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Original Research Article

Isolation and Purfication of Xylanase from *Bacillus Cereus* by Submerged State Fermentation

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A B S T R A C T

Enzymes are important classes of globular proteins of biological origin that act as biochemical catalysts. Xylanase catalyses the hydrolysis of complex sugars (xylan) to simpler sugars (xylose) and also important in bioconversion of hemicellulose into the constituents sugar. Xylanases shows great potential for industrial applications mainly for the bioconversion of lignocelluloses to sugars, ethanol and other substances, clarification of juices and wines, improving the nutritional quality of silage and green feed and the deinking process of waste papers. The soil samples were collected and the *Bacillus cereus* was isolated by spread plate method. The isolated organism was screened by xylan agar medium. The fermentation media was characterized based on effect of substrate feeding, effect of temperature, effect of initial pH, effect of incubation time and effect of inoculums. At optimised medium the pure culture was inoculated and shake flask and bioreactor were carried out. The germinated crude extract was collected and the enzyme activity was estimated. The crude enzyme was purified by ammonium sulphate precipitation, dialysis and ion exchange chromatography and the molecular mass of enzyme was determined by SDS PAGE. The purified enzyme was immobilized by sodium alginate entrapment method.

Keywords: *Bacillus cereus*, Xylanases, SDS PAGE

A R T I C L E I N F O

Contents

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1. Introduction

The major character of enzyme is its sensitivity to the conditions in which they operate are functional only within a specific range of p^H, temperature, and presence of inhibitors and cofactors[1]. A very useful property of enzyme as catalysts is that they are generally required in very small quantities. Celulose blended with xylanase in specific ratio is used for deinking newsprint. Xylanase is added to poultry feed material such as rice, wheat, bran along with down xylans, pectins and hemicelluloses present in fruits into simpler molecules[2].

2. Materials and methods

Isolation of Microorganism from Soil Sample by Serial Dilution Method

1g of soil sample were serially diluted from 10^{-1} to 10^{-9} from that 1ml of sample was taken from 10^{-9} dilution and spread plate technique was carried out. The plates were incubated at 37˚c for 24 hours.

Screening of Organisms

The individual colonies were transferred onto oat spelt xylan agar plates.

Composition of Oat Spelt Xylan Agar Medium

Xylan - 7g
K_2HPO_4 - 1g
NaCl - 5g
$MgSo_4$ - 0.2g
Na_2CO_3 - 10g
Yeast extract - 1g
$CaCl_2$ - 0.1g
Agar - 17g
Distilled water- 1litre

Sodium carbonate was sterilized separately and added to the rest of the medium to adjust the P^H to 9.The culture were grown 37˚c for 3 days.

Identification of Microorganisms by Staining Method

Gram staining were carried to identify whether the organism is gram positive or gram negative

Inoculum Preparation

The isolate *Bacillus cereus* was inoculated into broth. Plates were incubated at 37˚C for
48 hrs.

Downstream process Optimization for the production medium Composition of selective production medium

Urea	: 0.3g
Peptone	: 0.7g
Yeast Extract	: 0.25g
$(NH_4)_2SO_4$	: 1.4g
Magnesium sulfate	: 0.3g
KH_2PO_4	: 2g
Calcium chloride	: 0.3g
$FeSO_4.7H_2O$	: 0.005g
$MnSO_4.4H_2O$	: 0.0016g
$ZnSO_4.7H_2O$	: 0.0014g
$CoCl_2.6H_2O$	: 0.02g
Distilled water	: 1 litre

3. Results & Discussion

The isolated organism was identified as gram positive, rod shaped and spore forming organism by gram staining method. The *Bacillus cereus* had high activity at P^H 9.

Effect of Temperature

Bacillus cereus was inoculated into four flaks containing the medium. The flask was incubated at different temperature (17˚C, 27˚C, 37˚C, 47˚C) for 48 hours. The resulting cultures was subjected to centrifugation at 5000 rpm at 4˚C for 10 minutes [3]

Effect of PH

Bacillus cereus were prepared with different P^H (5, 6, 7, 8 , 9) by using sodium hydroxide and incubated at 37˚C for 48 hours on a rotator shaker at 170 rpm. The resulting culture was subjected to centrifugation at 5000 rpm at 4˚C for 10 minutes [4].

