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L-asparaginase Activity in Growing Conditions of *Streptomyces* spp. associated with *Therapon jarbua* and *Villorita cyprinoids* of Veli Lake, South India

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Streptomyces spp. isolated from the gut of fish, *Therapon jarbua* and *shellfish, Villorita cyprinoids* exhibited L-asparaginase activity under growing conditions in a liquid broth. *Streptomyces* AQBTJ 60 (isolate from fish) showed maximum enzyme activity at pH 8.0 whereas *Streptomyces* AQB VC67 showed it at pH 7.0. They showed optimum enzyme activity and growth at temperature 37°C. Carbon sources and amino acids had varying influence based on the constituents in the gut or viscera of the host organisms and heavy metals inhibited activity and growth. Similar trend was noticed with sodium chloride in the estuarine strains.

Key words: Streptomyces spp; enzyme activity; *T. jarbua; V. cyprinoids*

L-asparaginase (L-asparagine amino hydrolase Ec.9.5.1.1.) is an enzyme used for the treatment of tumors and acute lymphatic leukemia in human beings. This enzyme has been reported in bacteria, fungi and streptomycetes from Indian coastal waters (Selvakumar, 1979; Maya *et al.*, 1992 a,b; Annie *et al.*, 1994; Annie, 1995; Annie *et al.*, 1997). The survey on the association of streptomycetes with molluscs and fish and shellfish were carried out on the east and west coast. But for the report of Annie *et al.* (1997) and Dhevendaran & Annie (1999) there is a lack of available literature on streptomycetes having both antagonistic property and L-asparaginase activity. The aim of this work is to study the L-asparaginase activity in antagonistic streptomycetes associated with the gut of the fish, *T. jarbua* and that of shellfish *V. cyprinoids* under different cultural conditions like temperature, pH, sodium chloride, carbon sources, amino acids, heavy metals and inorganic phosphate and sulfate.

Materials and Methods

T. jarbua and *V. cyprinoids* were collected five times in a month using a cast net and hand picking respectively. They were brought to the laboratory alive for microbiological analysis. 16 streptomycetes strains were isolated using Kusters agar (Kuster, 1963) medium similar to the method employed previously by Dhevendaran & Annie (1999).

Purified streptomycetes cultures (eight from fish and eight from shellfish) were screened for L-asparaginase activity. The cells were suspended in distilled water to a concentration of 1 g wet weight. 1 g wet weight of fully grown individual streptomycete was inoculated into 5 ml of glycerol-asparaginase broth (Annie *et al.*, 1994). The medium was incorporated with 0.2 ml of 1% L-asparagine. They were individually incubated for 7 days at different cultural conditions such as varying pH (6 to 10), temperature (4 to 45°C), carbon sources (glucose, lactose, sucrose, raffinose and mannitol at 1% level), amino acids (methionine, threonine, glutamic acid and tryptophan at 0.8 mg/ml concentration), varying concentration of sodium chloride (0.1 to 5%), heavy metals (mercury, copper, zinc and arsenic at 2 ppm concentration) and inorganic phosphate and

sulfate (0.05 – 0.2 M concentration) At the end of the incubation period (7 days) the growth of the individual streptomycete was expressed as g dry weight by filtering through filter paper and drying in oven at 60°C.

L-asparaginase activity in the filtrate was measured by adding 0.5 ml of Nessler's reagent. Centrifuged supernatant for yellow coloured solution was read in UNICAM Spectrophotometer at 450 nm. Culture broth without the substrate was kept as control. Enzyme activity was expressed as µg ammonia/ml/h (Dhevendaran & Annie, 1999).

Result and Discussion

As it is difficult to obtain sufficient quantities of L-asparaginase from marine microorganisms, not many studies on this enzyme have been carried out. 16 streptomycetes associated with fish and shellfish showed different degree of enzyme activity and growth. Except the recent reports of Annie *et al.* (1997) and of Dhevendaran & Annie (1999) on L-asparaginase from antagonistic *Streptomyces plicatus* and *Streptomyces* spp. from sediment, fish and shellfish, no pertinent literature is available from South Indian Coast regions. The selected two cultures (*Streptomyces* AQBTJ60 and *Streptomyces* AQBVC67) exhibited L-asparaginase activity and growth at different degrees based on the cultural conditions. Streptomycetes showing maximum enzyme activities have been taken for the experimental studies.

