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BIOCHEMICAL CHARACTERISTICS OF XYLANASE-PRODUCING BACILLUS SPECIES BELONGING TO THE GENUS BACILLUS.

Mukhlisa Shodmonova, doctoral student.
Tohir Bozorov, senior researcher
[1] UZRFA scientific research Institute of Genetics and Experimental Plant Biology
e-mail: muxlisashodmonova1994@gmail.com

Abstract: *Microbial enzymatic agents represent a primary enzymatic process, although their production may pose environmental risks, requiring extraordinary catalysts to mitigate these effects. This new study aims to scrutinize xylanase yields from Bacillus strains and identify key factors influencing this enzymatic synthesis. Bacterial isolates were isolated from plants.*

The current study aims to isolate and optimize process parameters for xylanase overproduction through synergistic phenomena. Bacillus halotolerans, Bacillus safensis, Bacillus pumilus belonging to the genus Bacillus showed the highest xylanolytic potential. Although five different media were evaluated for xylanase production, our findings differed significantly from previous experiments. Recently, special attention has been paid to the ability of microorganisms to produce thermostable enzymes (eg, proteases, lipases, cellulases, xylanases, and DNA polymerases) that can be active even under extreme conditions [1]. Therefore, these enzymes can be used in high-temperature industrial fermenters, which accelerate biochemical reactions, reduce the level of contamination and facilitate product recovery. In addition, thermophilic bacteria have other biotechnological applications, including the production of bioactive compounds (eg, exopolysaccharides, antibiotics, and biosurfactants). In addition, they can be used for the removal of heavy metals by bioremediation, as well as for the biodegradation of plastic polymers [2]. Microbial xylanases (1,4-β-D-xylan xylanohydrolase, are preferred catalysts for xylan hydrolysis due to their high specificity, mild reaction conditions, negligible substrate loss, and byproduct formation. However, the cost of enzymatic hydrolysis of biomass is one of the factors limiting the economic feasibility of the process. Therefore, it is necessary to improve the production of xylanases by finding more potentt fungal or bacterial strains, or by developing mutant strains that secrete more enzymes, or both. Xylanases are produced by many organisms such as bacteria, algae, and fungi. Most of the bacteria

and fungi secrete extracellular xylanases which act on the hemicellulosic material to liberate xylose as a directly assimilable end product allowing the organisms to grow heterotrophically on xylan. Aspergillus, Penicillium, Bacillus, Trichoderma, Clostridium, Streptomyces, Penicillium and Fusarium species, Trichoderma sp., Aspergillus sp. A number of microorganisms known to produce xylanase, such as Bacillus species, are generally preferred for enzyme production because they are easy to isolate and identify, grow rapidly, have a short fermentation time, and absorb enzymes directly. environmentally friendly and low cleaning costs. Because of these advantages, Bacillus species have come to the fore in research on xylanase production, and they are safe and can be used in a variety of food processes and only some belong to the genus Bacillus.

METHODS

Isolation of bacteria from plants

The collected plant samples were homogenized and 1 ml of sterile PBS buffer (137 mM NaCl, 2.7 mM KCl, 1 mM Na2HPO4 and 1.8 mM KH2PO4; pH 7.4) was added and mixed. The solution was serially diluted to 10-6 with sterile buffer. Each diluted sample was placed on nutrient agar (NA) in a laminar flow cabinet (0.5% peptone, 0.3% beef extract, 1.5% agar, pH 6.8). The plates were placed in a thermostatic bacterial incubator. placed at 28 degrees Celsius for 48-96 hours until colonies appeared. The morphology of bacterial colonies differs from each other in their shape, size, border and height

Enzyme activity assay

Liquid enzyme activity was measured from relevant substrates, such as xylanase. Antagonists were produced from among the tested bacterial samples. Separated bacteria were cultured 3 times for each enzyme with substances harmful to bacteria.4-nitrophenyl beta-D-xylopyranoside 27.1 mg of dry mass was taken and each enzyme was dissolved in 10 ml of PBS buffer. This analysis process takes the active fractions in a microtitre with a total volume of 200 l for qualitative quality. Activity reactions to the above enzymes were evaluated by the development of bacterial colony color (yellow).

