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Isolation, Identification and Characterization of Amylase Producing Bacteria from Commercial Corn Starch

K.M. ELIZABETH * AND CH.V. MAHESWARA RAO KUDUPA
Department of Microbiology, College of Science
Gandhi Institute of Technology and Management
Visakhapatnam-530 045, India
E-mail: kmelizabeth@yahoo.com

Three different bacteria which have the ability to produce amylase enzyme have been isolated from commercial corn starch. They were identified as *Bacillus* species by performing several biochemical tests and named as *Bacillus* species I, II and III. The amylase production from these bacteria was assessed at various pH and temperatures. The effect of different substrates on amylase production and the effect of different temperatures on enzyme activity was studied.

Key Words: Amylase, Corn starch, *Bacillus* species, Enzyme activity.

INTRODUCTION

Amylase is ubiquitous in nature. Amylases are a family of endonucleases that catalyze the hydrolysis of α-1-4-glycosidic linkages in polymers of α-D-glucose and are thus needed for most organisms. The amylases are widely distributed in microorganisms, plants and animals. It plays a key role in the mobilization of starch reserves in the germination of cereals and many legumes[1] and in the mashing process of converting starch reserves to fermentable sugars[2]. In baking, α-amylases should be in adequate amounts to hydrolyze starch from starch granules to fermentable sugars. Microbial amylases find widespread application in starch processing industries[3].

A large number of bacteria and fungi are known to produce amylases. Among these thermophilic and thermotolerant species of genus *Bacillus* and other bacteria are reported to secrete large amounts of extra stable thermostable amylases[4–6]. It was reported that a thermophilic species F6 isolated from soil was characterized to produce α-amylase at 50°C under submerged conditions[1]. The optimum production was at 32 h and 50°C. The optimum pH for amylase production was 14.8. Novamyl was a thermostable five-domain maltogenic α-amylase that shows sequence and structural homology with the cyclodextrin glycosyl transferase[7]. The α-amylase family (glycoside hydrolase family 13: GH 13) contains enzymes with approximately 30 specificities[8]. Recently, it has been reported that a new starch binding domain (SBD) in α-amylase produced by three *Lactobacillus*

species: *L. amylovorus, L plantarum, L. manihotivorans.* SBD contains 100–500 amino acids and the α-amylase in *L. plantarum* was 10 times more efficient. Gycosyl hydrolases are industrially important. There was considerable interest in engineering the enzymes for optimal performance and maximum amylase synthesis by *Bacillus* species[6, 9].

In the light of the above literature, it was evident that most of the reports were based on amylase producing strains which have been isolated from soil or other sources. But the reports on isolation of amylase producing strains from commercial starch powders were absent. Therefore an attempt has been made to isolate, identify and characterize such bacteria from corn starch powder.

EXPERIMENTAL

Isolation of bacteria from corn starch: Bacteria were isolated from commercial starch. Corn starch was serially diluted and different dilutions were plated on nutrient agar. The plates were incubated at 37°C for 18–24 h. Three different bacterial colonies with different colony morphology were isolated.

Amylase test: To determine whether the isolates produce amylase or not the isolates were subjected to amylase test. These bacteria were line inoculated on starch agar plates and incubated at 37°C for 18–24 h. Later the plates were flooded with iodine solution. Formation of white zone along the line of inoculum was scored as positive for amylase test. The three amylase-positive cultures were maintained on nutrient agar medium at 4°C from which the fresh subcultures were made for experiments.

Identification of bacteria and culture conditions: In order to identify the isolated bacteria with amylase production, several biochemical tests were performed[10]. Later, in order to assess the effect of pH, temperature and different media, the three isolates were accordingly cultivated and subsequently amylase production was evaluated. The effect of various temperatures on amylase activity was also determined.

Amylase quantification: Amylase was quantified according to the method of Sawhney and Singh[11]. Maltose standard curve was plotted and the reaction was performed with dinitrosalicylic acid. The optical density of the solution was measured at 520 nm. The bacterial cultures which were cultivated for 24 h in different media at different pH and temperatures were spun at 5000 rpm for 15 min and the culture supernatant was subjected to amylase quantification. After completion of the reaction the O.D. at 520 nm was measured for the samples and the maltose released by the amylase was drawn from the graph.

