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Antimicrobial effect of nisin and carvacrol and competition between *Bacillus cereus* and *Bacillus circulans* in vacuum-packed potato puree

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Abstract

A study was undertaken to investigate the growth and competition between *Bacillus cereus* and *B. circulans* strains in both BHI and ready-to-eat potato puree. In addition, antimicrobial potential of supplemented nisin and carvacrol was evaluated against inoculated *B. cereus* and *B. circulans* strains. The accomplished growth inhibition was observed for both *B. cereus* and *B. circulans*, where *B. circulans* strains were more sensitive. MIC values were decreased by lowering the incubation temperature in separate applications of nisin and carvacrol, while the effect of combined application of nisin and carvacrol appeared to be more obvious at higher temperatures. The overall effect of interactions between spoilage and pathogenic *Bacillus* spp. was dependent on the psychrotrophic character of both cultures. The complete inhibition of *B. circulans* in the co-culture experiments corresponded to *B. cereus* population density of approximately 6 log units. Microbial challenge testing in potato puree showed that, at both 7 and 10 °C, *B. cereus* TZ415 and *B. circulans* 4.1 could grow to high counts when no antimicrobial substances (nisin and carvacrol) were applied, while no visible spoilage occurred. A more pronounced antimicrobial activity of nisin and nisin–carvacrol combination was observed in potato puree compared to the BHI medium.

Keywords: *Bacillus cereus*; *Bacillus circulans*; Psychrotrophic character; Nisin; Carvacrol; Microbial challenge testing

1. Introduction

Bacillus cereus is a spore forming foodborne pathogen associated with vegetable purees, soups, rice dishes and dairy products. Some *B. cereus* strains produce either a heat labile diarrhoeal enterotoxin or a heat stable emetic toxin and their involvement in foodborne outbreaks has been reported (Griffiths, 1990; Harmon and Kautter, 1990; Van Netten et al., 1990; Granum et al., 1993). Between 1980 and 1997, 2715 cases of *B. cereus* food poisonings in England and Wales were reported to the Public Health Laboratory Service (Ripabelli et al., 2000) and the annual totals varied between 27 and 418 cases per year. Due to the difficulties in diagnosis of bacterial foodborne intoxications, it is likely that published figures represent an underestimation of the actual situation (Tompkins et al., 1999). Growth of vegetative cells usually occurs within the temperature range of 10–50 °C. However, psychrotrophic variants of *B. cereus* have been identified (Dufrenne et al., 1994; Rusul and Yaacob, 1995), implying that the maintenance of the cold chain (⩽5 °C) is of the utmost importance. Mild preservation technologies are increasingly used in food industries, endorsing the safety problem related to the spore-forming micro-rganisms such as *B. cereus*. Refrigerated Processed Food of Extended Durability (REPFEDs) produced using mild heat technologies are recognized as a potential vehicle for *B. cereus* intoxications (Beuchat et al., 1997). Carlin et al. (2000) have isolated *B. cereus* from 80% to 100% of samples of

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cooked pasteurized and chilled vegetable purees of leek, zucchini, broccoli, split pea, carrot and potato purees subjected to the temperature abuse (10 °C). Del Torre et al. (2001) reported isolation of *B. cereus* from 33% of gnocchi in Italy.

Attempts have been made to identify processes and preservatives, either alone or in combination, that eliminate or control the growth of *B. cereus* in raw and pasteurized foods. Thomas et al. (1993) reported sorbate as the most effective preservative tested against *B. cereus*, whose inhibitory activity increased with an increase in acidity and NaCl concentration. Ababauch et al. (1992) tested 11 different fatty acids and their salts on their inhibitory activity against bacterial spores, reporting linolenic and lauric acid to be the most effective. Also, a garlic extract was reported to perform an antimicrobial activity against Gram-positive bacteria, such as *B. cereus* (Yoshida et al., 1999). An alternative approach to reduce the proliferation of micro-organisms is the use of essential oils. The antifungal and antibacterial effects of these components on different micro-organisms have been described in several studies (Conner, 1993; Juven et al., 1994; Kim et al., 1995; Sivropoulou et al., 1996; Ultee et al., 1998). Among the diverse group of chemical components in essential oils, carvacrol exerts a distinct antimicrobial action. Carvacrol is the major component of the essential oil fraction of oregano (60–74% carvacrol) and thyme (45% carvacrol) (Lagouri et al., 1993; Arrebola et al., 1994). With its hydrophobic characteristics it appears to have an influence on the biological membranes in bacterial cells (Ultee et al., 1999). Recently, Ultee et al. (1998) showed the effect of carvacrol on growth and diarrhoeal toxin production of *B. cereus* in Brain–Heart Infusion (BHI) and soup. To accomplish the effect in soup, 50 times higher concentration of carvacrol was needed.

