

reports have been published by many early workers (Harrington, 1972 and Rajendran, 1976). Therefore, it becomes imperative to consider both vigour

and germination together in deciding the safe storage period for individual clones.

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INFLUENCE OF CERTAIN CHEMICAL FACTORS IN GERMINATION OF SPORE CELLS OF *BACILLUS CEREUS*

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Among the various chemicals added glucose, casamino acid, alanine, aspartic acid and glycine showed higher germination of *B. cereus* spores. Among the positive germinants which were tested in milk all of them showed marked improvement in promoting the germination of spore cells of *B. cereus*. The most positive germinant among these was L-alanine which as a result of enhancing the germination of *B. cereus* increases the keeping quality of heat treated milk to 30 hours at room temperature.

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Among the predominance of microorganisms surviving laboratory pasteurisation of farm milk, *Bacillus cereus* as a major constituent of them (Thomas *et al.*, 1967). This micro-organism is associated with various defects in market milk (Franklin, 1969) and produces food poisoning (Boventre and Johnson, 1970; Bulyba *et al.*, 1973) and causes mastitis in cows and goats (Agarwal and Srinivasan, 1981; Akthar *et al.* 1982). These organisms have two forms - vegetative forms which are easily destroyed by heat and spore forms which are highly resistant. Hence, the effect of amino-acids, sugars, vitamins and nucleic acids on the germination of *B. cereus* spores are undertaken in the study.

MATERIALS AND METHODS

One of the isolates of *B. cereus* spores isolated from milk which was identified by morphological, cultural and biochemical tests as described by Buchanan and Gibbons (1974) was used in this study. The spores were inoculated in basal medium (Beef extract: 0.1 gm; Glucose - 1.0 gm; and Phosphate buffer 100ml, at pH 7.2).

The chemicals were added to the basal media were peptone, urea, case-amino acid, tryptone, L-alanine, phenylalanine, threonine, aspartic acid, proline, valine, glutamic acid, arginine, glycine, histidine, tryptophan, and serine at three levels of nitrogen viz. 0.1 mg N/ml; 0.3 mg N/ml, and 0.5 mg N/ml. The following vitamins were also added individually to the basal medium., thiamine (20 mg/100ml), nicotinic acid (100 mg/100ml), biotin (0.4 mg/100 ml),

folic acid (1 mg/100 ml), pyridoxin (100 mg/100ml), and calcium pantothenate (50 mg/100 ml). The nucleic acids, adenine, guanine and uracil each at 1 mg/100 ml were also added. The following sugars viz., glucose, sucrose starch were added at 0.5 and 1.0% level.

Milk was collected aseptically from a clean healthy cow. The udder of the cow was washed first with clean water and wiped dry, then it was disinfected using 200 ppm of chlorine. The teats were then dipped in 70% alcohol for a minute. Care was taken to see that the milker's hand was also clean and was further disinfected using alcohol. About one litre was thus collected in a sterilized can. 200 ml of the milk was taken in a sterilized bottle and cooled to 3° C immediately.

The low count milk collected was transferred into sterile glass flasks separately and the spores of *B. cereus* at a level of 1200 spores/ml was added and lab pasteurized and the milk was cooled and plated on yeast milk agar and incubated at 37° C. At 0 and 8 hours aliquot amounts were analysed and total count per ml was determined. Simultaneously the sample was heated to 80° C and plated. From this the percentage of germination was calculated for the basal media added milk samples.

RESULTS AND DISCUSSION

The rate of germination varied for the different nitrogen sources and

it is interesting to observe that casmino acids increased germination of spores to 64% (Table 1).

Table 1 Influence of different nitrogen sources in the basal medium on germination of spores of *B. cereus** at 37°C
[Average of 5 trials]

Basal Medium plus Nitrogen source	Concentration	Percentage of Germination Period of incubation (8 hours)
Control		20.0
Casamino acid	I	62.0
	II	62.0
	III	64.0
Urea	I	24.0
	II	24.0
	III	26.0
Peptone	I	56.0
	II	46.0
	III	58.0

*Spore cells of *B. cereus* were added to milk to give a concentration of spore cells between 1000 to 1500/ml.

- I : 0.1 mg N/ml
II : 0.3 mg N/ml
III : 0.6 mg N/ml

Among the various amino acids tried it was observed (Table 2) that maximum germination was obtained in the basal medium containing alanine, aspartic acid and glycine at the rate of 92.4: 93.0 and 91.0% respectively

irrespective of the concentration. This agrees with the work of other authors who have found the influence of L-alanine in promoting germination of spores of *B. cereus* cells (Church *et al.* 1954; Pulvertaft and Haynes, 1951 and Natarajan, 1980). Similar to the observation in this investigation these authors also came across negative results for germinating the spore cells of *B. cereus* by valine, leucine, isoleucine, serine, threonine, methionine and phenylalanine.

