In vitro antagonistic effect of nisin on faecal enterococci and staphylococci

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ABSTRACT: Enterococci and staphylococci, isolates from faecal samples of 46 different animals such as deer, chamois, European bison, zebra, camel, antelope, gazelle, horse, and piglets were treated by nisin (concentration 1 mg/ml). Only two strains (SX38 and EA163), isolates from the faeces of deer were not inhibited by nisin under *in vitro* conditions. It means 97.4% of target isolates were inhibited by nisin and 2.6% were resistant. The majority of microorganisms were inhibited by nisin under MIC 1.56–100 µg/ml. Twenty-two percent out of 77 isolates were inhibited by MIC of nisin 3.12 µg. *Enterococcus* sp. E6B strain was found the most sensitive (inhibition by MIC 1.56 µg of nisin). Although only a few staphylococci were tested, most of them were inhibited by nisin. Even though the effect of nisin on the individual species was not evaluated, its effect on the group of bacteria is already important. In general, the properties of nisin indicate a broad spectrum of its utilization.

Keywords: faeces; enterococci; staphylococci; nisin; inhibition

INTRODUCTION

Enterococci represent lactic-acid producing bacteria (LAB) that belong to the clostridial subdivision of Gram-positive bacteria (Devriese and Pot, 1995). They constitute a large group of autochthonous bacteria associated with the mammalian gastrointestinal tract. They are isolated not only from human faeces (Murray, 1990); they can also be found in the faeces of livestock such as cattle, sheep, calves and pigs (Lauková, 1996; Leclerc *et al*., 1996). Enterococci are also widely used as indicators of faecal contamination (Niemi *et al*., 1993; Lauková and Juriš, 1997). Staphylococci also belong to Gram-positive LAB. However, they belong to the family Micrococcacae. They are also widespread microorganisms that can colonize different tissues of humans as well as of animals (Lauková, 1993; Zakrzewska-Czerwinska *et al*., 1995). Moreover, enterococci as well as staphylococci are listed as the originators of nosocomial infections (Pulverer, 1993; Tailor *et al*., 1993), soft tissue infections, urinary tract or intra-abdominal infections (Lewis and Zervos, 1990). Because these bacteria can be cultured from different ecosystems, the way is searched how to improve their reduction most successfully. Generally, the treatment with antibiotics is recommended. However, in recent years, an emergence of resistance to an increasing number of antibiotics (aminoglycosides, macrolides, β-lactams, glycopeptides) was observed among these bacteria (Bell *et al*., 1998). That means new anti-microbial substances are tested to achieve higher inhibitory effectiveness. In the present study, an *in vitro* antagonistic effect of nisin was tested on the target enterococci and staphylococci isolated from faecal samples of different animals. Nisin is the most frequently studied anti-microbial low-molecular-weight protein – bacteriocin, lanthibiotic produced by some strains of *Lactococcus lactis* subsp. *lactis* (e. g. *Lactococcus lactis* MCFB497; Boziaris and Adams, 1999). The lanthibiotics were defined and named on the basis of their antibiotic action and their content of the thioether amino acids lanthionine and 3-methyllanthionine (Schnell *et al*., 1988; Nes and Tagg, 1996). The inhibitory effect of nisin was already used successfully against the above-mentioned species; however, from other sources (Lauková, 1995, 2000). Therefore, our interest was to test its effect also on the same species from various sources with the aim to extend possibilities of further nisin application because currently nisin is predominantly used as a food preservative (Delves-Broughton *et al*., 1996).

MATERIAL AND METHODS

Enterococci and staphylococci were isolated from faecal samples of 46 animals; they comprised isolates from deer (8), chamois (21), European bison (1), zebra (1),

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camel (1), antelope (2), gazelle (1), horse (1) and piglets (10). Samples from deer and chamois were collected in the Tatras National Park (Slovakia); the other samples came from wild animals living in a ZOO garden at Košice (Slovakia) and from piglets kept on the farm Grajciar (East Slovakia). The collection of 77 selected strains (68 enterococci and 9 staphylococci) was used. The strains from the faeces of deer, chamois, piglets are our own isolates. The strains isolated from wild animals in a ZOO garden were supplied by Dr. Štyriak. Enterococci were selected using M-Enterococcus agar and staphylococci were grown on Mannitol Salt Agar (Becton & Dickinson, Cockeysville, USA). Phenotypic determination of pure cultures was performed by help of BBL Crystal Gram-positive ID kit (Becton & Dickinson). The target strains that were treated with nisin are summarized in Table 1.

Pure nisin (a gift from Aplin and Barrett Ltd, Dorset, UK) was dissolved in nisin diluent that contains 0.02 N HCl. The stock solution of a commercial preparation Nisaplin containing 2.5% of nisin was prepared for use (calculated nisin 1 mg/ml) and stored at –20°C. The antimicrobial effect of nisin was analyzed by agar spot test (De Vuyst *et al*., 1996) in MRS agar and/or Brian Heart agar (Becton & Dickinson). Briefly, 200 µl of the overnight culture of the indicator organisms (target strains of enterococci and staphylococci) were inoculated into 4.5 ml of 0.7% agar formerly mentioned and Petri dishes were overlaid by them. Then the aliquots (10 µl) of nisin (appropriate twofold dilutions) were spotted onto the plates. After pre-diffusion at 4°C for 30 min, the plates were incubated at 37°C for 18 h. The highest dilution showing a definite zone of the growth of selected indicator organism was recorded. To quantify the inhibitory activity of nisin, the minimal inhibition concentration (MIC) was used expressing µg/ml of pure nisin that inhibited the growth of the target strains tested. Working cultures of enterococci and staphylococci were cultivated in MRS broth and/or Brian Heart Infusion (Becton & Dickinson).