Several bacteriocins have been investigated for their activity against *B. cereus* by Beuchat et al. (1997) and Wong et al. (1999) reporting nisin as the most widely effective. Nisin was effective in controlling both the growth of *B. cereus* in crumpets (a high-moisture, baked, flour-based product) (Jenson et al., 1994) and liquid egg (Delves-Broughton et al., 1996).

REPFEDs not only need control of *Bacillus* species for food safety reasons. From the food quality aspect, the main limitation and failure to meet the extended shelf-life is the growth of psychrotrophic *Bacillus* spp., such as *B. circulans*. Carlin et al. (2000) reported *B. circulans*, *B. macerans* and *B. polymyxa* to be dominating species in commercial vegetable based purees stored at 4 and 10 °C.

It was the aim of the present study to address both problems of food safety related to the outgrowth of *B. cereus* and economic losses as a consequence of food spoilage caused by development of psychrotrophic *Bacillus* spp. in REPFEDs. The potential of natural antimicrobial substances, namely carvacrol and nisin to inhibit the growth of *B. cereus* and *B. circulans* was investigated. Growth characteristics of *B. cereus* and *B. circulans* strains and the competitive effect that might occur between the two *Bacillus* species at refrigerated temperatures were also studied. Furthermore, microbial challenge testing (MTC) was performed in vacuum-packed potato puree supplemented with (or without) antimicrobial substances, to determine the ability of the food product to support the growth of spoilage organism, *B. circulans*, and the pathogen, *B. cereus*. Challenge testing may provide valuable information for risk assessment related to the food product and the pathogen of concern (Uyttendaele et al., 2004).

2. Materials and methods

2.1. Bacterial strains and enumeration

Four *B. cereus* strains, namely two potato puree isolates 1.94 and 4.9 (Laboratory for Food Microbiology and Food Preservation-LFMFP, Culture Collection, Ghent University, Belgium) and strains TZ415 and Z4222 isolated from minimally processed courgette puree, provided by dr. F. Carlin (Unité de Technologie des Produits Végétaux, INRA, France), were used throughout the study. In addition, *B. circulans* strains 2.4, 4.1 and A1 (potato puree isolates) and *B. circulans* strain W1 (carrot puree isolates) from LFMFP culture collection were used. Stock cultures, kept on Nutrient agar (NA, Oxoid, Basingstoke, England, UK) at 7 °C were activated in Nutrient broth (NB, Oxoid) by incubation at 30 °C for 24 h. Enumeration was done by plating appropriate 10 fold dilutions (in Peptone Physiological Solution, PPS) of the sample on NA, if working with the pure culture, or *B. cereus* agar (BCA, Oxoid) in the case of mixed cultures. *B. cereus* colonies on BCA gave typical pink color surrounded by a white zone as a consequence of the *B. cereus* lecithinase activity, while *B. circulans* remained yellow colored and without white surrounding zone. On NA plates *B. cereus* colonies were bigger and mat, comparing to smaller and slimy appearing *B. circulans* strains. The counts on NA and BCA were, for both *B. cereus* and *B. circulans* comparable, indicating no inhibition of strains on the selective BCA medium.

2.2. Determination of minimal inhibitory concentration (MIC) value of carvacrol and nisin

Nisin, in the form of Nisaplin was obtained from Aplin and Barret, Ltd., Trowbridge, UK and was dissolved in 50% ethanol (VWR, Leuven, Belgium), centrifuged and filter sterilized through 0.2 μm pore size