

Differentiation of *B. cereus* strains isolated from milk and milk products using rep-PCR

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Bacillus cereus is gram-positive microorganism producing endospores and manifests as two distinct food-poisoning syndromes: diarrheal type (heat-labile enterotoxin complex) causes abdominal pain with diarrhea and emetic type (heat-stable enterotoxin) which is associated with nausea and vomiting. Its occurrence in final products is mostly caused by raw milk contamination and by subsequent transfer of spores in the course of milk processing. There were investigated raw milk, pasteurized milk and final products (cheese, yoghurt and UHT milk) during milk processing. Samples were analyzed on MYP Agar (HiMedia) at temperature 30°C for 24-48 h. The suspect strains were confirmed on haemolysis, production of acetoin, growth at 7 and 40 °C, growth by anaerobic condition, growth in 10 % NaCl, production of catalase, phosphatase and urease, hydrolysis of gelatine, Tween 80, starch, casein, esculin, lecithin, ONPG, DNA and of tyrosine, arginine dihydrolase, fermentation of glucose, xylose and mannitol, reduction of nitrates, growth on commercial media Simon's Citrate Agar (Oxoid). 16 strains were identified as *Bacillus cereus* according to the biochemical and physiological tests. For confirmation we have used two rep-PCR methods, one based on (GTG)₅, the second on BOX-A1R primer. Each method amplifies different oligonucleotide DNA sequences. Rep-PCR with both of primers was consistent and divided the monitored *Bacillus* group into several clusters. This method is useful for strains identification in food-fermentation industry because both dendrograms were also identical with results of biochemical and physiological tests. From our results it is interesting that *Bacillus* strains are clustered according to their origin. The strains coming from UHT processed milk introduce a very homogenous group. All samples from yoghurt procedure are clustered into second group. Strains coming from cheese processing were dissimilar because they were collected from quite different places. These molecular rep-PCR methods seem to be useful for determination of bacteria origin. (This study was supported by project MZe NAZV QF4004)