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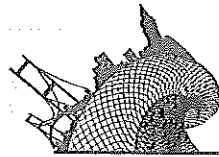
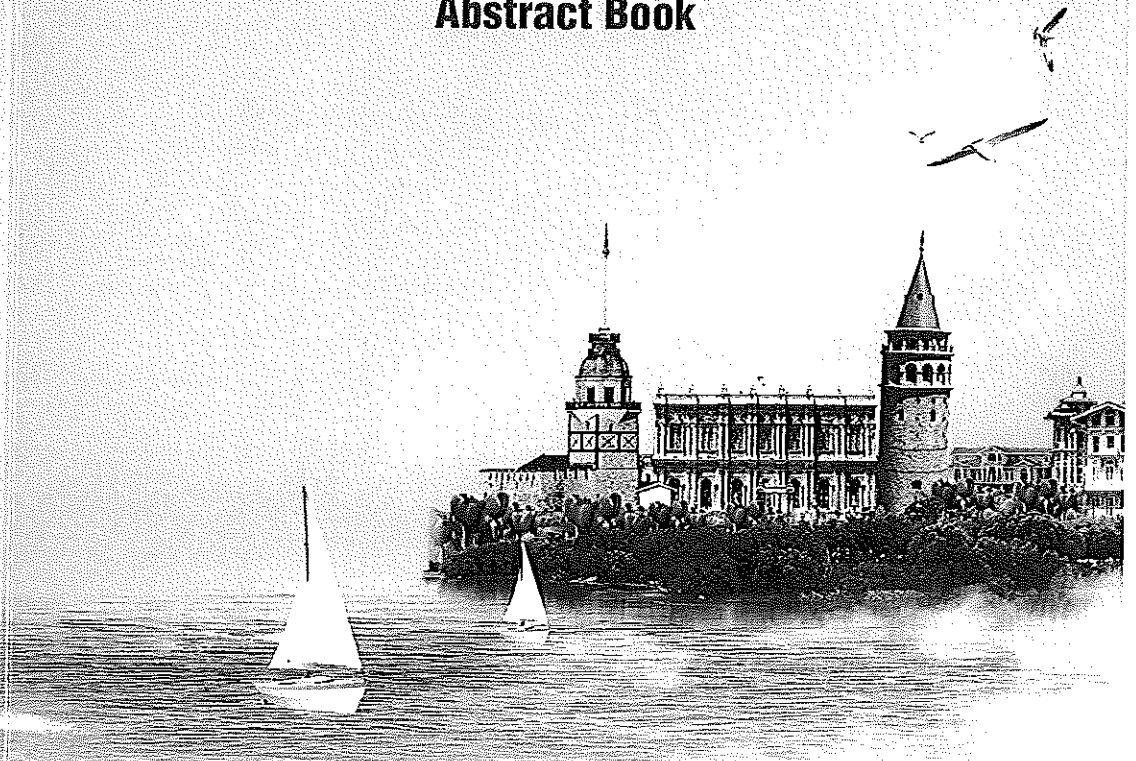


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Broad spectrum antimicrobial activity of *Bacillus amyloliquefaciens* strains isolated from traditional Bikalga, an African fermented food condiment

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Bikalga is a West African food condiment made from spontaneously fermented seeds of the plant *Hibiscus sabdariffa*. The fermentation is alkaline and dominated by *Bacillus* species. Three *Bacillus amyloliquefaciens* isolates (A4, I8, G3) belonging to the predominant aerobic sporeformers of bikalga were screened against a panel of 40 indicator organisms for their antimicrobial activity using agar spot and agar well diffusion assays. Antimicrobial activity in BHI medium was observed against several *Penicillium* spp. as well as a wide range of Gram-positive and Gram-negative food borne pathogenic bacteria, including *Bacillus cereus*, *Listeria monocytogenes*, *Salmonella* spp., *Escherichia coli*, *Shigella dysenteriae* and *Yersinia enterocolitica*. The investigated isolates further produced antimicrobial activity against *Bacillus cereus* in a *Hibiscus sabdariffa* seeds based medium. The antibacterial activity against *B. cereus* in BHI-broth was detected from the middle of the exponential phase, and maximum activity was observed during stationary phase. Treatment of cell free supernatants (CFS) to temperatures of 4°C and up to 80 °C, pH 3-11, and proteolytic, amyolytic and lipolytic enzymes did not affect the antimicrobial activity against *B. cereus*. Different extraction methods were used to obtain and further characterize the antimicrobial substance produced. No antimicrobial activity was observed for proteins > 2.5 kDa (precipitated from CFS using trichloroacetic acid) as deduced from SDS page gels overlaid with *B. cereus*. In comparison ethyl-acetate extracts of CFS showed antimicrobial activity against both the Gram-positive and Gram-negative indicator bacteria. HPLC-HRMS analysis of ethyl-acetate extracts with antimicrobial activity revealed the presence of iturins in addition to other compounds. Iturins are cyclic lipopeptides produced by various *Bacillus* spp. found to mainly inhibit yeast and fungi. Our results suggest that the three investigated *B. amyloliquefaciens* isolates produce more than one compound with antimicrobial activity. The antimicrobial activity against the Gram-positive and Gram-negative indicator bacteria might be associated to production of small lipophilic compounds other than iturins. In conclusion the *Bacillus amyloliquefaciens* strains of Bikalga show some interesting antimicrobial properties. They may have potential for use as starter cultures for the biological control of pathogenic and spoilage microorganisms during bikalga fermentation, though further investigations are needed.

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In situ production of Reuterin P572 in cheese

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Reuterin (β -hydroxypropionamide) produced by some strains of *Lactobacillus reuteri* (Gram-positive and Gram-negative) is considered an alternative for natural preservatives. *In situ* production of reuterin in cheese was considered an alternative for natural preservatives. Isolates of *L. reuteri* (INIA P572, P577 and INIA P579) that showed high reuterin production by gel electrophoresis (PFGE) were used. Reuterin concentrations obtained in cheese supplemented with 100 mM of glycerol and 100 mM of NaCl. Cheese was manufactured with different concentrations of 50, 100, 200 mM of glycerol. Commercial starter culture and control cheese was made from *L. reuteri* was observed during the 30 days of ripening. Reuterin was detected from the β -HPA. The highest reuterin concentrations were detected on day 1 in cheeses supplemented with 100 mM of glycerol. Reuterin concentrations corresponded to the indicator microorganism. In control cheese after 30 d, while the addition of glycerol led to reductions of 5.33 log units of reuterin and glycerol, counts of 6.09 log cfu of reuterin and reduction of *L. reuteri*, with values similar to levels in control cheese. Valued according to the ripening period. According to the development of a bioprotect