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On the red fluorescence emission of *Aggregatibacter actinomycetemcomitans*

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ABSTRACT

A number of studies have indicated that bacteria able to emit red fluorescence can be detected by light induced fluorescence technique and killed by photodynamic therapy. The objective of this study was to investigate the red fluorescence properties of the single gram negative capnophilic bacterium *Aggregatibacter actinomycetemcomitans*, ATCC 33384, and to investigate if these properties were related to the growth, morphology and size of the bacterial colonies. Time trend assessment was made with red fluorescence by QLF (Quantitative Light-induced Fluorescence), as well as with white light digital imaging. It was demonstrated that *A. actinomycetemcomitans*, a single capnophilic bacterium, is able to produce red fluorescence on its own, *i.e.* in the absence of other bacteria strains, and that blood agar is necessary to obtain red fluorescence from this bacterium on culture plates. This bacterium formed smooth circular, bell/dome like colonies increasing in size with time exhibiting various red fluorescence behaviors. A large variation in the fluorescence behavior points out an inhomogeneous distribution of red fluorescence within and between the colonies, *i.e.* the size of the investigated colonies did not correlate with the red fluorescence area, suggesting a dependence on the colony morphology such as the colony growth in height. To our knowledge this is the first study that have shown that *A. actinomycetemcomitans* on its own is able to produce fluorescence in the red spectral region.

Keywords: *Aggregatibacter actinomycetemcomitans*; Red Fluorescence; Porphyrin; Quantitative Light-induced Fluorescence; QLF

1. INTRODUCTION

Red fluorescence from dental plaque and calculus was first observed in the late 1920s [1] and in 1986 it was shown that anaerobic oral bacteria could emit red fluorescence [2]. This red fluorescence emission has been suggested to originate from porphyrins that are synthesized by and present in various microorganisms [2-4]. Red fluorescence emission has been demonstrated from *Actinomyces odontolyticus*, *Actinomyces naeslundi*, *Actinomyces israelii* and *Prevotella intermedia* present in cell cultures associated with dental caries, periodontal disease and dental plaque [3,5-7]. Red fluorescence has also been obtained from the black-pigmented obligate anaerobes *Porphyromonas gingivalis*, *P. intermedia*, *Prevotella melaninogenica*, as well as from *A. israelii* [8]. All these bacteria are associated with mature plaque [8] and it has been shown that areas displaying red fluorescence and areas of disclosed plaque correlate [6] and that red fluorescence increase with increasing disclosed plaque amount [9]. A clinical plaque assessment of removable dentures found that red fluorescence increased with an increase in plaque but no statistical correlation could be established between red fluorescent plaque and total plaque amount over time [10].

Obligate anaerobic bacteria have been suggested to be responsible for the red fluorescence due to their increasing numbers in mature biofilm [6,8]. Van der Veen *et al.* put forward that distinct red fluorescence from *Peptostreptococcus micros* only was observed when grown in close proximity of *P. gingivalis* [6] and proposed that it is the intrinsic bacterial mixes of the mature biofilm, rather than single bacterium species that are responsible for the red fluorescence. Other studies have supported this suggestion regarding the importance of the mature biofilm for the emission of red fluorescence [7-9]. One of the authors proposed that it is due to signaling and synergy between different bacterial species in the biofilm rather than the characteristics of the single species [8].

A. actinomycetemcomitans is a small, non-motile, gram negative, capnophilic rod that after incubation for 48 - 72 h generally forms domed, colorless, circular colonies

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with an irregular edge and a diameter of 1 - 3 mm [11]. On primary isolation, the colonies growing on serum-containing media will form rough, translucent colonies with a star like inner structure with a diameter of 0.5 to 1.0 mm [11,12]. *A*. *actinomycetemcomitans* is strongly associated with localized aggressive periodontitis (LAP) [13,14] and detection of *A*. *actinomycetemcomitans* in oral healthy children has been suggested as a risk marker for initiation of LAP [15]. In addition *A*. *actinomycetemcomitans* is a member of the HACEK microorganisms (*Haemophilus* spp., *A*. *actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* spp) which are associated with systemic diseases [13].

