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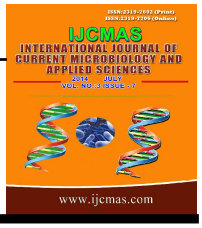

Original Research Article

A comparison on the production and characterisation of aminopeptidase from two species of *Aspergillus*

J.M.Benila Smily[1], R.Sivakami[2], G.Prem Kishore[3], P.Sumithra[1*]

[1]Department of Microbiology, Srimad Andavan Arts & Science College, Tiruchirappalli, Tamil Nadu, India
[2]P.G & Research Department of Zoology, Arignar Anna Govt. Arts College, Musiri, India
[3]Alan Feldman Public School and Junior College, Kazhakkuttm - 695 582, Kerala, India
**Corresponding author*

A B S T R A C T

K e y w o r d s

Aminopeptidase, *Aspergillus*, pH, Temperature, Carbon, Nitrogen

Aminopeptidase compare a large group of enzymes which are specially involved in metabolism of biologically active peptides. In addition aminopeptidases have many important commercial applications. Hence the present study was used to assess the production and characterization of aminopeptidase in two species of *Aspergillus (A. flavus* and *A. oryzae*) collected from Athivayal lake. Results show that aminopeptidase activity measured at different pH and temperature activity measured at different pH and temperature was found to be optimal at pH 7 and 45 °C for both the species. However, between both the species the activity was higher in *A. flavus* than *A. orvzae.*

Introduction

Aminopeptidases comprise a large group of enzymes that exist in animal and plant tissues as well as in microorganisms (Gonzalez and Boudouy, 1996; Strater and Hipscomb, 1998). Aminopeptidases catalyse the cleavage of amino acid residues from the N-terminals of peptides and proteins (Lin *et al*., 2008) and are specifically involved in the metabolism of biologically active peptides like hormones, neurotransmitters and peptides of food origin (Nampoothri *et al*., 2005).

Aminopeptidases have many important commercial applications. They are used in the food industry for debitessing and improving the functional properties of protein products and flavour development in cheese (Rao *et al*., 1998; Sanders *et al*., 1995; Ishihara *et al*., 1987; Nishimura *et al*., 1992; Okitani *et al*., 1981; Arima *et al*., 2006). In pharmaceutical industry inhibitors developing against their enzyme are used as drugs. They are also used for peptide sequencing and in the processing of recombinant proteins (Shiram *et al*., 2012).

The present study was conducted to assess the production and characterisation of amino peptidases in two different species of

Aspergillus that are commonly found in freshwater sediments of these parts of the region.

Materials and Methods

Sample Collection and Preparation

The freshwater sediment sample was collected from Aathivayal lake, Pudukkottai District, Tamil Nadu. The samples were kept in sterile polythene bags in refrigerator. One gram of sediment sample was mixed with 9 ml of sterile distilled water and serial dilution of the sample was made.

Isolation and Identification of Fungi

The technique of James and Natalie (2001) was adopted for identification of the unknown isolated fungi using cotton blue in lactophenol stain. The identification was achieved by placing a drop of the stain on a clean slide with the help of a mounting needle where a small portion of the mycelium from the fungal cultures was removed and placed in a drop of lactophenol. The mycelium was spread very well on the slide with the help of the needle. A coverslip was gently applied with little pressure to eliminate air bubbles. The slide was then mounted and observed and the species were identified in accordance with Chessbrough (1993).

Selection of the Fungus

As *Aspergillus oryzae* and *A. flavus* were dominant in collected sediment samples they were selected for further studies.

Optimization of pH for Aminopeptidase Activity

The activity of aminopeptidase was evaluated at different pH such as 5, 6, 7, 8 and 9 under assay condition. The fermentation media with each pH were prepared and inoculated with 0.2 ml of 2% fungus spore suspension. The flasks were then incubated at room temperature for 5 to 7 days (Felipe *et al.*, 2005).

Optimization of Temperature for Aminopeptidase Activity

To determine the effect of temperature on aminopeptidase activity, the reaction was carried out at different temperatures such as 25, 35, 45, 55 and 65 °C. The fermentation media were inoculated with 0.2 ml of 2% fungus spores suspension and the flasks were incubated at selected temperatures for 5 to 7 days.

Optimization of Carbon source for Aminopeptidase activity

The activity of aminopeptidase was evaluated with the use of different carbon sources, such as glucose, lactose, maltose, sucrose and arabinose. The fermentation media were prepared and carbon sources were added. After the sterilization, the prepared media were inoculated with 0.2 ml of 2% fungus spores suspension. The flasks were incubated at room temperature for 5 to 7 days.

Optimization of nitrogen source for aminopeptidase activity

The reaction was carried out using different nitrogen sources like ammonium chloride, ammonium nitrate, sodium nitrate, potassium nitrate and calcium nitrate. The inoculated flasks were incubated at room temperature for 5 to 7 days.

Estimation of Proteins

Protein concentration was determined according to Lowry *et al*. (1951).