Table 2 Influence of different amino acids in the basal medium on germination of spores of *B. cereus** at 37°C [Average of 3 trials]

Basal medium plus Amino acids	Concentration	Percentage of germination Period of incubation [8 hours]
Control		26.0
Alanine	I	92.0
	II	92.2
	III	92.4
Arginine	I	20.0
	II	19.0
	III	20.0
Aspartic Acid	I	92.0
	II	93.0
	III	92.0
Glutamic Acid	I	24.0
	II	28.0
	III	27.0

1	2	3
Histidine	I	20.0
	II	26.0
	III	22.0
Glycine	I	91.0
	II	90.0
	III	91.0
Phenylalanine	I	20.0
	II	22.0
	III	22.0
Proline	I	20.0
	II	22.0
	III	20.0
Serine	I	20.0
	II	14.0
	III	16.0
Threonine	I	16.0
	II	16.0
	III	18.0
Tryptophan	I	34.0
	II	34.0
	III	32.0
Valine	I	18.0
	II	20.0
	III	18.0

I : 0.1 mg N/ml II : 0.3 mg N/ml; 0.6 mg N/ml
 * Spore cells of *B. cereus* were added to milk to give a concentration of spore cells between 1,000 to 1,500 ml.

Though the vitamins were known to contribute positively to enhance

the growth of microorganisms in general it is interesting to note that (Table 3) in the present study only 40% of germination was observed in the case of thiamine and 36% in the case of folic acid.

The results of the studies on the influence of nucleic acids in basal medium is given in Table 4. It is noted that all the purine and pyrimidine bases showed similar rate of increase in germination ranging from 48-50% thereby indicating that these agents are not fully involved in the process of germination contradicting the observation of Pulvertaft and Haynes (1951) who have shown positive effect of adenosine.

Table 5 shows the results of the studies carried out on the influence of glucose, sucrose and starch on germination of spore cells of *B. cereus*. Out of these sugars glucose was found to favour the germination of spore to 82% at 1% level, while sucrose and starch exerted less influence as reported by Nakata and Halvorson (1960); Nakata (1963) and Natarajan (1980).

Some of the positive germinants that promotes germination of spore of *B. cereus* were tried in milk to find out whether there is a further enhancement of germination rate. (Table 6). It can be seen that casamino acid added to milk has shown a further increase in the rate of germination to the extent of 91%. Similar increase was obtained in the case of glucose added to milk. Alanine responded well

Table 3 Influence of different vitamins in the basal medium on germination of spores of *B. cereus** at 37°C (Average of 5 trials)

Sl. No.	Basal Medium plus Vitamins	Concentration	Percentage of germination
			Period of incubation [8 hours]
	Control		16 38
1.	Thiamine	200.00 mg/100 ml	40
2.	Biotin	0.04 mg/mL ml	24
3.	Nicotinamide	100 mg/100 ml	30
4.	Folic acid	100 mg/100 ml	36
5.	Pyridoxine	100 mg/100 ml	30
6.	Calcium Pantothenate	50 mg/100 ml	24

* Spore cells of *B. cereus* were added to milk to give a concentration of spore cells between 1000 to 1500/ml

Table 4. Influence of different constituents of purine and pyrimidine bases on germination of spores of *B. cereus** at 37°C (Average of 5 trials)

Sl. No. Basal medium	Percentage of germination
	Period of incubation [8 hours]
Control	46
1. Adenine	48
2. Guanine	54
3. Uracil	52

Concentration — 1 mg/100 ml

* Spore cells of *B. cereus* were added to milk to give a concentration of spore cells between 1000 to 1500/ml.

Table 5 Influence of different sugars on germination of spores of *B. cereus** at 37°C in the basal medium [Average of 5 trials]

Sl No.	Basal media plus sugars	Germination	Percentage of germination
			Period of incubation [8 hours]
1.	Glucose		82 92
2.	Sucrose		18 20
3.	Starch		18 20

*Spore cells of *B. cereus* added to milk to give a concentration of spore cells between 1,000 to 1,500 per ml.

Table 6 Influence of positive germinants added to milk on the germination of spores of *B. cereus** at 37°C

Positive germinants	Germination percentage after 8 hours	Keeping quality of heat treated milk [8 hours]
Casmino acid 0.6 mg N/ml	91	24
Glucose 1%	97	28
Alanine 0.6 mg N/ml	96.36	30
Aspartic acid	96.80	26
Glycine	90.00	24

* approximately 1200 spore cells were added to low count milk (less than 30,000 bacteria/ml).

1. Keeping quality of heat treated milk at room temperature.

to give a germination rate of 98.3%. Glucose, casamino acid and alanine were found influence germination of the greater proportion of *B. cereus* cells. The keeping quality of heat treated milk is maximum (30 hours) in the milk samples to which alanine

was added. From this investigation it is evident that addition of one or combination of the above chemicals in raw milk helps to maximise the the germination of spores resulting in the destruction of vegetative cells during pasteurization which

ultimately helps in the keeping quality of milk.

The amino acid alanine added at a level of 0.6 mg N/ml has shown better keeping quality and this evidence reveals addition of this amino acid before pasteurization will help in increasing the keeping quality.

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