There are only a few publications so far about bacteria associated with periodontal disease and their ability to emit red fluorescence thus, and it is of interest to investigate which periodontopathogens that are able to emit red fluorescence since according to the literature this property could be used for diagnosis and treatment [3,4,16]. To our knowledge there are no specific studies on the fluorescence of *A*. *actinomycetemcomitans*. This is a capnophilic bacterium and the major hypothesis is that red fluorescence is due to the obligate anaerobes, bacteria which are indicative for mature plaque. As has been discussed above, the majority of studies made the last decade have proposed that the intrinsic characteristics of the mature biofilm, rather than the single species are responsible for the fluorescence in the red spectral region. The aim of this basic research study was to investigate the red fluorescence properties of *A*. *actinomycetemcomitans* and to investigate if these properties are related to the growth, morphology and size of the bacterial colonies.

2. MATERIALS AND METHODS

In this study *A*. *actinomycetemcomitans* was grown up to 12 days under aerobic conditions on Trypticase Soy agar with Bacitracin and Vancomycin (TSBV) and Columbia agar with 5% defibrinated horse blood agar (CB) while red fluorescence and white light photographs were captured daily of selected colonies.

2.1. Microbiological Procedures

A. *actinomycetemcomitans* strain ATCC 33384, serotype c was obtained from the Department of Clinical Bacteriology at the University of Gothenburg. Cultured bacteria were identified by Gram, oxidase and catalase reactions, and verified on the basis of the bacterium morphology. *A*. *actinomycetemcomitans* samples were subcultured on both TSBV agar as well as on CB agar and incubated for 12 days in 37˚C in a 5% CO_2 atmosphere. Agar plates were inoculated with verified subcultures of *A*. *actinomycetemcomitans*. Care was taken to select clear and distinct colonies for the analysis with Quantitative Light induced Fluorescence (QLF) and white light imaging to make it possible to compare if there were any correlations between QLF red fluorescence area and the physical colony area. Assessment started on day four post inoculation and continued for eight days. During this period of growth the colonies also were assessed regarding their morphology. On three selected plate QLF assessment was started at day one post inoculation in order to observe the entire growth cycle.

2.2. The QLF Technique

The instrument for Quantitative Light-induced Fluorescence (QLF) used for this project was the Inspektor™ Pro (Inspector Research Systems BV, Amsterdam, The Netherlands) consisting of an intra-oral fluorescence camera and PC based software. The agar plates with the bacteria cultures were illuminated with blue light from a xenon arc lamp equipped with an optical interference band pass transmission filter having maximum intensity at λ = 370 nm and a full-width at half-maximum of 80 nm. QLF images of the bacteria colonies were captured by a color micro-CCD sensor, equipped with a yellow high-pass filter (λ > 520 nm) to exclude scattered light. The images were registered, stored and processed using the Inspektor™ Pro 2.0.0.38 computer software operating in red fluorescence mode. A fixed set-up of the QLF instrument was constructed to obtain reproducible imaging of the agar plates. An internal reference system was developed in order to be able to adjust each culture plate for the QLF assessments and to compare images from the QLF with white light digital images. The reference marks were made of triangular shaped, hard, matt, white card board placed on the surface of the agar substrate.