Figs. 1a & 1b showed optimum growth for both the cultures at pH 8.0 but the L-asparaginase activity was maximum in *Streptomyces* AQBTJ60 at pH 8.0 and for *Streptomyces* VC67 at pH 7.0. In addition to this the latter culture has an additional peak at pH 6.0. This clearly indicates that the growth and metabolic activity preferably L-asparaginase activity were independent. This observation confirmed the earlier findings of Maya *et al* (1992a & b) observed in Bacillus and Moraxella of retting ground. In *Penaeus indicus* AQB 104 the enzyme activity and growth were maximum at pH 7.0

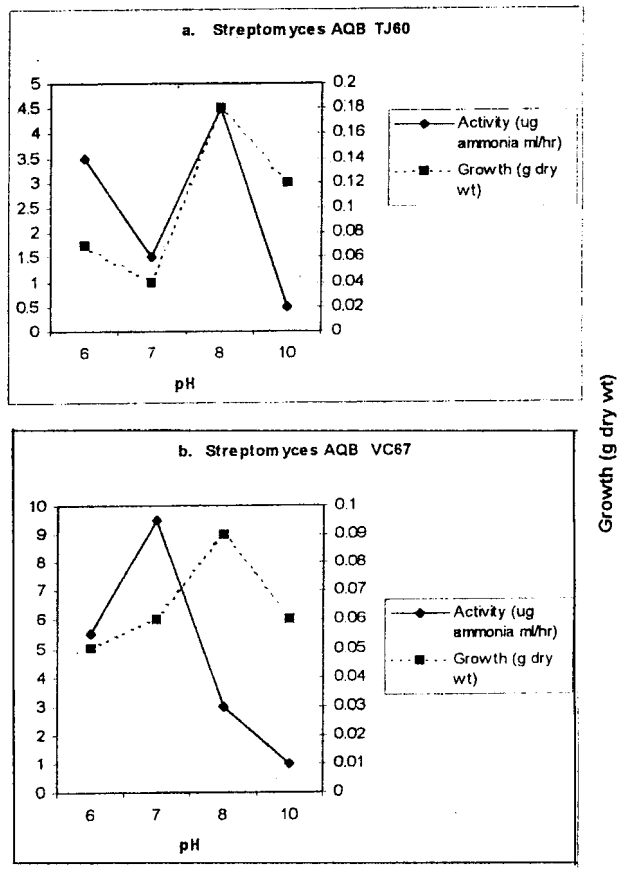

Fig. 1. Effect of pH on the L-asparaginase activity and growth

(Dhevendaran & Annie, 1999). It is presumed that extra cellular secretion or release of L-asparaginase enzyme after the rupture of the cell wall of the host streptomycetes may be the possible reason for differential peaks as well as additional peaks also.

Since the cultures were isolated from aquatic organisms the study of the influence of temperature on the enzyme activity and growth of streptomycetes is imperative. It was observed (Figs. 2a & 2b) that optimum activity and maximum growth were at 37°C for both the cultures thereby implying that they might have been transported from terrestrial environment as suggested by Imada & Okami (1995). Their adaptation to the aquatic environment was due to metabolic change and the production of L-asparaginase may be unique. It has already been reported by Maya *et al* (1992a & b) that optimum activity and maximum growth in *Bacillus* sp. and *Moraxella* sp. were at 37°C

and *Vibrio* sp. exhibited (Selvakumar, 1979) maximum L-asparaginse activity at 68°C eventhough it was isolated from shellfish *Telescopium telescopium* of Vellar estuary. Recently Dhevendaran & Annie (1999) observed maximum activity of this enzyme in *Streptomyces* AQB PI 104 at 37°C isolated from *Penaeus indicus* of Veli lake region stating that it was mesophilic in nature. Further, the temperature of the water column at the time of collection of specimen was around 32±2°C.

