0–1.0 M NaCl. The bound proteins were eluted, and the fractions of 5 ml each were collected and analyzed for protein content and xylanase activity. The fraction with highest enzyme activity was then concentrated using Amicon ultrafiltration cell membrane (10 kDa cut off). Each of the experiments was performed in a triplicate set.

2.6. Effect of physicochemical factors on xylanase stability

To assess thermal stability, the residual xylanase activity was measured after preincubating the enzyme preparations at 30–90°C in 0.1 M sodium phosphate buffer (pH 7.0) without substrate for 1 h. Similarly, pH stability was measured after 24 h of incubation of enzyme preparations in pH buffers ranging from 4.0 to 10.0 at 25°C. Later, the residual activity was quantified under ideal assay conditions.

Table 1
Factors and levels of independent variables in BBD for xylanase production.

Factor name (g/L)	Low value (−1)	Median (0)	High value (+1)
Carbon source	2	6	10
Nitrogen source	0	0.5	1
K_2HPO_4	0	0.5	1
$NaNO_3$	0	2.5	5.0
Temperature (°C)	35	40	45
Time (h)	24	48	72

2.7. Kinetic parameters

The xylanase's *Km* and *Vmax* values were ascertained by evaluating the enzyme's activity with varying xylan substrate concentrations. The activity was measured under the assay conditions as described previously. To find *Km* and *Vmax*, the Lineweaver-Burk and Michaelis-Menton plots were obtained.

2.8. Genome annotation of B. safensis FB03

Genome annotation and identification of the position of different xylanase genes in *B. safensis* FB03 were accomplished by Prokssee with default parameters [29]. Carbohydrate-Active Enzyme Database (CAZy) was also annotated to identify carbohydrate-active enzyme domain in the genome of FB03 [30].

3. Results

3.1. Xylanase activity and protein concentration

On xylan agar plates, 10 bacterial strains out of 64, isolated from soil of the study site, were discovered to create holos around their colonies. Isolate 9 (*B. safensis* FB03) was found to produce hollo zone of about 21 mm around its growth on a screening agar plate. When these isolates were allowed to synthesize xylanase on MSM, iso9 came out with the highest enzyme activity 14.95 ± 1.31 U/ml (Fig. 1).

3.2. Optimization of key factors for the synthesis of xylanase

During media optimization by BBD statistical design, a total of 54 runs were conducted to ascertain the optimal xylanase production conditions of *B. safensis* FB03. The obtained results for BBD runs (Table 2) show that the lowest activity was 0.12 U/ml in run no. 22 and the highest activity of 25.24 U/ml was provided by run 2 where the factors were 2 g/L banana rachis, 1 g/L yeast extract, 1 g/L K_2HPO_4, 5 g/L $NaNO_3$, 35°C and 72 h of incubation time. The software determined the predicted value using a quadratic equation (Table 2). According to the R^2 or coefficient of determination (0.936), 93% of the variability can be explained by the model. The adjusted R^2 value of 0.931 expressed the degree of closeness between the actual and the predicted values.

The observed value curve as a function of predicted values (Fig. 2) displays the correlation as the points are distributed around the regression line. Fig. 2 shows that the estimated xylanase production was within the limits of the experimental factors since the actual values and the predicted response values coincided well. As a result, the model is deemed to be of adequate quality, having a 93.36% probability of explaining the observed response variability.

3.3. Pareto chart and interaction of variables

The Pareto chart of standardization histogram graph indicated that time (E), temperature (F), yeast extract (B), the interaction between temperature and $NaNO_3$ (DF), yeast extract and temperature (BF), and banana rachis and yeast (AB) were significant since they all crossed the *p*-value (<0.05) (Fig. 3). The response surface plot was created with the Z-axis (xylanase activity) versus any two variables while keeping the other variables at hold values (Figs. 4A, 4B, 4C).

Fig. 4A illustrates the contour plot of carbon and yeast extract vs enzyme activity. High activity was obtained at low (2 g/L) to high (10 g/L) concentrations of banana rachis in 1 g/L yeast extract. Fig. 4B shows that the minimum activity was attained at 35°C to 37°C at all concentrations of carbon source. Fig. 4C demonstrates

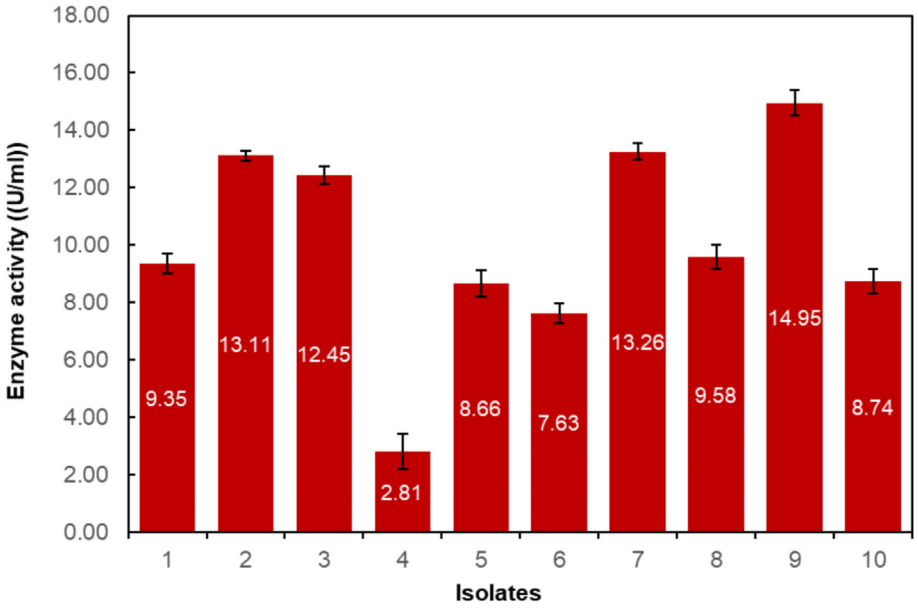


Fig. 1. Enzyme activity of 10 isolates on MSM media.

that at the highest carbon concentration, xylanase activity started to increase after 50 h of incubation.