Selected colonies in the QLF images were processed by the software. The capture level for the reference mark showed ≥99% repeatability from day to day. When continuing the QLF assessment past day nine, the repeatability in some cases decreased to 97% which was due to the reference mark had started to sink down in the agar substrate. The software returned the marked area (RF area, mm^2) and the average fluorescence intensity (ΔR) of this reference area. ΔR is the difference (dR) between the value of the red component (Rp) of the RGB value of a CCD pixel, and the average Rp-value of all pixels in the reference area (Ra), averaged over all pixels within the marked area that have a dR larger than a threshold value (typically 120% of Ra). $\Delta R = \Sigma\left(Ra - Rp(i)\right)/n$ where n is the number of pixels where $Rp(i) > 1,2 * Ra$ and the summation is for i = 1 to n. Reproducibility tests of the measurements were performed by letting a second operator capture three images on two different agar plates. The capture reproducibility was shown to be ≥99%.

2.3. White Light Photographs

White light photographs were obtained to compare with the images of the selected colonies obtained with the QLF instrument. A digital camera (Coolpix 4500, Nikon Corporation, Japan), without flashlight, was installed in a light microscope at ×6.4 magnification. The white light photo images were adjusted to the same size as the QLF images using the internal reference system. Thus, the area of both types of images could be compared. Digital image analysis of selected colonies, regarding morphology, was performed with the Image J computer program (Image Processing and Analysis in Java, version 1.44 n, National Institutes of Health, USA).

2.4. Assessment of Bacterial Growth Time Series

Time series of QLF fluorescing colonies in growth were studied as follows: TSBV agar plates and CB agar plates were inoculated and the cultures were undisturbed for 96 hours. The culture plates were taken out of incubation, subjected to QLF assessment and white light photography in a dark room and then put back into incubation. Measurements were repeated in 24 hour cycles up to and including the twelfth day of incubation. Furthermore twenty-one well defined single colonies exhibiting clear and distinct QLF fluorescence on three CB agar plates were selected for the time trend assessment. Nine colonies exhibiting no increasing fluorescence with time were used as fluorescence blanks to obtain a background reference value. In order to investigate fluorescence behavior during all twelve days of incubation three additional blood agar plates were assessed in the same way as above but starting after 24 hours. All colonies grown on TSBV agar showed very low red fluorescence of which five well defined single colonies on two different plates were selected for a time trend study. All cultures had undergone subculturing prior to time trend assessment in order to obtain strong, active cells.

2.5. Data Analysis

Statistical analyses using ANOVA with post hoc t-tests using Bonferroni correction, t-test for equal and unequal variances, and bivariate linear regression were applied using the WinStat 3.0 statistical software (Analytical Chemistry, Stockholm University, Sweden).

3. RESULTS

3.1. Plate Adjustment Reference Material

The reference marks were triangular shaped pieces cut from card board with the short side being approximately 3 mm. Hard, matt, white card board showed to be a suitable material to use as reference marks on the agar plates. It can be sterilized by autoclavation, it stayed on top of the agar substrate during twelve days of time trend studies, and showed no disturbing light reflections. The matt white surface was visible both in the red light fluorescence measurements as well as in the white light images.

3.2. Culturing Substrate

Two different types of agar substrates were investigated for culturing the *A*. *actinomycetemcomitans* strain ATCC 33384. Physical growth structures and growth rate on both the CB agar and the TSBV agar plates were similar when observed in white light. In red fluorescence assessed by QLF the blood agar substrate itself exhibited some scattered red fluorescence as a kind of weak background luminescence, and the intensity level in all colonies were above zero intensity from day one to day twelve. Colonies cultured on the CB blood agar plates displayed a strong red fluorescence increasing with growth time, while when grown on TSBV agar the colonies showed very low red fluorescence with no significant increase in red fluorescence intensity or red fluorescence area in any colony during the twelve day growth period. This "baseline" fluorescence (ΔR = 28.3% (RSD 13%, n = 30) was similar to the blank colonies, *i.e*. colonies showing no increasing fluorescence with time, on the CB blood agar plates. Thus, culturing of *A*. *actinomycetemcomitans* for further experiments was made using the CB blood agar substrate.

