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The Ability of Selected Oral Microorganisms to Emit Red Fluorescence

Á.M. Lennon[a] W. Buchalla[a] L. Brune[a,b] O. Zimmermann[b] U. Gross[b] T. Attin[a]

[a]Department of Operative Dentistry, Preventive Dentistry and Periodontology, and
[b]Institute of Medical Microbiology, Georg August University Göttingen, Göttingen, Germany

Key Words
Dentin · Fluorescence · Microorganisms

Abstract
Some novel caries detection and excavation devices rely on the ability of bacteria to produce red fluorescing compounds. The aim of this study was to examine the ability of selected oral microorganisms to emit red fluorescence. *Streptococcus mutans, S. oralis, S. salivarius, S. sobrinus, Lactobacillus fermentans, L. casei, L. rhamnosus, Actinomyces naeslundi, A. israelii, Prevotella intermedia,* and *Fusobacterium nucleatum* were inoculated onto Columbia agar with haemin and vitamin K and incubated anaerobically for up to 7 days in the dark. The resulting bacterial colonies were excited using filtered xenon light (405 ± 20 nm) and digitally photographed through a 530-nm high-pass filter. The red and green portions of the colony fluorescence were analyzed using a computer program and the red/green ratio was calculated. All colonies emitted both red and green fluorescence. The green outweighed the red portion for the following species (in descending order) *S. oralis, S. salivarius, S. mutans, F. nucleatum* and *S. sobrinus*. The red portion was higher for the following species (in descending order) *P. intermedia, A. naeslundi, A. israelii, L. fermentans, L. rhamnosus* and *L. casei*. With all the bacteria examined, one color portion generally outweighed the other, giving the visual impression of either red or green fluorescence. We conclude that red fluorescence is well suited to detection of the bacteria which cause dentin caries but it is not suitable as an indicator of the presence and activity of the streptococci involved in initial caries.

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The phenomenon of red fluorescence in carious dental hard tissue and in dental plaque and calculus was first reported in the 1920s [Bommer, 1927; van den Bergh, 1928; Benedict, 1928]. Since then, spectrographic studies have shown that carious dentin fluoresces more intensely in the red portion of the visible spectrum than sound dentin [Buchalla et al., 2004; Alfano and Yao, 1981].

Caries detection by laser fluorescence functions by detecting increased fluorescence intensity in carious dental tissue as opposed to sound in the near infrared spectral region [Hibst and Gall, 1998]. A caries excavation method (Fluorescence Aided Caries Excavation) using orange-red auto-fluorescence as a marker for infected dentin during caries excavation has recently been developed [Lennon, 2003; Lennon et al., 2002]. Using this method the

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Fax +41 61 306 12 34
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Dr. Áine M. Lennon
Klinik für Präventivzahnmedizin, Parodontologie und Kariologie
Zentrum für Zahn-, Mund- und Kieferheilkunde der Universität Zürich
Plattenstrasse 11, CH–8032 Zürich (Switzerland)
Tel. +41 44 634 32 71, Fax +41 44 634 43 08, E-Mail aine.lennon@zzmk.unizh.ch

cavity is excited with violet light, so that red fluorescing carious areas are visible through a highpass filter and can be selectively removed.

Recent spectroscopic investigations using multiple excitation wavelengths across the ultraviolet and visible portions of the electromagnetic spectrum show distinct fluorescence bands in carious tissue between 600 and 700 nm, which are typical for porphyrin compounds [Buchalla et al., 2004; Buchalla, 2005]. Red fluorescence is thought to be emitted by porphyrin compounds synthesized by oral microorganisms in the caries lesion [Hibst and Paulus, 1999; König and Schneckenburger, 1994]. Because the fluorescence is believed to be emitted by bacterial metabolites, it has also been proposed that red fluorescence may be used to assess caries activity [Ribeiro et al., 2005].

So far, very little has been published about which oral bacteria can indeed produce red fluorescing compounds and which do not. These data are necessary for development of fluorescence-based caries detection systems in the future.

The aim of this study was to examine the ability of selected oral microorganisms to emit red fluorescence.

Materials and Methods

Microbiology

The following species (strain number in parentheses) were obtained as dried cultures from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (Braunschweig, Germany):

- *Streptococcus mutans* (20523), *S. oralis* (20627), *S. salivarius* (20560) and *S. sobrinus* (20742)
- *Lactobacillus fermentans* (20053), *L. casei* (20011) and *L. rhamnosus* (20021)
- *Actinomyces naeslundi* (43013) and *A. israelii* (43320)
- *Prevotella intermedia* (20706)
- *Fusobacterium nucleatum* (20482)

The cultures were rehydrated and inoculated into 0.1% thioglycolate broth and incubated at 36°C. An aliquot of each culture was plated in duplicate on Columbia agar with vitamin K and haemin. These plates were immediately placed in anaerobic jars covered with aluminum foil to exclude light and incubated at 36°C for 4 days. *P. intermedia* was incubated for 3 days longer, prior to fluorescence imaging, because of its slower growth.

