Dept. of Food Control,
Faculty of Veterinary Medicine, Zagazig University

PREVALENCE OF AEROBIC SPORE FORMING BACILLI WITH SPECIAL REFERENCE TO BACILLUS CEREUS IN YOGHURT AND RICE BUDDING

(With 4 Tables)

By

E.I. AWAD

(Received at 4/9/2008)

تواجد العصويات الهوائية المتحوصلة مع إشارة خاصة الى ميكروب الباسيلس سيريس فى الزبادى والارز باللبن

عصمت ابراهيم السعيد

أجريت الدراسة علي ثلاثون عينة من كل من الزبادي والأرز باللبن المجمعة من أماكن مختلفة بمدينة الزقازيق وذلك بهدف استبيان مدي تواجد البكتيريا الهوائية المتجرثمة خاصة الباسيلس سيرس وقد أسفرت النتائج علي أن متوسط العدد الكلي للبكتيريا الهوائية المتجرثمة $5.3x10^4$، $4.6x10^5$ في الزبادي والأرز باللبن علي التوالي بينما كان نسبة تواجد الباسيلس سيرس في الزبادي والأرز باللبن علي التوالي 40% و 73% وعند تصنيف 60 عتره من البكتيريا الهوائية المتجرثمة المعزولة من الزبادي كان الباسيلس ليشينيفورميز هو أكثر المعزولات ويمثل 38% من جملة المعزولات ولكن عند تصنيف عتره معزولة من الأرز باللبن تبين أن الباسيلس سيرس هو الأكثر تواجدا ويمثل 42% وعند اختبار قدرة ميكروب الباسيلس سيرس علي تحليل البروتين تبين الأتي 80% من معزولات ميكروب الباسيلس سيرس المعزولة من الأرز باللبن لهل القدرة علي تحليل البروتين بينما 55.5% من الميكروبات المعزولة من الزبادي لها القدرة علي إفراز الإنزيم المحلل للبروتين وقد دلت النتائج علي أن عدد و نسبة تواجد ميكروب الباسيلس سيرس في الأرز باللبن أكثر من الزبادي مما يشير إلي أن مصدر تلوث اللبن الخام بهذا الميكروب هو الأرز هذا وقد تمت مناقشة النتائج لبيان أهميتها الصحية والاقتصادية.

SUMMARY

A total of 60 samples including (30 plain yoghurt and 30 rice budding) were collected from dairy shops, supermarkets and groceries in Zagazig city, Egypt and analyzed for enumeration of their *Aerobic spore-formers* and *Bacillus cereus* counts. The proteolytic activity of isolated *Bacillus cereus* was also investigated. The results obtained showed that mean counts of Aerobic *spore-formers* in the examined yoghurt and rice

budding samples respectively were $5.3x10^4$ and$4.6x10^5$, while *Bacillus cereus* could be isolated from 12 (40%) and 22 (73%) with mean counts of $1.3x10^3$ and $1.6x10^5$of the examined samples, respectively. Out of 60 *Bacillus spp.* isolated from examined yoghurt samples, Bacillus licheniformis was predominating and comprising 23(38%) while 9 (15%) isolates proved to be *Bacillus cereus* and on the other hand *Bacillus cereus* was predominating other *Bacillus spp.* isolated from rice budding samples 25(42%). 20 (80%) out of 25 *Bacillus cereus* obtained from rice budding samples possessed proteolytic activity compared to 5 (55.5%) isolates from yoghurt samples. Results were discussed in the context of present microbiological specifications and the need for its implementation by regulatory agencies to ward off possible health hazards arising from these microorganisms.

Key words: *B. cereus, yoghurt, rice budding*

INTRODUCTION

Fermented milk is the most popular fermented dairy products produced in Egypt and world wide. The value of fermented milk in human nutrition is based not only on the nutritive value of milk from which it is made and increased digestibility due to changes of milk constituents during fermentation period but also on the beneficial effect of intestinal micro flora , prophylactic and healing effect (buttress, 1997; Hussein and Kebary, 1999 and Zedan *et al.*, 2001).

Fermented milk has a positive effect on human defense system and cardiovascular diseases (Piaia, 2001) and high intake of fermented products was associated with decrease risk of ulcer (Elmstahl *et al.*, 1998).

Yoghurt is highly nutritious food of useful therapeutic value as it prevents the intestinal putrefaction resulting from anaerobic decomposition, prevents the gastrointestinal disorder, prevents coronary heart disease, reduces the risk of colon cancer, exerts a hypocholesterolaemic effect and produces antibiotics as acidophilin, lactocidin, nicin and lactoline that inhibit the growth of many pathogens (Robinson, 1991).