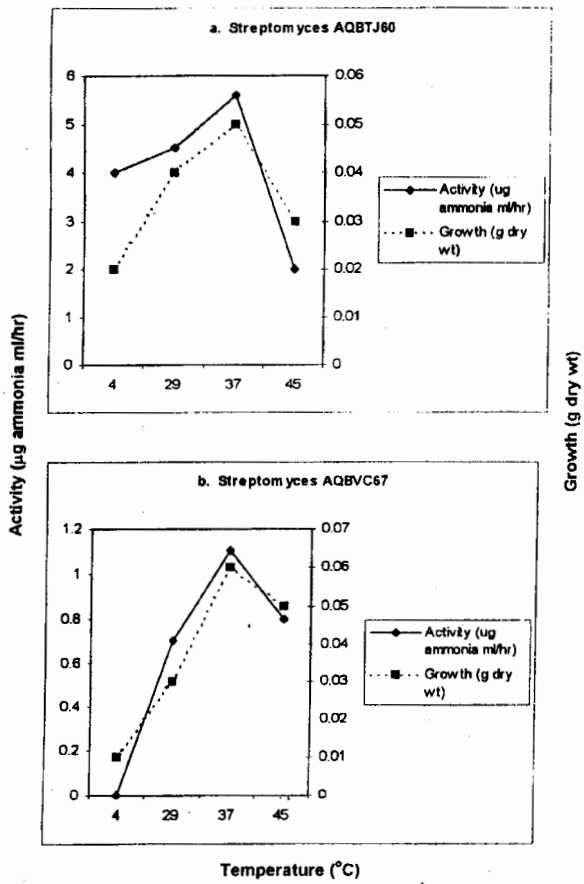

Fig. 2. Effect of Temperature on the L-asparaginase activity and growth

Since streptomycetes were isolated from the estuarine fish and shellfish, it was necessary to understand the tolerance of enzymatic activity and growth at various concentration of sodium chloride (Fig. 3a & 3b). *Streptomyces* AQBTJ60 showed maximum enzyme activity at 0% and optimum growth at the range of 1-2% sodium chloride concentrations stating that they were independent to sodium chloride concentration as

Table 1. Effect of carbon sources on the L-asparaginase activity and growth of *Streptomyces* AQBTJ60 and *Streptomyces* AQBVC67

Sl. No.	Carbon source (1%)	Enzyme activity (µg am/ml/h)		Growth of streptomycetes (g dry wt.)	
		AQBTJ 60	AQBVC 67	AQBTJ 60	AQBVC 67
1	Control	1.40	0.75	0.68	0.61
2	Glucose	0.50	1.50	0.04	0.01
3	Sucrose	1.20	5.30	0.06	0.01
4	Lactose	13.00	0.50	0.03	0.03
5	Raffinose	0.50	3.30	0.01	0.02
6	Mannitol	0	2.50	0.03	0.02

suggested by Maya *et al* (1992a) and by Dhevendaran & Annie (1999). Among carbon sources (Table 1) lactose enhanced L-asparaginase activity in strain AQBTJ60 and it inhibited in *Streptomyces* AQBVC67. However, sucrose induced maximum growth in *Streptomyces* AQBTJ60 and the growth was inhibited in *Streptomyces* AQBVC67.