3.4. Purification and characterization of enzyme

After purification and concentration, the enzyme from FB03 showed the maximal activity of 143.6 U/ml, roughly ten times than the crude one, where the protein content was 2.3 mg/ml. When enzyme characterization was studied, the findings demonstrated that, after 60 min of incubation from 30 to 60°C, there was no discernible drop-in xylanase activity (Fig. 5A). However, at 60°C, xylanase activity was 134.77 U/ml and at 70°C, the enzyme showed sensitivity, maintaining 40.86 U/ml. Above that, the stability of xylanase was found to steadily decrease with the rise of temperature. Similarly, the stability of xylanase was observed throughout a wide range of pH values, from 6 to 10, ranging from neutral to alkaline (Fig. 5B).

3.5. Scaled-up fermentation in optimized conditions for further study

Scaled-up fermentation was carried out to compare the enzyme activity with the smaller-scale production. Under run 2 conditions in 1L medium (2 g/L banana rachis, 1 g/L yeast extract, 1 g/L K_2HPO_4, 5 g/L $NaNO_3$, 25°C, and 72 h of incubation time), the measured activity was 136.90 U/ml, which was close to the smaller-scale enzyme production (134.77 U/ml).

3.6. Kinetic analysis

As determined by Lineweaver-Burk plot using beechwood xylan as substrate, the *Km* and the *Vmax* were 0.64 mg/ml and 156.25 µmol/min/mg (Fig. 6).

3.7. Genomic annotation of xylanase and other carbohydrate-active enzymes genes

Proksee-based annotation of enzyme domain facilitated the identification of six different genes xynA, xynB1, xynB2, xynB3, xylP, and xylT on FB03 associated with xylan breakdown and utilization (Table 3). xynA is identified as the key gene that encodes an endo-β-1,4-xylanase that acts to hydrolyze the β-1,4-glycosidic bonds in xylan. xynB1, xynB2, xynB3, other genes of this operon, work synergistically with xynA to facilitate the breakdown of xylan. While xylT encodes for xylose transporter protein to facilitate the uptake of xylose into the cell from the extracellular environment, xylP encodes xylose permease, another transporter protein involved in the movement of xylose across the cell membrane. According to the findings of the CAZy database annotation results (Fig. 7), six different functions—glycoside hydrolases (GHs), glycosyl transferases (GTs), polysaccharide lyases (PLs), carbohydrate esterases (CEs), auxiliary activities (AAs), and carbohydrate binding modules (CBMs)—have been identified. The percentages of enzyme functions with annotations are 38.58%, 34.51%, 1.01%, 12.69%, 2.03%, and 11.17%, in that order. Moreover, 76, 68, 2, 25, 4, and 22 genes are involved in the indicated functions, correspondingly.

4. Discussion

The global demand of xylanases in the industrial sector for a range of biotechnological applications has recently made it necessary to expand the field's research logically. While the isolation of endophytic organisms for the synthesis of industrially significant enzymes has been documented previously [31], little research has been done on *B. safensis*, a firmicute bacterium with enormous potency. Therefore, through careful selection of dozens of bacteria, here, a distinctive endophytic strain of *B. safensis* was isolated from the crop field of featured Bahrind soil to evaluate its efficacy as xylanase synthesizer. Parallelly, a novel carbon source was utilized to ensure a sustainable cost-effective bioprocess for large-scale enzyme production. Although selection of the appropriate strain is crucial for a successful industrial enzyme factory, it will not ensure maximal production unless the process is adjusted correctly [32]. Therefore, the current study also focused on media optimization for maximizing enzyme production. Besides, the whole genome of FB03 was annotated to shed light on its inherent ability to produce xylanase and other extracellular enzymes so that genetic engineering can be done in future to turn this isolate as a valuable industrial workhorse. To the best of our knowledge, it is the first ever reported work that marks the number and position

Table 2
Result of BBD matrix for six significant independent variables tested with predicted and observed responses for xylanase production.

Run order	Experimental value (coded value) g/L						Enzyme activity (U/ml)	
	Carbon	Yeast extract	K_2HPO_4	$NaNO_3$	Time	Temperature	Observed	Predicted
1	10	1	1	5	72	45	2.70	2.63
2	2	1	1	5	72	35	25.24	20.02
3	2	0	0	5	72	35	11.88	11.85
4	6	0.5	0.5	3	48	40	4.15	4.40
5	10	1	1	1	24	45	13.32	11.21
6	10	1	0	1	24	35	10.27	10.58
7	10	1	0	1	24	35	11.58	10.58
8	6	0.5	0.5	3	48	40	4.29	4.40
9	6	0.5	0.5	3	48	40	4.20	4.40
10	10	1	0	0.5	24	45	8.13	8.54
11	10	0.5	0.5	3	48	40	4.02	4.51
12	2	1	0	5	24	35	13.75	14.41
13	2	1	1	1	72	45	2.70	2.78
14	10	1	0	5	72	35	16.26	14.89
15	10	0	1	5	72	35	4.92	4.84
16	6	0.5	0.5	3	48	40	5.67	4.40
17	2	0	0	1	24	35	6.11	5.65
18	6	0.5	−0.68	3	48	40	3.83	3.33
19	6	0.5	0.5	3	48	40	3.61	4.40
20	2	1	0	1	72	35	6.50	7.18
21	10	0	0	5	72	45	1.00	0.41
22	10	0	1	1	72	45	0.12	−1.08
23	6	0.5	0.5	7.75	48	40	3.16	4.95
24	6	0.5	0.5	3	105	40	2.60	2.30
25	2	0	0	1	72	45	4.46	4.34
26	6	0.5	0.5	3	48	40	3.13	4.40
27	6	0.5	0.5	3	48	51	1.11	1.22
28	2	0	1	5	24	35	13.60	13.01
29	2	0.5	0.5	1	72	40	2.05	2.55
30	10	1	0	1	72	45	4.31	4.31
31	6	0.5	0.5	3	48	40	4.68	4.40
32	6	−0.68	0.5	3	48	40	8.15	7.46
33	10	0	0	1	72	35	1.00	0.15
34	2	0	1	1	72	35	4.34	5.99
35	2	0	1	5	72	45	0.40	0.38
36	6	0.5	1.6	3	48	40	2.42	6.04
37	6	0.5	0.5	3	48	40	1.62	4.40
38	6	0.5	0.5	3	48	40	3.27	4.40
39	6	0.5	0.5	3	48	40	4.33	4.40
40	10	0	0	1	24	45	7.02	7.74
41	2	0	0	5	24	45	7.82	7.26
42	2	1	1	5	24	45	3.14	3.74
43	2	1	1	1	24	35	14.84	15.07
44	2	0	1	1	24	45	5.46	6.30
45	6	0.5	0.5	3	48	35	8.36	8.85
46	2	1	0	5	72	45	2.40	2.17
47	6	1	0.5	3	48	40	8.58	8.23
48	2	1	0	1	24	45	3.18	3.04
49	10	1	1	1	72	35	14.58	15.00
50	6	0.5	0.5	−1.7	48	40	2.36	1.13
51	10	0	1	1	24	35	8.84	6.33
52	6	0.5	0.5	3	24	40	5.06	6.03
53	10	0	1	5	24	45	1.18	0.58
54	10	0.5	1	3	48	35	9.47	10.29