The rice budding and custard are the most popular varieties of Egyptian dairy desserts used not only in villages but also in cities .Rice budding ingredients are milk sugar, rice and other additives such as vanillin, raisins and shaved coconut to enhance its nutritive value and improve its flavor (Ray, 1996).

Heat resistant Psychrotrophs predominating *Bacillus spp.* can be introduced into milk supplies from water, udder, teat surfaces, soil or from milk stone deposits on farm bulk tanks, pumps, pipelines, gaskets and processing equipment and these organisms produce proteinases which result in the development of objectionable flavor and quality defects in dairy products (Meer *et al.*, 1991).

Bacillus cereus had been reviewed in food poisoning cases linked with milk and dairy products and there are two clinical forms of *Bacillus cereus* food poisoning , one is called diarrheal form that resemble to *Clostridium perfringens* food poisoning while the second form is called emetic syndrome food poisoning (Mossel, 1982). Infection of human by *Bacillus cereus* may be complicated by bronchopneumonia and otitis (Eley, 1996).

The unique combination of both thermoduric and Psychrotrophic properties of *Bacillus cereus* represents recurring problems for dairy industries such as spoilage of pasteurized milk (sweet curdling) and cream (bitty cream) under the effect of proteolytic and lipolytic actions (Griffiths, 1990).

In recognition of the public health and economic significance of *Aerobic spore-formers* especially *Bacillus cereus*, therefore the present study was undertaken to investigate its presence in milk and dairy products.

MATERIALS and METHODS

A total of 60 samples including (30 plain yoghurt and 30 rice budding) were collected from dairy shops, supermarkets and groceries in Zagazig city, Egypt.

Preparation of samples (*A.P.H.A.*, 1992):

The sample containers were mixed thoroughly using sterile spatula before examination. Eleven gram of the prepared samples were added to 99 ml of peptone water in a sterile bottles and thoroughly mixed to make a dilution of 1/10, from which decimal serial dilutions were prepared.

Methodology (*A.P.H.A.*, 1992):

1- Enumeration and identification of *Aerobic spore-formers* and *Bacillus cereus*.

Dextrose tryptone agar medium (Oxoid, 1999) and *Bacillus cereus* medium (Biolife, 1993) were used for enumeration of *Aerobic*

spore-formers and *Bacillus cereus* respectively. Isolated colonies were purified and identified according to Cowan and Steel (1974)

2-Determination of proteolytic activity of isolated *Bacillus cereus* (Harrigan and McCance, 1976)

Overnight cultures were spot inoculated onto pre-poured plates of standard plate count agar with 10% added skim milk. The inoculated plates were incubated at 25Ĉ for 48 hours and subsequently flooded with 10% W/V acetic acid, appearance of clear zone around the colonies after one minute exposure were regarded as positive.

RESULTS

Table 1: *Aerobic spore-formers* count/gram of examined samples (n=30).

Samples	Positive samples		Minimum	Maximum	Mean	±S.E.M
	No	%				
Yoghurt	26	87.0	$3.0x10^3$	$4.2x10^6$	$5.3x10^4$	$2.1x10^4$
Rice budding	28	93.0	$2.8x10^4$	$2.6x10^7$	$4.6x10^5$	$2.5x10^5$

*-ve samples >100

Table 2: *Bacillus cereus* count/gram of examined samples (n=30)

Samples	Positive samples		Minimum	Maximum	Mean	±S.E.M
	No	%				
Yoghurt	12	40.0	$1.0x10^2$	$3.2x10^4$	$1.3x10^3$	$0.4x10^3$
Rice budding	22	73.0	$1.8x10^4$	$2.2x10^6$	$1.6x10^5$	$0.5x10^5$

*-ve samples >100

Table 3: Frequency distribution of *Bacillus spp.* isolated from examined samples.

