molecular oral microbiology

molecular oral microbiology

Streptococcus anginosus L-cysteine desulfhydrase gene expression is associated with abscess formation in BALB/c mice

Y. Takahashi[1], A. Yoshida[1], E. Nagata[2], T. Hoshino[3], T. Oho[2], S. Awano[1], T. Takehara[1] and T. Ansai[1]

1 Division of Community Oral Health Science, Kyushu Dental College, Kitakyushu, Japan
2 Department of Preventive Dentistry, Faculty of Dentistry, Kagoshima University, Kagoshima, Japan
3 Department of Pediatric Dentistry, Faculty of Dentistry, Nagasaki University, Nagasaki, Japan

Correspondence: Akihiro Yoshida, Division of Community Oral Health Science, Kyushu Dental College, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu 803-8580, Japan Tel.: +81 93 285 3091; fax: +81 93 591 7736; E-mail: akihiro@kyu-dent.ac.jp

Keywords: hydrogen sulfide; *lcd*; L-cysteine desulfhydrase; odontogenic abscess; streptococci; βC-S lyase
Accepted 20 October 2010
DOI: 10.1111/j.2041-1014.2010.00599.x

SUMMARY

***Streptococcus anginosus*, an anginosus group bacterium, is frequently isolated from odontogenic abscesses, and is the oral bacterium that is primarily responsible for producing hydrogen sulfide from L-cysteine through the action of its L-cysteine desulfhydrase (βC-S lyase) enzyme. However, the relationship between its production of hydrogen sulfide and abscess formation has not been investigated. To elucidate the etiological role of hydrogen sulfide in abscess formation, we initially measured, using specific primers, expression of the *lcd* gene, which encodes βC-S lyase, in the pus of abscesses that formed in BALB/c mice following subcutaneous injection of *S. anginosus* into the dorsa. Expression of *lcd* was >15-fold higher when L-cysteine was present than when it was absent. A mouse virulence assay revealed that the mean diameter of abscesses caused by *S. anginosus* FW73 plus L-cysteine was greater than that of abscesses caused by *S. anginosus* FW73 in the absence of L-cysteine. These findings demonstrate that the *lcd* gene of *S. anginosus* is upregulated in mouse abscesses and that hydrogen sulfide, the product of a reaction catalyzed by βC-S lyase, plays an etiological role in odontogenic abscess formation.**

This article was first published online 10 January 2011. An error was subsequently discovered and the following corrections have been made 31 January 2011: figure 4 as well as portions of text from the Summary, Results and Dsicussion sections of the paper have been removed.

INTRODUCTION

Streptococcus anginosus, *Streptococcus constellatus* and *Streptococcus intermedius*, commonly referred to as the *Streptococcus milleri* group, were recently designated the *Streptococcus anginosus* group. They are generally considered to be part of the commensal microflora of various body sites, including the mouth and genitourinary and gastrointestinal tracts (Ruoff, 1988). They are frequently associated with purulent infections of the internal organs, including the liver, brain and lungs (Bateman *et al.*, 1975; de Louvois, 1980; Legrand *et al.*, 1989). In the oral cavity, members of the anginosus group of bacteria have been repeatedly isolated from odontogenic abscesses (Fisher & Russell, 1993; Robertson & Smith, 2009).

Streptococcus anginosus has been reported to produce hydrogen sulfide from L-cysteine in a reaction catalyzed by the enzyme L-cysteine desulfhydrase, which converts L-cysteine to pyruvate and ammonia (Guarneros & Ortega, 1970). The gene encoding

L-cysteine desulfhydrase in *S. anginosus* has been identified as *lcd* (Yoshida *et al.*, 2002). Furthermore, the L-cysteine desulfhydrase is reported as a C-S lyase that catalyzes α,β-elimination (αC-N and βC-S) reactions (Hori *et al.*, 1996).