of xyl genes in the genome of *Bacillus safensis* along with other genomic traits.

When the strain was grown on banana rachis for 2 d at 37°C and pH 7.00, maximum enzyme production was noted for Iso9, that is 14.96 U/ml. This enzyme activity suggests further optimization of growth parameters and production conditions to avail the maximum enzyme activity by FB03. Despite the fact that a number of studies have documented the sustainability of a range of lignocellulosic materials—including sugarcane bagasse, rice straw, rice bran, wheat straw, corn cob, and ragi bran—as raw materials for the production of xylanase [33,34], this is the first ever reported use of banana rachis in this instance for the synthesis of xylanase from *B. safensis*. A similar study reported the value 15.21 ± 0.58 U /ml in relation to sawdust's aptitude for producing xylanase from *B. safensis* [18].

To significantly increase xylanase production, medium and culture parameters must be optimized, a goal that can be achieved through Response surface methodology (RSM) [35]. In contrast to the other methods, this approach thoroughly analyzes the interactive effects of every independent element in a fermentation process and looks at the particular interactions between a response variable and a number of design-independent variables [36]. To maximize FB03′s xylanase production, the Box–Behnken design (BBD), one of the best RSM designs, was used [37], taking into account five factors. According to regression analysis, the high R^2 value (0.936) indicates that the model fits the data well rendering only 6.4% of the total variances unexplained. Additionally, the importance of the model was confirmed by the high corrected R^2 (0.936). The maximum xylanase activity was obtained at 2 g/L banana rachis, 1 g/L yeast extract, 1 g/L K_2HPO_4, 5 g/L $NaNO_3$,

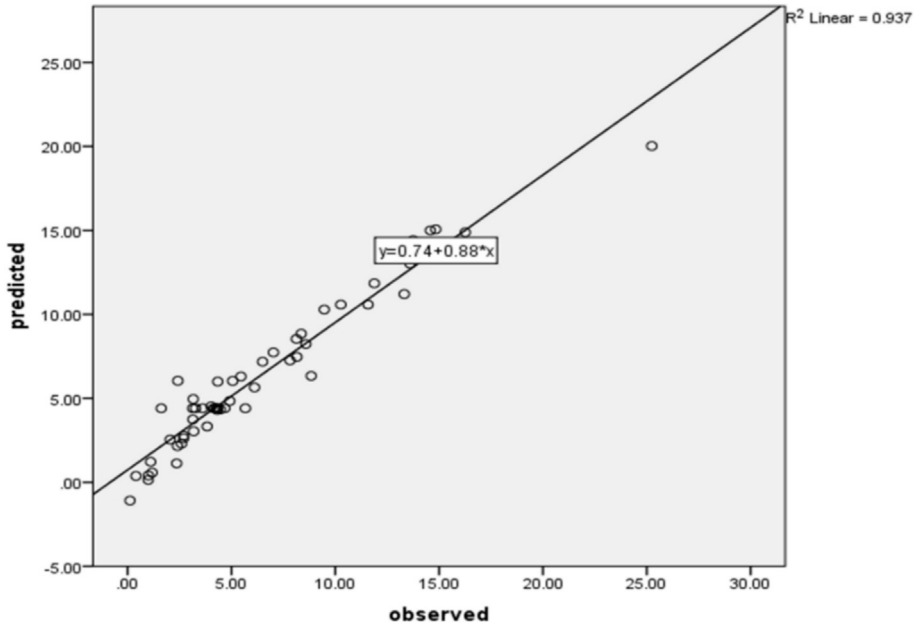


Fig. 2. Regression correlation between observed and predicted values.