Fluorescence Imaging

The plates were opened in a dark room. The colonies were excited using violet light ($\lambda = 405 \pm 20$ nm) produced using a 100-watt Xenon discharge lamp (Linos Photonics, Göttingen, Germany) and an infrared filter and band pass filter with peak transmission at 370 nm (Schott, Mainz, Germany). The plates were viewed through a 530-nm high-pass emission filter (Schott, Mainz, Germany). A single examiner (L.B.) made a visual assessment and captured a digital image of the autofluorescence of each plate (two plates per bacteria). The camera used was a Canon EOS 300D with a Canon EF-S 18- to 55-mm lens (Canon Inc., Tokyo, Japan).

Image Analysis

The visual impression of either red, green or orange fluorescence was noted for each species. The digital images were analyzed by measuring the red and green values at a central point in the colony using a computer program (Adobe Photoshop version 6.0, Adobe Systems Inc., San Jose, Calif., USA). The red and green values were measured for five randomly chosen colonies per image. These values were used to calculate the average red/green ratio for each species.

Results

The growth medium used was only very weakly fluorescent in comparison with the colonies under the experimental conditions and did not interfere with the fluorescence assessment.

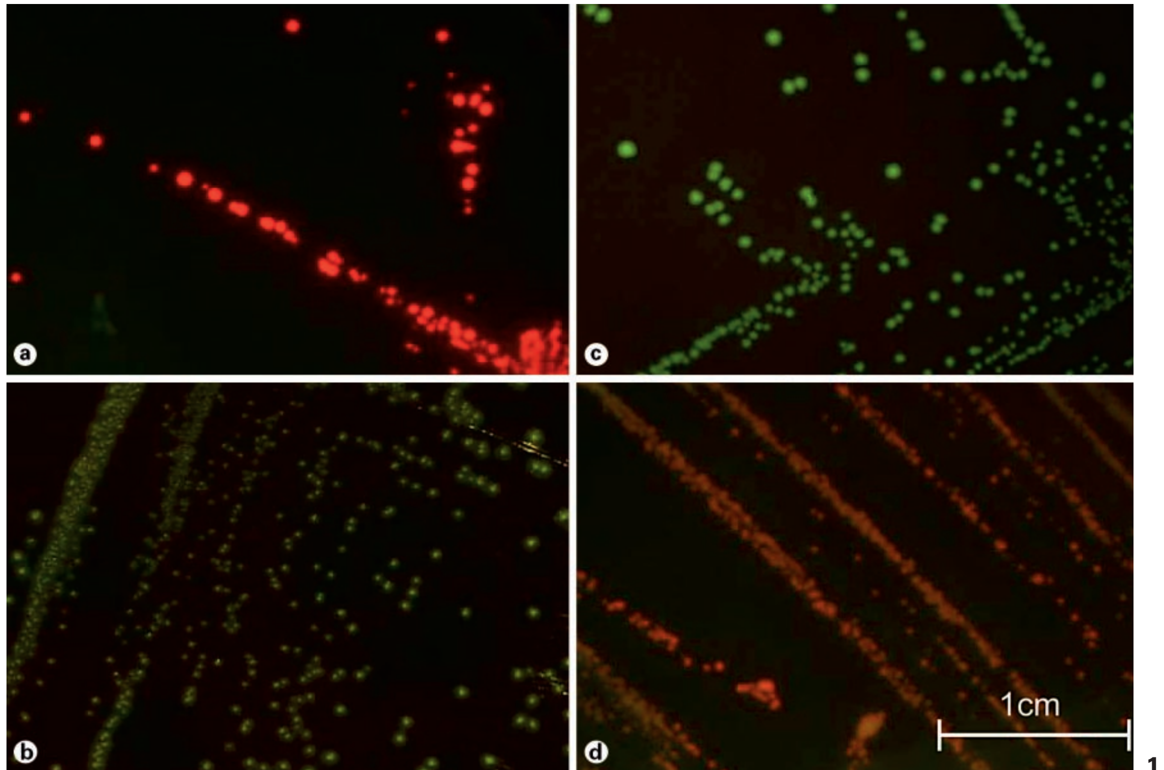
A visual assessment of the colony fluorescence showed that *F. nucleatum* and the streptococci fluoresced green (fig. 1). Lactobacilli fluoresced orange-red. The actinomycetes fluoresced red and *P. intermedia* emitted spectacular red fluorescence (fig. 1).

