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OCCURRENCE AND ENUMERATION OF *BACILLUS CEREUS* IN EGYPTIAN DAIRY DESSERTS

(With 3 Tables)

By

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تواجد الباسيليس سيريس فى الحلويات اللبنية المصرية

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تضمن البحث فحص ٢٠٠ عينه من الحلويات اللبنيه المصريه (٥٠ عينه لكل من الأيس كريم والمهلبيه والكسترد والأرز باللبن) جمعت بطريقة عشوائية من محلات الألبان والسوبر ماركت بمدينة المنصوره محافظة الدقهلية - جمهورية مصر العربية ، وذلك لمعرفة تواجد ميكروب الباسيليس سيريس بها بإستخدام طريقة الفرد السطحي على مستنبت MYP . وأثبتت النتائج عن تواجد الميكروب في عينات الأيس كريم والمهلبية والكسترد والأرز باللبن بنسب ٣٨٪، ٤٤٪، ٤٢٪، ٦٤٪ على التوالي . وكان متوسط العدد الكلي للباسيليس سيريس ٦٨ر٩ x ١٠^٤ ± ٤٣ر٣ x ١٠^٤ ، ٦٥ر١ x ١٠^٥ ± ٥٦ر٦ x ١٠^٥ ، ٧١ر١ x ١٠^٥ ± ٠ر٦ x ١٠^٥ ، ٣٩ر٢ x ١٠^٧ ± ٥ر١ x ١٠^٧ على التوالي . وبدراسة تأثير المضادات الحيويه على العترات المعزولة من الميكروب أوضحت النتائج أن جميع العترات أظهرت حساسية بنسبه ١٠٠٪ لكل من الإستربتومايسين والكلورمفينكول والتتراسيكلين وباستراسين والكاناميسين والجنتاميسين والإرثرومايسين والنيومايسين. وعلى عكس ذلك جميع العترات غير حساسه للكولستين سلفات. هذا وقد نوقشت التوصيات الواجب مراعاتها في إنتاج وتداول الحلويات اللبنية.

SUMMARY

A total of 200 random samples of Egyptian dairy desserts including flavoured ice cream; mehallabia, custard and rice pudding (each 50 samples) were collected from different dairy shops and supermarkets in El-Mansoura City,

El-Dakahlia province, Egypt. The samples were examined bacteriologically to enumerate and isolate *Bacillus cereus* using surface plating technique on Mannitol egg youlk phenol red polymyxin (MYP) agar plates. *Bacillus cereus* was found in 19 (38%); 22 (44%); 21(42%) and 32(64%) samples of flavoured ice cream, mehallabia, custard and rice pudding with a mean values of $9.68 \times 10^4 \pm 3.43 \times 10^4$; $1.65 \times 10^5 \pm 0.56 \times 10^5$; $1.71 \times 10^5 \pm 0.60 \times 10^5$ and $2.39 \times 10^7 \pm 1.5 \times 10^7$ cells/g. respectively . All *B. cereus* strains were highly sensitive to streptomycin, chlormphenicol, tetracycline, kanamycin, gentamycin, neomycin and erythromycin. Whereas all the isolates were resistante to colistin . The suggestive measures to minimize contamination of dairy desserts with *B. cereus* are discussed.

Key words: *Bacillus cereus Egyptian diary desserts*

INTRODUCTION

Bacillus cereus is widely distributed in the environment and is frequently found in milk. The unique combination of both thermoduric and psychrotrophic properties of *B. cereus* represents recurring problems of dairy industries (Meer et al., 1991). It had been shown to cause spoilage of pasteurized milk (sweet curdling) and cream (bitty cream) under the effect of exoenzymtic action (Collins, 1981). Moreover, even at low count (10^5 cells/ml) *B. cereus* was capable to cause off flavours of milk products such as unclean, fruity, bitter, putrid, rancid and yeasty (Stadhouders 1993).

Bacillus cereus had been reviewed in food poisoning cases linked with milk and dairy products (Christiansson, 1992). It was implicated in two distinct forms of food poisoning, a rapid onset emetic syndrome and a delayed onset diarrhoeal syndrome (Van Netten et al. , 1990). The number of colony forming units of *B. cereus* required to produce disease are generally of the order 10^8. However, in compromised consumers a much smaller dose may cause illness (Gianella & Braisle, 1979). It was also reported that the organism may be the causative agent of bovine mastitis (Jones and Turnbull 1981).