Previous studies have reported that hydrogen sulfide is highly toxic to mammalian cells (Beauchamp *et al.*, 1984) and induces the modification and release of hemoglobin in erythrocytes (Chu *et al.*, 1999; Kurzban *et al.*, 1999). Indeed, the production of hydrogen sulfide in the subgingival sulcus is thought to contribute to periodontal diseases and dental abscess formation (Persson *et al.*, 1990; Carlsson *et al.*, 1993; Yoshida *et al.*, 2009). In the present study, we examined the expression of the *lcd* gene of *S. anginosus* in dorsal abscesses in mice, formed as a result of *S. anginosus* infection, and its relationship with hydrogen sulfide production. This is the first investigation to identify a relationship between the expression of the *S. anginosus* L-cysteine desulfhydrase gene and abscess formation in the BALB/c mouse.

METHODS

Bacterial strains and growth conditions

To identify primers specific for the *lcd* and *gyrB* genes of *S. anginosus* for use in real-time polymerase chain reaction (PCR) analysis, 29 bacterial strains were used to test the specificity of real-time PCR assays (Table 1). The *S. anginosus* strain FW73 was grown in brain–heart infusion broth (Becton Dickinson, Sparks, MD) at 37°C in a 5% CO_2 atmosphere (Yoshida *et al.*, 2002).

Design of specific primers for use in real-time reverse transcription-PCR

Oligonucleotide primers specific for the *lcd* (target) and *gyrB* (internal control) genes of *S. anginosus* were designed according to sequence data obtained from the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/) (accession numbers: *lcd*, AB326998; *gyrB*, GU982987) using PRIMER EXPRESS 3.0 software (Applied Biosystems, Foster City, CA). Primer specificity was initially confirmed by BLAST analysis and then by conventional PCR performed using chromosomal DNA from various oral bacteria (Table 1).

Table 1 Strains and amplification results

Strain	Amplification with the primers for *lcd*	*gyrB*
Streptococcus anginosus FW73	+	+
Streptococcus constellatus ATCC 27823	–	–
Streptococcus intermedius ATCC 27335	–	–
Streptococcus sobrinus OMZ176	–	–
Streptococcus salivarius HT9R	–	–
Streptococcus gordonii DL1	–	–
Streptococcus oralis ATCC 10557	–	–
Streptococcus mutans UA159	–	–
Streptococcus mitis 903	–	–
Streptococcus sanguinis OMZ9	–	–
Aggregatibacter actinomycetemcomitans Y4	–	–
Treponema denticola ATCC 35405	–	–
Treponema vincentii ATCC 35580	–	–
Treponema pectinovorum ATCC 33768	–	–
Porphyromonas gingivalis ATCC 33277	–	–
Prevotella bivia ATCC 29303	–	–
Prevotella corporis ATCC 33547	–	–
Prevotella intermedia ATCC 25611	–	–
Prevotella loescheii ATCC 15930	–	–
Prevotella melaninogenica ATCC 25845	–	–
Prevotella nigrescens ATCC 25261	–	–
Prevotella oralis ATCC 33322	–	–
Prevotella oris ATCC 33573	–	–
Prevotella pallens ATCC 700821	–	–
Prevotella veroralis ATCC 33779	–	–
Tannerella forsythia ATCC 43037	–	–
Lactobacillus rhamnosus ATCC 7469	–	–
Enterococcus faecalis ATCC 19433	–	–
Haemophilus aphrophilus NCTC 5908	–	–
Escherichia coli DH5α	–	–

These PCR assays were performed using the following thermal conditions: 94°C for 5 min, followed by 25 cycles of 94°C for 30 s, 48°C for 30 s, and 72°C for 1 min (for *lcd*); 94°C for 5 min, followed by 25 cycles of 94°C for 30 s, 62°C for 30 s and 72°C for 1 min (*gyrB*).

DNA and RNA sample preparation

Isolation of DNA and RNA from bacterial cultures
Genomic DNA was isolated and purified using a Pure gene DNA Isolation Kit (Gentra Systems, Minneapolis, MN). Total RNA was isolated from *S. anginosus* FW73 using Isogen (Wako Pure Chemical Industries, Saitama, Japan).

Isolation of RNA from clinical specimens

Coat samples were collected from the dorsal surfaces of the tongues of four *S. anginosus*-positive patients who visited the Department of Preventive Dentistry, Kyushu Dental College Hospital, in accordance with ethical guidelines established by the Ethics Committee of Kyushu Dental College Hospital. All patients who participated in the study understood the nature of the research and provided their informed consent. Pus was obtained from dorsal abscesses 2–6 days after the injection of mice with *S. anginosus* FW73 and/or human tongue coat. Tongue coat and pus were washed with diethylpyrocarbonate-treated water, and bacterial precipitates were obtained. Total RNA was isolated from tongue coat and/or pus using Isogen.

