

HYDROPHOBICITY TEST IN *MUTANS STREPTOCOCCI*

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ABSTRACT

Kinetic hydrophobic measurements were performed by confronting 40 *mutans streptococci* from thirty 10- to 20-year-old patients with 200 ml hexadecane (Sigma). Fourteen patients had high dental caries risk (Group A), *dmft* + *DMFT* > 5 with 3 or more active caries, and 16 had low dental caries risk (Group B), *dmft* + *DMFT* < 3 without active caries. Twenty bacteria from Group A and 20 bacteria from Group B were typed using De La Higuera's procedure and confirmed by API strip (bio-Merieux). From the 14 patients in Group A we obtained 12 *S. mutans* (8 hydrophobic/ 4 non-hydrophobic), 5 *S. sobrinus*

(4 hydrophobic/ 1 non-hydrophobic) and 3 *S. rattus* (hydrophobic). From the 16 patients in Group B we obtained 11 *Streptococcus mutans* (10 non-hydrophobic/ 1 hydrophobic), 7 *Streptococcus sobrinus* (6 non-hydrophobic/ 1 hydrophobic) and 2 *Streptococcus rattus* (hydrophobic). Patients with high dental caries risk have a higher prevalence of hydrophobic bacteria than patients with low dental caries risk ($p=0.0003$). All typed *S. rattus* were hydrophobic.

Key words: hydrophobicity, *Streptococcus mutans*, dental caries, *n*-hexadecane.

PRUEBA DE HIDROFOBICIDAD EN *STREPTOCOCCUS* GRUPO *MUTANS*

RESUMEN

Con el objeto de evaluar una posible relación entre hidrofobicidad y caries dental, se estudiaron 40 cepas de *Streptococcus* grupo *mutans* provenientes de 30 pacientes de entre 10 y 20 años, 14 pacientes con tres o más caries activas e índice *ceod* más *CPOD* > 5 (Grupo A) y 16 pacientes sin caries activas, con *ceod* más *CPOD* < 3 (Grupo B). Las cepas fueron aisladas a partir de muestras de saliva en AMS-BT y tipificadas por pruebas bioquímicas y API-strep realizándose la medición cinética de hidrofobicidad, enfrentándolas con 200 ml de hexadecano (Sigma). En el grupo A se caracterizaron 12 cepas *Streptococcus mutans*: 8 hidrofóbicas y 4 no hidrofóbicas, 5 cepas de *Streptococcus sobrinus*: 4 hidrofóbicas y 1 no hidrofóbica y 3 cepas *Streptococcus rattus* hidrofóbicas. En el grupo B, se caracteri-

zaron 11 cepas de *S. mutans*, 10 no hidrofóbicas y 1 hidrofóbica, 7 cepas de *S. sobrinus*: 6 no hidrofóbicas y 1 hidrofóbica y 2 cepas de *S. rattus* hidrofóbicas.

Todos los pacientes del grupo A presentaron al menos 1 cepa hidrofóbica. En las cepas aisladas de estos pacientes se demostró la existencia de una alta prevalencia de *Streptococcus* grupo *mutans* con características hidrofóbicas $p=0,0003$. Estos resultados indicarían la relación entre la capacidad de adherencia a hexadecano y caries activa. Todas las cepas tipificadas como *S. rattus* fueron hidrofóbicas independientemente del grupo de origen.

Palabras clave: hidrofobicidad, *Streptococcus mutans*, caries dental, *n*-hexadecano.

INTRODUCTION

Dental caries is a multifactorial disease. Its development may be influenced by factors that are inherent to the host, habit-related or genetic, such as oral hygiene, age, dental crowding and saliva pH; and others related to the patient's diet and colonization of dental biofilm and saliva by cariogenic microorganisms. *Mutans streptococci* and oral hygiene play a major part in the caries process, together with the aggressiveness of the bacterial strains involved. Some subjects may have a high *mutans streptococci* count and yet not suffer from or be predisposed

to suffer from dental caries, even if they do not maintain adequate oral hygiene or clean their teeth according to the frequency advised by the dentist. Other subjects may have active caries despite a low salivary *mutans streptococci* count and good oral hygiene habits. Therefore, in order to contribute to determining the risk of caries, it is necessary not only to know the number of streptococci in the saliva but also to investigate the virulence factors of the species present in the patient's ecological system¹⁻⁷. S. Mudd and E.B.H. Mudd⁸ (1924) noted that some bacteria had a tendency to separate at the water-oil

interface. These observations were the starting point for the study of the role of microbial adhesion to hydrocarbons. The hydrocarbon adherence test shows that some microorganisms tend to adhere to the surface of this kind of substance. This may be compared to the bacterial mechanism for adhering to various surfaces, including catheters, implants, teeth, prostheses, red blood cells and mucosa cells. The aim of the present work was to contribute to the knowledge of whether there is a relationship between the adherence capacity of *mutans streptococci* and the development of dental caries, by evaluating groups of patients with and without active caries.