Results and Discussion

By direct plating from the lake sediment, a total of 11 *Aspergillus* sps. could be isolated. The list of species isolated are provided in Table-1. Among these, as *Aspergillus oryzae* and *Aspergillus flavus* were the dominant organisms, both of them were chosen for the present investigation. The result of the optimization of pH for aminopeptidase activity are presented in Table-2. As evident from the Table for both the species, the maximum production was recorded at pH 7 and the minimum at pH 5. However, among the two species, *A. flavus* recorded higher production (0.38 ± 0.06 IU/ml) when compared to *A. oryzae* (0.34 ± 0.4 IU/ml).

The optimization temperature for aminopeptidase activity at various temperature are presented in Table-3. Results indicate that the aminopeptidase activity peaked at 45 °C for both the species with *A. flavus* recording higher activity (4.9 ± 0.2 IU/ml) when compared to *A. oryzae* (4.7 ± 0.46 IU/ml). Nevertheless for both the species, the aminopeptidase activity increased with increase in temperature till 45 °C followed by a decrease in activity with further increase in temperature.

The optimization of carbon source for aminopeptidase activity is presented in Table-4. Among the various sources used, maltose appeared to be the best as the highest activity was noticed in both the species with this source. However, among the two *A. flavus* recorded higher levels of activity (3.8 ± 0.31 IU/ml) when compared to *A. oryzae* (3.9 ± 0.28 IU/ml). Further, for both the species minimal activity was noticed when the source was arbinose.

Table-5 records the optimization of different nitrogen sources for aminopeptidase activity. As evident from the Table, both the species recorded maximal activity with sodium nitrate with *A. flavus* recording higher levels (0.5±0.9 IU/ml) than *A. oryzae* (0.52 ± 0.62 IU/ml). Further, both the species recorded minimal activity with Ammonium sulphate.

Table-6 records the estimation of protein at different pH of aminopeptidase production. Results indicate that among the various pH analysed, both species recorded maximal production at pH 7 with *A. flavus* again recording higher levels (0.23 ± 0.28 IU/ml) than *A. oryzae* (0.20 ± 0.28 IU/ml). The minimal production was noticed in both the species at pH 5.

Table-7 shows the estimation of protein at different temperatures of aminopeptidase production. Here also for both the species, maximum protein production took place at 45 °C with *A. flavus* recording higher levels (0.46 ± 0.64 IU/ml) than *A. oryzae* (0.42 ± 0.24 IU/ml). However, the minimal activity again for both the species was noticed at 25 °C.

Table-8 records the estimation of protein with different carbon sources for aminopeptidase production. Results indicate that maximal production for both the species were recorded when the source was maltose. However, *A. flavus* recorded higher production (0.29 ± 0.72 IU/ml) when compared to *A. oryzae* (0.262 ± 0.42 IU/ml). Table-9 records the estimation of protein with different nitrogen sources for aminopeptidase production. Results indicate that maximal production for both to species were recorded when the source was potassium nitrate. However, *A. flavus* higher production (0.43 ± 0.54 IU/ml) when compared to *A. oryzae* (0.39 ± 0.26 IU/ml).

Table.1 List of *Aspergillus* from the sediment of the lake

S. No.	Name of the Species	Percentage Contribution
1.	*Aspergillus flavus*	26.4
2.	*A. fumigatus*	6.8
3.	*A. nidulans*	5.6
4.	*A. terrus*	8.4
5.	*A. versicolar*	7.2
6.	*A. clavatus*	4.5
7.	*A. carneus*	5.6
8.	*A. japonicas*	2.8
9.	*A. niger*	9.8
10.	*A. flavipes*	1.2
11.	*A. sydowii*	1.4
12.	*A. oryzae*	20.1

Table.2 Optimization of aminopeptidase production by *Aspergvillus oryzae* and *A. flavus*

S. No.	pH	Enzyme activity (IU/ml) mean/SD	
		Aspergillus oryzae	***Aspergillus flavus***
1.	5	0.12 ± 0.03	0.14 ± 0.02
2.	6	0.17 ± 0.05	0.20 ± 0.04
3.	7	0.34 ± 0.04	0.38 ± 0.06
4.	8	0.26 ± 0.03	0.29 ± 0.07
5.	9	0.30 ± 0.36	0.31 ± 0.08

Table.3 Optimization of aminopeptidase production by *Aspergillus* species at different temperature

S. No.	Temperature °C	Enzyme activity (IU/ml) mean/SD	
		Aspergillus oryzae	***Aspergillus flavus***
1.	25	3.4 ± 0.40	3.6 ± 0.32
2.	35	3.6 ± 0.38	3.9 ± 0.81
3.	45	4.7 ± 0.46	4.9 ± 0.82
4.	55	3.6 ± 0.98	3.8 ± 0.72
5.	65	2.2 ± 0.82	2.6 ± 0.82

Table.4 Optimization of different carbon sources for aminopeptidase activity of *Aspergillus* species

S. No.	Carbon source	Enzyme activity (IU/ml) mean/SD	
		Aspergillus oryzae	***Aspergillus flavus***
1.	Glucose	1.1 ± 0.62	1.3 ± 0.62
2.	Maltose	3.8 ± 0.31	3.9 ± 0.28
3.	Lactose	3.2 ± 0.44	3.3 ± 0.48
4.	Sucrose	1.2 ± 0.33	1.4 ± 0.82
5.	Arabinose	0.6 ± 0.26	0.8 ± 0.46