3.3. Assessment Time

Assessment of *A*. *actinomycetemcomitans* cultures on the CB agar plates in white light showed an increasing growth in colony size from day one. At day eight post inoculation the blood agar substrate started to get increasingly darker and the colony growth seemed to come to an end. On the eleventh day post inoculation the colonies displayed a dry look.

Red fluorescence was observed in all the investigated colonies from day one to day twelve. During the first four days red fluorescence intensity and area remained on the same low "baseline" level. From day four the fluorescence increased with time, but after nine and up to day twelve there were no significant changes in the red fluorescence, **Figure 1**.

3.4. Red Fluorescence Time Trends

Twenty-one solitary colonies in close vicinity to the plate reference marks on three different blood agar plates were selected. These isolated, clear and distinct colonies were selected for the time trend assessment with QLF red fluorescence and white light digital imaging. The red fluorescence from the colonies grown on CB agar exhibited a

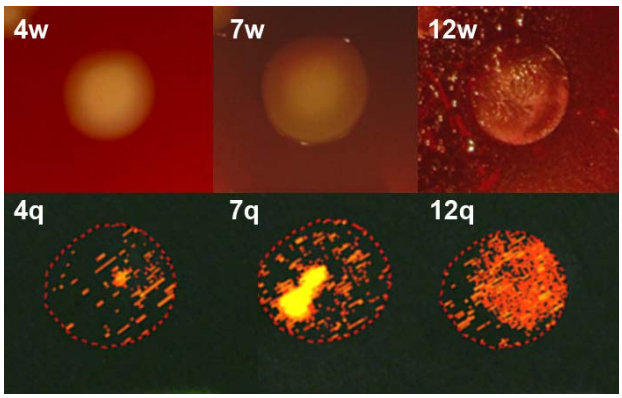


Figure 1. A solitary, distinct colony of *A. actinomycetemcomitans* after 4, 7 and 12 days of growth. Upper row: White light images (w). Lower row: QLF images (q). From day four the fluorescence increased with time, and at day seven the colony reached maximum total red fluorescence intensity. From day nine and to day twelve there was a decrease in the total red fluorescence intensity.

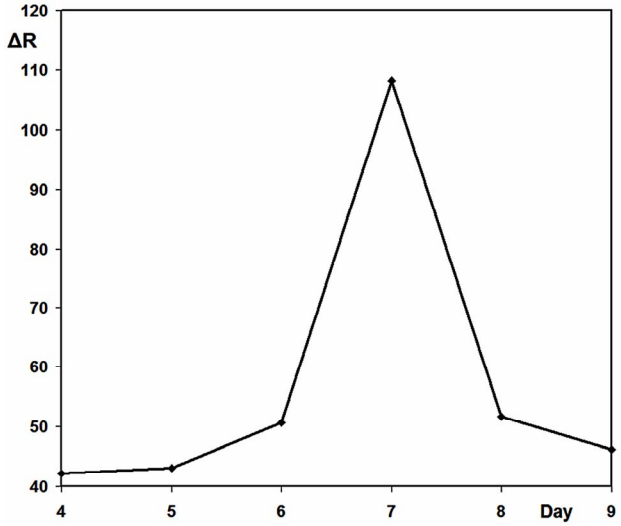


Figure 2. Time trend of red auto fluorescence intensity (ΔR) as the mean of twelve colonies of the bacterium *A. actinomycetemcomitans* as function of the growth day after inoculation.

wide span in intensity. Nine of the assessed colonies with a physical growth observed in white light, only exhibited a constant red fluorescence level (ΔR = 41.5%, RSD 16%, n = 54) from day four to nine after inoculation. These colonies were used as a fluorescence background level constituting a baseline for the red fluorescence measurements.