Isolates	Yoghurt		Rice budding	
	NO	%	NO	%
Bacillus cereus	13	22.0	25	42.0
Bacillus licheniformis	23	38.0	16	27.0
Bacillus coagulans	12	20.0	14	23.0
Bacillus mycoides	12	20.0	5	8.0
Total	60	100.0	60	100.0

Table 4: Proteolytic activity of isolated *Bacillus cereus*

Samples	No of isolates	Positive isolates	%
Rice budding	25	20	80.0
Yoghurt	13	5	38.5

DISCUSSION

The results given in Table 1 showed that out of the examined 30 yoghurt samples 26 (87%) were positive for *Aerobic spore-formers*. The high level of *Aerobic spore-formers* contamination was $4.2x10^6$, the low level was $3.0x10^3$ and the mean value was $5.3x10^4$ while *Aerobic spore-formers* could be isolated from 28 (93%) of the examined rice budding samples and the count ranged from $2.8x10^4$ to $2.6x10^7$ with a mean value of $4.6x10^5$ .Nearly similar finding was reported by Rangasamy *et al.* (1993) and Said and Fahmy (1991)

Information derived from the results reported in Table 2 revealed that *Bacillus cereus* could be isolated from 12 (40%) and 22 (73%) with a mean counts of $1.3x10^3$ and $1.6x10^5$of the examined yoghurt and rice budding samples respectively. These findings were in agreement with those reported by Sallam *et al.* (1991), Rangasamy *et al.* (1993), Notermans *et al.* (1997) and Warke *et al.* (2000) while Giffel *et al.*, (1996) recorded lower values.

The results presented in Table 2 declared that the incidence and count of *Bacillus cereus* in examined rice budding samples are higher than yoghurt. The *Bacillus cereus* spores are often found in cereals especially rice and some spores are surviving cooking and subsequently germinated into *Bacilli* which, under warm storage condition in cooked food grow and produce toxins (Hobbs and Roberts, 1993). *Bacillus cereus* can be introduced into milk via sources other than raw milk and the equipment is probably an important source of contamination. *Bacillus cereus* spores were predominantly transmitted from feed, via feces to farm bulk tank milk (Vissers *et al.*, 2007). In fermented milk and cheese vegetative *Bacillus cereus* are rapidly eliminated during production process (Schoder *et al.*, 2007)

Out of 60 *Bacillus spp.* isolated from examined yoghurt samples, Bacillus licheniformis was predominating and comprising 23(38%) while 13 (22.0%) isolates proved to be *Bacillus cereus* and on the other hand *Bacillus cereus* was predominating other *Bacillus spp.* isolated

from rice budding samples 25(42%) (Table, 3). These findings were in agreement with those reported by Meer *et al.* (1993) and Zhou *et al.* (2008). *Bacillus cereus* is a common cause of two types of food-borne illnesses, the diarrheal type is characterized by abdominal cramps and diarrhea 8-16 hours following ingestion while emetic type is characterized by vomiting 1-5 hours after ingestion of incriminated food (Schoder *et al.*, 2007 and Svensson *et al.*, 2007). Other spore-forming organisms such as *Bacillus licheniformis* have also been reported as agents of food poisoning with similar characteristics to those of *Bacillus cereus* (Hobbs and Roberts, 1993).

The results given in Table 4 showed that out of 25 *Bacillus cereus* obtained from rice budding samples, 20 (80%) were possessed proteolytic activity while only 5 (38.5%) out of 13 *Bacillus cereus* isolates isolated from yoghurt samples. Nearly similar finding was reported by Awad (1996). The thermoduric bacteria do not influence on the keeping quality of either raw and heat treated milk except those produced spores. The later are responsible for spoilage of pasteurized (sweet curdling) and occasionally raw milk which has been kept at low temperature as cream (bitty cream) under the effect of proteolytic and lipolytic action (Schoder *et al.*, 2007)

REFERENCES

A.P.H.A "American public health Association" (1992): Standard method for the examination of dairy products. 16th Ed., NewYork.

Awad, E.I. (1996): Study of proteolytic psychrotrophic microflora in raw buffaloe's milk. M.V.Sc., Faculty of Vet. Medicine, Zagazig Univ., Egypt.

Biolife, (1993): Quality Assurance Laboratory. Milano, Italy.

Buttress, J. (1997): Nutritional properties of fermented milk products. Int. J Dairy Technol., 50: 21.

Cowan, S.T. and Steel, K.J. (1974): Manual for the identification of medical bacteria. 2nd, Cambridge Univ., Press, England.

Eley, A.R. (1996): Microbiological food poisoning. 2nd Ed., Chapman and Hall, London.

Elmstahl, S.; Svensson, U. and Berglund, G. (1998): Fermented milk products are associated ulcer disease. Results from across sectional populattion study. Eur. J. Clin. Nutr., 52(9): 668-674.

