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Journal of Taibah University for Science 8 (2014) 207–215

Journal of Taibah University for Science

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# Lecithinase-producing bacteria in commercial and home-made foods: Evaluation of toxic properties and identification of potent producers

Eman F. Sharaf [a,*], Wael S. El-Sayed [a,b], Roaa M. Abosaif [a]

[a] *Department of Biology, Faculty of Science, Taibah University, Al Madinah Al Munawarah, Saudi Arabia*
[b] *Microbiology Department, Faculty of Science, Ain Shams University, Cairo, Egypt*

Available online 8 April 2014

**Abstract**

The aim of this study was to determine bacterial contamination in 28 refrigerated and unrefrigerated, commercial and home-made food items in Al Madinah Al Munawarah, Saudi Arabia. Of the 60 bacterial strains isolated, 53 were recovered from food samples refrigerated for 7 days and 7 from fresh, unrefrigerated food. The isolated bacteria were screened for lecithinase enzyme (toxin) production by the cup plate clearing zone technique. All isolates were positive on the basis of their ability to grow aerobically on egg yolk agar, giving black colonies as a characteristic feature of lecithinase production and opaque zones with diameters of 4–27 mm. Isolates were categorized into low, moderate and high lecithinase producers. Potent lecithinase-producing strains isolated from refrigerated green pepper (PS1) and lettuce (LS1) had a remarkably large zone measuring $27 \pm 1.9$ mm. All isolates also had additional toxic properties, including caseinase and haemolytic activities. Genotypic characterization by amplified ribosomal DNA restriction analysis showed that strains PS1 and LS1 were from same genus. Phenotypic characterization with biochemical tests and the Phoenix identification system suggested their affiliation to the *Bacillus* group. 16S rDNA sequence analysis of both strains showed them to be *Bacillus cereus*, with 99% sequence similarity to *B. cereus* strain J8B-67.


*Keywords:* Food contamination; Bacteria; Lecithinase; *B. cereus*

## 1. Introduction

Raw and cooked foods have been reported in various countries to be rich media for contamination by pathogenic organisms, resulting in foodborne disease. Food samples can easily be contaminated by spoilage and toxic bacteria, rendering them unsafe for consumption [1]. Bacterial contamination of raw vegetables and fruits can occur in the field or during post-harvest treatment, while cooked food can be contaminated during moderate heat treatment, inadequate refrigeration or poor hygiene during processing, with the production of toxins [2,3]. Toxin production by foodborne bacteria has raised public concern because of direct and associated food poisoning.

Of all the foodborne bacteria, *Bacillus cereus* is the most widely distributed. *Some B. cereus* strains cause severe local and systemic human infections, posing a public health problem [4]. The pathogenesis and virulence of *B. cereus* varies according to strain, some being regarded as lethal or highly toxic [5]. *Although*

* Corresponding author at: Biology Department, Faculty of Science, Al Jameaat Street, PO Box 344, Taibah University, Al Madinah Al Munawarah, Saudi Arabia. Tel.: +966 569929160; +966 541443086; fax: +966 48454770.
*E-mail address:* emanfsharaf@yahoo.com (E.F. Sharaf).
Peer review under responsibility of Taibah University

ELSEVIER Production and hosting by Elsevier

1658-3655 
http://dx.doi.org/10.1016/j.jtusci.2014.03.006

gastroenteritis caused by *B. cereus* is generally considered to be mild, fatal bloody diarrhoea and emetic poisoning have been reported [6], and the emetic toxin has been detected in *B. cereus*-contaminated food products such as milk, rice and pasta [4]. Molecular and genetic characterization of *B. cereus* strains have revealed the presence of genes encoding at least one of the known diarrhoeal toxins [7]. In the gastrointestinal tract, vegetative cells ingested as viable cells or spores produce and secrete a protein enterotoxin and induce a diarrhoeal syndrome. Tissue-destructive haemolysins are known to be produced by pathogenic intestinal and non-intestinal strains of *B. cereus*, including phospholipase, an emesis-inducing toxin, and the pore-forming enterotoxins haemolysin BL, non-haemolytic enterotoxin and cytotoxin K [5,8,9].

