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SUMMARY

The importance of bacterial adhesion to solid substrata in various applications of environmental, medical and industrial interest is pointed out in **chapter 1** of this thesis. Adhesion of oral bacteria to teeth can be followed by bacterial growth and metabolic activity and causes dental caries and periodontal diseases. An understanding of the mechanism of bacterial adhesion contributes to the development of new preventive measures in dentistry; this requires a detailed knowledge of the chemical composition and physico-chemical properties of the bacterial cell surfaces.
The aims of this study were:
(1) to characterize oral streptococcal cell surfaces by their zeta potential, surface free energy and elemental and molecular composition
(2) to explain possible relations between the different surface parameters
(3) to determine the influence of saliva treatment of oral streptococci on the surface properties.
As a secondary aim, the surface properties of oral streptococci were compared with those of non-encapsulated and encapsulated coagulase-negative staphylococci. This was done to estimate the general validity of the previous results.
Chapter 2 presents a literature survey on bacterial cell wall properties. Special attention has been given to a compilation of the physico-chemical surface properties of bacteria relevant for adhesion (zeta potential, hydrophobicity and chemical composition). Despite the abundant literature on the bacterial cell wall and the physico-chemical surface properties of these cell walls, no detailed comparisons between the four physico-chemical surface properties mentioned (relevant for adhesion) have been made.
In **chapter 3** an overview of the major experimental techniques employed in this study is presented: electrophoresis, contact angle measurements, X-ray photoelectron spectroscopy and transmission infrared spectroscopy. The latter two techniques were applied to freeze-dried cells; the electrophoresis was used on fully hydrated cells, whereas the contact angle measurements were employed on partly dehydrated bacteria.
In **chapter 4** physico-chemical surface characteristics and adhesive properties of a series of mutants of *S. salivarius* HB, with defined cell surface structures are described and discussed. Zeta potentials related neither with the presence or absence of specific antigens on the bacterial cell surface nor with the adhesive properties of the cells. The removal of fibrils from these cell surfaces resulted in a more hydrophilic character (higher surface free energy). Bacterial adhesion to the polymer polymethylmethacrylate increased with increasing hydrophobicity of the mutants.
The results obtained by several methods, commonly employed in microbiology to determine bacterial cell surface hydrophobicity including the adsorption to hexadecane, hydrophobic interaction

chromatography, salt aggregation and contact angle measurements are compared in **chapter 5.** No correlations were observed between the results of the various tests when applied to a wide variety of oral streptococcal strains. However, good correlations between the mentioned hydrophobicity tests were obtained for *S. salivarius* HB and the range of mutants deprived of surface structures. It is concluded that it is impossible to define the surface "hydrophobicity" of a bacterium otherwise than on a comparative level in closely related strains. Furthermore, we conclude that the expression "hydrophobicity" should not be used without explicitely mentioning of the technical details of the measurements.

Chapter 6 describes the influence of saliva coating on the surface free energy of a range of oral streptococci. The low surface free energy strains showed a significant increase in this parameter after saliva treatment. The high surface free energy strains, however, were not significantly affected by the saliva treatment.

The determination of bacterial cell surface free energies from contact angles measurements is tedious and delicate, as emphasized in **chapter 7.** Especially excessive drying of cell surfaces can cause deviations from the physiologically relevant results due to collapse of hydrophobic surface structures. This can be concluded from a comparison of water contact angles measured on partly dehydrated and freeze-dried oral streptococci.

Chapter 8 presents the elemental surface compositions of *S. salivarius* HB and mutants as measured by X-ray photoelectron spectroscopy. An increasing loss of proteinaceous fibrillar surface antigens was concurrent with a decrease in the N/C (nitrogen/carbon) surface concentration ratio and a decrease in the isoelectric point.

In **chapter 9**, the zeta potentials, surface free energies and elemental surface compositions of a much broader variety of oral streptococcal strains are compared. A decrease in the N/C surface concentration ratio was found to be concurrent with an increase in the O/C (oxygen/carbon) ratio. Simultaneously, with the decrease in the N/C concentration ratio the isoelectric point decreased and the surface free energy increased. It is concluded that zeta potentials, surface free energies and elemental compositions of bacterial cell surfaces show clear relationships, despite the fact that these parameters were measured in different hydration states of the surface.

The influence of saliva treatment of oral streptococci on their zeta potentials, surface free energies and elemental surface compositions is presented in **chapter 10.** The adsorption of salivary constituents was detectable with all techniques applied. Similar relationships as indicated above for the untreated strains (described in chapter 9) were also observed for the saliva-treated strains. An increase in the N/C surface concentration ratio was concurrent with an increase in the isoelectric point, a decrease in the O/C surface concentration ratio and a decrease in the surface free energy.

In **chapter 11**, the molecular composition of oral streptococci

is determined by transmission infrared spectroscopy. All strains investigated show similarly shaped spectra. They differed however in absorption band ratios. Two positive relationships could be established between the AmII/CH (N-H bending in amide bonds) absorption band ratio and the N/C surface concentration ratio determined by X-ray photoelectron spectroscopy as well as between the AmI/CH (C=O stretch in amide bonds) and the fraction of carbon atoms at the surface involved in amide bonds. These relationships indicate that, despite the fact that transmission infrared spectroscopy is a bulk technique, it reflects the surface composition of freeze-dried bacteria.

Additional evidence for this surface sensitivity of transmission infrared spectroscopy in the study of bacterial cell surfaces is provided in **chapter 12**, where it was found that the amide absorption bands of the cells significantly increased after adsorption of salivary constituents.

In an attempt to determine whether the relationships between the zeta potentials, surface free energies and elemental and molecular compositions observed for oral streptococci have any general validity, physico-chemical properties of non-encapsulated and encapsulated coagulase-negative staphylococci are determined in **chapter 13** and compared with those of oral streptococci. Within the relationships between surface properties observed, an increase in the O/C surface concentration ratio was always concurrent with a decrease in the N/C surface concentration ratio and an increase in the surface free energy. The N/C and P/C (phosphorus/carbon) surface concentration ratios showed positive correlations with the AmII/CH and PI/CH (phosphate) absorption band ratios. The staphylococci could be distinguished from the streptococci by their different absolute values.

In the general discussion (**chapter 14**) of this thesis the physico-chemical surface properties of the oral streptococcal strains are discussed in relation with their adhesion to solid substrata. It is concluded that all parameters investigated show relations with bacterial adhesion under *in vitro* and *in vivo* conditions.

Summarizing, it can be stated that the four physico-chemical techniques used in this thesis to study bacterial cell surface properties are unique in the sense that they show comprehensible relationships.