Effect of Carbon Source

The *Bacillus cereus* flask were prepared with varies concentration (0.1, 0.15, 0.25 and 0.3)g of carbon source and incubated at 27˚C for 48 hours and kept on a shaker at 170 rpm. The resulting culture was subjected to centrifugation at 5000 rpm at 4˚C for 10 minutes.[5,6]

Effect of Nitrogen Source

The *Bacillus cereus* flask were prepared with varies concentration (0.05, 0.1, 0.15 and 0.2)g of nitrogen source and incubated at 27˚C for 48 hours and kept on a shaker at 170 rpm. The resulting culture was subjected to centrifugation at 5000 rpm at 4˚C for 10 minutes [7,8]

Enzyme assay by DNS method Production of enzyme Shake flask method

1ml of inoculum was inoculated into optimized medium and incubated at 27˚C for 0-48 hours at 120 rpm in shaker. After incubation the medium was centrifuged at 5000 rpm for 20 minutes. The supernatant was collected and screened for xylanase enzyme activity.

Purification of Xylanase by Ammonium Sulphate Precipitation Method

The supernatant ammonium sulphate was added to 30% saturation and undisturbed for overnight incubation at 0 to 4˚C and then the precipitate was removed by centrifugation at 8000rpm for 20 minutes at 4˚C and precipitate was dissolved in phosphate buffer for storage. The stored enzyme was subjected to dialysis and ion exchange chromatography and then it subjected to SDS-PAGE. The purified protein was entrapped in sodium alginate gel.

Effect of P^H

Sample P^H	Enzyme assay						OD	Enzyme Activity (IU)
	Enzyme Sample (ml)	Acetate Buffer (ml)	Substrate Xylose (ml)	DNS (ml)	Distilled Water (µl)	Xylan (µl)		
5	1	1	1	2	500	-	0.82	8.31
6	1	1	1	2	500	-	0.95	11.1
7	1	1	1	2	500	-	1.02	12.97
8	1	1	1	2	500	-	1.12	15.3
9	1	1	1	2	500	-	1.30	21.15
Blank		1	1	2	500	500	0.60	

Effect of Temperature

Sample Temperature (˚C)	Enzyme assay						OD	Enzyme Activity (IU)
	Enzyme (ml)	Acetate Buffer (ml)	Substrate Xylose (ml)	DNS (ml)	Distilled Water (ml)	Xylan (µl)		
17	1	1	1	2	500	-	0.70	6.0
27	1	1	1	2	500	-	0.75	6.82
37	1	1	1	2	500	-	1.70	6.2
47	1	1	1	2	500	-	1.70	6.0
Blank		1	1	2	-	-	0.60	

Sample Incubation Time(hrs)	Enzyme assay						OD	Enzyme Activity (IU)
	Enzyme Sample (ml)	Acetate Buffer (ml)	Substrate (xylose) (ml)	DNS (ml)	Distilled Water (µl)	Xylan (µl)		
24	1	1	1	2	500	-	1.20	17.9
28	1	1	1	2	500	-	1.25	19.07
Blank	-	1	1	2	-	500	0.60	

Effect of Substrate (Carbon Source)

Sample (Carbon Source)yeast extract	Enzyme assay						OD	Enzyme Activity (IU)
	Enzyme (ml)	Acetate Buffer (ml)	Substrate xylose (ml)	DNS (ml)	Distilled Water (µl)	Xylan (µl)		
0.0005	1	1	1	2	500	-	0.66	5.31
0.010	1	1	1	2	500	-	0.70	6.0
0.015	1	1	1	2	500	-	1.76	6.02
0.020	1	1	1	2	500	-	1.78	7.5
Blank		1	1	2		500	0.60	

Effect of Substrate (Nitrogen Source)

Sample (nitrogen Source) Urea	Enzyme assay						OD	Enzyme Activity (IU)
	Enzyme (ml)	Acetate Buffer (ml)	Substrate xylose (ml)	DNS (ml)	Distilled Water (µl)	Xylan (µl)		
0.010	1	1	1	2	500	-	0.67	5.31
0.015	1	1	1	2	500	-	0.68	6.0
0.025	1	1	1	2	500	-	1.77	6.02
0.030	1	1	1	2	500	-	1.82	7.5
Blank		1	1	2	-	500	0.60	

Effect of Fermenter and Shake Flask Sample

Source	Enzyme Assay						OD	Enzyme Activity (IU)
	Enzyme Sample (ml)	Acetate Buffer (ml)	Substrate Xylose (ml)	DNS (ml)	Xylan (µl)	Distilled Water (µl)		
Fermenter	1	1	1	2	-	500	1.94	10.51
Shake flask	1	1	1	2	-	500	1.62	4.35
Blank	-	1	1	2	500	-	0.60	

In purification process part both are centrifuged supernantant was treated with ammonium per sulphate and collected for precipitate fractions. All treated samples was dialysed. In both samples single band exhibiting a xylanolytic activity revealed by sds analysis indicates that this enzyme 46KDa is relatively compared with marker.

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