Production and Optimization of Xylanase Enzyme from *Bacillus cereus* BSA1 by Submerged Fermentation

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ABSTRACT

Xylanases are hydrolytic enzymes that depolymerise hemi-cellulosic polysaccharide xylan, a major component of plant cell walls. The production of xylanase often necessitates a cost-effective manufacturing procedure. In the current study, synthesis of extracellular xylanase by *Bacillus cereus* BSA1 in presence of different amino acids was optimized under submerged fermentation. The growth of bacteria and its enzyme production was optimum in the presence of beef extract. The maximum enzyme production was achieved in the presence of the amino acids L-proline and L-cystine. This study focuses on achieving a 38% enhancement in xylanase production through the use of amino acids, suggesting the presence of regulatory mechanisms influencing enzyme biosynthesis.

Introduction:

Xylanase, an industrially used enzyme, has become increasingly important, especially in the paper industry for the bio-bleaching process. Enhancing enzyme production is becoming increasingly crucial due to rising demand for industrially utilized enzymes. Improvement of fermentation technology remains an empirical process to obtain large quantity of desired metabolites. Study thus primarily focuses on the optimization procedure to scale up the synthesis of a desired metabolite. Generally, xylanases are produced by different microorganisms like fungi and bacteria through processes like solid- (Amare and Gashaw, 1999; Battan *et al*., 2006) semi solid- and submerged-fermentation (Gomes *et al*., 2000). Amino acids have been used as the enhancer for microbial growth and hence the production of the enzyme as well (Ikura and Horikoshi, 1987). α-Larabinofuranosidase, α-D-glucuronidase, galactosidase, and acetyl xylan esterase hydrolyze the side groups of xylan (Figure 1).

Previously a bacterial strain, *Bacillus cereus* BSA1 was isolated as xylanase producer and the optimum physico-chemical features of the media for xylanase production by it was reported (Mandal *et*

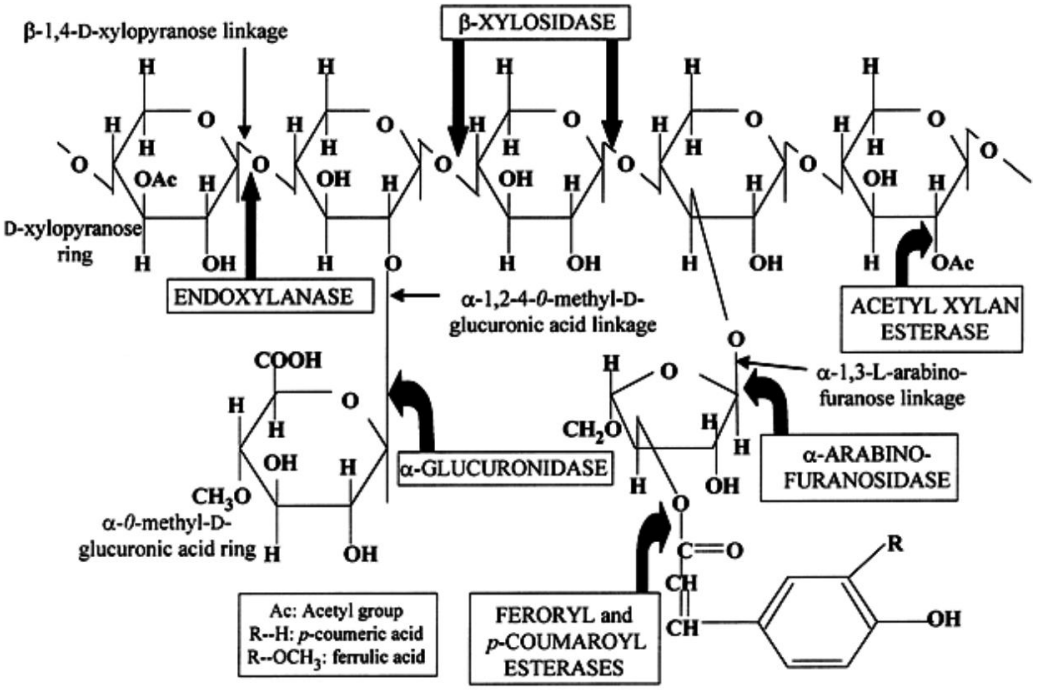

Figure 1: Mode of action of microbial xylanases on xylan compound

al., 2008). In the present case, role of different amino acids for enhanced xylanase production by *Bacillus cereus* BSA1 is studied.