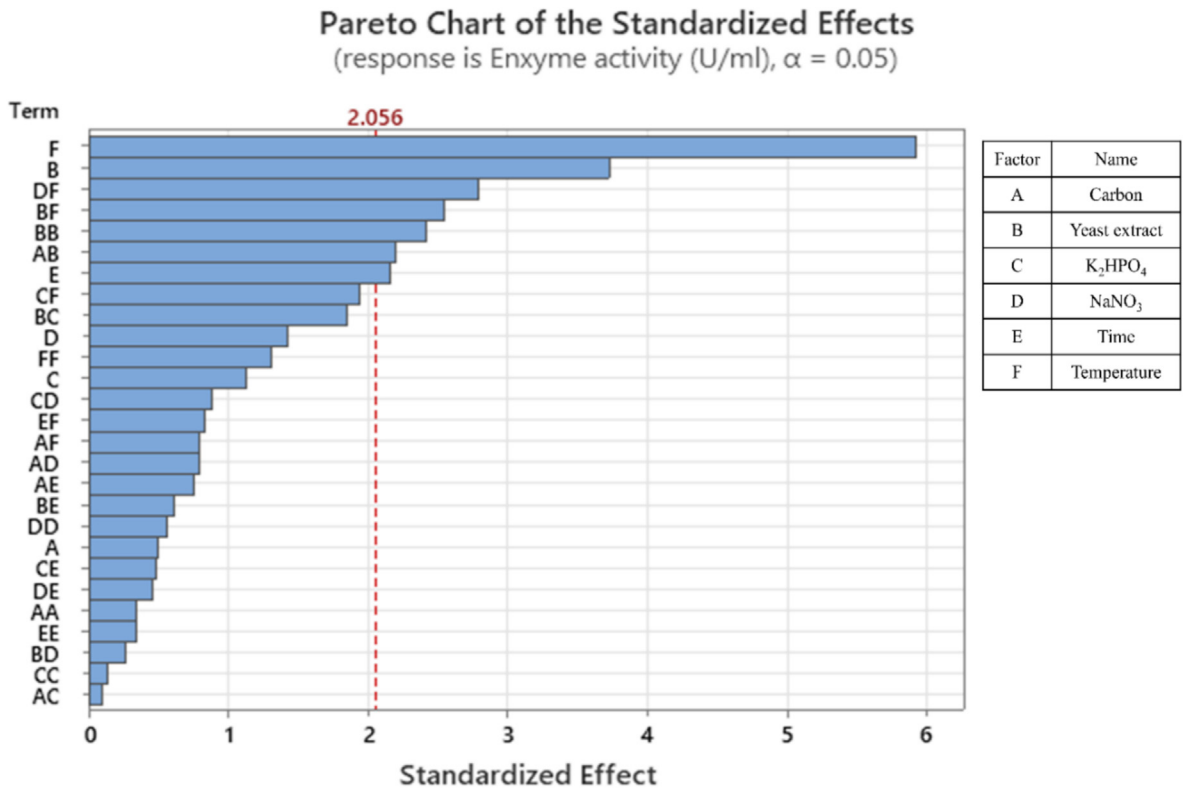


Fig. 3. The Pareto chart of standardization ($p < 0.05$).

35°C and 72 h of incubation time where a substrate concentration efficacy of banana rachis as a potential carbon source for bacterial enzyme production even at a low concentration. At pH 7, with 3 g/L xylan and 5 g/L peptone, and after 24 h of incubation at 40°C, the maximum amount of xylanase activity of *Bacillus haynesii* was 35.02 U/ml [38]. As the optimal conditions for the production of xylanase differ from strain to strain, therefore, it is crucial to optimize parameters for each strain of *Bacillus*. So far, the optimization of ideal conditions for xylanase production by *Bacillus safensis* using BBD has not been reported. Considering collectively, this research may represent a new finding on the optimization of xylanase production from the *Bacillus safensis* species.

Due to the presence of impurities, protein quantification, physicochemical testing, and activity assays may become challenging [39]. Hence, purification of xylanases is required to eliminate contamination from proteins, other enzymes, including cellulases,

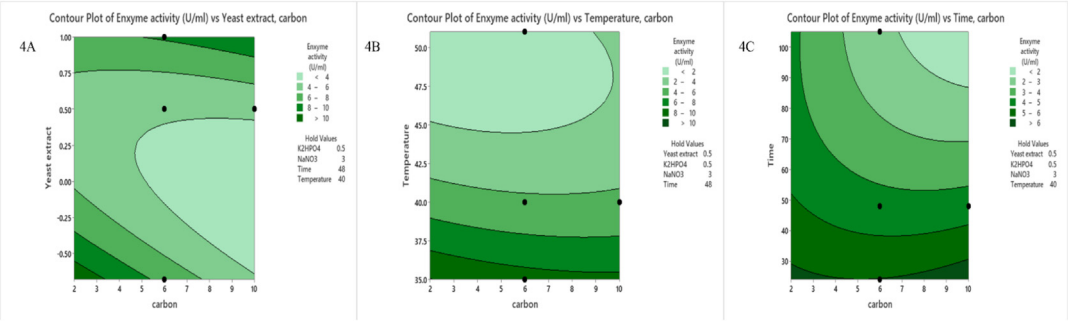


Fig. 4. Contour plot showing the interactive effect of (A) carbon, yeast extract; (B) carbon, temperature; (C) carbon, time on the enzyme activity of *Bacillus safensis* FB03.