MATERIALS AND METHODS

Study Group

A saliva sample was taken from each of 30 patients, of both sexes, aged 10 to 20 years, with different risks of caries development. Caries in primary and permanent teeth were counted. There were 14 patients with three or more active caries, with dmft + DMFT > 5 (Group A), and 16 without active caries, with dmft + DMFT < 3 (Group B).

Isolation and characterization of isolated species

Strains were isolated on 20% mitis salivarius sucrose-potassium tellurite-bacitracin agar (MSA-BT) and picked onto Columbia Agar (bio Merieux) and triptein-soy agar (TSA). They were typed using the method of De la Higuera⁹ and the results checked with bioMerieux API strip. Bacteria were kept on Skim milk BD[®] at -30° C, for less than one month.

Bacteria processing

Strains were recovered on Schaedler/ sheep blood agar plates, ensuring that there was no contamination in the recovery vial. They were cultured on Schaedler broth (bioMérieux 51076) for 24 hours under microaerophilic conditions and 24 hours under aerobic conditions, at 36 ± 1°C.

Hydrophobicity study

The Microbial Adhesion to Hydrocarbons (MATH)¹⁰ method was selected to establish the tendency of certain microorganisms to adhere to a hydrocarbon surface. The hydrocarbon used was Hexadecane (Sigma), which is photosensitive, hygroscopic (< 0,005% H₂O), irritant, toxic to humans, and highly sensitive to temperature. It was kept in darkness at 25°C, free from any impurities

that might alter the results. Hydrophobicity tests were performed on strains obtained which had undergone fewer than five passages, in view of the results of the experiments by G. Westergren^{11,12}.

Method

Baseline Reading. Bacteria were washed twice with 0.4M phosphate buffer PO₄ H₂ Na / PO₄ H Na₂ (pH 7.2), which is considered optimum for the viability of the *mutans streptococci*. The optical density (OD) of the bacterial suspension was adjusted to between 0.8 and 1.0 at 450 nm in a glass cuvette (Makro, 10mm, Schichttiefe N° 6030) with a Boeco S-20 spectrophotometer.

1st Reading. 2.5 ml of the bacterial suspension (OD 0.8 - 1.0) were transferred to a Kant tube, 200 ml hexadecane were added and the tube was vortexed (V1 Boeco) at speed 4 - 5 for 2 minutes. Phases were left to separate for 2 minutes and the aqueous (lower) phase was collected for the first reading.

2nd Reading. The tube was vortexed again for 2 minutes, the phases left to separate and a second reading taken, following the same procedure as for the first reading.

Hydrophobicity controls

Negative hydrophobicity control: *Bacillus subtilis* var. Níger (ATCC 6633), a strain cited in literature² as non-hydrophobic, which aggregates in clumps that are very difficult to resolve. In order to prevent aggregation, it was cultured in soy triptein broth (STB) under continuous stirring with a magnetic stirrer set at level one (Hanna Instruments) for 24 hours at 37°C^{13,14}.

Positive hydrophobicity control: *Gemella haemolysans*.

Statistical evaluation

Pearson's Chi-square test was performed to determine whether the result of the hydrophobicity test is related to the level of dental caries risk in patients from whom the strain was isolated. An Odds ratio was calculated to determine the strength and direction of the evidential relationship.

RESULTS

A total 40 *mutans streptococci* strains were typed. Patients with dmft + DMFT > 5 with 3 or more active caries were assigned to Group A, and those

Table 1: Characterization and distribution of strains according to caries activity.

Species	Group A	Group B	Total
<i>S. mutans</i>	11	12	23
<i>S. rattus</i>	2	3	5
<i>S. sobrinus</i>	7	5	12
Totals	20	20	40

Table 2: Hydrophobicity in isolates from patients without active caries (group B).

Species	Positive hydrophobicity (+)	Negative hydrophobicity (-)	Total
<i>S. mutans</i>	1	10	11
<i>S. rattus</i>	2	--	2
<i>S. sobrinus</i>	1	6	7
Totals	4	16	20

Table 3: Hydrophobicity in isolates from patients with active caries (group A).