Materials and Methods:

Microorganism:

Bacterial strain *Bacillus cereus* BSA1, (Mandal *et al*. 2008) was used in this study.

Production of enzyme:

Enzyme productions was made in 250 ml Erlenmeyer flasks containing 50 ml of sterilized liquid media [(g/l): $(NH_4)_2SO_4$ 1.0; $MgSO_4$ 0.2; K_2HPO_4 0.2; $CaCl_2$ 0.2; $MnCl_2$ 0.02; yeast extract 0.1, xylan 10.0] Before sterilization, xylan was completely dissolved in water by sonication (7.0 hz, 2 min) and then pH of the medium was adjusted to 7.0. The medium was sterilized for 15 minutes at 121ºC. Fermentation was carried out in a rotatory shaker (120 rpm) at 37ºC for 72 h. The cell-free supernatant after centrifugation (5000gÍ 5min) was used as the source of crude enzyme. Growth of the organism was determined according to the formation of colony forming unit (c.f.u./ml).

Optimization of fermentation process using different amino acids:

Different types of amino acids and their concentration were added separately to the media. The media before adding amino acids were sterilized and filtrate solution of each amino acid was added at a concentration of

0.20% (w/v).All the experiments were done in triplicate and data were presented here as mean ± SE.

Enzyme assay:

Xylanase activity was assayed by measuring released reducing sugar from birch wood xylan (Fluka) with 3, 5- dinitrosalicylic acid (Miller, 1959). The reaction mixture containing 0.4 ml phosphate buffer (0.2M, pH 7.0), 0.3 ml of 5% (w/v) xylan and 0.3 ml enzyme solution. The enzymatic reaction was carried out at 50ºC and after 30 min 1ml of DNS (3%w/v) was added to stop the reaction. The solution was incubated in a boiling water bath for 15 min for colour development and the absorbency was measured at 540 nm (Systronic spectrophotometer 105) against the enzyme blank. The xylanase activity was determined by using a standard calibration curve of D-xylose (Sigma).

One unit of xylanase activity (U/ml) was defined as the amount of enzyme required to produce 1 ìmol of reducing sugars as xylose by hydrolyzingxylan per minute under the above assay condition.

Result:

Effect of different amino acid:

Maximum enzyme production was noticed in presence of proline and cysteine (Table 1). Tyrosine, alanine, ornithine, arginine and methionine also had significant results. Different concentrations of proline and cysteine (Table 2, 3) were also tested and the most effective result was found in 0.25% (w/v) of both the amino acids.

Table 1: Effect of amino acids on xylanase production by *Bacillus cereus* BSA1. Different amino acids were mixed separately with the media and the organism was grown 35ºC and pH 6.0 under shaking (120 rev/min) condition for 84h.

Amino acid	Relative activity (%)
DL- Alanine	115
DL- Aspertic acid	111
L- Histidine	113
L-Leucine	108
DL-Tryptophan	101
Glycine	109
L-Glutamic acid	111
L-Tyrosine	116
L-Ornithine	115
L-Proline	138
L-Arginine	115
L-Cysteine	138
DL-Metheonine	115
L-Isoleucine	112
L-Lysine	110
L-Phenyl alanine	106
DL-Valine	108
DL-Threonine	103
Basic media	100

Table 2: Effect of concentration of proline on xylanase production by *Bacillus cereus* BSA1.The bacteria was grown for 84 h under shaking (120 rev/min) condition (temperature: 35ºC pH: 6.0).

Proline concentration (%)	Xylanase (U/ml)
0.1	5.23 ± 0.15
0.15	5.75 ± 0.17
0.20	6.02 ± 0.21
0.25	7.12 ± 0.31
0.30	6.83 ± 0.21
0.35	6.30 ± 0.22

Table 3: Effect of concentration of cystine on xylanase production by *Bacillus cereus* BSA1 at 35ºC and pH 6.0 under shaking (120 rev/min) condition (bacteria was grown for 84h).