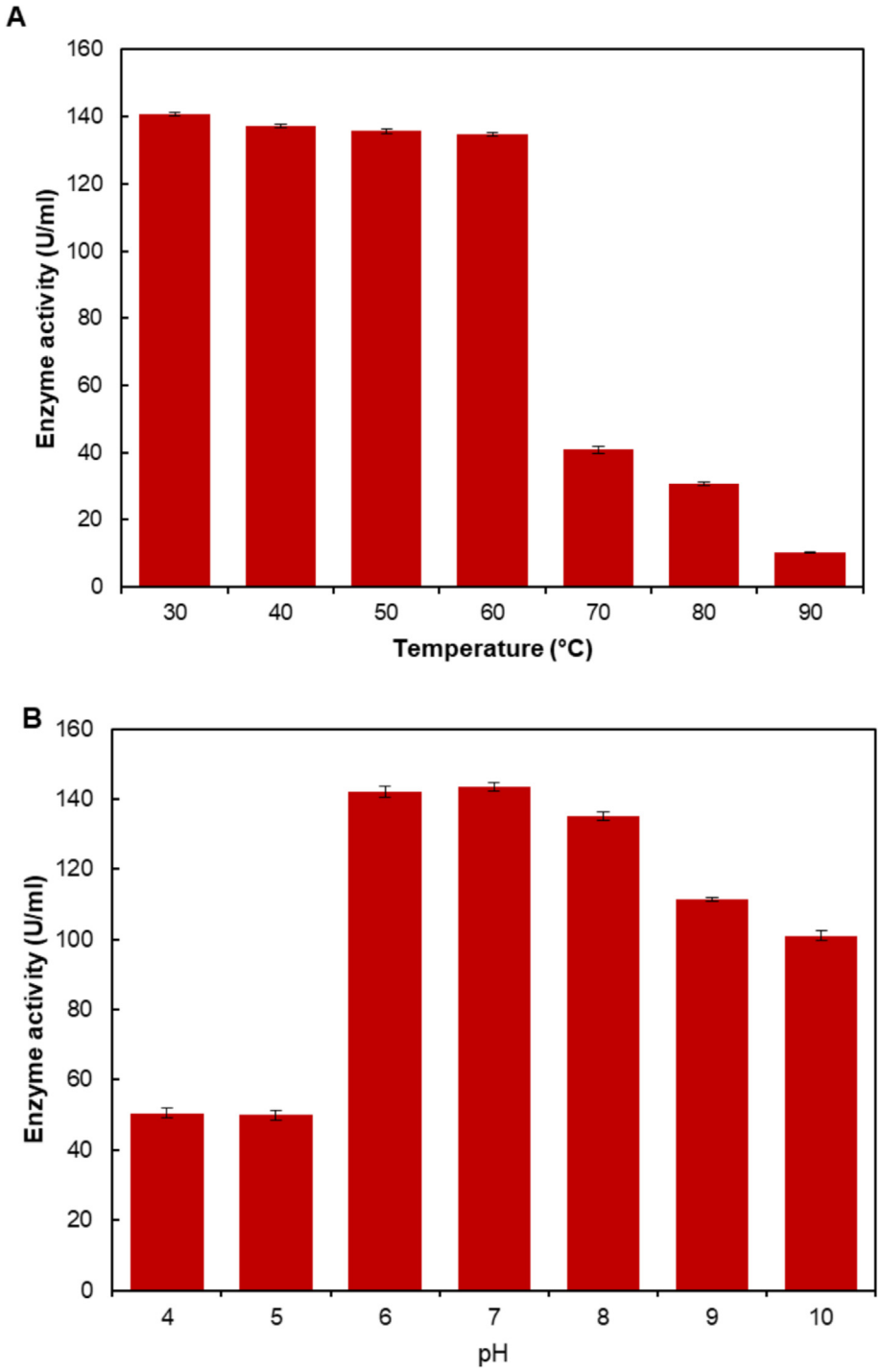


Fig. 5. (A) Temperature, (B) pH stability of xylanase from isolate FB03.

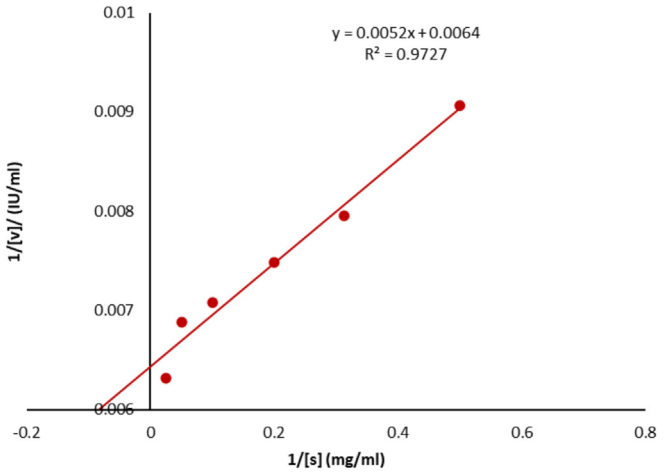


Fig. 6. Double reciprocal plot of the purified xylanase from *B. safensis* FB03 on beechwood xylanase. Data point represents the mean ± SD (n = 3).

Table 3
Position and length of different xyl genes on the genome of FB03.

Gene	Contig	Position		Length (bp)
		Start	Stop	
xynA	NODE_03	28,985	30,322	1338
xynB1	NODE_01	170,488	171,965	1518
xynB2	NODE_03	27,450	28,949	1500
xynB3	NODE_03	471,806	473,431	1626
xylP	NODE_03	33,573	34,764	1392
xylT	NODE_03	468,244	469,584	1341

and substances produced during substrate hydrolysis in the culture media [40]. The xylanase of FB03 increased in activity by more than tenfold compared to the crude form after undergoing a one-step purification process. A similar study concluded that *Bacillus subtilis* xylanase purification in one step increased its activity by 10.5 times [41]. When examining the properties of enzymes, the most crucial aspect is their stability. So, pre-incubating xylanase for 60 min at a temperature range of 30–90°C was done in order to conduct thermal stability tests. An enzyme's industrial significance increases with decreasing temperature input's effect on its peak activity. In this context, our isolated strain might be a valuable resource for biotechnological uses as it maintained its activity up to 60°C. According to the work of Ketsakhon et al., most bacterial xylanases show their maximum activity in the range of 50 to 60°C [42], where Saleem et al. discovered that 50°C is the ideal temperature for *Bacillus* xylanases [43]. Another feature that makes an industrial enzyme appealing is its ability to remain active over a wide pH range. Fungal xylanases are more productive in acidic pH ranges of 4.0–6.0 [44], whereas bacterial xylanases are more inclined towards environments with a pH of higher than 6 [45] and when doing the current study, comparable outcomes were noted. Elevated xylanase activity was also seen at pH 7.0 in *Bacillus pumilus* [46] and at pH 8 in *Bacillus mojavensis* AG137 [47]. Studies revealed that variations in pH and temperature stability for extracellular xylanases might be explained by glycosylation and other post-transcriptional changes in the xylanase excretion pathway [48].

The Michaelis constant, Km, can be calculated by measuring the substrate concentration at half the maximum velocity. In every given enzyme and substrate concentration, *Km* remains constant and therefore, a low *Km* represents high substrate affinity of the enzyme [49]. The *Vmax* of xylanase from *B. safensis* was 156.25 μ mol/min/mg, and the *Km* was found to be 0.64 mg/ml. In a similar study, the *Km* and *Vmax* values of the isolated *Pseudomonas* sp. XPB-6 xylanase were 0.60 mg/ml and 144.92 IU/mg protein, respectively [50]. This finding reveals that with a high *Vmax* and low *Km* value, *B. safensis* xylanase exhibits a strong substrate affinity for beechwood xylan and can catalyze it more rapidly and effectively. Six genes namely xynA, xynB1, B2, and B3, xylP, xylT have been detected on the genome of FB03. This set of genes is involved in both the xylan breakdown and transportation of xylose into the cell to convert them into simple sugar that the organism can use for energy and growth. xynA encodes the xylanase enzyme which breaks down xylan into smaller sugar components like xylose. xynB codes a different form of xylanase enzyme, often involved in the same biochemical process as xynA but with different specificities or optimal conditions [51]. Once xylanase enzymes break down xylan into smaller sugar units like xylose, xylT ensures that the xylose can be taken up by the microorganism to be used as an energy source or metabolized further. Additionally, in this route, xylP encodes xylose permease, a membrane protein that is involved in the facilitated diffusion of xylose across the cell membrane [51]. Finding the exact locations of genes provides a valuable foundation for additional genetic alteration and cloning research

