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Short Communication: Thermostability of α-amylase produced by *Bacillus* sp. E2—a thermophilic mutant

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An α-amylase from a hyper-producing strain of *Bacillus* (sp. E2) was stable at 70°C for 30 min but was quickly inactivated at higher temperatures. In the presence of 10 mM Ca^{2+} and starch (20% w/v), however, the enzyme was stable at 90°C for 10 min and after 30 min at 100°C still retained 26% of its initial activity.

Key words: α-Amylase, *Bacillus* sp., thermostability.

Table 1. Effect of 10 mM $CaCl_2$ and soluble starch on the thermostability of the α-amylase produced by *Bacillus* sp. E2.

Additions	Temperature (°C)	Residual α-amylase activity (%) after heating for:*		
		5 min	10 min	30 min
None	70	100	100	100
	80	62	41	11
	90	36	10	ND
	100	0	ND	ND
Ca^{2+}	70	100	100	100
	80	72	67	21
	90	2	ND	ND
	100	0	ND	ND
Ca^{2+} and 5% (w/v) starch	70	100	100	100
	80	100	100	85
	90	74	63	19
	100	31	11	2
Ca^{2+} and 20% (w/v) starch	70	100	100	100
	80	100	100	86
	90	83	81	32
	100	41	41	26

*The enzyme was heated as indicated, cooled and assayed at 70°C. 100% activity = 4300 units/ml.
ND—Not detectable.

An amylolytic, thermophilic *Bacillus* sp. has been isolated from a hot-water spring. Although it resembled *Bacillus stearothermophilus* in many characters, it showed some significant differences from it and other thermophilic *Bacillus* spp. Accordingly, the isolate was designated as *Bacillus* sp. MK 716. The isolate has been mutated with ethyl methane sulphonate and a hyper α-amylase-producing mutant, E2, obtained. The enzyme yield of E2 was 4800 units/ml compared with 140 units/ml from MK 716. The thermostability of the α-amylase has now been examined.

Materials and Methods

For enzyme production, the organism was grown in M9 broth (Maniatis *et al.* 1982) containing 1% soluble starch for 20 h at

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60°C and 200 rev/min in a shaker. The cell-free supernatant was used as enzyme source. The α-amylase assay was modified from that of Fuwa (1954): 0.25 ml enzyme was mixed with 0.25 ml 0.2% soluble starch in 0.1 M phosphate buffer pH 6.5, and kept at 70°C for 10 min. The reaction was stopped by adding 0.25 ml 1M HCl; 0.25 ml 0.2% I_2 (in 2% KI) and 4 ml water were added and the absorbancy measured at 690 nm. In the control, enzyme was replaced with buffer. Activity was calculated as described by Fuwa (1954).

Results and Discussion

The activity of the α-amylase from *Bacillus* sp. mutant E2 was as stable as the enzyme from the wild-type (MK 716), being unchanged after 30 min at 70°C but inactivated at higher temperatures (Table 1). However, addition of $CaCl_2$ (10 mM) and soluble starch (20% w/v) significantly improved the thermostability of the enzyme at 80, 90 and 100°C. The enzyme, which had been completely inactivated within 5 min at 100°C, retained 26% activity after 30 min at 100°C when mixed with Ca^{2+} and starch. The increased stability of the enzyme correlated with the concentration of starch added. Moreover, no residual starch was detected after 10 min with the highest concentration (20%) of starch that was used. This property of the enzyme may be useful for industrial liquefaction and hydrolyses which are carried out in the presence of 35% starch.

References

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