The twelve colonies demonstrating an increase in fluorescence intensity during cultivation all showed a similar behavior. The fluorescence time trend from day four to nine as a mean of the twelve assessed colonies is shown in **Figure 2**. At day four these colonies exhibited a red fluorescence intensity (average ΔR = 40.3%, RSD 14.7%, n = 12) similar to the baseline fluorescence of the colonies with no increase in fluorescence described above. When looking individually at each selected colony, the red fluorescence started to increase slightly at day five and six raising above the fluorescence baseline of the fluorescing colonies, but the average increase was not statistically significant (day six average ΔR = 46.4%, RSD 32.0%, n =12). At day seven all colonies reached maximum red fluorescence (average ΔR = 108.2%, RSD 49.7%, n = 12) followed by a decrease to the endpoint at day nine (average ΔR = 46.2%, RSD 27.5%, n = 12).

Statistical analysis using double sided t-test for unequal variances showed that ΔR of day 7 was significantly different from all other days ($p < 0.003$, t (11)) as well as the fluorescence baseline ($p < 0.002$, t (11)). The fluorescence intensity for all the other days of fluorescence measurement was not statistically different from the baseline at a 95% confidence level. The total fluorescence (ΔR × RF area, *i.e.* fluorescence intensity x fluorescing area) showed a similar behavior as the ΔR, having a significant maximum on day 7, $p < 0.04$, t (11) using double sided t-test for unequal variances. There was no significant difference in the RF area between day 4 and day 9 even ($p > 0.2$). This shows that the variation in total fluorescence mainly was due to the variation in ΔR, *i.e.* the fluorescence intensity. Looking at the fluorescing area of the individual colonies reveals a large variation, from an area increase of 4% up to an increase of 3000%. Linear regression analysis showed that there was no correlation ($r^2 < 0.20$) between red fluorescence intensity and red fluorescence area of the twelve colonies exhibiting a change with time of the fluorescence intensity.

3.5. Size Assessment of the Selected Bacterial Colonies

The QLF red fluorescence area and the physical colony areas obtained from digital images in white light were compared, but no significant correlation was found. It was also noted that there were large differences between the fluorescing area and the physical area of a colony. As an example, the physical size of one of the assessed colonies was 3.66 mm^2 on day seven, while the corresponding red fluorescence area only was 0.98 mm^2. Size assessment of the selected twelve colonies on the CB agar were carried out in a light microscope and were punctate to 0.9 mm - 1.9 mm in diameter after 4 days incubation, increasing to a maximum between 1.1 mm - 2.3 mm in 8 days.

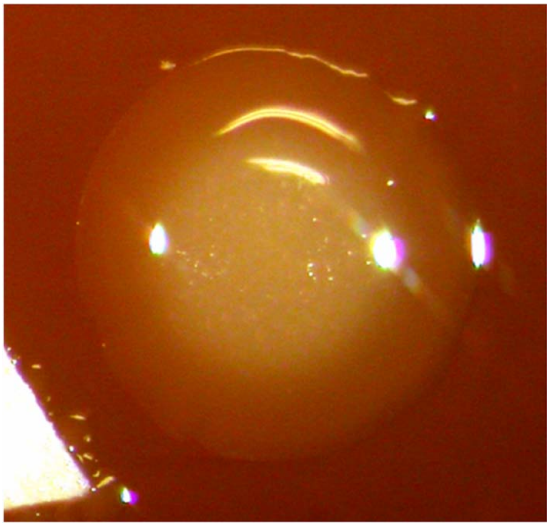
3.6. Analysis of the Selected Colonies Regarding their Morphology