Table.5 Optimization of different nitrogen sources for aminopeptidase activity of *Aspergillus* species

S. No.	Nitrogen source	Enzyme activity (IU/ml) mean/SD	
		Aspergillus oryzae	***Aspergillus flavus***
1.	Ammonium nitrate	0.42 ± 0.72	0.43 ± 0.28
2.	Ammonium sulphate	0.28 ± 0.60	0.31 ± 0.38
3.	Sodium nitrate	0.50 ± 0.44	0.54 ± 0.64
4.	Potassium nitrate	0.52 ± 0.62	0.57 ± 0.75
5.	Calcium nitrate	0.32 ± 0.82	0.34 ± 0.64

Table.6 Estimation of protein at different pH amino peptidase production by *Aspergillus* species

S. No.	pH	Estimation of protein (IU/ml) mean/SD	
		Aspergillus oryzae	***Aspergillus flavus***
1.	5	0.06 ± 0.21	0.07 ± 0.28
2.	6	0.15 ± 0.32	0.16 ± 0.24
3.	7	0.20 ± 0.28	0.23 ± 0.28
4.	8	0.13 ± 0.18	0.14 ± 0.32
5.	9	0.04 ± 0.28	0.07 ± 0.42

Table.7 Estimation of protein at different temperature of amino peptidase production by *Aspergillus* species

S. No.	Carbon source	Estimation of protein (IU/ml) mean/SD	
		Aspergillus oryzae	***Aspergillus flavus***
1.	25	0.20 ± 0.28	0.22 ± 0.62
2.	35	0.32 ± 0.42	0.34 ± 0.21
3.	45	0.42 ± 0.24	0.46 ± 0.64
4.	55	0.28 ± 0.32	0.30 ± 0.32
5.	65	0.24 ± 0.42	0.27 ± 0.28

Table.8 Estimation of Protein with different carbon sources for aminopeptidase production by *Aspergillus* species

S. No.	Carbon source	Enzyme activity (IU/ml) mean/SD	
		Aspergillus oryzae	***Aspergillus flavus***
1.	Glucose	0.031 ± 0.62	0.042 ± 0.62
2.	Maltose	0.262 ± 0.42	0.290 ± 0.72
3.	Lactose	0.094 ± 0.38	0.120 ± 0.68
4.	Sucrose	0.122 ± 0.28	0.140 ± 0.60
5.	Arabinose	0.083 ± 0.62	0.090 ± 0.42

Table.9 Estimation of Protein with different nitrogen sources for aminopeptidase production by *Aspergillus* species

S. No.	Nitrogen source	Enzyme activity (IU/ml) mean/SD	
		Aspergillus oryzae	***Aspergillus flavus***
1.	Ammonium nitrate	0.21 ± 0.16	0.23 ± 0.18
2.	Ammonium sulphate	0.28 ± 0.18	0.38 ± 0.50
3.	Sodium nitrate	0.24 ± 0.24	0.24 ± 0.52
4.	Potassium nitrate	0.39 ± 0.26	0.43 ± 0.54
5.	Calcium nitrate	0.19 ± 0.64	0.21 ± 0.54

Literature reveals that *Aspergillus* cultures have been used for non-specific aminopeptidase production (Blinkousky *et al*., 2000). For the uptake of any short chain peptides, *Aspergillus* species has not been reported to have any transport system and therefore the proteolytic enzyme should contain a range of different peptidases to enable the organism to degrade the various proteins to free amino acids (Garraway and Evans, 1984).

In the present study, the production of aminopeptidase by assay of enzymes at different temperatures revealed that for both the species, the ambient temperature was 45 °C. Literature reveals that Blimkovsky *et al*. (2000) reported maximum aminopeptidase production to occur in *A. oryzae* strain AICC 2036 at 55 °C while Sriram *et al*. (2012) suggested maximum production for *A. flavus* to occur at 45 °C and for *A. oryzae* at 30 °C. Nampoothri *et al*. (2005) suggested that optimal temperature of the enzyme assay often coincides with the optimum temperature for the growth of the microorganism from which the aminopeptidase enzyme originated. With regard to pH, aminopeptidase showed maximal activity for both the species when the pH was 7. Chein *et al*. (2012) reported that *A. sojae* (ATCC42249) was most active at pH 9 while Blinkocky *et al*. (2006) recorded maximal activity to occur in *A. oryzae* (ATCC2036) at pH 9.5. Sriram *et al.* (2012) reported that maximal activity occurred at pH 7 for *A. flavus* which is in line with the present observation. Nevertheless, Vitale *et al*. (1986) reported that aminopeptidase activity is strongly influenced by pH, with the optimal pH generally to be in the range of 6-9. The study further shows that both carbon and nitrogen sources are important not only for the growth but also for the production of aminopeptidase. In the present study, maltose and potassium nitrate recorded maximal activity for both the species.

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