Real-time reverse transcription-PCR

Single-stranded complementary DNA was synthesized in reactions containing 10 U Transcriptor Reverse Transcriptase, 20 U Protector RNase Inhibitor, 1 mM each deoxynucleoside triphosphate (dNTP), 1 μM antisense primer, 1 × Transcriptor Reverse Transcriptase Reaction Buffer (Roche Diagnostics GmbH, Germany) and 1.0 μg total RNA; reactions were performed at 55°C for 30 min. The resulting complementary DNA and a negative control sample were amplified using LightCycler FastStart DNA Master^{PLUS} SYBR Green I (Roche Diagnostics). Mixtures of DNA template, 5 × Master Mix, and forward and reverse primers (500 nM each) were applied to a LightCycler Capillary (Roche Diagnostics). Amplification and detection of specific products were performed using the LightCycler Carousel-based System (Roche Diagnostics), with the following thermal conditions: 95°C for 10 min, followed by 45 cycles of 95°C for 10 s, 53°C for 10 s and 72°C for 12 s. To confirm the formation of a single PCR product, melting curve analysis was performed using the following conditions: 95°C for 1 min, 55°C for 1 min, and then an increase in temperature from 55.0 to 95.0°C (with a heating rate of 0.5°C per 10 s). The PCR products were additionally separated on 2% agarose gels to confirm that they were of the predicted sizes. Fold-changes in *lcd* gene expression (relative to a control sample) were calculated using the Pfaffl equation (Livak & Schmittgen, 2001):

$$\text{Expression ratio} = (E_{\text{target}})^{\Delta C_t \text{target(control}-\text{experimental)}} / (E_{\text{ref}})^{\Delta C_t \text{ref(control}-\text{experimental)}}$$

This equation normalizes the expression of the *S. anginosus lcd* gene (target) to that of the reference gene (*gyrB*) based on PCR efficiency (E) and threshold cycle (C_t) values. Efficiency of the PCR itself was calculated using the following equation (Livak & Schmittgen, 2001):

$$E = 10^{-1/\text{slope}}$$

The slope is calculated from a logarithmic plot of cycle number derived from a series of 10-fold dilutions of complementary DNA template. Theoretically, a slope of −3.3 represents a PCR efficiency of 100% (two fold amplification per cycle) (Livak & Schmittgen, 2001). The *lcd*/*gyrB* expression ratios were compared.

Mouse virulence assays

The virulence of *S. anginosus* and human tongue coat were determined by the method of Okayama *et al.* (2005). Bacteria were grown in brain–heart infusion broth to an optical density at 600 nm (OD_{600}) of 1.0. Cells were then pelleted by centrifugation, washed three times with phosphate-buffered saline (PBS), and resuspended at a concentration of 1.0×10^9 colony-forming units (CFU) ml^{-1}. The number of bacteria was adjusted using the CFU–OD_{600} graph and diluted to a concentration of 1.0×10^9 CFU ml^{-1} (for *S. anginosus*) and 2.8×10^8 CFU ml^{-1} (for tongue coat). L-cysteine was added to a final concentration of 100 mM. One hundred-microliter aliquots of each bacterial cell suspension were injected subcutaneously into the dorsa of 4-week-old female BALB/c mice, and abscess size and consistency, ulceration, the presence and locations of secondary lesions, and general health status were monitored daily. To test the effect of human tongue coat on the abscess-forming ability of *S. anginosus*, a suspension of tongue coat in PBS was used. Human tongue coat obtained from four donors was mixed, centrifuged and washed three times with PBS. The 100-μl aliquots, each containing 2.8×10^7 CFU of oral bacteria, were injected into mouse dorsa, and abscess formation was monitored. Approval to conduct these studies was obtained from the Animal Care Committee of Kagoshima University, Kagoshima, Japan (No. D09016).