Some of the most important contaminants involved in food toxicity are lecithinase, caseinase and haemolysin activity. Lecithinase (phospholipolytic) activity is therefore used as an indicator of food toxicity. *B. cereus*, *B. mycoides* and *B. thuringiensis* have all been characterized as lecithinase producers [10]. Lecithinase hydrolyses lecithin on egg yolk agar plates [11], resulting in an opaque zone [12,13]. Phospholipase C (lecithinase C) hydrolases ester bonds, and phosphoric monoester hydrolase (EC 3.1.4.3) hydrolases the link between glycerol and phosphate in lecithin. The bacterial enzyme is a zinc protein [14]. The phospholipid lecithin is one of the main components of cell membranes and can be degraded by lecithinase to produce diglyceride and phosphorylcholine, hence causing toxicity [15]. Lecithinase can damage reproductive tract tissues [16] and cause haemolysis [17] and membrane disruption leading to cell lysis [18].

Relatively few microbial toxins have been definitely implicated in disease, and the modes of action have been identified for even fewer of these toxins [19]. Therefore, effective monitoring of bacterial contamination and detection methods is necessary to identify the organisms in processed foods, ingredients and the processing environment. The goal of the present study was to isolate lecithinase-producing bacteria from some commercial and homemade food samples and to characterize and evaluate their toxic properties, including haemolytic and caseinase activities.

## 2. Materials and methods

### 2.1. Food samples

Twenty-eight samples of commercial and home-made foods were collected in Al Madinah Al Munawarah, Saudi Arabia. The home-made, refrigerated food samples included cooked food (green vegetables, potatoes, beans and rice) and freshly purchased vegetables (cucumber, turnip, lettuce and green pepper), while the commercial foods were rocket, yoghurt, frozen meat, processed milled salad, sandwiches, Romano cheese, white cheese, sardines and luncheon meat. The unrefrigerated food samples included fresh vegetables (aubergine, carrot, tomato, cucumber and green pepper) and commercial food samples (raw goat milk, raw meat and yoghurt). All food samples were transported in sterile plastic boxes.

### 2.2. Isolation of lecithinase-producing bacteria

The culture medium used was made up of egg-yolk agar [3] with egg-yolk tellurite emulsion (BBL, USA) replacing the egg yolk powder at a concentration of 20 ml/L; NaCl, 5 g/L; and agar, 20 g/L. The pH was adjusted to 7.8, and the medium was sterilized at 121 °C for 15 min. To isolate bacteria, 10 g of each food sample were placed in flasks containing 100 ml sterile distilled water, shaken for 10 min and then filtered through a Whatman no. 1 filter. Then, 1 ml of each filtrate was spread aseptically on egg-yolk agar plates in triplicate and incubated aerobically for 24 h at 37 °C. Bacterial colonies were counted, purified and characterized [11], preserved on nutrient agar medium (HIMEDIA, India) and stored at 6 °C.

### 2.3. Screening for toxicity

#### 2.3.1. Lecithinase activity

Lecithinase production was tested on a modified medium according to Oladipo et al. [20], in which 10% corn millet replaced the *ogi* in the original broth. Lecithinase was detected according to the method described by Nandy et al. [21], in which 1 ml of each bacterial isolate at a cell density of $6 \times 10^8$ CFU/ml was inoculated into test tubes containing corn millet broth and incubated for 24 h at 37 °C. After incubation, the cultures were centrifuged at 2500 rpm for 15 min (MPW-350R High Speed Brushless centrifuge) to obtain a cell-free filtrate, and 100 μl of the filtrate were transferred into 10-mm wells made centrally in the egg-yolk agar plates and incubated for 24 h at 37 °C. Opaque zones were measured as indicators of lecithinase production, and the means were used as a criterion of lecithinase activity.

#### 2.3.2. Caseinase activity

Caseinase was identified according to the method of Gudmudsdo [22]. The isolated bacteria were streaked