

PROTEOLYTIC ACTIVITY OF *BACILLUS* SPECIES ISOLATED FROM MILK AND DAIRY PRODUCTS

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ABSTRACT

One hundred and eighty two *Bacillus* species isolated from milk and dairy products were analyzed for their ability to induce proteolysis in sterilized milk. The proteolysis expressed as percentage and measured in terms of a decrease in casein nitrogen to total protein was the highest in *Bacillus subtilis* (4.82%), followed by *Bacillus cereus* (4.15%), *Bacillus licheniformis* (4.08%), *Bacillus megaterium* (3.52%), *Bacillus circulans* (2.89%), *Bacillus coagulans* (2.79%), *Bacillus sphaericus* (2.71%) and *Bacillus pumilus* (2.56%) respectively. *B.subtilis*, *B.cereus*, *B.licheniformis* and *B.megaterium* produced off flavours at the end of 30 h of incubation at 37°C with curdling of milk.

Key words: *Bacillus*, milk, dairy products, proteolysis obtained from milk and dairy products.

INTRODUCTION

Advances in dairy technology have resulted in improved reliance on refrigeration to maintain raw milk quality during storage and transportation. Psychrotrophic thermophilic bacteria like *Bacillus* produce extracellular enzymes which can cause extensive proteolysis in milk (Cousin, 1982). The dairy industry is concerned about the damage to milk components due to proteolysis and lipolysis. Increased proteolysis reduces the economic value of milk by its negative impact on protein functionality especially, casein nitrogen. Proteolysis can reduce cheese yield and cause bitter off flavours in dairy foods (Ma *et al.*, 2000). The objective of this study was to estimate the extent of proteolysis induced by *Bacillus* isolates

MATERIALS AND METHODS

The total viable count and aerobic spore count were carried out as per the standard methods for examination of dairy products, APHA, (1978). After incubation, the representative colonies that had developed on the petri dishes were isolated and cultured in the nutrient broth. Morphological, cultural and biochemical tests were carried out to identify the isolates as per the methods described by Buchanan and Gibbons (1974).

The estimation of proteolysis was done as per AOAC (2000) by Kjeldahl method by determining the total nitrogen, non protein nitrogen and non casein nitrogen. Decrease in casein nitrogen / total protein (CN/TP) was used as an index of proteolysis as described by Ma *et al.* (2003). The data obtained in the study were

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analyzed statistically as per the methods described by Snedecor and Cochran (1989).

RESULTS AND DISCUSSION

One hundred and eighty two isolates were identified as *Bacillus* species based on the cultural and morphological characteristics. Of the total *Bacillus* isolates, 27 of 51 *Bacillus subtilis* (53%), 23 of 39 *Bacillus megaterium* (59%), 18 of 28 *Bacillus cereus* (64%), 14 of 32 *Bacillus licheniformis* (44%), all the 7 *Bacillus coagulans* (100%), 6 of 8 *Bacillus sphaericus* (75%) and 7 of 9 *Bacillus pumilus* (78%) and 6 of 8 *Bacillus circulans* (75%) induced proteolysis.

The growth and proteolytic activity of *Bacillus* species at different duration is presented in Table 1 and the proteolytic activity of the *Bacillus* isolates, expressed as a percentage of decrease in casein nitrogen to total protein is presented in Table 2. *Bacillus subtilis* produced the highest proteolytic activity followed by *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus circulans*, *Bacillus coagulans*, *Bacillus sphaericus* and *Bacillus pumilus* at the end of 30 h of incubation at 37°C.

Meer *et al.* (1993) concluded that 56% of the *Bacillus* isolates obtained from 59 Grade A milk samples in Oregon, US were proteolytic which was lower than the findings of the present study (68.5%). Isolates of *Bacillus cereus* had significantly higher proteolytic activity than other isolates, which fairly agreed with the findings of the present study.

Garcia *et al.* (1994) found that proteinases obtained from *Bacillus subtilis* were highly active and produced bitterness because of their intense proteolytic action on b-casein. In the present study, the *Bacillus subtilis* isolates produced the highest proteolytic activity at 6 h, 12 h, 18 h, 24 h and 30 h duration when compared to other *Bacillus* isolates.

Almeida *et al.* (2000) concluded that 92% of the *Bacillus* isolates were proteolytic and / or lipolytic in nature. In this study, on an average 68.50% of total *Bacillus* species isolated were found to be proteolytic. Santos *et al.* (2003) concluded that decrease in casein, as a percentage of true protein (CN/TP), of low and high proteolysis skim milk was 0 and 4.76% respectively. As presented in Table 2, the net CN / TP decrease expressed in percentage was fairly within the range obtained in the present study.

Ma *et al.* (2000) predicted the sensory threshold for detection of off flavour due to proteolysis would be about a 4% decrease in CN / TP, which correlated well with the findings of the present study wherein the isolates of *Bacillus subtilis*, *Bacillus cereus*, *Bacillus licheniformis* and *Bacillus megaterium* produced off flavour at the end of 30 h of incubation with curdling of milk.

