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Comparison of α -amylase from bacterial and plant sources

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Abstract

14 plant and bacterial species were extensively investigated for α-amylase enzyme present in them. Comparison was made on the basis of optimum pH, optimum temperature, Km, Vmax, pH stability range and thermal stability of enzyme. It was found that the plant enzyme is equally good than the bacterial enzyme in terms of characteristics.

Keywords: *Dolichos biflones*, *Phaseolus vulgaris*, *α- amylex*, *enzyme*

Introduction

The α-amylase industry generally focuses on bacterial species for production of the enzyme. Among plants, the Family Poaceae is considered for enzyme production in food industry to a certain extent. The present research focuses on two new sources of α-amylase from the family leguminoceae. It is of considerable interest that if a comparison is made between the properties of α-amylase obtained from bacterial sources and that from leguminous sources, then the industrial feasibility of α-amylase of leguminous plants can be explored to a certain extent. In the present paper studies on two 14 α-amylase sources have been compared.

Materials and Method

Kinetic characteristics of *Dolichos biflorus* and *Phaseolus vulgaris* were studied according to standard protocols adopted by Goyal and Dobriyal, 2010. Optimum pH, optimum temperature, pH stability, thermal stability, Km and Vmax were studied according to standard protocol of Miller (1959). Studies on *Eleusine coracana* Indaf- 15 were done according to Nirmala and Muralikrishna (2006). *Sorghum bicolor* was studied according to Nour and Yaqoub (2010); *Triticum aestivum* according to Mohammed *et al.* (2009); *Glycine max* according to Kumar *et al*. (2010) and *Zea mays* according to Biazus (2009). Among bacteria, the *Bacillus subtilis* (2 strains) and *Bacillus amyloliquefaciens* strain F, α- amylase were investigated according to Welker and Campbell (1967), Gangadharan *et al*. (2009), Femi-Ola and Olowe (2011). *Bacillus cereus* α- amylase was studied according to Anto *et al*. (2006); *Thermomonospora curvata* was studied according to Glymph and Stutzenberger (1977); X*anthomonas campestris* K-11151 according to Abe *et al*. (1994) and *Streptococcus bovis* JB1 was studied according to Freer, 1993.

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Results and Discussion

α-amylase of *Dolichos biflorus* and *Phaseolus vulgaris* (Family Leguminoceae) showed complete instability in highly alkaline pH range. Optimum pH of Rajma came out to be 6.5 while that of Kulath came out to be 6.1. These were completely unstable above pH 7.5. However, these were compared with α-amylase of the family Poaceae, which were stable in acidic pH range only, and become completely unstable above pH

7. *Eleusine coracana* Indaf-15, *Sorghum bicolor*, *Zea mays* and *Triticum aestivum* were studied. All these have pH stability range and optimum pH below 7 (Table 1). Among bacteria, 6 bacterial strains were studied, of which 3 belonged to the genus *Bacillus*. All *Bacillus species* have an optimum pH below 6.5, and are unstable in alkaline range. *Thermomonospora curvata* and *Streptococcus* had showed slight stability in alkaline range, only up to pH 8.5. It was thus concluded that that optimum pH of almost all bacterial and plant amylases range between pH 4 to 8.

The thermal stability of all amylases is not the same. It was deduced that the legume family has a high thermal stability, and the amylase is stable almost up to 80˚C. However, the grass family has a relatively low thermal stability with amylase stability up to a temperature of 50˚C. Among bacteria, the thermal stability is varying. Strains of *Bacillus* spp. are highly thermo-stable, while *Streptococcus* and *Xanthomonas* have low thermal stability. Generally, the optimum temperature ranges between 40-55˚C (Table 2).

Table.1: Table showing comparison between temperature and pH for plant amylases

S.No	Property / α-amylase source	Common Name	pH stability range	Optimum pH	Thermal Stability (˚C)	Optimum temperature (˚C)
1	*Dolichos biflorus*	Kulath	5.5 -7	6.1	40-75	45
2	*Phaseolus vulgaris*	Rajma	4 -7.5	6.5	30-80	50
3	*Glycine max*	Soyabean	4-6	5.5	25-85	50
4	*Eleusine coracana* Indaf-15	Ragi or Finger millet	5-5.5	5.3	45-50	47
5	*Sorghum bicolor*	Jowar	4.5-6	5.5	30-70	70
6	*Triticum aestivum* (5 isozymes)	Wheat	5.5-7	5.5-7	40-50	50
7	*Zea mays*	Corn	4-6.5	5.5	50-90	55

Table 2: Table showing comparison between temperature and pH for bacterial amylases

S.No	Property / α-amylase source	pH stability range	Optimum pH	Thermal Stability (˚C)	Optimum temperature (˚C)
1	*Bacillus amyloliquefaciens* strain F	5.5 -6	5.9	0-70	65
2	*Bacillus subtilis Strains* (W23 and BS5)	5.7 -6	6.3	0-70	65
3	*Bacillus cereus*	4-6	5	35-75	55
4	*Thermomonospora curvata*	6 -8	7.5	40-70	53
5	*Xanthomonas campestris* K-11151	4.5-5	4.5	45-55	45
6	*Streptococcus bovis* JB1	5.5-8.5	5-6	Below 50	Below 50

Further comparison was made on the basis of size. Among plants, it was found that the plant amylases are small in size in comparison to bacterial amylases. Such small sized proteins are beneficial in food industry. Recent researches conducted by food industries proved that small protein molecules are easily digestible in the small intestine, and provide very high surface area for reaction (Table.3 and 4). Table 5 gives a very close comparison of km value between plant and bacterial enzyme. Low km values of plant alpha amylases studied indicate high affinity of the enzyme for starch substrates, like some bacterial strains. Not all workers have stated the Vmax values in their work owing to the fact that, Vmax values of different enzymes are difficult to compare as they depend on the substrate used and the reaction conditions.

The investigation simply proves that the kinetic characteristics of plant and bacterial amylases are similar. It was finally concluded that the plant enzyme is as good as bacterial enzyme in terms of characteristics. The study also proves that the enzyme can be better produced commercially by legume sources.

Table 3: Molecular weight of amylase from Bacterial sources

Bacterial Source	Molecular weight (KDa)	Reference
B. stearothermophilus	43 to 46	Yutani (1973)
Thermomonospora curvata	62	Glymph and Stutzenberger (1977)
Bacillus subtilis AX20	149	Najafi *et al.* (2005)
Bacillus subtilis BS5	63	
Xanthomonas campestris K-11151	55	Abe *et al.* (1994)
Streptococcus bovis JB1	77	Freer 2010

Table 4: Molecular weight of amylase from Plant sources

S. No	Plant Source	Molecular weight (KDa)	Reference
1.	*Dolichos biflorus*	Below 30	Goyal and Dobriyal, 2010
2	*Phaseolus vulgaris*	Below 30	Goyal and Dobriyal, 2010
3	*Eleusine coracana* Indaf-15	22	Nirmala and Muralikrishna, G (2003)

Table 5: Comparison of Km value of α-amylase from plant and bacterial sources

S.No	Source of α-amylase	Km value (mg/ml)
1	*Dolichos biflorus*	1.95
2	*Triticum aestivum* (5 isozymes)	1.42-1.7
3	*Bacillus amyloliquefaciens strain* F	3.076
4	*Bacillus subtilis* BS5	16.67
5	*Thermomonospora curvata*	0.39
6	*Streptococcus bovis* JB1	0.88

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