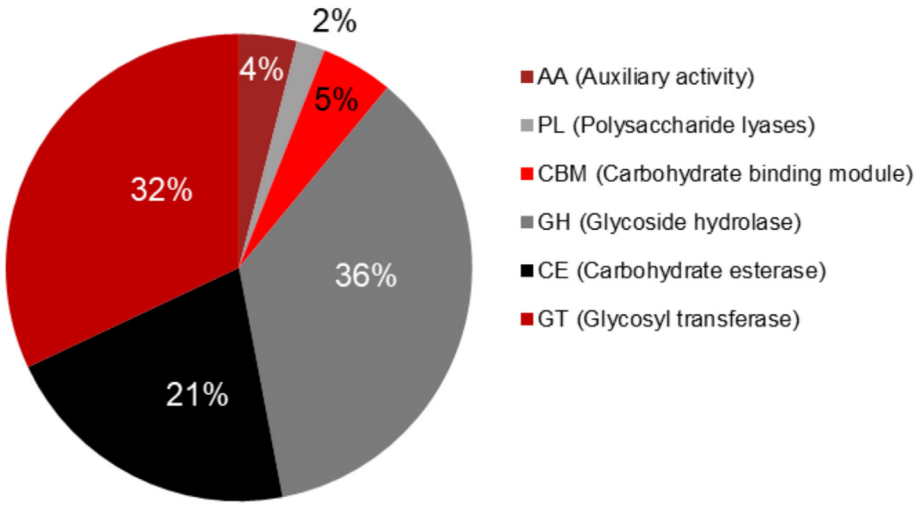


Fig. 7. Carbohydrate active enzymes produced by *B. safensis* FB03: CAZy database annotation.

aimed at improving enzyme output. An essential class of enzymes known as glycoside hydrolases is often involved in the hydrolysis of carbohydrates' glycosidic bonds. According to earlier research, hundreds of distinct glycosyltransferases are involved in the manufacture of polysaccharides, oligosaccharides, and disaccharides, which helps to create glycosidic linkages [52]. The active enzymes GHs and GTs, which are encoded by genes in the FB03 genome, allow the bacteria to manufacture carbohydrates and metabolize sugars. Thus, from this study, a new avenue for the biotechnological use of the native strain of *B. safensis* FB03 as an industrially significant microbe has been opened.

5. Conclusions

Through the use of a medium containing banana rachis in place of high-cost xylan, this study demonstrates that xylanase from *B. safensis* FB03 can be produced in a cost-effective way utilizing banana rachis as a fermentation medium. FB03 is an effective xylanase producer, with a maximum productivity of 25.24 U/ml which upon purification became 143.6 U/ml. Enzyme from this isolate can retain activity up to 60°C in a wide range of pH from 6 to 10. Besides, the whole genome data analysis opens a new door of research for further genetic manipulation to enhance its biosynthetic capabilities for industrial-scale application.

CRediT authorship contribution statement

Farhana Boby: Data curation, Writing – original draft, Formal analysis, Methodology. **Md Nurul Huda Bhuiyan:** Conceptualization, Supervision, Writing – review & editing. **Md Mashud Parvez:** Formal analysis, Methodology. **Md Jahidul Islam:** Data curation, Writing – review & editing. **Ifrat Jannati:** Formal analysis.

Financial support

The financial support for this work was provided by Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka, Bangladesh. It was conducted as a part of research and development project at BCSIR, Rajshahi.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

Md Mahmudur Rahman, BCSIR, Rajshahi is acknowledged for providing the raw material utilized in this study.

Supplementary material

https://doi.org/10.1016/j.ejbt.2025.05.004.

Data availability

All data are available in the manuscript

References

[1] Rastogi M, Shrivastava S, Shukla P. Bioprospecting of xylanase producing fungal strains: Multilocus phylogenetic analysis and enzyme activity profiling. J Basic Microbiol 2022;62(2):150–61. https://doi.org/10.1002/jobm.202100408. PMid: 34783043.

[2] Chaudhary R, Pandey A, Tiwari R, et al. Current status of xylanase for biofuel production: A review on classification and characterization. Biomass Convers Biorefin 2021;13(10):8773–91. https://doi.org/10.1007/s13399-021-01948-2.

[3] Walia A, Guleria S, Mehta P, et al. Microbial xylanases and their industrial application in pulp and paper biobleaching: A review. 3 Biotech 2017;7(1):11. https://doi.org/10.1007/s13205-016-0584-6. PMid: 28391477.

[4] Shrivastava S. Introduction to glycoside hydrolases: Classification, identification and occurrence. In: Industrial Applications of Glycoside Hydrolases. Singapore: Springer; 2020. p. 3–84. https://doi.org/10.1007/978-981-15-4767-6_1.

[5] Dias LM, dos Santos BV, Albuquerque CJB, et al. Biomass sorghum as a novel substrate in solid-state fermentation for the production of hemicellulases and cellulases by *Aspergillus niger* and *A. fumigatus*. J Appl Microbiol 2018;124(3):708–18. https://doi.org/10.1111/jam.13672. PMid: 29253315.

[6] Bhardwaj N, Kumar B, Verma P. A detailed overview of xylanases: An emerging biomolecule for current and future prospective. Bioresour Bioprocess 2019;6(1):40. https://doi.org/10.1186/s40643-019-0276-2.

[7] Parab P, Khandeparker R. Xylanolytic enzyme consortia from *Bacillus* sp. NIORKP76 for improved biobleaching of kraft pulp. Bioprocess Biosyst Eng 2021;44(12):2513–24. https://doi.org/10.1007/s00449-021-02623-6. PMid: 34402971.

[8] Contesini FJ, de Melo RR, Sato HH. An overview of *Bacillus* proteases: From production to application. Crit Rev Biotechnol 2018;38(3):321–34. https://doi.org/10.1080/07388551.2017.1354354. PMid: 28789570.

[9] Rastogi M, Shrivastava S. Recent advances in second generation bioethanol production: An insight to pretreatment, saccharification and fermentation processes. Renew Sustain Energy Rev 2017;80:330–40. https://doi.org/10.1016/j.rser.2017.05.225.