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Table 2.
Proteolysis (net decrease in CN / TP) by different *Bacillus* isolates

S.No	Name of the isolate	Extent of Proteolysis {% decrease in (CN / TP)*}
1	<i>Bacillus subtilis</i> (27)	4.82
2	<i>Bacillus megaterium</i> (23)	3.52
3	<i>Bacillus cereus</i> (18)	4.15
4	<i>Bacillus licheniformis</i> (14)	4.08
5	<i>Bacillus coagulans</i> (7)	2.79
6	<i>Bacillus sphaericus</i> (6)	2.71
7	<i>Bacillus pumilus</i> (7)	2.56
8	<i>Bacillus circulans</i> (6)	2.89

Figures in parenthesis indicate total number proteolysis positive isolates.

Table 1.
Growth and proteolytic activity of *Bacillus* species

S.No	Name of aerobic spore former	Treatment	Duration of incubation at 37°C					
			0 h	6 h	12 h	18 h	24 h	30 h
1	<i>Bacillus subtilis</i> (51)	SPC (Log ₁₀ cfu / ml)	3.59 ± 0.031	4.44 ± 0.087	5.20 ± 0.026	6.59 ± 0.075	7.51 ± 0.095	7.76 ± 0.104
		Proteolytic activity (CN/TP) %	80.147 ± 0.007	79.802 ^a ± 0.043	79.410 ^a ± 0.041	78.157 ^a ± 0.058	76.257 ^a ± 0.064	75.330 ^a ± 0.149
2	<i>Bacillus megaterium</i> (39)	SPC (Log ₁₀ cfu / ml)	3.61 ± 0.034	4.55 ± 0.038	5.21 ± 0.017	6.31 ± 0.021	7.35 ± 0.100	7.69 ± 0.084
		Proteolytic activity (CN/TP) %	80.173 ± 0.018	79.930 ^{ab} ± 0.043	79.713 ^c ± 0.032	78.705 ^{cd} ± 0.045	77.205 ^b ± 0.091	76.658 ^c ± 0.136
3	<i>Bacillus cereus</i> (28)	SPC (Log ₁₀ cfu / ml)	3.63 ± 0.036	4.48 ± 0.059	5.28 ± 0.037	6.72 ± 0.069	7.73 ± 0.053	7.94 ± 0.008
		Proteolytic activity (CN/TP) %	80.178 ± 0.022	79.892 ^{ab} ± 0.025	79.500 ^{ab} ± 0.038	78.308 ^{ab} ± 0.038	77.145 ^b ± 0.123	76.032 ^b ± 0.180
4	<i>Bacillus licheniformis</i> (32)	SPC (Log ₁₀ cfu / ml)	3.60 ± 0.025	4.46 ± 0.053	5.17 ± 0.020	6.54 ± 0.111	7.47 ± 0.092	7.82 ± 0.108
		Proteolytic activity (CN/TP) %	80.165 ± 0.026	80.077 ^c ± 0.038	79.672 ^{bc} ± 0.053	78.467 ^{bc} ± 0.091	76.890 ^b ± 0.116	76.082 ^b ± 0.119
5	<i>Bacillus coagulans</i> (7)	SPC (Log ₁₀ cfu / ml)	3.66 ± 0.030	4.53 ± 0.056	5.14 ± 0.030	6.43 ± 0.084	7.31 ± 0.117	7.54 ± 0.115
		Proteolytic activity (CN/TP) %	80.237 ± 0.031	79.963 ^{bc} ± 0.042	79.588 ^{abc} ± 0.042	78.760 ^d ± 0.064	77.907 ^c ± 0.077	77.445 ^d ± 0.101
6	<i>Bacillus sphaericus</i> (8)	SPC (Log ₁₀ cfu / ml)	3.64 ± 0.021	4.63 ± 0.055	5.19 ± 0.022	6.67 ± 0.070	7.61 ± 0.070	7.92 ± 0.018
		Proteolytic activity (CN/TP) %	80.228 ± 0.043	79.928 ^{ab} ± 0.031	79.522 ^{ab} ± 0.037	78.653 ^{cd} ± 0.057	77.867 ^c ± 0.143	77.522 ^d ± 0.137
7	<i>Bacillus pumilus</i> (9)	SPC (Log ₁₀ cfu / ml)	3.60 ± 0.031	4.59 ± 0.044	5.22 ± 0.042	6.63 ± 0.081	7.67 ± 0.028	7.90 ± 0.017
		Proteolytic activity (CN/TP) %	80.227 ± 0.046	79.912 ^{ab} ± 0.035	79.590 ^{abc} ± 0.063	78.705 ^{cd} ± 0.063	78.193 ^c ± 0.089	77.663 ^d ± 0.064
8	<i>Bacillus circulans</i> (8)	SPC (Log ₁₀ cfu / ml)	3.63 ± 0.025	4.63 ± 0.054	5.23 ± 0.030	6.54 ± 0.064	7.57 ± 0.114	7.85 ± 0.082
		Proteolytic activity (CN/TP) %	80.243 ± 0.039	79.965 ^{bc} ± 0.043	79.598 ^{bc} ± 0.062	78.687 ^{cd} ± 0.075	77.922 ^c ± 0.143	77.357 ^d ± 0.090

Means bearing different superscripts within a column differ significantly (P < 0.01);

Figures in parenthesis indicate the total number of isolates.

SPC and proteolytic values indicate mean value of number of samples given in parenthesis.