Evaluation of Automated Ribotyping for Characterization of *Streptococcus mutans*

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Introduction

Streptococcus mutans belonging among the oral streptococci is assigned as a member of 'mutans group' streptococci. There is a great interest in *S. mutans* among oral clinical microbiologists because it plays a crucial role in the dental caries development and is considered to be the principal etiological agent involved in human dental caries initiation. The aim of this work was to evaluate automated ribotyping with *Eco*RI restriction enzyme (RiboPrinter® microbial characterization system) for typing and identification of *Streptococcus mutans* strains. Only a few studies dealing with manual ribotyping with *Eco*RI and/or *Hin*dIII restriction enzymes for epidemiological typing of dental *S. mutans* isolates have been published (Alaluusua et al. 1994, 1996; Saarela et al., 1996; Grönroos et al., 1998), however, according to our best knowledge, they are no more papers dealing with application of the RiboPrinter® system for characterization of *S. mutans* available.
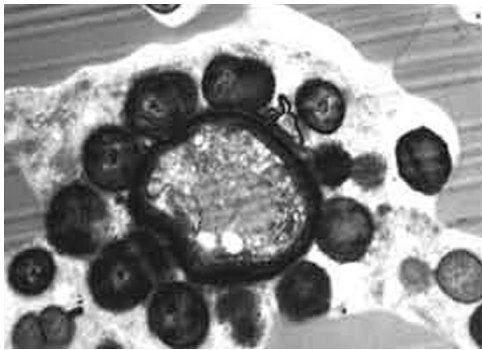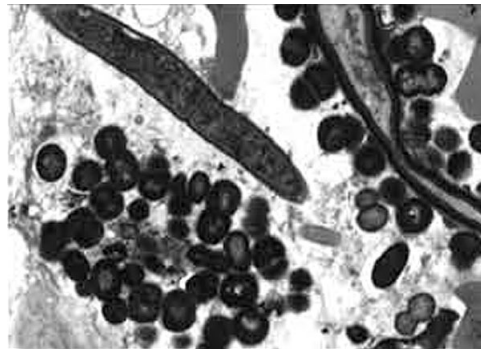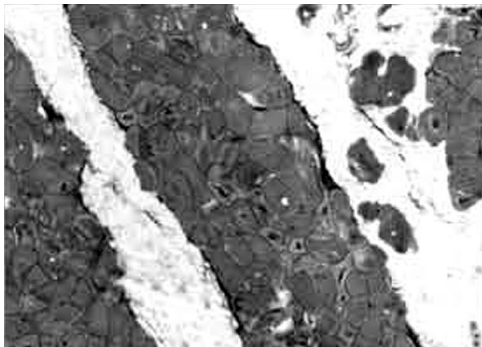

Fig. 1

Fig. 2

Fig. 3

Objectives

The aim of the study was to evaluate the automated ribotyping for characterization of *Streptococcus mutans*.

Material and Methods

Bacterial strains

In total, 29 tested clinical strains were isolated from dental plaque of early childhood caries (ECC) affected children treated in the Department of Pedodontics (Children's Teaching Hospital, Brno, Czech Republic). All strains presented in this study were isolated from different patients. Reference strains were obtained from the Czech Collection of Microorganisms (http://www.sci.muni.cz/ccm). Identification of analysed strains was achieved by biotyping (API 20 Strep identification kit, conventional tests), rep-PCR fingerprinting using the (GTG)5 primer (Svec and Sedlacek, 2008) and whole-cell protein fingerprinting (Pot et al., 1994).

