Characterization of Lactococcus lactis subsp. lactis isolated from surface waters

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Introduction
Lactococci are Gram-positive, non-motile, coccus-like bacteria belonging among the lactic acid bacteria group. The genus Lactococcus consists of five species inhabiting various environments. The most important species of the genus is Lactococcus lactis that is traditionally considered as a milk-product associated bacterium. L. lactis plays an important role in dairy industry as a common part of many fermented products. Isolation of Lactococcus lactis from other sources is reported less commonly.

This study deals with a group of L. lactis subsp. lactis strains isolated from different surface waters on enterococcal selective media during a routine microbiological water analysis.

Material and methods

Bacterial strains
Studies were isolated by inoculation of analyzed water samples on斜orth-Bartley agar (Oxoid) and/or on Kanamycin esculin azide agar (Merck). Analyzed water samples were collected in the Czech Republic from the following sources: P1:58, peloid bath; P149, storage reservoir in a lime and cement factory; P1943, brook Radějovice; P1944, unwarmed pond; P1946, intake of the hydrogen sulphide water into the reservoir; P2011, swimming beach Retkov; P2012, swimming beach Sobotka; P2016 and P2019 two different unwarmed little ponds. Reference strains were obtained from the Czech Collection of Microorganisms, Masaryk University, Brno, Czech Republic (Centre for Microorganisms, Brno).

Repetitive sequence-based PCR

The rep-PCR fingerprinting using the (GTG)5 primer (5'-GTG GTG GTG GTG GTG-3') was performed as described by Gereze et al. (2001) with the following modifications. Bacterial DNA was isolated by alkaline lysis extraction procedure: a 1 ml loopful of bacterial cells was homogenized in 20 ml of lysis buffer (0.25 % SDS + 0.05 M NaOH) and heated at 95°C for 15 minutes. Obtained cell lysates were diluted by adding of 180 µl of sterile water, centrifuged at 13,000 rpm for 5 minutes and maintained at -20°C. Totally, 1 µl of cell lysates and 24 µl of a PCR mixture were inletted into PCR reactions performed in Tepsonal thermocycler (Biometra). Obtained PCR products were separated in 1.5 % agarose gels for 18 h at 60 V (approx. 1.7 V/cm). Resulting fingerprints were digitized and processed using Bionumerics v. 4.601 software (Applied-Maths).

Phenotypic characterization

Phenotypical testing for catalase production, growth at 10, 42 and 45°C and in 6.5 % NaCl was performed in BHI broth. Pymidoxyl arylamine production and Voges-Proskauer reaction were tested using PYRA test strip (both from PLWA, Brno). Group D antigen was tested using a Streptococcus grouping kit (Oxoid). Biochemical tests were performed using API 20C kit according to the manufacturer’s instructions (bioMérieux). Identification was achieved using the Internet-identification tool ApeWeb (bioMérieux, https://apeweb.biomerieux.com).

Ribotyping

Automated ribotyping with EcoRI restriction enzyme was carried out using a Riboprinter microbial characterization system (DuPont Qualicon). Standard procedure intended for lactic acid bacteria was performed in accordance with the protocol provided by the manufacturer. Numerical analysis of obtained ribopatterns and dendrogram construction was performed with Bionumerics v. 4.601 software (Applied-Maths).

Results and discussion

All nine strains were catalase negative. The results of the ribotyping and the phenotypic and biotyping tests are summarized in Table 2. The ribotyping of EcoRI restriction enzyme of each strain was performed using the Bionumerics software (Applied-Maths). The results were compared with the reference strain (L. lactis subsp. lactis 64/641) and the strain isolated from the water sample (strain P1:58). The results showed that the L. lactis subsp. lactis 64/641 strain had the same ribotype as the strain isolated from the water sample (strain P1:58). The results also showed that the L. lactis subsp. lactis 64/641 strain had a different ribotype from the strain isolated from the water sample (strain P1:58), indicating that the strain isolated from the water sample (strain P1:58) was a new isolate.

Conclusions

Further characterization using rep-PCR with the (GTG)5 primer generated DNA fragments ranging from 200 to 2400 bp and were separated by electrophoresis. The results from all enterococci were included in the INHUSEN database (data not shown). All strains revealed visually nearly identical fingerprints very close to the L. lactis subsp. lactis representatives and clustered them into a homogeneous group at the similarity level more than 62 % (Fig. 1).

Fig. 1. Dendrogram based on cluster analysis of Lactococcus lactis subsp. lactis strains isolated from surface waters on enterococcal selective media.

All nine strains were catalase negative and oxidase arranged in groups, pairs and short chains and grew at 10°C and weakly at 42°C. Growth at 45°C was negative (except strain P1946). Variable reaction revealed for growth in 6.5 % NaCl (weakly positive P1944, P1946, P2011) and pymidoxyl arylamine production (weakly positive P1944, P2016, P2019). Aesculin production (Voges-Proskauer reaction) was positive, group D antigen was negative. Evaluation of API 20C gallery results using the ApeWeb identification tool assigned all strains as L. lactis subsp. lactis.

Rep-PCR using the (GTG)5 primer has been successfully used for identification of different lactic acid bacteria (e.g. Gereze et al. 2001; Švec et al. 2005) including lactobacillus (Diamond et al. 2005; Huyts et al. 2006; Zamfir et al. 2006). Our results confirmed this simple and fast method as a good taxonomic tool for identification of L. lactis subsp. lactis and may (GTG)5-PCR as a suitable method for identification of other lactobacillus species as well as for their differentiation from enterococcus.

Identification of lactococcus bacteria using biochemical tests is not often straightforward and reliable. Lactococcus can be most often confused with enterococcus due to possible combination of positive as so-called “classical” tests used for differentiation of the genera of Gram-positive, catalase negative lactococcus. Lactococcus strains can be positive for Voges-Proskauer reaction, esculin hydrolysis, pymidoxyl arylamine production and growth at 10°C and 45°C and in 6.5 % NaCl (Dennisee et al. 1993; Facklam and Elliot 1995). Especially in a case of analysis of bacteria isolated from non-dairy sources it is possible to misidentify lactobacillus as enterococcus (Elliot and Facklam 1996).

Automated ribotyping with EcoRI restriction enzyme clustered reference and water L. lactis subsp. lactis strains into a single group separated from all other lactococcus taxa at the similarity level of >96 % (Fig. 2). Totally, six ribopatterns were shown among water isolates. All strains (except P1946) shared two typical bands (9.3 and 9.7 kb) separating clearly all L. lactis subsp. lactis from other lactococcus except L. lactis subsp. lactis hordaei CCM 4545, which revealed the closest ribopattern to L. lactis subsp. lactis strains (48 % similarity).

Conclusions

Obtained results showed that L. lactis subsp. lactis is not rare in different surface waters as generally considered, but it can be commonly recovered during a routine microbiological water analysis. Although lactococcus are fastidious microorganisms they can survive in specific natural niches providing sufficient nutrition (Kijne et al. 1996).

This short study demonstrated that L. lactis subsp. lactis strains isolated from surface waters can be confused with enterococcus due to their growth on selective enterococcus media as well as positive “classical” tests commonly used for identification of the genus Enterococcus. Molecular methods such as rep-PCR used in this work or extensive ribotyping should be used for reliable differentiation of these two genera.

References


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