[10] Lateef A, Adelere IA, Gueguim-Kana EB. The biology and potential biotechnological applications of *Bacillus safensis*. Biologia 2015;70(4):411–9. https://doi.org/10.1515/biolog-2015-0062.

[11] Devi S, Dwivedi D, Bhatt AK. Utilization of agroresidues for the production of xylanase by *Bacillus safensis* XPS7 and optimization of production parameters. Fermentation 2022;8(5):221. https://doi.org/10.3390/fermentation8050221.

[12] Rekik H, Jaouadi NZ, Gardouri F, et al. Production, purification and biochemical characterization of a novel detergent-stable serine alkaline protease from *Bacillus safensis* strain RH12. Int J Biol Macromol 2019;121:1227–39. https://doi.org/10.1016/j.ijbiomac.2018.10.139. PMid: 30352229.

[13] Patel K, Parikh S. Identification, production, and purification of a novel lipase from *Bacillus safensis*. J Appl Biol Biotechnol 2022;10(4):73–6. https://doi.org/10.7324/JABB.2022.100410.

[14] Pandya U, Saraf M. Purification and characterization of antifungal chitinase from *Bacillus safensis* MBCU6 and its application for production of chito-oligosaccharides. Biologia 2015;70(7):863–8. https://doi.org/10.1515/biolog-2015-0112.

[15] Sharma H, Singh J, Batra N. Isolation and molecular identification of α-amylase producing bacteria from hot water spring by 16S rRNA gene sequencing. Indian J Exp Biol 2019;57:937–44.

[16] Singh RS, Singh RP. Response surface optimization of endoinulinase production from a cost effective substrate by *Bacillus safensis* AS-08 for hydrolysis of inulin. Biocatal Agric Biotechnol 2014;3(4):365–72. https://doi.org/10.1016/j.bcab.2014.05.002.

[17] Nath A, Sarkar S, Maitra M, et al. An experimental study on production of intracellular β-galactosidase at different conditions by batch process using isolated *Bacillus safensis* (JUCHE 1) and characterization of synthesized β-galactosidase. J Inst Engineers (India): Ser E 2012;93(2):55–60. https://doi.org/10.1007/s40034-013-0011-z.

[18] Al-Shawi AH. Enhanced xylanase production from *Bacillus safensis* MABS6 using sorghum straw substrate: Optimization, characterization, and biotechnological applications. Catrina: Int J Environ Sci 2024;29(1):9–24. https://doi.org/10.21608/cat.2023.239574.1209.

[19] Lateef A, AdelereI A, Gueguim-Kana EB. *Bacillus safensis* LAU 13: A new source of keratinase and its multi-functional biocatalytic applications. Biotechnol Biotechnol Equip 2015;29(1):54–63. https://doi.org/10.1080/13102818.2014.986360. PMid: 26740788.

[20] Azeem MA, Shah FH, Ullah A, et al. Biochemical characterization of halotolerant *Bacillus safensis* PM22 and its potential to enhance growth of maize under salinity stress. Plants 2022;11(13):1721. https://doi.org/10.3390/plants11131721. PMid: 35807673.

[21] Zagorchev L, Kamenova P, Odjakova M. The role of plant cell wall proteins in response to salt stress. Sci World J 2014;2014(1):764089. https://doi.org/10.1155/2014/764089. PMid: 24574917.

[22] Wu PS, Liu CH, Hu SY. Probiotic *Bacillus safensis* NPUST1 administration improves growth performance, gut microbiota, and innate immunity against *Streptococcus iniae* in Nile tilapia (*Oreochromis niloticus*). Microorganisms 2021;9(12):2494. https://doi.org/10.3390/microorganisms9122494. PMid: 34946096.

[23] Kwon EH, Adhikari A, Imran M, et al. Novel melatonin-producing *Bacillus safensis* EH143 mitigates salt and cadmium stress in soybean. J Pineal Res 2024;76(4). https://doi.org/10.1111/jpi.12957. PMid: 38803089.

[24] Priyalaxmi R, Murugan A, Raja P, et al. Bioremediation of cadmium by *Bacillus safensis* (JX126862), a marine bacterium isolated from mangrove sediments. Int J Curr Microbiol App Sci 2014;3(12):326–35.

[25] Kamble RD, Jadhav AR. Isolation, purification, and characterization of xylanase produced by a new species of *Bacillus* in solid state fermentation. Int J

