Enterocin NA: A Bacteriocin Produced by *Enterococcus faecalis* BFE 1057 Isolated from a Nigerian Fermented Dairy Product

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Abstract

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Enterococcus faecalis BFE 1057 isolated from a Nigerian fermented dairy product ('nono') produced a bacteriocin designated enterocin NA. It was found inhibitory towards *Lactobacillus*, *Enterococcus* and *Listeria* strains. Enterocin NA was inactivated by proteolytic enzymes, and resistant to heating at 80 °C for 30 min and active at pH 2.0 to 8.0. Its molecular weight was estimated by ultrafiltration to be above 3000 daltons. The ability of this bacteriocin to inhibit *Listeria* is of potential interest in food safety and enterocin NA may have future applications as a food preservative.

Key words: bacteriocin; *Enterococcus faecalis*; fermented foods; enterocin; nono

Many of the African fermented foods are prepared in a traditional way in small-scale and home-level processes. As a result, these indigenous foods are often associated with problems such as inconsistent quality, hygienic risks and short shelf-life (ONYEKWERE *et al.* 1989). The preparation of these indigenous foods generally depends on a "spontaneous" fermentation by naturally occurring lactic acid bacteria (LAB) and the use of starter cultures is rare. Hence, to improve the quality and safety of these foods, the use of bacteriocin-producing LAB in fermentation was suggested (OLASUPO *et al.* 1995). Extensive reports are available on the production of bacteriocins by lactic acid bacteria isolated from fermented and other food products in the temperate regions of the world. However, the availability of such information is still searce from African countries.

The present report contributes to further expansion of knowledge on bacteriocin production among lactic acid bacteria associated with fermented African foods. In this study it was attempted to isolate and identify bacteriocin-producing LAB from traditional African fermented foods and to characterise the inhibitory substance.

MATERIAL AND METHODS

Bacterial Cultures and Media

A bacteriocin-producing LAB was isolated from 'nono', a Nigerian fermented liquid cow milk product. The strain was identified as *Enterococcus faecalis* using methods of SCHILLINGER & LÜCKE (1987) and the key described by HARDIE (1986) and DEVRIESE & POT (1995). Indicator bacterial strains and their sources are listed in Table 1. All LAB were grown in MRS broth (Merck) at 25 °C for 24 hrs, while food-borne pathogens were grown in Standard 1 Nutrient broth (Merck) at 30 °C for 24 hrs.

Detection and Measurement of Bacteriocin Activity

Two hundred LAB strains were isolated from a variety of actively fermenting traditional African foods by plating onto MRS agar (Merck). The fermented foods included *fufu* (fermented cassava product), *ogi* (fermented maize), *kunun-zaki* (fermented sorghum or millet), *ugba* (fermented African oil bean), *iru* (fermented African locust bean), *nono* (fermented liquid cow milk), *wara* (fermented solid cow milk), *burukutu* (fermented alcoholic cereal beverage) and *kenkey* (fermented maize), all obtained from the southern part of Nigeria. Isolates were all screened for ability to produce bacteriocin on a bacteriocin screening medium (BSM) (TICHACZEK *et al.* 1992) using the agar spot assay and *Lactobacillus sake* DSM 20017 as the primary indicator strain.

Cell-free culture supernatant fluid was concentrated 10-fold by ultrafiltration with a 3000 Mr exclusion membrane (Amicon Corp., Beverly USA), and the microtitre plate assay system (MØRTVEDT & NES 1990) was used to quantify bacteriocin activity in both filtrate and retentate.

Determination of Bacteriocin Activity Spectrum

The spectrum of bacteriocin activity was determined using the spot-on-the-lawn method (SCHILLINGER *et al.* 1993) in which BSM plates were spot inoculated with 8 µl of an overnight culture of *E. faecalis* BFE 1057 and 5 µl of proteinase K (25 mg/ml) was spotted adjacent to the growing colony. After incubation at 25 °C for 24 hrs, the plates were overlaid with LAB and food-borne pathogen indicator strains (Table 1). Following a 24 hrs incubation at 25 °C for LAB and 30 °C for pathogenic indicator strains, plates were read for zones of inhibition.