Species	Positive hydrophobicity (+)	Negative hydrophobicity (-)	Total
<i>S. mutans</i>	8	4	12
<i>S. rattus</i>	3	--	3
<i>S. sobrinus</i>	4	1	5
Totals	15	5	20

with $dmft + DMFT < 3$ without active caries were assigned to Group B⁷.

Of the 40 strains characterized, 20 were from patients in Group A and 20 from patients in group B. Table 1 shows the species typed and the number of strains isolated in each group (A with active caries and B without caries).

Hydrophobicity of isolated strains

The 40 strains were tested for hydrophobicity following the technique described above. Table 2 summarizes the results obtained with the strains recovered from patients without active caries (Group B), and Fig. 1, 2 and 3 are graphs plotting their absorbance (nm) as a function of time. Table 3 shows the results of the hydrophobicity test for strains from patients with active caries (Group A), and Fig. 4, 5 and 6 are graphs plotting their absorbance as a function of time.

Of the twenty isolates from patients without active caries, 80% (16 strains) produced negative results in the hydrophobicity tests, while of the twenty isolates from patients with active caries, 75% (15 strains) had positive hydrophobicity, as shown by a decrease in absorbance.

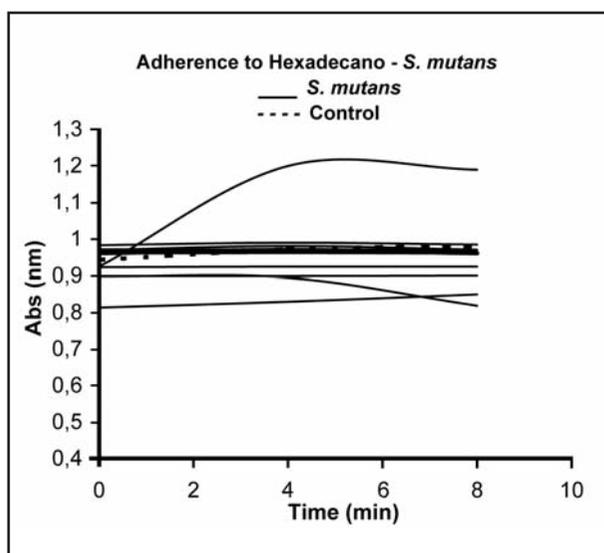


Fig. 1: Adherence to Hexadecane from 10 strains of *S. mutans*. Patients without active caries.

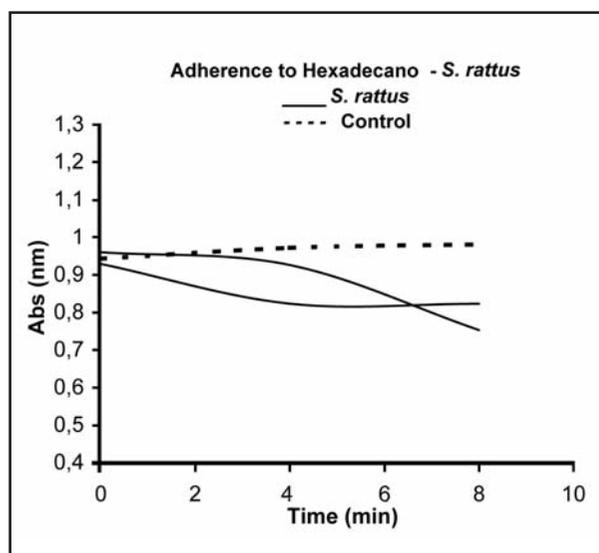


Fig. 2: Adherence to Hexadecane from 2 strains of *S. rattus*. Patients without active caries.

The five *S. rattus* isolates had positive hydrophobicity, regardless of the caries-forming capacity of the patient they were obtained from.

Table 4 shows the results of the hydrophobicity test for *mutans streptococci*. The 5 *S. rattus* strains were omitted because they were all positive for the hydrophobicity test, regardless of the group they came from.

The relationship between caries-forming capacity and hydrophobicity was found to be statistically

Table 4: Hydrophobicity test for the *mutans streptococci*.