Automated ribotyping

Automated ribotyping with *Eco*RI restriction enzyme was carried out using a RiboPrinter® microbial characterization system (DuPont Qualicon) in accordance with the protocol provided by the manufacturer. Obtained ribopatterns were normalized, automatically categorized into ribogroups and compared to a DuPont Qualicon database DUP 2004 containing 6448 different ribotype profiles (including two *S. mutans* representatives) by using the RiboExplorer v. 2.1.4216.0 operating software (DuPont Qualicon). There is a sliding treshold of ≥ 93% for seeding ribogroup on down to 90 % for additional samples to join an existing ribogroup. In addition, manual correction of the automatic categorization into ribogroups was performed after careful examination of the resulting riboprints as proposed by Brisse et al. (2002). Numerical analysis of obtained ribopatterns and dendrogram construction was performed with Bionumerics software v. 4.601 (Applied-Maths). The dendrogram was calculated with Pearson's correlation coefficients using UPGMA clustering method. Optimization value of 1 % was allowed for the densitometric curves. Import of the ribopatterns into the Bionumerics software was achieved by using the Load samples import script obtained from Applied-Maths.
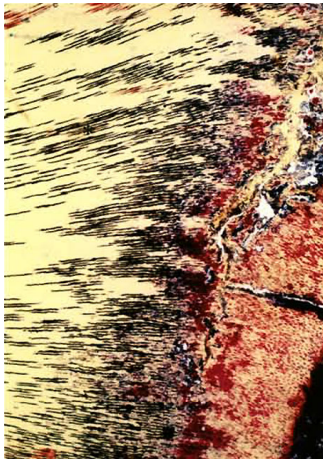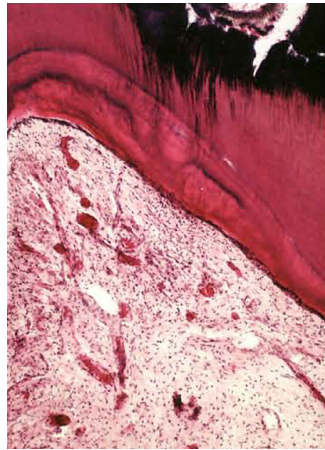

Fig. 4

Fig. 5

Results

Automated ribotyping with *Eco*RI restriction enzyme generated bands ranging from approx. 6 to 60 kbp from all analysed strains except strain CCM 7410 revealing an extra 0.7 kbp band. Similarities between individual patterns ranged from 42.3 to 97.6 %. The automatic characterization process separated analysed strains into 24 ribogroups; however automatic identification performed by the RiboPrinter system did not assign any strain to the species level. Cluster analysis of obtained riboprints in the BioNumerics software and manual inspection and correction of the automatic ribopatterns categorization clustered, in addition to the automatic characterization results, four individual strains into two groups (group P2080, P2129 and group P1561, P2084) and included two single strains (CCM 7409T, P2090) into already existing ribogroups (Fig. 6).
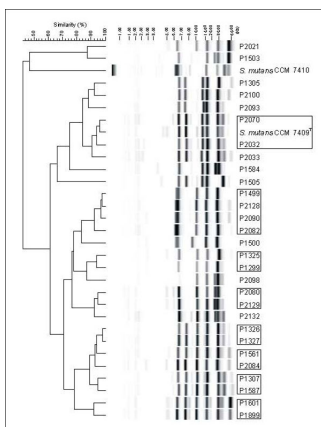

Fig. 6: Dendrogram based on cluster analysis of *S. mutans Eco* RI ribotype patterns obtained using the RiboPrinter® microbial characterization system. Ribotypes represented by multiple strains are highlighted by squares.

Conclusions

The RiboPrinter system failed to identify analysed strains; the automatic identification performed by the system using a DuPont Qualicon database DUP 2004 did not assign any *S. mutans* strain to the species level.

In total, 20 ribotypes were revealed among 31 strains. These results imply RiboPrinter microbial characterization system as a satisfactory tool for *S. mutans* intraspecies typing purposes although careful inspection of obtained automatic characterization results is needed to obtain reliable outcomes.

Explanation to figures:
Illustrative pictures of microbes in plaque (figs. 1-2) and in dentine (figs. 3-5).

Literature

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This Poster was submitted by Dr. Jarmila Kuklová.

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Poster Faksimile:

Evaluation of automated ribotyping for characterization of *Streptococcus mutans*

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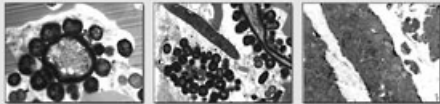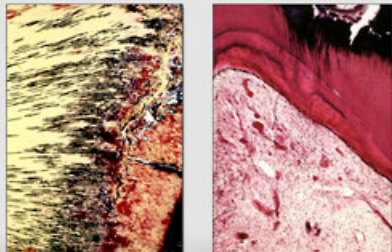
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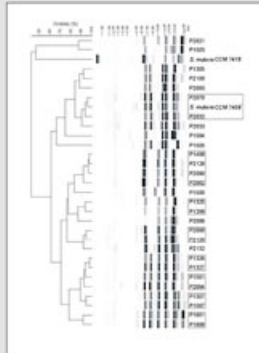

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