

Role of *Fusobacterium nucleatum* in volatile sulphur compound production on the dorsum of the tongue

D.Doudoux¹, C.Neut², D. Wils³, A. Bourgeois¹, E. Deveaux¹

¹ Faculté d'Odontologie, Département d'Odontologie Conservatrice Endodontie, place de Verdun 59000 Lille cedex, ² Faculté des Sciences Pharmaceutiques et Biologiques, Service de Bactériologie Clinique, 3 rue du Professeur Laguesse 59006 Lille cedex, ³ Société Roquette Frères, Service de Toxicologie, 62080 Lestrem cedex

INTRODUCTION: Oral malodor is mainly associated with volatile sulphur compounds (VSC), particularly hydrogen sulphide (H₂S) [1]. These compounds are highly toxic. The dorsum of the tongue is the major site of oral malodor production [2]. One possible pathway of forming VSC involves the microbial degradation of sulphur-containing amino acids. Methionine and cysteine are the substrates for this reaction, but the best characterized enzyme implicated in H₂S formation (L-cysteine desulphydrase) shows the best affinity for cysteine [3]. The aim of this study is to investigate the VSC metabolism and its regulation by tongue microbiota. We also evaluate a medium (CSH agar) able to count bacteria releasing H₂S from cysteine.

METHODS: Ten healthy adult males aged 18 to 55 years and non-smokers for three months were recruited by the Clinical Investigation Center of the University Hospital of Lille. All subjects gave their informed consent. Samples were taken by dorsum tongue scraping using a sterile plastic loop (1 µl), immediately immersed in prerduced transport medium (8 ml cysteinated 1/4 strength Ringer solution- CR). All samples were transported to the laboratory and processed in less than 4 hours. 10 fold dilutions in CR were plated on Columbia blood agar to establish the total anaerobic counts and on Columbia cysteine agar (CSH) to establish counts of -SH liberating bacteria. -SH liberating bacteria produce identifiable black colonies on this medium. All plates were incubated for seven days under anaerobic conditions. From plates with 15 to 150 CFU, the total number of colony forming units was determined. From CSH plates, black colonies were presumptively identified. Pure strains with -SH producing ability were analyzed by *in vitro* assays. H₂S release was quantified using a portable sulphide monitor (Halimeter® - RH 17 Interscan).

RESULTS: The mean count under anaerobic conditions was 8.53 log CFU/ml. -SH producing bacteria counts were 2 log lower than total anaerobes (Fig. 1).

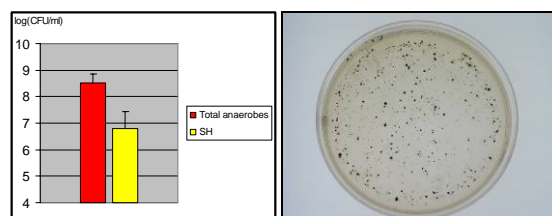


Fig. 1 Counts of total anaerobes and -SH producing bacteria (left); -SH-releasing black colonies on Columbia cysteine agar plate (right).

From CSH plates (Fig.1), *Fusobacterium nucleatum* was detected in 9 subjects and *Prevotella sp.* in 1 subject.

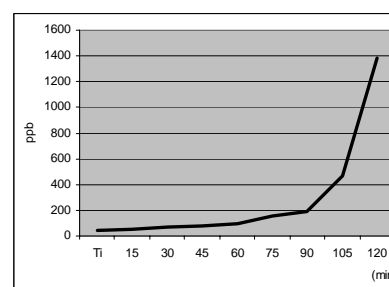


Fig. 2: Halimeter® measurements: *in vitro* H₂S production by a *F. nucleatum* strain (starting after 23 hours of culture).

H₂S was rapidly released by *F. nucleatum* strains after 24 hours of incubation (Fig.2). Measurable amounts of H₂S were formed when viable counts exceed 7 log CFU/ml.

DISCUSSION & CONCLUSIONS:

Fusobacterium nucleatum is the most frequent -SH producer (by proteolysis) on the dorsum of the tongue. *In vitro* assays show a release in end logarithmic growth phase. Tongue cleaning should be recommended to avoid thick biofilms.

REFERENCES: ¹CH. Lee, HS. Kho et al (2003), *J Periodontol* **74**:32-37. ²MM. Danser, SM. Gomez et al (2003), *Int Dent Hygiene* **1**: 151-158. ³ H. Fukamachi, Y. Nakano et al (2002), *FEMS Microbiology Letters* **215**: 75-80.