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IN VITRO PRODUCTION OF PROTEOLYTIC ENZYMES BY *ASPERGILLUS SYDOWII VAR. AGRAII* SHARMA ET SHARMA

The microorganisms are known for the production of proteolytic enzymes by which they hydrolyse proteins (Hofmann and Shaw, 1964, Koch and Dedic, 1957). In the present investigation the production of the proteolytic enzymes has been studied *in vitro* by *Aspergillus sydowii* var. *agraii* Sharma et Sharma var. nov (Sharma and Sharma, 1978) to know the behavior of this fungus in comparison to *A. sydowii* (Bain & Sart.) Thom & Church and the specificity of the enzymes.

The enzyme production by these fungi was studied on different broth media. Each medium (40 ml) was taken in 250 ml Erlenmeyer flask and sterilized at 12 lb. pressure for 30 minutes. The media were inoculated with 1 0 ml of spore suspensions (10-15 spores/drop) of pure cultures of test fungi and incubated for 7 days at 30±1°C. A control was also maintained for each case. The effect of pH and temperature was also determined on enzyme production by growing these organisms on medium selected on the basis of maximum yield of enzymes.

The assessment of enzyme production was made following colorimetric method of Penner and Aston (1967) in combination with the method of Lowry *et al.* (1951). The enzyme units were calculated from the standard graph prepared by taking tyrosine in various concentrations ranging from 0.0001 to 0.1 mg/ml. The protease activity is expressed in units; one unit is equal to the amount of enzyme that liberates 0.001 mg of tyrosine from 1.0 ml of 0.1 per cent casein solution by the action of protease in 1.0 ml of culture filtrate at 35°C in one hour.

The results (Table-1) indicated that no proteolytic activity was obtained in the control sets. Medium composition considerably influenced the enzyme production. Out of seven media taken, the maximum production was recorded on gelatin broth medium and hence selected for further studies.

Table 1. *Effcet of media, pH and temperature on the production of proteolytic enzymes by test-fungi*

	Quantity of enzymes in units	
	A. sydowii var. agrail	*A. sydowii*
Media		
Peptone water	16.0	20.3
Peptone leather	15.5	35.1
Glucose yeast extract nutrient broth	35.2	35.0
Nelatin broth	54.7	50.0
Nutrient broth	30.0	35 2
Czapek's broth	48.2	40.2
Beef extract peptone broth	35.3	30.1
Control	0.0	0.0
p H		
4.0	15.3	9.4
5.0	16.0	10.0
6.0	60.2	48.2
7.0	67.2	51.7
8.0	44.2	30.1
9.0	10.0	15.2
Control	0.0	0.0
Temperature°C		
25	35.2	30.0
30	54.7	50.0
35	40.2	35.3
40	20.3	15.2
45	10.2	10.2
Control	0.0	0.0

One Unit = 0.001 mg tyrosine released by the hydrolysis of casein.

It can clearly be seen from Table 1 that the optimum pH and temperature for the maximum enzyme production were 7.0 and 30°C respectively. The low as well as high temperatures retarded the enzyme production. Production of these enzymes has been studied by various workers (Wetter, 1952, Yoshida *et al*, 1959, Satyanarayan & Jain, 1978) in the case os different fungi. But as regards the production and activity are concerned, variable behaviour has been recorded so far.

The qualitative specificity of enzymes in culture filtrates of these fungi was estimated on different substrates (casein, gelatin, eggalbumun and collagen) because of their presence in finished leather. The 0-1 per cent solution of each substrate was mixed separately in reaction tubes with 1.0 ml of culture filtrate of the test fungi and adjusted to pH 7.0 using phosphate buffer. The tubes were incubated at 35°C for one hour. At the end of incubation period, the reaction mixtures were tested for the release of amino acids; 0.1 ml of aliquot was separated on paper chromatograms (Block *et al*., 1955). After the spray of 0.3% alcoholic solution of ninhydrin, distinct spots were obtained on chromatograms which were identified with the corresponding spots of known pure amino acids.

The observations revealed that the crude culture filtrates of these fungi could hydrolyse all these proteins liberating amino acids as hydrolysis products. Tyrosine was found to be released by the hydrolysis of casein, gelatin and egg albumin and hydroxyproline in the case of collagen. *Aspergillus sydowii* var. *agraii* exhibited high activity on casein, gelatin and egg albumin whereas *A. sydowii* showed high specificity on casein and moderate on gelatin and egg albumin. Both these test organisms were slow in their action when collagen was used as substrate.

The over all assessment of results indicated that the new variety *A. sydowii* var. *agraii* Sharma et Sharma is an active producer of proteases and produces comparatively more units than *A. sydowii* (Bain & Sart.) Thom & church (Table-1). These proteases have wide range of specificity and may play an important role in the degradation of leather proteins.

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