Group	Hydrophobicity		Total
	Positive	Negative	
A (with active caries)	12	5	17
B (without active caries)	2	16	18
Total	14	21	35

significant ($p = 0.0003$). An Odds ratio was calculated to determine the strength and direction of the relationship found, and it showed that the odds that

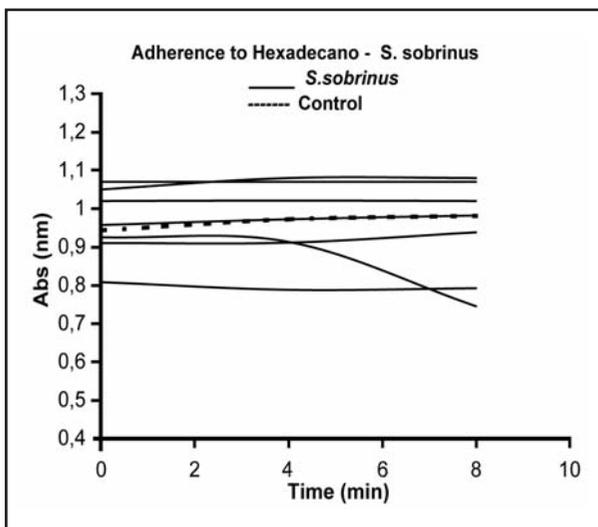


Fig. 3: Adherence to Hexadecane from 6 strains of *S. sobrinus*. Patients without active caries.

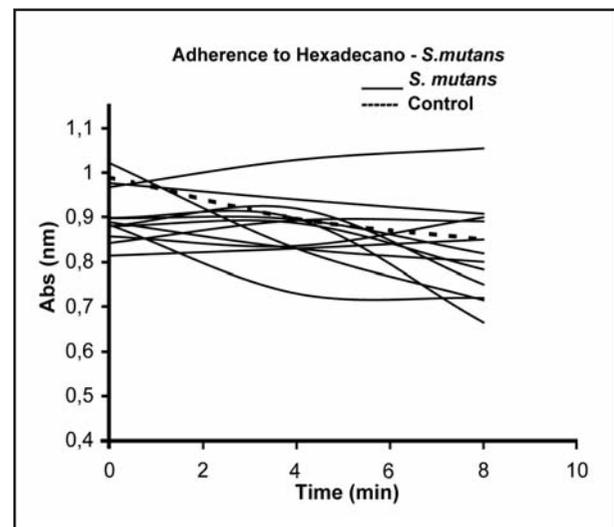


Fig. 4: Adherence to Hexadecane from 12 strains of *S. mutans*. Patients with active caries.

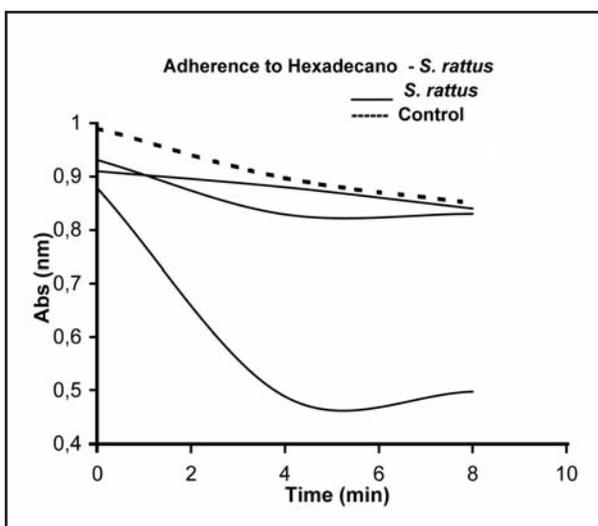


Fig. 5: Adherence to Hexadecane from 3 strains of *S. rattus*. Patients with active caries.

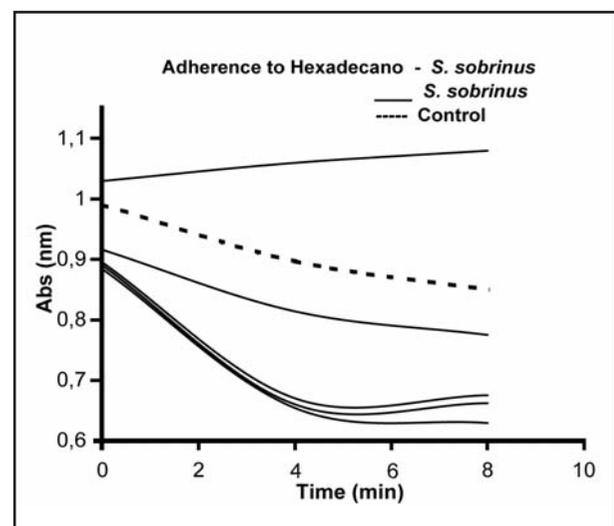


Fig. 6: Adherence to Hexadecane from 5 strains of *S. sobrinus*. Patients with active caries.

the hydrophobicity test would be positive in isolates from patients with active caries are 19 times greater than for isolates from patients without active caries.

