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AMYLASE SYNTHESIS IN THE PARENT AND THE MUTANT STRAIN OF A THERMOPHILIC *BACILLUS STEAROTHERMOPHILUS*[1].

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ABSTRACT

The parent strain of *Bacillus stearothermophilus* has been mutated using physical and chemical mutagens in order to get a mutant that produces high amount of thermostable extracellular amylases. Only one mutant, obtained as a result of physical mutagenic agent, showed comparable activity with the parent one. However, a mutant strain, obtained as a result of chemical mutagen (NTG), was obtained that produced more amylase comparable to the parent one but the heat stability was comparable with the parent one.

INTRODUCTION

Extracelluler amylases from bacteria have been found to be more thermostable than amylases from other sources (Hartman *et al.*, 1955). Among bacteria, *Bacillus stearothermophilus* and *Bacillus subtilis* (Ogasahara *et al.*, 1970) have been reported to produce amylases that had greater thermostability. To further increase the thermostability and/or the production of extracellular amylases, mutation studies are made. Shinke *et. al.*, (1977) have used this technique to obtain a mutant strain of *Bacillus cereus* with high production of amylase. With a view to increase the production and/or heat stability of amylases, mutation studies have been reported in this paper using a thermophilic *Bacillus* sp. (Srivastava *et al.*, 1980).

MATERIALS AND METHODS

The bacterium and the culture media used in the present study were the same as used earlier (Srivastava, *et al.*, 1980 ; Srivastava *et al.*, 1981 a, 1981 b).

For isolation of mutant *Bacillus stearothermophilus* was grown on the defined medium (Srivastava *et al.*, 1980) and the growing cell suspension spread on the agar medium was irradiated with UV light from a 10 watt germicidal lamp placed at a distance of 35 cm for 5 min. After incubation, the isolates from colonies on the plate were subjected to amylase measurement in the liquid medium. The mutant showing, better amylase activity was compared with the parent bacterium.

Mutation caused by chemical mutagen : N-Methyl-N-nitrosoguanidine (NTG) (Sigma chemical Co. St. Louis, U.S.A.) in different concentrations (20, 40, 60, 80 μg/ml) which served as chemical mutagen were added to 0.1 ml of the culture in 5 ml of the nutrient broth and was shaken for 30 min. at 30°C. NTG was washed out with fresh nutrient broth by centrifugation and washed cells were

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resuspended in the nutrient broth (5 ml) and were shaken at 50°C for 2h. The culture (0.1 ml) was plated on nutrient agar plates and pure colonies with good growth were picked up with the help of an inoculation needle on the starch agar slants. The isolates were examined for their capability to produce high heat stable amylase using the liquid medium (Srivastava *et al.*, 1981 a). Isolates were compared with the parent bacterium and if found suitable were further put on various serial experiments.

RESULTS AND DISCUSSION

Mutation with UV irradiation showed comparable amylase production and heat stability when compared to the parent strain (Table I). Mutant no. 2 produced extra-cellular amylase and showed therostability above 80°C. None of the mutants was, therefore, found to be better than the parent one. In another study of chemical mutation by NTG, the mutants showed characteristics similar to that of parent (Table II). Mutant no. 6, however, produced more amylase in the extracellular fluid, but its heat stability

TABLE I

BACILLUS STEAROTHERMOPHILUS: COMPARISON OF AMYLASE PRODUCED FROM MUTANTS AND THE PARENT STRAIN (MUTANTS OBTAINED WITH PHYSICAL MUTAGEN, UV).

	Parent strain	Selected mutants				
		1	2	3	4	5
Optimum temperature	82	78	80	76	70	77
Optimum *pH*	6.8	6.6	6.8	7.0	7.2	6.4
Heat stability* at 90° C %)	56	27	39	24	14	19

*Heat stability has been calculated taking 100% activity in optimum conditions.

was not better than the parent strain (Table II). Its optimum temperature for amylase production was found to be 82°C, but retained only 37% of its total activity at 90°C, whereas the parent strain retained about 50% of its total activity under identical conditions. (Table II). For the production of large amounts of amylase, mutant no. 6, could be of

TABLE II

BACILLUS STEAROTHERMOPHILUS- COMPARISON OF AMYLASE PRODUCED BY SELECTED MUTANTS AND THE PARENT (MUTANTS OBTAINED WITH CHEMICAL MUTAGEN, NTG).

Properties	Parent Strain	Selected mutants							
		1	2	3	4	5	6	7	8
Optimum temp.	82	79	80	81	78	80	82	81	78
Optimum pH	6.8	7.0	6.9	6.8	6.4	6.6	6.8	7.1	6.7
Heat stability* at 90 C (%)	56	37	41	40	42	35	37	29	32
pH stability range	5—12	6.5—11	6—10	5—11	5—9.5	5—9.5	6—12	6—11	6—10

*of the maximal activity in optimum conditions.

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CONIDIAL ONTOGENY IN *CERCOSPORA CANESCENS* ELLIS & MARTIN[1]

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ABSTRACT

The conidial ontogeny in *Cercospora canescens* Ellis & Martin, pathogenic to blackgram (*Phaseolus mungo* L.), was studied. Conidial development starts with prolongation of the conidiophore from one side of the distal scar, nine hours after the previous crop of conidia was removed. After some growth for four hours the distal part of the conidiophore completely transforms into a conidium which separates. This type of conidial development which may be described as 'arthrosporc-like' differs from the ealier reports.

INTRODUCTION

The conidial ontogeny is extensively used in classification of hyphomycetes since the classical work of Hughes (1953) on this aspect. He included the genus *Cercospora* Fresenius in section II of hyphomycetes, which is characterized by production of conidia from successively formed new growing points. Apart from the general description of conidial production, ontogeny was studied for a single species *C. kikuchii* in culture (Yeh and Sinclair, 1979). Because of the paucity of information on conidiogenisis in *Cercospora* species, our observations on this aspect in *Cercospora canescens*, a pathogen on backgram (*Phaseolus mungo* L.) are presented in this paper.

MATERIALS AND METHODS

Leaves of blackgram infected with *C. canescens*, were collected from the plots raised in the botanical experimental garden of Nagarjuna University. The infected leaves very thoroughly washed to remove the preformed conidia, and then incubated in humid petriplates. One or more incubated leaves were taken at hourly intervals and the infected portions cut from these leaves were fixed in FAA. The fixation was done upto 15 hours, since preliminary observations showed the conidia are fully formed by that time. Thin freehand sections were taken from the fixed material, stained with trypon blue, mounted in lactophenol and sealed with nail polish. The sections were observed under high power (10×40×) and camera lucida diagrams were drawn.

OBSERVATIONS

No development of conidiophore was observed upto 8 hours, after previously formed conidia were removed. A finger like projection (6 μm length) of conidiophore from one side of the distal scar was observed after 9 hours. By 10th hour (Fig. 1 a-c) this was slightly pushed to a side as the conidiophore continues to grow. The newly forming portion measured about 12-15 μm and it is thin

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