RESULTS AND DISCUSION

The results are distributed in Tables 1–6. The biochemical tests (Table-1) revealed that the three bacterial isolates from corn starch were identified as *Bacillus* species. They were named as *Bacillus* species-I (BS-I), *Bacillus* species-II (BS-II) and *Bacillus* species-III (BS-III). The production of amylase by these bacterial species when cultured in nutrient broth at different pH (Table-2) showed that there was a range of 20–280 μg/mL amylase production by BS-I, maximum

production at pH 2 and lowest production at pH 10. *Bacillus* species-II produced a maximum of 2000 μg/ mL of amylase at pH 7. *Bacillus* species-III could secrete 180 μg/mL of amylase in nutrient broth at pH 6 indicating the highest production.

TABLE-1
MORPHOLOGICAL AND BIOCHEMICAL CHARACTERIZATION OF BACTERIAL ISOLATES FROM CORN STARCH

Test	*Bacillus* species-I	*Bacillus* species-II	*Bacillus* species-III
Gram staining	+	+	+
Motility	+	+	–
Colony morphology	White, circular	Large yellow, circular	Small, rod-shaped
Spore forming ability	+	+	+
Indole	–	–	–
Methyl red	+	+	–
Voges Proskeur	–	–	+
Citrate	–	–	+
Catalase	+	+	+
Starch hydrolysis	+	+	+
Protein hydrolysis	–	+	–
Lipid hydrolysis	–	–	–

TABLE-2
AMYLASE PRODUCTION BY *BACILLUS* SPECIES CULTURED IN NUTRIENT BROTH

pH	Amylase production (μg/mL)		
	Bacillus species-I	*Bacillus* species-II	*Bacillus* species-III
2	280	0	0
4	0	40	40
6	200	0	180
7	0	2000	40
8	0	0	40
10	20	20	60

When these isolates were cultured in amylase production medium (Table-3) there was the highest enzyme production (1120 μg/mL) by BS-I at pH 4. BS-II and BS-III produced 80 and 200 μg/mL enzymes respectively at pH 7. There was a range of 0–1120 μg/mL production of amylase by BS-I in LB broth (Table-4) and 40–3000 and 20–140 μg/mL by BS-II and BS-III respectively, indicating that BS-II was the hyper amylase producer at pH 6. The effect of temperature on amylase production showed (Table-5) that there was the highest amylase produc-

tion (2000 and 2600 μg/mL) by *Bacillus*-II in nutrient broth and LB broth respectively. When cultured in amylase production medium BS-I produced maximum amylase, *i.e.*, 260 μg/mL at 37°C. The amylase activity was highest (Table-6) at 40°C in case of BS-I (260 μg/mL) and 460 μg/mL by BS-II at 30°C and 160 μg/mL by BS-III. These results clearly indicate that all three species which were isolated from corn starch were amylase producers. Luria bertain broth was the most effective medium for amylase production especially for *Bacillus* species-II. The effect of temperature on amylase activity showed that there was maximum activity at 40°C.

TABLE-3
AMYLASE PRODUCTION IN AMYLASE PRODUCTION MEDIUM

pH	Amylase production (μg/mL)		
	Bacillus species-I	*Bacillus* species-II	*Bacillus* species-III
2	800	40	0
4	1120	40	60
6	100	20	20
7	260	80	200
8	480	20	60
10	0	60	40

TABLE-4
AMYLASE PRODUCTION IN LURIA BERTAIN MEDIUM

pH	Amylase production (μg/mL)		
	Bacillus species-I	*Bacillus* species-II	*Bacillus* species-III
2	140	240	20
4	40	40	40
6	940	3000	120
7	880	2600	120
8	1120	2160	140
10	0	220	140

TABLE-5
EFFECT OF TEMPERATURE ON AMYLASE PRODUCTION

Temp. (°C)	Nutrient broth			Amylase production medium			Luria Bertain broth		
	BS-I	BS-II	BS-III	BS-I	BS-II	BS-III	BS-I	BS-II	BS-III
4	0	0	40	0	180	20	260	20	320
29	120	20	40	40	20	20	0	600	100
37	0	2000	40	260	80	200	880	2600	120

BS: *Bacillus* species.

TABLE-6
EFFECT OF TEMPERATURE ON AMYLASE ACTIVITY

Temp.	*Bacillus* species-I	*Bacillus* species-II	*Bacillus* Species-III
30	20	460	60
35	0	60	0
40	260	180	160
45	80	140	80
50	80	60	40

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