Giffel, M.C.; Beumer,R.R.; Bonestroo, M.H. and Rombouts, F.M. (1996): Incidence and characterization of Bacillus cereus in two dairy processing plants. Netherlands Milk and Dairy Journal 50(4): 479-492.

Griffiths, M.W. (1990): Bacillus cereus in liquid milk and other milk products. Bulletin of the International Dairy Fedration 275: 36-39.

Harrigan, W.F. and McCane, M.E. (1976): Laboratory methods in food and dairy microbiology. Academic Press London, New York, San Francisco**.**

Hobbs, B.C. and Roberts, D. (1993): Food poisoning and Food Hygiene 6th Edition. Singular publishing, Group INC. San Diego, California.

Hussein, S.A. and Kebary, K.M.K. (1990): Improving viability of Bifidobacteria by microenterapment and their effect in stirred yoghurt. Acta Alimentaria, 28: 113.

Meer, R.R.; Baker, J.; Bodyfelt, F.W. and Griffiths, M.W. (1991): Psychotrophic Bacillus spp. In fluid milk products: A review J. of Food Protection 54 (12): 969-979.

Meer, R.R.; Wodburn, M.J. and Bodyfelt, F.W. (1993): Identification and characterization of heat resistant Psychotrophic bacteria in Oregon Grade A raw milk. Dairy, Food and Enviromental sanitation 13 (11): 631-637.

Mossel, D.A.A. (1982): Microbioogy of foods. 3rd Edition. The Univ. of Utrecht, the Netherlands.

Notermans, S.; Dufrenne, J.; Teunis, P.; Beumer, R.; Giffel, M. and Weem, P.P. (1997): A risk assessment study of Bacillus cereus present in pasteurized milk. Food Microbiology 14 (2): 143-151.

Oxoid, (1990): The oxoid manual. 6th Edition, Unipath Ltd., United Kingdom.

Piaia, M. (2001): Fermented milk and successful aging. Danone World Newsletter No. 22: 1-14.

Rangasamy, P.N.; Iyer, M. and Roginski, H. (1993): Isolation and characterization of Bacillus cereus in milk and dairy products manufactured in Victoria. Australian J. of Dairy Tech. 48 (2): 93-95.

Ray, B. (1996): Fundamental Food Microbiology CRC Press, Inc., Tokyo, New York.

Robinson, R.K. (1991): Therapeutic properties of fermented milks. Elsevier applied scientific publishers, London.

Said, M.R. and Fahmy, M. (1991): A survey of incidence of Staphylococcus aureus, Esherichia coli and Bacillus cereus in some types of Egyptian cheeses in Assiut city. Assiut J. of Agricultural Science 22 (2): 239-246.

Sallam, S.S.; Hafez, N.M.; El-Kholy, A.M. and Amer, I.H. (1991): Incidence of Bacillus cereus in milk and some dairy products in Egypt. J. Egypt. Vet. Med. Ass. 51 (1&2): 153-159.

Schoder, D.; Zangerl, P.; Manafi, M.; Wagner, M.; Lindner, G. and Foissy, H. (2007): Bacillus cereus – A foodborne pathogen of different risk potential for the dairy industry. Wiener Tierarztliche Monatsschrift 94 (1&2): 25-33.

Svensson, B.; Monthan, A.; Guinebretiere, M. and Nguyen, C. (2007): Toxin production potential and the detection of toxin genes among strains of Bacillus cereus group isolated along the dairy production chain. International Dairy J.17 (10): 1201-1208.

Vissers, M.M.M.; Giffel, M.C.; Driehuis, F.; De Jong, P. and Lankveld, J.M.G. (2007): Minimizing the level of Bacillus cereus spores in farm tank milk. J. Dairy Sci.90: 3286-3293.

Warke, R.; Kamat, A.; Kamat, M. and Thomas, P. (2000): Incidence of pathogenic Psychrotrophs in ice creams sold in some retail outlets in Mumbai, India. Food Control 11: 77-83.

Zedan, M.A.; Zedan, A.N.; Kebary, K.M.K. and Mahmoud, S.F. (2001): Effects of fortification of cows milk with acetylated whey protein concentrates on the quality of set yoghut. Egyptian J. Dairy Sci., 29: 285-297.

Zhou, G.; Liu, H.; He, J.; Yuan, Y. and Yuan, Z. (2008): The occurrence of Bacillus cereus, Bacillus thuringiensis and Bacillus mycoides in Chinese pasteurized full fat milk. International J. of food Microbiol.121(2): 195-200.