A number of colonies were examined by applying image

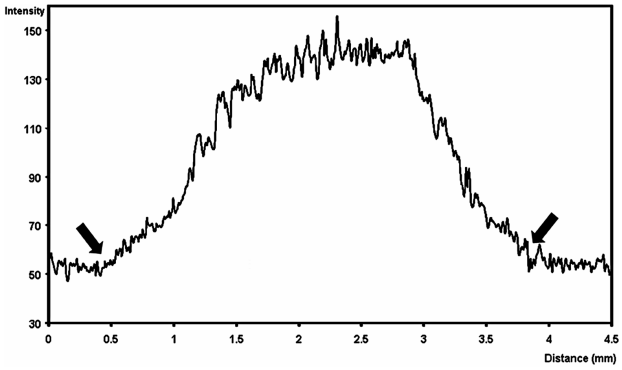
analysis to the white light digital images. The images were first converted to a grey scale image. A typical example is shown in **Figure 3(a)**. In the photo several light reflections can be seen on the colony. These reflections reveal the features of the colony three-dimensional structure. As it rises above the agar substrate surface of the plate the uppermost part of the colony exhibit an arched, thin reflection revealing an almost entire margin with minor irregularities. A second arched reflection below the first, reveals a steeper increase in the height towards the center of the colony. A third arched reflection indicates the rim of the top of the colonies, further marked by the two intense, slightly oval reflections. It is also to be noted that the margin of the colony is slightly transparent, but going towards the center the opacity increases. Together this indicates a kind of bell- or dome-like shape where an increase in opacity, resulting in an increasing whiteness in the image, shows an increasing thickness of the smooth circular colony. Since reflections strongly distort the grey scale image, a colony without light reflections was selected to create a 3D image of a colony. A profile analysis of the grey scale image over a transect through the center is shown in **Figure 3(b)**. It demonstrates the bell shaped profile of the whiteness intensity/opacity of the colony. The arrows in the grey scale intensity profile indicate the physical boundary of the colony as measured on the original image. This structure is similar irrespectively of the direction of the transect. By using this grey scale a three dimensional image was created, **Figure 3(c)**. For a number of colonies the red fluorescence area was compared with their physical dimensions. It was noted that the colony area with the highest fluorescence intensity did not necessarily coincide with the center of the colony, *i.e*. the thickest part of the colony.

4. DISCUSSION

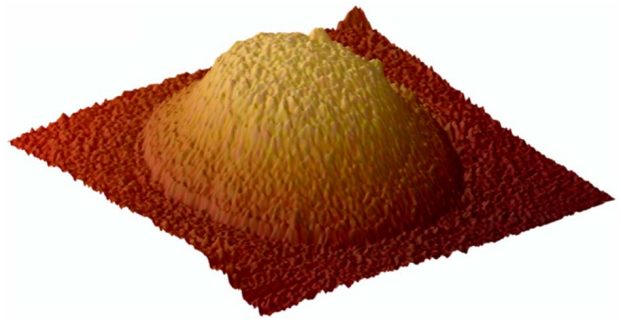
The present study shows that the single gram negative, capnophilic bacterium *A*. *actinomycetemcomitans* is able to emit red fluorescence. A significant correlation in time between increasing red fluorescence intensity (ΔR) and growth in colony size was demonstrated for this bacterium. Maximum in fluorescence emission coincided with maximum in the bacterial physical growth curve on agar plates [17]. That red fluorescence started to increase in day four suggests a behavior in accordance to statements that mature plaque is the source of red fluorescence emission in the oral cavity [6-9]. In addition, according to the literature, it is important not to disturb growing cultures during the initial "lag phase" when they are becoming acclimatized to the environment [17]. As a conclusion, the time trend experiments for the QLF assessment were restricted to a maximum of nine days of culture growth, with red fluorescence QLF assessment start-



(a)



(b)



(c)

Figure 3. (a) A converted white light digital image of an *A. actinomycetemcomitans* colony. The white areas exhibited by the colony is light reflections which distinctly indicates the three dimensional structure; (b) The whiteness intensity at a cross-section of an *A. actinomycetemcomitans* colony when the digital grey-scale image has been analyzed with the ImageJ software. The abscissa shows the distance in mm and the ordinate shows the whiteness intensity; (c) A 3D image of the same colony as in 3(b). The image was created in ImageJ using the 2D format of the digital photo for the base of the image and the whiteness intensity to visualize a 3D structure. The "*in vivo*" colony as well as the agar substrate has smooth surfaces. The 3D image surfaces gets rugged since the whiteness intensity is use to create the third dimension from the image.

ing at day four post inoculation. The behavior of the fluorescence intensity is in agreement with the expected growth cycle of bacterial colonies cultured on agar plates according to literature [17]. First there is a "lag phase" followed by a "fast (logarithmic) growing phase", a "stationary phase" during which essential nutrients becomes limiting and some bacteria dies, and finally the "decline phase" where some of the bacteria remains viable.