Although sweet dairy desserts seem to be excellent substrates for growth of *B. cereus*, there is very little information on this subject in the literature. Therefore this work was planned to know the incidence and level of contamination of *B. cereus* in Egyptian dairy desserts (Flavoured ice cream, mehallabia, custard and rice pudding). Trials were also made to examine the antibiotic sensitivity of isolated B. sereus strains .

MATERIALS and METHODS

Two hundreds random samples of dairy desserts including flavoured ice cream, mehallabia, custard and rice pudding (each 50 samples) were collected from different dairy shops and supermarkets in El - Mansoura City, El-Dakahlia province, Egypt. The samples were transferred in sample cases and delivered promptly to the laboratory.

The samples were prepared according to the method recommended by Anon, (1985).

Enumeration and isolation of *B. cereus*

The surface plating technique was used for enumeration and isolation of *B. cereus* 0.1ml of each dilution was transferred and evenly spread onto a dry surface of Mannitol egg yolk phenol red polymyxin (MYP) agar plates (Mossel, et al., 1976) and incubated at 30°C for 48 hours. Suspected colonies showing pink colouration and surrounded by precipitated zone of lecithinase activity were counted. The countable colonies were picked up, purified and identified according to Cowan and Steel (1974) and Shinogawa (1993).

Antibiotic sensitivity of isolated *B. cereus* strains

The diffusion method described by Cruickshank et al. (1975) was performed using different types of sensitivity discs obtained from BioMerieux France.

RESULTS

The results are presented in Tables 1, 2 and 3.

DISCUSSION

Results reported in Tables (1) and (2) reveal that *B. cereus* was isolated from 38% of flavoured ice cream samples in counts ranging from 4×10^3 - 1.2×10^6 cells/g with a mean value of $9.68 \times 10^4 \pm 3.43 \times 10^4$ cells/g. The highest frequency distribution (63.16%) lies within the range of 10^3-10^5 cells/g. The results obtained are nearly similar to that reported by Nagah (1985) and Roy (1993). On the other hand these results are lower than there reported by Ahmed et al. (1983) and higher than the finding recorded by Nikodemusz (1979).

The incidence of *B. cereus* in mehallabia samples was 44% with a minimum count 1×10^2 cells/g. and maximum count 1.8×10^6 cells/g. The

mean value was $1.65 \times 10^5 \pm 0.56 \times 10^5$ cells/g. The highest frequency percentage (50%) lies within the range of 10^5-10^6 cells/g. Tables (1) and (2). As no available literature could be traced dealing with the incidence of *B. cereus* in Egyptian dairy desserts (mehallabia), therefore, it was hard to discuss the formentioned results. However, there are relatively a few papers describing the role of vanilla sauce as a major source of *B. cereus* implicated with food poisoning. Hauge (1950 and 1955) observed the development of symptoms of *B. cereus* food poisoning among four human volunteers who drank 155-27 ml of vanilla sauce containing 3.6×10^7 cells/ml. The author consumed 200ml of the same product carrying 9.2×10^7 cells/ml, and he suffered from sever abdominal pains, diarrhoea and rectal tenesmus after 13 hour from ingestion which persisted 8 hours.

B. cereus was also found in 21 (42%) custard samples with a count varying from 9×10^2 to 2.4×10^6 cells/g. with a mean value of $1.71 \times 10^5 \pm 0.60 \times 10^5$ cells/g. The highest frequency distribution (71.43%) lies within the range of 10^4-10^6 cells/g.

The findings recorded in Tables 1 and 2 exhibit that *Bacillus cereus* was detected in 32 (64%) samples of rice pudding with a count varying from 1×10^3 to 7.2×10^8 cells/g. The mean value was $2.39 \times 10^7 \pm 1.5 \times 10^7$ cells/g. The highest frequency distribution (53.12%) lies within the range of 10^4-10^6 cells/g. Nygren (1962) isolated *B. cereus* from food samples including pudding with a percentage of 43.8% and the level of microorganisms was less than 1×10^2/g. Moreover Iversen et al. (1982) detected *B. cereus* from semolina pudding with a count of 6×10^6 cells/g which caused an outbreak of food poisoning at a Norwegian geriatric and health care center.

The highest incidence percentage of *B. cereus* in rice pudding samples among the other products undoubtedly results from the rice which was the major ingredient in preparation and very likely was contaminated with soil and consequently with bacterial spores .