Figures 7 and 8 are graphs plotting absorbance as a function of time, which show that the five species typed as *S. rattus* had positive hydrophobicity. Because of the small number *S. rattus* of strains isolated, further studies are needed to establish whether positive hydrophobicity is a general characteristic of the species. It is important to point out the lack of data on this topic in the literature reviewed.

DISCUSSION

The advantages of the method used for studying adherence are that it is economical, fast, sensitive, simple and its results correlate well with those obtained using other methods⁴. However, it has the following disadvantages:

- It is difficult to perform spectrophotometer readings with bacteria that self-agglutinate.
- Electrostatic interactions are not considered¹⁵.
- It is advisable not to run the test on strains that have undergone more than 5 passages. G. Westergren^{9,10} showed that streptococci hydrophobicity changes after 18 successive passages on sheep blood agar.

Regarding adherence evaluated in strains typed from patients with and without active caries, the results obtained using the hydrophobicity test must be considered highly significant, as they showed that at least one *S. mutans* isolate and/or one *S. sobrinus* isolate with hydrophobic characteristics, as well as three *S. rattus* isolates were recovered from all patients with active caries.

Our results allow us to conclude that there is high prevalence of *S. mutans* with hydrophobic characteristics in patients with active caries.

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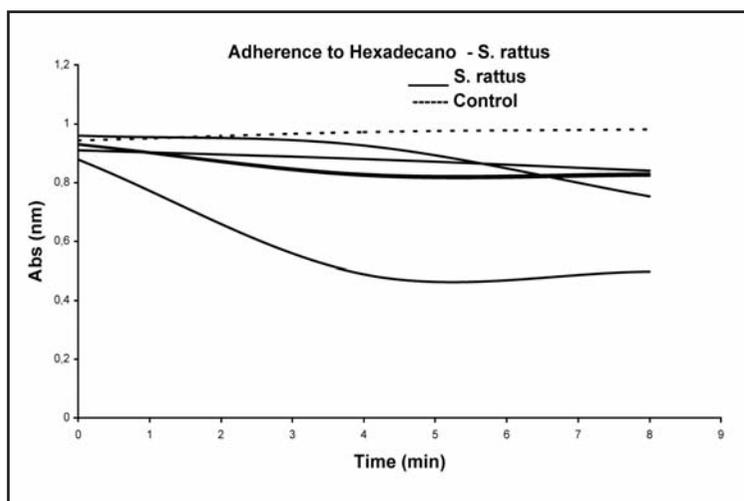


Fig. 7: Positive hydrophobicity from 3 strains of *S. rattus*. Patients with active caries.

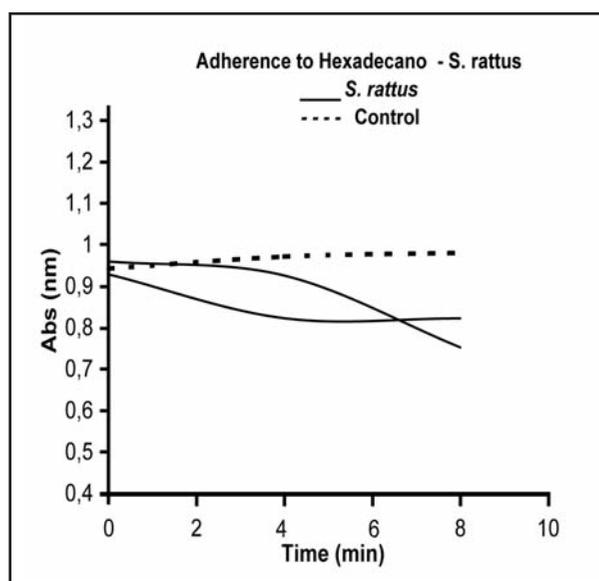


Fig. 8: Positive hydrophobicity from 2 strains of *S. rattus*. Patients without active caries.

This may be considered as a test that can be transferred to the peripheral laboratory, which is able to provide relevant results to contribute to defining an at-risk patient, and could be advised in cases in which patients have a high frequency of dental caries even though they perform adequate oral hygiene.

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