Studies have suggested that obligate anaerobic bacteria are the source of red fluorescence emission and especially the black pigmented e.g. *Prevotella intermedia* and *Porphyromonas gingivalis* [3,5,6,8,9]. In addition the majority of these studies havealso suggested that the red fluorescence emission not is directly caused by a single bacterium species, but "by the intrinsic characteristics of a mature biofilm" [6-9]. That a single strain of the gram negative capnophilic bacterium *A. actinomycetemcomitans* emits red fluorescence is in contrast to these studies.

In the present study the physical area obtained from the image analysis of the white light digital photos of the colonies did not correlate with the red fluorescing area obtained by the QLF instrument. As mentioned in the results, an example, the physical size of one of the assessed colonies was 3.66 mm^2 on day 7 while the corresponding red fluorescence area was 0.98 mm^2. Physical diameter of the selected colonies on CB agar was punctate to 0.9 mm - 2.3 mm with a maximum growth peak at day 8. A similar result was obtained for the colonies on the TSBV agar plates were the diameters were punctate to 0.8 mm after 4 days and were increasing to 2.1 mm in diameter after 7 days. Similar results of size assessment has been reported in other studies [11,18]. This coincides with the red fluorescence time trends analysis of the twelve colonies demonstrating an increase in fluorescence intensity during cultivation that showed a maximum red fluorescence at day seven.

Nine of the twenty-one selected *A. actinomycetemcomitans* colonies on the CB agar did not show any change in fluorescence intensity during the growth period, even though their physical area was increasing. Two studies have indicated that not every colony of a bacterium strain produces red fluorescence [2,19] andtheir suggestion was that red fluorescence could be absent due to rapid fading on exposure to air or to the absence of horse and/or sheep blood in the culture media. In the present study the cultivation as well as the measurements was performed in a dark room and the Columbia agar contained defibrinated horse blood. This indicates that different colonies of the same bacterium strain have different biosynthesis of the fluorescing substances and/or different uptake of these substances or their precursors from the agar substrate. During the measurements the CB agar substrate itself only exhibited a low background level of scattered red fluorescence. When using TSBV agar, a substrate without any blood, there was no fluorescence background detected from the substrate itself, and the growing colonies only showed a week baseline red fluorescence intensity at half the base line intensity exhibited by the colonies cultured on the CB agar substrate. This indicates that there are essential components for the bacterial fluorescence in the blood. Still, the question is if the fluorescence is due to the porphyrin content or some other constituent. However, it has been shown that the spectra from red fluorescing bacteria is very similar to the emission spectra of porphyrins [3,4,16].

There is also a question regarding fluorescence and the viability of the bacteria. In the literature it has been reported that there exist two types of bacterial cells; active and inactive. Active cells grow, perform cell division and move actively while inactive cells do none of these [20]. In this study we observed red fluorescence even on day twelve from the selected colonies and we can only speculate that bacteria can produce red fluorescence even if they are non-viable or perhaps viable but not at a non-culturable state. Are there dead or inactive cells, showing no fluorescence, unevenly distributed within the colony?