RESULTS

Bacterial strains showing antagonistic properties were isolated from among the tested bacterial samples. Nutrient agar was selected as the optimal medium for growing bacterial samples.Antagonistic bacteria were carefully transplanted into 30 petri dishes. Each bacterial sample was transferred to 3 nutrient mediums in the same way for 3

different enzymes. 4-Nitrophenyl β-D-chloropyranoside enzymes were taken in the amount of 10 mM and dissolved in PBS buffer.4-Nitrophenyl β-D-chloropyranoside - 27,122 mg. Enzymes were released by acting on separately cultivated bacteria in the amount of 0.3 µM. Bacterial strains were left in the thermostat for 8 hours.As a result, the enzyme activity of our isolated isolates was determined by the change in the color of the colony.Antagonist bacteria take the leading place among bacteria in terms of enzyme activity.

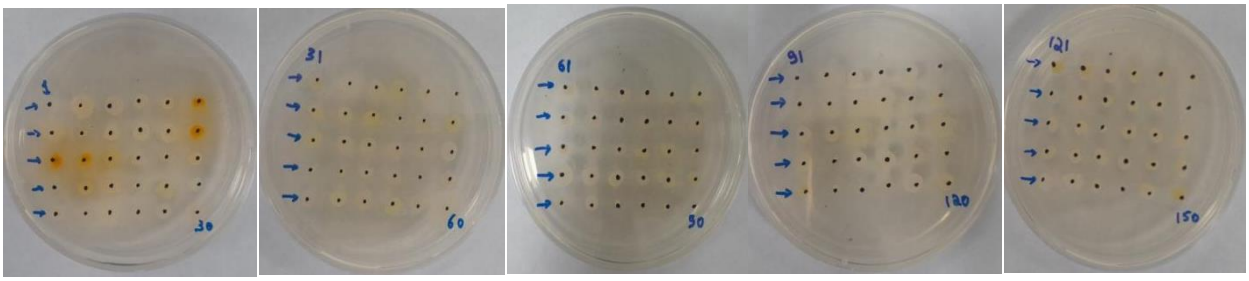

Fig. 1. Colony color change of bacteria tested for xylanase enzyme

The colony color of 16 species of Bacillus species belonging to the Bacillus family was determined by the change of color. Representatives of the Bacillus genus showed different levels of enzyme activity.Among the tested bacteria, 2 - Bacillus atrophaeus and Bacillus mycoides did not show sensitivity to enzyme activity. Bacillus stercoris and Bacillus inaquosorum bacterial species showed higher sensitivity than other species.

table 1

Sample	**Xlyosidase Activity**	**Sample**	**Xlyosidase Activity**
Bacillus altitudinis	+	Bacillus mycoides	-
Bacillus amyloliquefaciens	+	Bacillus pumilus	+
Bacillus atrophaeus	-	Bacillus safensis	+
Bacillus cereus	+	Bacillus stercoris	++
Bacillus halotolerans	+	Bacillus subtilis	+
Bacillus haynesii	+	Bacillus tequilensis	+
Bacillus inaquosorum	++	Bacillus toyonensis	+
Bacillus mojavensis	+	Bacillus wiedmannii	+

+++: Strong activity, ++: moderate activity, +: mild activity, −: no activity,

In these studies, the effects of xylanase production parameters such as pH of the initial medium, inoculation ratio, age of the inoculum, incubation temperature, incubation duration, composition of the medium, type of carbon and nitrogen source, agitation speed and aeration ratio were investigated. This indicates that there are various variables for the fermentation process, especially the species of xylanase-producing microorganisms. Since there are many parameters that affect the process conditions, it is complicated and time-consuming to determine the crucial parameters individually. Therefore, the process parameters that play a role in experimental studies must be determined practically. In addition, the optimization of the process conditions is important for both the yield and the economic efficiency of the process.

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