RESULTS

Only two strains (*Staphylococcus xylosus* SX83 and *Enterococcus avium* EA163) isolated from the faeces of deer were not inhibited by nisin under *in vitro* conditions.

Table 1. Faecal isolates used as the indicator organisms and MIC of active nisin in µg

Species	Number	Source	MIC*
Enterococcus faecium	9	faeces of deer	100–12.5
Enterococcus casseliflavus	1	faeces of deer	12.5
Enterococcus faecalis	1	faeces of deer	12.5
Enterococcus faecium	14	faeces of chamois	100–12.5
Enterococcus casseliflavus	3	faeces of chamois	100–12.5
Enterococcus faecium	12	faeces of piglets	100–12.5
Enterococcus casseliflavus	2	faeces of piglets	50
Enterococcus avium	3	faeces of piglets	100–50
Enterococcus sp.	2	faeces of piglets	100, 25
Enterococcus durans	1	faeces of camel	3.12
Enterococcus hirae	1	faeces of camel	3.12
Enterococcus sp.	2	faeces of camel	12.5–3.12
Enterococcus faecium	1	faeces of horse	3.12
Enterococcus sp.	3	faeces of horse	3.12
Enterococcus faecium	2	faeces of E. bison	3.12
Enterococcus sp	3	faeces of E. bison	3.12
Enterococcus durans	2	faeces of antelope	3.12
Enterococcus sp.	2	faeces of antelope	3.12
Enterococcus durans	1	faeces of gazelle	3.12
Enterococcus sp.	1	faeces of gazelle	6.25
Enterococcus hirae	1	faeces of zebra	12.5
Enterococcus sp.	1	faeces of zebra	1.56
Staphylococcus xylosus	4	faeces of deer	25, 12.5, 6.25
Staphylococcus lentus	5	faeces of deer	25–6.25

Staphylococcus xylosus SX83, *Enterococcus avium* EA163 were not inhibited by nisin

*MIC – minimal inhibition concentration of pure nisin (µg/ml) that inhibited the strains tested

That means nisin was active against 97.4% of indicator organisms used in our experiment and only 2.6% of strains were not inhibited. However, nisin was active towards the majority of tested target strains by its minimal inhibition concentration (MIC) 1.56 – 100 µg/ml (Table 1). Among the strains inhibited by nisin, *Enterococcus* sp. E6B (isolate from the faeces of zebra) was reduced by MIC of nisin 1.56 µg. That means this strain was the most sensitive to nisin treatment among the target strains tested. MIC 3.12 µg was active to inhibit 17 strains among all strains tested. It represents 22% among the target 77 strains tested. Two staphylococci, isolates from the faeces of deer were inhibited by MIC 6.25 µg of nisin. Among 12 enterococci from the faeces of deer, six strains were inhibited by nisin in MIC 12.5 µg, four strains were inhibited by MIC of nisin 25 µg and one strain by MIC 100 µg of nisin. Among enterococci isolated from the faeces of chamois (17), 64.7% were inhibited (MIC 12.5 µg/ml). The growth of the remaining strains was reduced and inhibited by nisin in MIC 25 µg and/or 100 µg. The lowest inhibition of nisin was detected among enterococcal isolates from the faeces of piglets. In contrast, the majority of enterococci, isolates from the faeces of wild animals, were inhibited by MIC of nisin 3.12 µg. Although only a few staphylococci were included in testing, most of them were inhibited by nisin.

DISCUSSION

The fact that most bacteria tested in our experiment were inhibited by nisin confirms the general knowledge concerning nisin; it has bactericidal effects on a broad spectrum of Gram-positive bacteria (Jack *et al*., 1995). It was formerly mentioned in our previous study where all (59) ureolytic ruminal staphylococci and enterococci were inhibited in the same conditions (Lauková, 1995). Although the effect of nisin on the individual species was not evaluated, its effect on the group of bacteria is already important. Nisin has been used as a food preservative for over 30 years. However, recent developments in the production of pure nisin preparation as well as results of testing its effects on microorganisms in the different ecosystems have resulted in nisin re-evaluation for veterinary, pharmaceutical uses and for further i.e. post-treatment of animal waste (Delves-Broughton *et al*., 1996; Lauková, 2000). The examples of successful use of nisin e.g. as a therapeutic agent in the treatment of bovine mastitis were reported by Delves-Broughton *et al*. (1996). Because nisin is non-toxic and readily inactivated by digestive enzymes in the guts, it should be possible to demonstrate that the agent and its peptide residues pose no hazard to the milk supply. Howell *et al*. (1993) reported the use of nisin to prevent gingival inflammation in a dog model. In human medicine nisin was found to be effective against *Helicobacter pylori* (Blackburn and Projan, 1994). There is also much information on nisin use in combination with chelating agents or with ultrahigh hydrostatic pressure or pulsed electric fields to improve its inhibitory effect (Stevens *et al*., 1992; Ponce *et al*., 1998; Calderón-Miranda *et al*., 1999). In general, the properties of nisin indicate its broad spectrum for utilization and further studies are underway in our laboratory aimed at its use with other bacteriocins (enterocins) to reduce sanitary-important species in animal waste.

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