The difference in fluorescing and physical area as well as the differences in the fluorescence intensity demonstrates an irregular occurrence of the fluorescence within the colonies. In 1979, Whimpenny [21] stated that bacterial colonies show differences in growth with respect to height and diameter when cultured on agar plates. The interior region often increases in height and sometimes with an upward curve [21]. Image analysis of the white light digital photos of the colonies of *A. actinomycetemcomitans* in this study revealed circular smooth surface and bell- or dome-like shapes with smooth, thinner, transparent rims and an increasing opacity and thickness towards the center of the colonies, when growing on both TSBV and CB agar, **Figure 3(a)**. These results are similar to that reported in other studies where, besides that on primary isolation, bacterial colonies of *A. actinomycetemcomitans* appear translucent with a rough surface and a star like inner structure. However, repeated subculturing can result in loss of the star structure and the rough surface become more smooth [11,12,18]. In the present study we have used cultures that had undergone subculturing at least twice. An explanation to why fluorescence area and fluorescence intensity showed no correlation is the colony morphology, e.g. the growth in height of the colonies. Their elevation was convex and bell- or dome-like, so the colony increase in thickness could give an increase in intensity of the emitted fluorescence. This suggests that there is a three-dimensional structure of the fluorescence, a fluorescing volume, within the colonies dependent on the growth pattern and viability of each individual colony. This is however not possible to meas-

ure with the QLF instrument.

Colony growth rate has been suggested to be dependent on concentration gradients of nutrients in the agar plates [22]. Studies of *Veillonella* species cultured on Brain Heart Infusion Agar plates (BHIA) have shown that red fluorescence was produced only when horse or sheep erythrocytes were present in the agar substrate [19]. They suggested that the substrates contained heme and/or precursors for the heme biosynthetic pathway. Thus, if the red fluorescence shown by bacteria is due to porphyrins as suggested by several authors [3,4,16], this would then be dependent on the supply of porphyrins, or porphyrin precursors, from the blood agar substrate. Such behavior was demonstrated by *A. actinomycetemcomitans* in this study. When grown on TSBV agar substrate, a substrate lacking erythrocytes, *i.e*. lacking heme and heme related porphyrins, no red fluorescence emission was observed. This implies a connection with the red fluorescence emission from *A. actinomycetemcomitans* and the presence of porphyrin related material. Thus, it is of interest to conduct further research in order to verify this hypothesis and to identify porphyrins present in *A. actinomycetemcomitans*.

The specific aim in this study was to investigate if *A. actinomycetemcomitans* colonies could exhibit red fluorescence. Further, we wanted to investigate if there were any correlation between the QLF red fluorescence area and the physical colony area of the colonies. We also wanted compare the red fluorescence properties with morphology and size of the selected colonies.

In this context is also of interest to mention photodynamic therapy (PDT). This could be an alternative therapy for treating periodontal disease, since it have a low toxicity and a rapid effect [24,25]. The suggestion is that bacteria that can emit red fluorescence can be killed by photodynamic therapy [16]. PDT is based on the concept of a photo sensitizer, e.g. selected porphyrins, absorbing light of appropriate wavelength and inducing the generation of cytotoxic singlet oxygen and free radicals. Due to the increasing number of antibiotic resistant strains of micro organism there is an increased interest for alternative treatments of microbial infections [16,24], making research regarding bacterial fluorescence very interesting.

5. CONCLUSIONS

To our knowledge this is the first study that have shown that *A. actinomycetemcomitans* on its own is able to produce fluorescence in the red spectral region. We have found that:

- It is possible for a single bacterium strain to produce red fluorescence which is in contrast to several other studies.
- A capnophilic bacterium is able to produce red fluorescence, which is in contrast to the major hypothesis.
- The size of the investigated colonies do not correlate with the red fluorescence area, but depends on the colony morphology.

We have found indications that the fluorescence from *A. actinomycetemcomitans* is due to porphyrins, but this has to be established further. This makes it interesting to continue research on if the red fluorescence from *A. actinomycetemcomitans* is due to intrinsic fluorescent molecules such as porphyrins, and if different bacterial species produces different kinds of fluorophores. A study of the porphyrin composition of this bacterium strain is in progress.

6. ACKNOWLEDGEMENTS

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