Cystine concentration (%)	Xylanase (U/ml)
0.1	4.20 ± 0.11
0.15	5.45 ± 0.12
0.20	6.03 ± 0.15
0.25	6.90 ± 0.13
0.30	6.57 ± 0.15
0.35	6.18 ± 0.22

Discussion:

The *Bacillus* genus of bacteria is highly versatile, capable of producing a broad spectrum of products without heavy reliance on petrochemicals. Their resilience in diverse and challenging environments, including agro-industrial wastes (Chattaraj *et al*. 2024), enables the production of various beneficial and environmentally friendly products including feeds (Ganguly *et al*. 2024), foods (Ganguly *et al*. 2018a; Ganguly *et al*. 2018b), probiotics (Ganguly *et al*. 2018c; Chattaraj *et al*. 2023a; Ganguly & Ganguly 2016), plant growth regulators (Samantaray 2024), enzymes (Nad *et al*. 2024; Bhattacharjee *et al*. 2018), biocides (Chattaraj *et al*. 2022), and bioactive chemicals (Chattaraj *et al*. 2023b). Prior studies have optimized xylanase production in several *Bacillus* species incuding *B. licheniformis* (Malhotra *et al*., 2022), *B. subtilis* (Yardimci *et al*., 2018), and *B. safensis* (Devi *et al*., 2022). This current study marks the first attempt to enhance the cultural conditions for xylanase production utilizing *Bacillus cereus* as inoculum.

The high cost of enzyme production can be a limiting factor in meeting industrial demands. Therefore, the development of a low-cost growth medium for microbial cultivation and enzyme production is crucial to make the process more economically feasible. Submerged fermentation technology offers a significant advantage in xylanase production, enabling high yields to be achieved in shorter production periods while keeping costs low. However, the success of this process heavily relies on two crucial elements: the composition of the nutrient medium and the culture conditions. The production of xylanase is sensitive to various physical and chemical factors, including temperature, pH levels, incubation duration, the type and concentration of

carbon and nitrogen sources, and agitation speed (Dhaver *et al*. 2022). The present study exhibited a significant level of stimulation of xylanase production by a number of amino acids like L-cysteine, L-proline, L-histidine, L-arginine, DL-alanine and L-ornithine. L-cysteine and L-proline stimulated about 1.4 fold increase in the enzyme synthesis whereas DL-threonine, L-tyrosine, L-leucine, L-lysine, DL-tryptophan, DL-valine, DL-aspertic acid L-glutamic acid showed only a marginal or no effect on enzyme production. There are many reports about the stimulating effect of amino acids or their derivatives. Beg *et al*. (2000) reported DL-norleucine, L-leucine, DL-isoleucine, L-lysine monohydrochloride and DL-phenylalanine stimulated the xylanase production up to 3.72-fold in *Streptococcus* sp. Ikura and Horikoshi (1987) reported that 0.5% glycine enhanced 1.8-fold enzyme production. Balakrishnan *et al*. (1997) also reported 2 to 5 fold increased xylanase production in *Bacillus* sp. in presence of glycine and DL-norvaline and casamino acids. Okafor *et al*. (2007) isolated a strain of *Penicillium chrysogenum* PCL501 from wood wastes and found that after 4 days of fermentation, wheat bran produced the highest xylanase activity at 6.47 U/ml. Thomas *et al*. (2016) obtained increased xylanase production utilizing *Aspergillus* sp. within 4 days of fermentation. Dhaver *et al*. (2022) utilized *Trichoderma harzianum* strain to significantly enhance xylanase production using the Plackett–Burman Design, achieving a 4.16-fold improvement compared to the preliminary one-factor-at-a-time (OFAT) approach. The distinctive feature of the enzyme characterized in this study is its exceptional stability across a broad range of physical parameters, including temperature, pH, and solvent tolerance, thereby conferring robustness and versatility as a biocatalyst. This stability profile expands its potential utility in various industrial and biotechnological applications.

In the present study the cell density remained more or less same in all cases, which indicated that xylanase production was not a direct function of cell growth, instead there might be some switching on or off phenomena present in the organism for xylanase production which are operating not only in the presence of xylan but also in the presence of some specific amino acids (Gupta *et al.,* 1999). Bakry *et al*. (2024) reported that *Bacillus haynesii* strain K6 exhibited significant enhancement in xylanase production when the media is supplemented with amino acids proline and cysteine.

Conclusion

In this study, it was found that *Bacillus cereus* BSA1 strain efficiently produces extracellular xylanase under specific conditions: 84 h incubation period at 35ºC, at pH 6, supplemented with L-proline and

L-cystine. This mesophilic soil bacterium, *Bacillus cereus* BSA1, exhibited substantial xylanase production in response to these amino acids, suggesting potential regulatory mechanisms. Further investigation is needed to delve deeper into these mechanisms and uncover additional details.

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