

Searching for Genes of *Lactococcus lactis* subsp. *lactis* Encoding the Bacteriocin Nisin using DNA/DNA Hybridisation

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Abstract: In this work, PCR primers P8 and P9 were used for amplification of a 320 bp long PCR product specific to the nisin gene. The PCR product was labelled with digoxigenine during amplification and used as a DNA probe for the screening of homologous DNA sequences in 7 *Lactococcus lactis* subsp. *lactis* strains from the Culture Collection of Dairy Microorganisms (CCDM). Dot blot hybridisation and hybridisation of colonies were used for DNA/DNA hybridisation. It was shown that 6 tested strains of *Lactococcus lactis* subsp. *lactis* have genes encoding nisin. One strain had probably a defective gene encoding nisin.

Keywords: *Lactococcus lactis* subsp. *lactis*; screening; nisin; gene; PCR probe; DNA/DNA hybridisation

INTRODUCTION

Food-grade lactic acid bacteria produce many bacteriocins, antimicrobial peptides. One of the most studied bacteriocins is nisin. Nisin from the group of lanthibiotics (DELVES-BROUGHTON *et al.* 1996) has been in use not only in human and veterinary medicine but also in the pharmaceutical and food industries for more than 30 years (DE VUYST & VANDAMME 1994; JACK *et al.* 1995). Nisin produced by certain strains of the bacteria *Lactococcus lactis* subsp. *lactis* is one of the major bacteriocins with lethal effect on bacteria of the genera *Listeria*, *Staphylococcus*, *Micrococcus*, *Lactococcus*, *Streptococcus*, *Mycobacterium*, *Bacillus*, and *Clostridium* (MONTVILLE *et al.* 1999). The nucleotide sequence of the gene encoding its production is known including PCR primers for its amplification (MEGHROUS *et al.* 1999).

The aim of this work was to prepare a nisin probe using the strain *Lactococcus lactis* subsp. *lactis* CCDM 416. *Lactococcus lactis* subsp. *lactis* strains collected in the Culture Collection of Dairy Microorganisms (CCDM) (Laktoflora, Tábor, Czech

Republic) were screened for presence of the nisin gene using DNA/DNA hybridisation.

MATERIAL AND METHODS

The following CCDM strains of *Lactococcus lactis* subsp. *lactis* were used in the study: 71, 414, 418, 731, 416, S32, and HMM4. The strain *Lactococcus lactis* subsp. *lactis* CCDM 416 was known as a producer of bacteriocin (DRÁB – unpublished results) and its DNA was used for preparation of a DNA probe. Strains S32 and HMM4 were used as negative controls. The DNA probe was prepared from PCR products labelled with digoxigenine using 4 kits: PCR DIG Labeling Mix (Roche, CZ) (kit 1); PCR DIG Probe Synthesis Kit (Enzo-Bioehringer, Mannheim, D) (kit 2); PCR DIG Probe Synthesis Kit (Roche, CZ) (kit 3), and DIG DNA Labeling and Detection Kit (Roche, CZ). DNA probe was denatured (5 min/boil) before DNA/DNA hybridisation.

Dot blot hybridisation and colony hybridisation were tested. Purified (phenol extraction) and

denatured DNA (10 µl, in concentrations of 5, 15, and 25 ng/µl) was spotted on a nitrocellulose membrane for dot blot hybridisation. Individual bacterial colonies were placed on an agar plate, cultivated overnight, transferred to the hybridisation membrane, and lysed in lysis buffer containing lysozyme (10mM Tris.HCl, pH 7.8, 5mM EDTA, lysozyme 3 mg/ml). DNA was immobilised on membranes using UV light irradiation (2 min/15 cm). Digoxigenine-labelled lambda DNA (5 µl) in concentrations of 100 and 10 ng/µl was spotted on each membrane for the control of immunological detection of the hybridisation product (see later).

DNA/DNA hybridisation was performed at 68°C or 53°C overnight (high or low stringency of hybridisation). Different amounts of the DNA probe added to 1 ml of hybridisation solution were tested (PCR product: 10 and 20 µl; DNA: 1.5, 2.0, 2.5, and 4.0 µl). Hybridisation products were detected immunologically using antidigoxigenin-AP antibody and visualised with the colour reaction substrate NBC/BCIP according to the recommendation of the DIG DNA Labeling and Detection Kit (Roche, CZ) producer.