However, analysis of the red and green values of the fluorescence images showed that all bacteria emitted both red and green fluorescence. The red/green ratio was less than 1 in all of the 10 points measured for *F. nucleatum* and the streptococci, and over 1 in all points measured for Lactobacilli, actinomycetes and *P. intermedia*. The mean red/green ratios are given in figure 2. The dominant color portion agreed with the visual impression of either red or green fluorescence.

Discussion

A selection of cultivable bacteria associated with caries and periodontal disease [Marsh and Martin, 1992] were chosen in order to carry out an initial investigation of their potential for fluorophore production. Studies relying on culture methods are limited in that a great proportion of caries bacteria cannot be grown in vitro [Nadkarni et al., 2002; Martin et al., 2002].

Columbia agar with added vitamin K and haemin was chosen as a growth medium to provide optimal growth conditions for all of the bacteria. Vitamin K and haemin, which are metabolized by black pigmented anaerobes, were added to provide the bacteria with a possible substrate for porphyrin synthesis. The excitation



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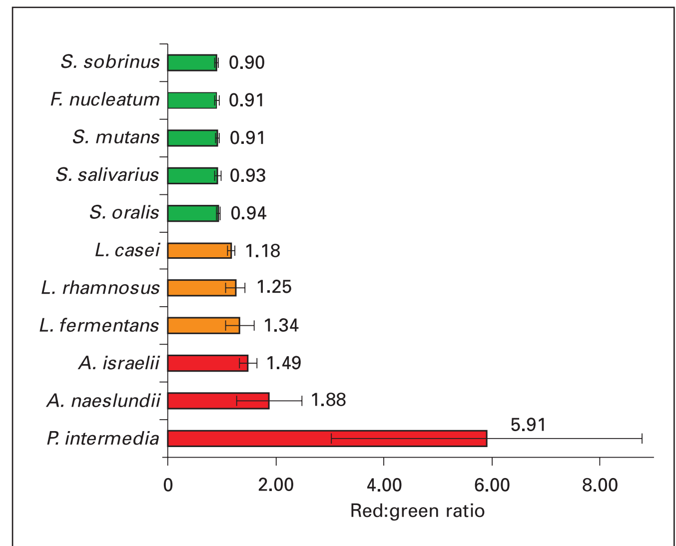

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Fig. 1. Fluorescence images of *P. intermedia* (**a**), *S. mutans* (**b**), *F. nucleatum* (**c**) and *A. naeslundii* (**d**) grown on Columbia agar with vitamin K and haemin.

Fig. 2. Mean red:green ratio with standard deviation for all samples. The results of the visual assessment are given in the color of the bars (green, orange or red).

wavelength and the emission filter used were chosen on the basis of recent spectroscopic studies which determined the optimal excitation and emission wavelengths for red fluorescence in caries [Buchalla et al., 2004; Buchalla, 2005].

Absence of red fluorescence in streptococci and lactobacilli has been reported [König and Schneckenburger, 1994]. It is difficult to compare the studies because König and Schneckenburger did not sufficiently describe the methods they used. While we also found that streptococci did not produce red fluorescence, the present investigation showed more red than green fluorescence for lactobacilli.

Interestingly, the visual impression of either red or green fluorescence, depending on which color portion was dominant, also roughly divided the bacterial genera into two groups which occupy different niches in the oral cavity. The visibly green fluorescing streptococci play an important role in the initial colonization of the tooth surface and lesion initiation. *S. salivarius* is isolated only occasionally from carious sites and is not considered a significant opportunistic pathogen, and *S. oralis* plays an important role in the early formation of plaque [Marsh and Martin, 1992]. The mutans streptococci are both highly saccharolytic and acidophilic and predominate in initial caries lesions [Marchant et al., 2001]. While mutans streptococci are also found in dentin caries, lactobacilli dominate in deeper lesions [Bjørndal and Larsen, 2000; Martin et al., 2002]. The red fluorescing lactobacilli, actinomycetes and *P. intermedia* are found in dentin caries and at the advancing front of the dentin lesion [Banerjee et al., 2002; Wicht et al., 2004; Munson et al., 2004].

F. nucleatum is primarily associated with periodontal disease but can also be found in dentin caries [Martin et al., 2002].

This means that, for visual methods, red fluorescence in plaque or at the tooth surface is probably not a good indicator of presence and activity of streptococci involved in initial caries lesions. On the other hand, under optimal excitation and emission conditions, visible red fluorescence is probably a good indicator of the presence and activity of bacteria in dentin caries. The use of bacterial green fluorescence for caries detection would be difficult because of the intense green background fluorescence of dental hard tissues [Stübel, 1911; Kvaal and Solheim, 1989; Armstrong, 1963]. The results of the present study are limited in that they were found in vitro; the fluorescence properties of oral microorganisms still need to be investigated in the clinical situation. Further studies are also necessary to identify the relevant fluorophores and analyze whether they play a role in the pathogenesis of caries.

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