Generally *B. cereus* can gain access to dairy desserts from different sources during its manufacturing, handling and directly from contaminated ingredients of animal (milk) and/or vegetable origin. *B. cereus* gain entery into milk by variety of routes. Contamination via the udder can occur either by infection with *B. cereus* causing acute mastitis (Jones and Turnbull, 1981) or by sticking dirt from soil, bedding, dung, fodder, pasture and water (Becker and Terplan 1989). Other possible sources may be milk handling equipment, transport vessels and processing equipment (Heddeghem and Vlaemynck 1992).

It is evident from Table 3 that all isolates of *B. cereus* were highly sensitive to 8 of 13 antibiotics (streptomycin, chlormphenicol, tetracycline, bacitracin, kanamycin gentamycin, erythromycin and neomycin). The only antibiotic with uniform resistance between all the isolates was colistin, while 80% of isolates were resistant to pencillin. Chopra et al. (1980) reported that all tested isolates of *B. cereus* (25 strains) were sensitive to streptomycin and gentamycin. While Schiemann (1978) reported that all tested *B. cereus* strains were sensitive to erythromycin, streptomycin, kanamycin, clindamycin and tobramycin, while all isolates were resistant to colistin.

It is apparent from the results obtained that the dairy desserts sometimes would contain *Bacillus cereus* strains with various levels of contamination. Furthermore if the conditions are suitable, multiply rapidly and produce sufficient toxin to induce symptoms of food poisoning . Therefore, the effective measures should be applied to reduce the number of *B. cereus* in the ingredient through; proper heating (temperature/time) during preparation and filling of the product, adequate refrigeration temperature of the product after the preparation, during distribution and in the house hold and finally prevention of the contamination of raw product by the equipment in relation to the scale of production.

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Table (1): Incidence of *Bacillus cereus* in Egyptian dairy desserts

Product	Positive samples		Count /g			
	No./50	%	Minimum	Maximum	Mean	S.E.M.
Flavoured ice cream	19	38	4×10^3	1.2×10^6	9.68×10^4	$\pm 3.43 \times 10^4$
Mehallabia	22	44	1×10^2	1.8×10^6	1.65×10^5	$\pm 0.56 \times 10^5$
Custard	21	42	9×10^2	2.4×10^6	1.71×10^5	$\pm 0.60 \times 10^5$
Rice pudding	32	64	1×10^3	7.2×10^8	2.39×10^7	$\pm 1.50 \times 10^7$

Table (2): Frequency distribution of different positive samples based on the *B. cereus* count

Count range	Flavoured ice cream		Mehallabia		Custard		Rice pudding	
	Frequency							
	No.	%	No.	%	No.	%	No	%
10^2-<10^3	0	0	1	4.54	1	4.76	0	0
10^3-<10^4	6	31.58	4	18.18	2	9.52	4	12.50
10^4-<10^5	6	31.58	3	13.64	5	23.81	5	15.62
10^5-<10^6	5	26.32	11	50.00	10	47.62	12	37.50
10^6-<10^7	2	10.52	3	13.64	3	14.29	3	9.38
10^7-<10^8	0	0	0	0	0	0	6	18.75
10^8-<10^9	0	0	0	0	0	0	2	6.25
Total	19	100.00	22	100.00	21	100.00	32	100.00

Table (3): Antibiotic susceptibility of 145 strains of *Bacillus cereus* isolated from dairy desserts .

Antimicrobial agent	Disk content	Resistant		Intermediate		Sensitive	
		No	%	No	%	No	%
Streptomycin	10 mcg	—	—	—		145	100
Chlormephenicol	30 mcg	—	—	—		145	100
Tetracycline	30 mcg	—	—	—		145	100
Ampicillin	10 mcg	41	28.27	2	1.38	102	70.53
Naladixic acid	30 mcg	15	10.34	5	3.45	125	86.21
Bacitracin	10 ui	—	—	—		145	100
Cloxacillin	5 mcg	46	31.72	—		99	68.28
Kanamycin	30 mcg	—	—	—		145	100
Gentamycin	10 mcg	—	—	—		145	100
Erythromycin	15 mcg	—	—	—		145	100
Colistin Sulphate	10 mcg	145	100	—		—	—
Pencillin	10 ui	116	80	29	20	—	—
Neomycin	30 mcg	—	—	—		145	100