Microbiol 2012;2012(1):683193. https://doi.org/10.1155/2012/683193. PMid: 22315613.
[26] Samanta AK, Kolte AP, Senani S, et al. A simple and efficient diffusion technique for assay of endo β-1,4-xylanase activity. Braz J Microbiol 2011;42 (4):1349–53. https://doi.org/10.1590/S1517-83822011000400016. PMid: 24031763.
[27] Bhalla A, Bischoff KM, Sani RK. Highly thermostable xylanase production from a thermophilic *Geobacillus* sp. strain WSUCF1 utilizing lignocellulosic biomass. Front Bioeng Biotechnol 2015;3:84. https://doi.org/10.3389/fbioe.2015.00084. PMid: 26137456.
[28] Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976;72(1–2):248–54. https://doi.org/10.1016/0003-2697(76)90527-3. PMid: 942051.
[29] Grant JR, Enns E, Matinier E, et al. Proksee: In-depth characterization and visualization of bacterial genomes. Nucl Acids Res 2023;51(W1):W484–92. https://doi.org/10.1093/nar/gkad326. PMid: 37140037.
[30] Drula E, Garron ML, Dogan S, et al. The carbohydrate-active enzyme database: Functions and literature. Nucl Acids Res 2022;50(D1):D571–7. https://doi.org/10.1093/nar/gkab1045. PMid: 34850161.
[31] Lodi RS, Dong X, Jiang C, et al. Antimicrobial activity and enzymatic analysis of endophytes isolated from *Codonopsis pilosula*. FEMS Microbiol Ecol 2023;99 (8):fiad071. https://doi.org/10.1093/femsec/fiad071. PMid: 37365694.
[32] Tarafdar A, Sirohi R, Gaur VK, et al. Engineering interventions in enzyme production: Lab to industrial scale. Bioresour Technol 2021;326:124771. https://doi.org/10.1016/j.biortech.2021.124771. PMid: 33550211.
[33] Marimuthu M, Sorimuthu A, Muruganantham S. Production and optimization of xylanase enzyme from *Bacillus subtilis* using agricultural wastes by solid state fermentation. Int J Pharm Investig 2019;9(4):169–73.
[34] Al Mousa AA, Abo-Dahab NF, Hassane AMA, et al. Harnessing *Mucor* spp. for xylanase production: Statistical optimization in submerged fermentation using agro-industrial wastes. BioMed Res Int 2022;2022(1):3816010. https://doi.org/10.1155/2022/3816010. PMid: 35496057.
[35] Abdella A, Segato F, Wilkins MR. Optimization of process parameters and fermentation strategy for xylanase production in a stirred tank reactor using a mutant *Aspergillus nidulans* strain. Biotechnol Rep 2020;26:e00457. https://doi.org/10.1016/j.btre.2020.e00457. PMid: 32420050.
[36] Güler F, Özçelik F. Screening of xylanase producing *Bacillus* species and optimization of xylanase process parameters in submerged fermentation. Biocatal Agric Biotechnol 2023;51:102801. https://doi.org/10.1016/j.bcab.2023.102801.
[37] Prabha B, Ramesh D, Sriramajayam S, et al. Optimization of pyrolysis process parameters for fuel oil production from the thermal recycling of waste polypropylene grocery bags using the Box–Behnken design. Recycling 2024;9 (1):15. https://doi.org/10.3390/recycling9010015.
[38] Bakry MM, Salem SS, Atta HM, et al. Xylanase from thermotolerant *Bacillus haynesii* strain: Synthesis, characterization, optimization using Box-Behnken Design, and biobleaching activity. Biomass Convers Biorefin 2024;14 (8):9779–92. https://doi.org/10.1007/s13399-022-03043-6.
[39] Salmanizadeh H, Beheshti-Maal K, Nayeri H, et al. Optimization of xylanase production by *Pichia kudriavzevii* and *Candida tropicalis* isolated from the wood product workshop. Braz J Microbiol 2024;55(1):155–68. https://doi.org/10.1007/s42770-023-01171-3. PMid: 37957443.
[40] Yadav P, Maharjan J, Korpole S, et al. Production, purification, and characterization of thermostable alkaline xylanase from *Anoxybacillus kamchatkensis* NASTPD13. Front Bioeng Biotechnol 2018;6:65. https://doi.org/10.3389/fbioe.2018.00065. PMid: 29868578.
[41] Sanghi A, Garg N, Gupta VK, et al. One-step purification and characterization of cellulase-free xylanase produced by alkalophilic *Bacillus subtilis* ash. Braz J Microbiol 2010;41(2):467–76. https://doi.org/10.1590/S1517-83822010000200029. PMid: 24031518.
[42] Ketsakhon P, Thammasittirong A, Thammasittirong SNR. Adding value to rice straw waste for high-level xylanase production using a new isolate of *Bacillus altitudinis* RS3025. Folia Microbiol 2023;68(1):87–99. https://doi.org/10.1007/s12223-022-00998-x. PMid: 35945409.
[43] Saleem A, Waris S, Ahmed T, et al. Biochemical characterization and molecular docking of cloned xylanase gene from *Bacillus subtilis* RTS expressed in *E. coli*. Int J Biol Macromol 2021;168:310–21. https://doi.org/10.1016/j.ijbiomac.2020.12.001. PMid: 33309670.
[44] Dhaver P, Pletschke B, Sithole B, et al. Optimization, purification, and characterization of xylanase production by a newly isolated *Trichoderma harzianum* strain by a two-step statistical experimental design strategy. Sci Rep 2022;12(1):17791. https://doi.org/10.1038/s41598-022-22723-x. PMid: 36273028.
[45] Gupta GK, Dixit M, Kapoor RK, et al. Xylanolytic enzymes in pulp and paper industry: New technologies and perspectives. Mol Biotechnol 2022;64 (2):130–43. https://doi.org/10.1007/s12033-021-00396-7. PMid: 34580813.
[46] Nagar S, Gupta VK, Kumar D, et al. Production and optimization of cellulase-free, alkali-stable xylanase by *Bacillus pumilus* SV-85S in submerged fermentation. J Ind Microbiol Biotechnol 2010;37(1):71–83. https://doi.org/10.1007/s10295-009-0650-8. PMid: 19859753.
[47] Sepahy AA, Ghazi S, Sepahy MA. Cost-effective production and optimization of alkaline xylanase by indigenous *Bacillus mojavensis* AG137 fermented on agricultural waste. Enzyme Res 2011;2011(1):593624. PMid: 21904670.
[48] de Carvalho LM, Borelli G, Camargo AP, et al. Bioinformatics applied to biotechnology: A review towards bioenergy research. Biomass Bioenergy 2019;123:195–224. https://doi.org/10.1016/j.biombioe.2019.02.016.
[49] Deshmukh RA, Jagtap S, Mandal MK, et al. Purification, biochemical characterization and structural modelling of alkali-stable β-1,4-xylan xylanohydrolase from *Aspergillus fumigatus* R1 isolated from soil. BMC Biotech 2016;16(1):11. https://doi.org/10.1186/s12896-016-0242-4. PMid: 26847222.
[50] Sharma PK, Chand D. Purification and characterization of thermostable cellulase-free xylanase from *Pseudomonas* sp. XPB-6. Adv Microbiol 2012;2 (1):17–25. https://doi.org/10.4236/aim.2012.21003.
[51] Halmschlag B, Hoffmann K, Hanke R, et al. Comparison of isomerase and Weimberg pathway for γ-PGA production from xylose by engineered *Bacillus subtilis*. Front Bioeng Biotechnol 2020;7:476. https://doi.org/10.3389/fbioe.2019.00476. PMid: 32039180.
[52] Mazmanian SK, Round JL, Kasper DL. A microbial symbiosis factor prevents intestinal inflammatory disease. Nature 2008;453(7195):620–5. https://doi.org/10.1038/nature07008. PMid: 18509436.