Effect of Enzymes, Heat and pH on Bacteriocin Activity

Concentrated, filter-sterilised culture supernatant fluid was pH adjusted (pH 6.5) and heated at 70, 80, 90, 100 °C for 30 min and 121 °C for 15 min. For enzyme inactivation, culture supernatant fluids were treated with the enzymes listed in Table 2 according to methods of UHLMAN *et al.* (1992), using untreated samples as control. Bacteriocin stability at pH values between 2 and 10 was tested according to methods of VAN LAACK *et al.* (1992). As a control, to correct for inhibition due to pH, a proteinase K inactivated supernatant fluid at the same pH was tested against the same indicator strain. Bacteriocin activity following heat, enzyme and pH treatment was assayed using the microtitre plate assay system.

Table 1. Inhibitory spectrum of the bacteriocin-producing strain *Enterococcus faecalis* BFE 1057 isolated from 'nono', a fermented dairy product from Nigeria

Indicator strain	Source	Bacteriocin activity
Lactobacillus sake 20017	DSM	++
Lactobacillus plantarum 20174	DSM	–
Lactococcus lactis ssp. *lactis* 20384	DSM	++
Lactobacillus fermentum 20052	DSM	–
Lactobacillus plantarum 20205	DSM	–
Lactobacillus viridescens 20410	DSM	++
Lactobacillus brevis 20054	DSM	–
Enterococcus faecalis 20380	DSM	++
Leuconostoc paramesenteroides 20193	DSM	–
Leuconostoc carnosum 5576	DSM	–
Leuconostoc mesenteroides 20343	DSM	–
Leuconostoc paramesenteroides 20288	DSM	–
Pedicoccus acidilactici 20333	DSM	–
Pedicoccus pentosaceus 20336	DSM	–
Listeria monocytogenes 2250	WS	+
Listeria monocytogenes 2247	WS	–
Listeria monocytogenes 125	WS	+
Listeria innocua 2258	WS	+
Listeria welshimeri 2254	WS	+
Bacillus cereus 2010	CCM	–
Staphylococcus aureus 14438	ATCC	–

++ inhibition zone of 3.0–9.0 mm; + inhibition zone of 2.0 mm; – no inhibition zone

DSM – Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany

WS – Technical University, München-Weihenstephan, Germany

CCM – Czechoslovak Collection of Microorganisms, Brno, Czech Republic

ATCC– American Type Culture Collection, USA

RESULTS AND DISCUSSION

Small-scale preparation of traditional African fermented foods is often faced with problems of hygienic status and poor shelf-life. The use of starter cultures is not common, but chance inoculation or "back-slopping" is usually encouraged. The isolation of a bacteriocin-producing LAB strain in the present study, from an African fermented food, could possibly contribute to current efforts to develop means for improving the safety and quality of African fermented foods. A novel approach to the use of bacteriocins, and preferably the producer strains to control undesirable microorganisms in food, would be to produce a fermented food that would "naturally" contain a significant amount of a particular bacteriocin. This product might also be used as an additive containing a natural microbial inhibitor, to control both spoilage and potentially pathogenic bacteria in other foods. This approach might be of great help to improve the preservation of foods in the African environment. Especially, it might contribute to reduce food-borne infections related to underprocessed fermented foods.

Out of a total of two hundred LAB isolates obtained from a variety of African fermented foods and screened for bacteriocin activity, only four strains were found to produce inhibition zones against *Lactobacillus sake* DSM 20017 in the agar spot assay. These include one isolate from 'nono', a Nigerian fermented dairy product, which was identified as *Enterococcus faecalis*. This strain BFE 1057 produced an inhibitory substance active against some strains of *Lactobacillus*, *Enterococcus* and *Listeria* (Table 1). The activity of this substance, called enterocin NA, was completely inactivated by α-chymotrypsin, trypsin, pepsin and proteinase K (Table 2), thus confirming the proteinaceous nature of the substance, a key criterion in the identification of a substance as a bacteriocin. In ultrafiltration experiments, the antimicrobial activity was concentrated using a 3000 daltons molecular weight exclusion membrane. As the filtrate did not contain any bacteriocin activity, the antimicrobial substance, enterocin NA, is suggested as a protein with a molecular weight above 3000 daltons. Recently OLASUPO *et al.* (1994) demonstrated the antimicrobial activity of *E. faecium* NA01 against *Listeria* spp. whilst some other reports have dealt with bacteriocin-like substances from *E. faecalis* (BOTTONE *et al.* 1971; HARASAWA *et al.* 1980; GÁLVEZ *et al.*