RESULTS AND DISCUSSION

Different quantities of nisin probes prepared with the use of different kits were used for the hybridisation (Table 1). Hybridisation products were often detected at lower intensities. The best results were

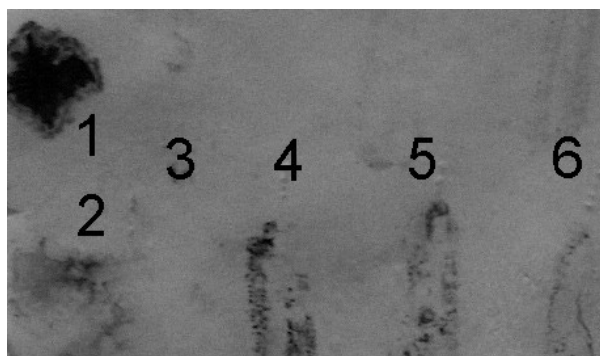


Figure 1. Membrane with hybridisation products. No. 1 and 2: lambda DNA labeled with digoxigenine 200 ng and 20 ng, No. 3–6: *Lactococcus lactis* subsp. *lactis* S32, *Lactococcus lactis* subsp. *lactis* HMM4, *Lactococcus lactis* subsp. *lactis* CCDM 731, *Lactococcus lactis* subsp. *lactis* CCDM 416

achieved with probes prepared from labelled PCR products and used in amounts of 10 to 20 µl/ml of the hybridisation solution. Hybridisation took place efficiently with probe prepared using kit 1. In dot blot hybridisation, the intensity of hybridisation products was affected by DNA concentration spotted on the membrane; the highest intensity was achieved with 250 ng of DNA.

The same procedure was used for the screening of tested strains of *Lactococcus lactis* subsp. *lactis*. DNA/DNA hybridisations demonstrated that 6 tested strains carried genes encoding nisin (Figure 1 and Table 2), including one negative control strain (HMM4). Production of bacteriocin

Table 1. Nisin gene identification in *Lactococcus lactis* subsp. *lactis* CCDM 416 strain. Results of colony hybridisation and dot blot hybridisation with probes prepared using kits 1–4

Kit	Colony hybridisation		Dot blot hybridisation	
	probe volume (µl/ml)	hybridisation product	probe volume (µl/ml)	hybridisation product
Kit 1 PCR product	10	+	10	+
	20	+/-	20	-
Kit 2 PCR product	20	+	20	+/-
Kit 3 PCR product	20	+	20	-
Kit 4 DNA	1.5	+/-	1.5	-
	2.0	+/-	2.0	-
	2.5	+/-		

+ hybridisation product of higher intensity was detected; +/- hybridisation product of low intensity was detected; - hybridisation product was not detected

Table 2. The results of DNA/DNA hybridisation of colonies and dot blots

Strain <i>Lactococcus lactis</i> subsp. <i>lactis</i>	Colony hybridisation	Dot blot hybridisation
CCDM 71	n.d.	+
CCDM 414	+	–
CCDM 418	+	–
CCDM 416 *	+	+
CCDM 731	+	+
S32	–	n.d.
HMM4	+	–

*DNA from *Lactococcus lactis* subsp. *lactis* 416 and kit 1 were used for probe preparation; + hybridisation product of higher intensity was detected; +/– hybridisation product of low intensity was detected; – hybridisation product was not detected; n.d. = not detectable (weak growth)

was demonstrated in 5 strains. For the bacterial strain HMM4 hybridisation with the nisin probe was positive but the gene was non-functional and did not produce nisin as the negative control strain S32 (DRÁB – unpublished results). Phenotypically, the strains *Lactococcus lactis* subsp. *lactis* HMM4 and *Lactococcus lactis* subsp. *lactis* S32 are manifested as non-nisinogenic strains of bacteria. The presence of a non-functional nisin gene in *Lactococcus lactis* subsp. *lactis* was described in the literature (MOSCHETTI *et al.* 2006).

Hybridisation was performed under conditions of high stringency (68°C) when complementary sequences of DNA probe and chromosomal DNA renature. The intensities of hybridisation products were lower in comparison with low stringency conditions (53°C). The low intensity of hybridisation products can be caused by the small length of the DNA probe (320 bp) which hybridised to one copy of the gene in the bacterial genome. Using a low stringency (53°C), the intensity of hybridisation products increased in all strains of *Lactococcus lactis* subsp. *lactis*, since hybridisation can occur between two ssDNA molecules which may not be

completely complementary. This allows to detect the related sequence too. MEGHROUS *et al.* (1999) described hybridisation at low stringency and the emergence of hybridisation products with non-nisinogenic strains. Low signal intensity may be caused by the worse quality of DNA probes. The intensity of hybridisation products of the dot blot hybridisation was affected by the concentration of DNA spotted on the membrane. The highest intensity of hybridisation products was detected with 250 ng of DNA.

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