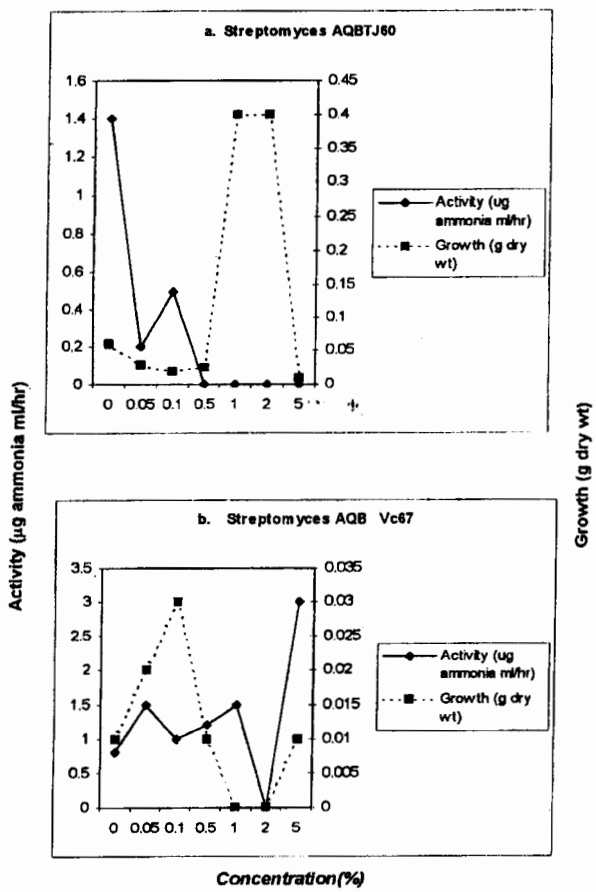

Fig. 3. Effect of NaCl Concentration on the L-asparaginase activity and growth

Table 2. Effect of amino acids on L-asparaginase activity and growth of *Streptomyces* AQBTJ 60 and *Streptomyces* AQBVC 67

Sl. No.	Amino acids (0.8 mg/ml)	Enzyme activity (µg am/ml/h)		Growth of streptomycetes (g dry wt.)	
		AQBTJ 60	AQBVC 67	AQBTJ 60	AQBVC 67
1	Control	1.40	0.75	0.68	0.61
2	Methionine	1.50	0.40	0.04	0.01
3	Tryptophan	0	1.10	0.11	0
4	L-Glutamic acid	0	0.75	0.03	0.02
5	Threonine	2.00	0.80	0.02	0

Table 2 shows the effect of added amino acids on L-asparaginase activity and growth of streptomycetes selected. As far as the growth was concerned in both the cultures the added amino acids do not have influence, thereby indicating the naturally available amino acids in the host organisms were sufficient for their maximum growth as observed by Annie *et al* (1994).

Table 3. Effect of heavy metals on the L-asparaginase activity and growth of *Streptomyces* AQBTJ 60 and *Streptomyces* AQBVC 67

Sl. No.	Heavy metals (2 ppm)	Enzyme activity (µg am/ml/h)		Growth of streptomycetes (g dry wt.)	
		AQBTJ 60	AQBVC 67	AQBTJ 60	AQBVC 67
1	Control	1.40	0.75	0.68	0.61
2	Mercury	0	0	0.03	0.01
3	Zinc	0	0.10	0.06	0.02
4	Copper	0	0.80	0.02	0.02
5	Arsenic	0	0.10	0.06	0.02

Since the streptomycetes were isolated from organisms exposed to industrial effluents, it is advisable to understand the added divalent cations on L-asparaginase activity and growth (Table 3) of them. It was very clear that added cations have strong inhibiting effect on both of them. Similar results were noticed in *Vibrio* sp. (Selvakumar, 1979) in *Bacillus* sp., *Moraxella* sp. (Maya *et al.*, 1991a & b) and in *Streptomyces* sp. associated with shellfish of Veli lake region (Annie *et al.*, 1994). Tables 4 & 5 showed influence of

Table 4. Effect of sulfate on the L-asparaginase activity and growth of *Streptomyces* AQBTJ 60 and *Streptomyces* AQBVC 67

Sl. No.	$MgSO_4$ concentration (M)	Enzyme activity (µg am/ml/h)		Growth of streptomycetes (g dry wt.)	
		AQBTJ 60	AQBVC 67	AQBTJ 60	AQBVC 67
1	Control	1.40	0.75	0.68	0.61
2	0.05	1.70	2.50	0.05	0.01
3	0.10	1.80	0.80	0.04	0
4	0.20	2.00	0.50	0.02	0.02

Table 5. Effect of phosphate on the L-asparaginase activity and growth of *Streptomyces* AQBTJ 60 and *Streptomyces* AQBVC 67

Sl. No.	$MgSO_4$ concentration (M)	Enzyme activity (µg am/ml/hr)		Growth of streptomycetes (g dry wt.)	
		AQBTJ 60	AQBVC 67	AQBTJ 60	AQBVC 67
1	Control	1.40	0.75	0.66	0.61
2	0.05	0.20	1.10	0.03	0.02
3	0.10	0	2.00	0.05	0.02
4	0.20	0	2.25	0	0.03

inorganic sulfate (0.2M) and phosphate (0.2M) on L-asparaginase activity and growth of selected streptomycetes.

From the above observations it is possible to optimize the growth conditions and the maximum L-asparaginase enzyme synthesis under laboratory conditions. Further, the indigenous constituents of the host organisms and the environmental factors have influence on the metabolic activity of microorganisms also.

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