Table 2. Effect of different enzymes, heat treatment and pH on the inhibitory effect of the bacteriocin produced by *E. faecalis* BFE 1057 isolated from 'nono', a fermented dairy product from Nigeria

Treatment	Activity
Enzyme:	
α-chymotrypsin (Serva, 17160)	–
Trypsin (Merck, no. 8367)	–
Pepsin (Merck, no. 7189)	–
α-amylase (Sigma, type VII-A)	+
Lipase (Serva, no. 28262)	+
Lysozyme (Serva, no. 28262)	+
Proteinase K (Sigma, P-03090)	–
Papain (Merck, no. F593147)	+
Heat:	
70 °C for 30 min	+
80 °C for 30 min	+
90 °C for 30 min	–
100 °C for 30 min	–
121 °C for 15 min	–
pH:	
2.0	+
4.0	+
6.0	+
8.0	+
10.0	–

+ indicates full activity of the bacteriocin
– indicates loss of bacteriocin activity

1989; ARIHARA *et al.* 1991; VILLANI *et al.* 1993; JOOSTEN *et al.* 1996; MAISNIER-PATIN *et al.* 1996). However, only few authors (ARIHARA *et al.* 1991; VILLANI *et al.* 1993; MAISNIER-PATIN *et al.* 1996) reported antimicrobial activity of *E. faecalis* against *Listeria* strains. Listeriosis of man or animals caused by *Listeria monocytogenes* is an important disease which has spread in recent years. Hence the detection of *E. faecalis* BFE 1057 with ability to produce a bacteriocin against *L. monocytogenes* in this study is interesting and increases the state of knowledge on bacteriocins with antilisterial effects. In contrast to enterocin 226 NWC produced by *E. faecalis* 226, which was stable at 100 °C for 30 min (VILLANI *et al.* 1993), enterocin NA was found to be stable only at 80 °C for 30 min. The inhibitory activity of enterocin NA was stable at pH between 2.0 and 8.0 (Table 2).

Enterococci have caused food-borne infections only in rare instances, but may be associated with opportunistic infections, e.g. endocarditits. Evidence on their role as food-borne pathogens is however scanty or missing (JOHNSON 1990). On the other hand, the technical use of *Enterococcus* strains in the production of fermented foods (e.g. of typical Italian and Greek cheeses) has a long safety record (KALANTZOPOULOS 1993; TSAKALIDOU *et al.* 1993). The use of this bacteriocin-producing *E. faecalis* BFE 1057 may therefore provide a natural means of preservation and safety improvement of African fermented foods with the perennial problem of short shelf-life due to poor hygiene. Since strains of *E. faecium* and *E. faecalis* are also commercially available in "probiotic" products (FULLER 1989), the potential of this organism as a probiotic in such foods and as a stabilising agent of the gut, would justify further study.

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Souhrn

OLASUPO N. A., SCHILLINGER U., FRANZ C., HOLZAPFEL W. H. (1998): **Enterocin NA: bakteriocin produkovaný** *Enterococcus faecalis* BFE 1057 **izolovaným z nigerijského kvašeného mléčného produktu.** Czech J. Food Sci., **17**: 1–4.

Enterococcus faecalis BFE 1057 izolovaný z nigerijského kvašeného mléčného produktu („nono") vytvářel bakteriocin, který byl označen jako enterocin NA. Enterocin NA vykazoval inhibiční účinky vůči kmenům *Lactobacillus*, *Enterococcus* a *Listeria*, byl inaktivován proteolytickými enzymy a byl rezistentní po dobu 30 min při teplotě 80 °C a aktivní při pH 2,0–8,0. Pomocí ultrafiltrace byla jeho molekulová hmotnost odhadnuta na více než 3 000 daltonů. Inhibiční schopnost tohoto bakteriocinu vůči kmenům *Listeria* může být zajímavá z hlediska nezávadnosti potravin; v budoucnosti by mohl být používat jako konzervační látka v potravinářství.

Klíčová slova: bakteriocin; *Enterococcus faecalis*; fermentované potraviny; enterocin; „mono"

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