Statistical analysis

Mann–Whitney *U*-tests were used to evaluate differences in gene expression and abscess size between mice infected with *S. anginosus* FW73, alone or with L-cysteine, or human tongue coat with or without L-cysteine ($n = 5$).

RESULTS

Specificity of *S. anginosus lcd* and *gyrB* primers

To examine *S. anginosus lcd* gene expression in tongue coat and mouse pus by real-time reverse transcription–PCR, we designed the specific oligonucleotide primers *Sa-lcd*-F: 5′-TTCGTTTGATAAAGAAGA-3′ and *Sa-lcd*-R: 5′-ACTGCAAAGAAGGTACAA-3′, whose specificities were confirmed by testing their ability to amplify chromosomal DNA from various oral bacteria in conventional PCR (Table 1). These primers only amplified the *S. anginosus lcd* gene (Table 1). Primers specific for the *gyrB* gene of *S. anginosus* (*Sa-gyrB*-F: 5′-GCACGTCGTAGCGGATCTG-3′, *Sa-gyrB*-R: 5′-GGGTCTGGTGTAAAATGAACAGTTG-3′) were used as an internal control. Their specificities were confirmed as described above. The presence, in tongue coat and pus, of substances that inhibit RT-PCR assays was assessed using lysates spiked with *S. anginosus*. Levels of inhibition were negligible (data not shown).

Quantification of *S. anginosus lcd* gene expression

To assess *S. anginosus lcd* gene expression, we initially evaluated the effects of L-cysteine concentration. We first evaluated the effect of L-cysteine concentration on the expression of the *S. anginosus gyrB* gene. We detected no significant differences in *gyrB* gene expression at concentrations of L-cysteine ranging from 10 mM to 1 M (data not shown). Therefore, *lcd* gene expression levels were normalized to those of *gyrB*. To identify a suitable concentration of L-cysteine for use in *lcd* gene expression analysis, *S. anginosus* FW73 cells were treated with various concentrations of L-cysteine. Expression of the *lcd* gene was approximately nine-fold higher in cells treated with 100 mM and 1 M L-cysteine than in those treated with 10 mM L-cysteine (data not shown). We therefore used L-cysteine at a concentration of 100 mM to induce *S. anginosus lcd* gene expression.

Detection of *S. anginosus lcd* gene expression in tongue coat

Real-time PCR analysis directly confirmed expression of the *S. anginosus lcd* gene in tongue coat, both before and after rinsing the mouth with 100 mM L-cysteine. After rinsing, *S. anginosus lcd* gene expression was induced between 7.4-fold and 19.7-fold in tongue coat from four donors (data not shown).

Streptococcus anginosus lcd gene expression in mice

We hypothesized that hydrogen sulfide produced by *S. anginosus* may play a crucial role in oral abscess formation and tested this hypothesis using mouse virulence assays. BALB/c mice were challenged through subcutaneous dorsal injection with *S. anginosus* FW73 suspension (1.0×10^8 CFU per animal) with or without 100 mM L-cysteine ($n = 5$). Within 2 days, *S. anginosus* FW73 (with and without L-cysteine) induced the formation of dorsal abscesses in all mice. Pus was collected when abscess size peaked, and total bacterial RNA was purified. Using specific primers, *S. anginosus* FW73 *lcd* gene expression from pus samples was analyzed. Expression of *lcd* from pus was >15-fold higher in mice treated with L-cysteine than in those not treated with L-cysteine (Fig. 1). In addition, tongue coat containing *S. anginosus* was inoculated subcutaneously into the dorsa of mice with or without 100 mM L-cysteine ($n = 5$). Expression of *lcd* in the dorsal pus formed as a result was >10-fold higher in mice treated with L-cysteine than in those not treated with L-cysteine (Fig. 1).

Pathogenicity of *S. anginosus* in mice

Dorsal abscess sizes were evaluated. Abscess size peaked between days 1 and 6. On days 1 and 4, the mean diameter of the abscesses formed following injection of *S. anginosus* FW73 was greater in mice treated with L-cysteine than in those not treated with L-cysteine (Mann–Whitney *U*-test, $P < 0.05$; Fig. 2A). Similarly, the mean diameter of abscesses formed following injection of tongue coat was greater in mice treated with L-cysteine than in those not treated with

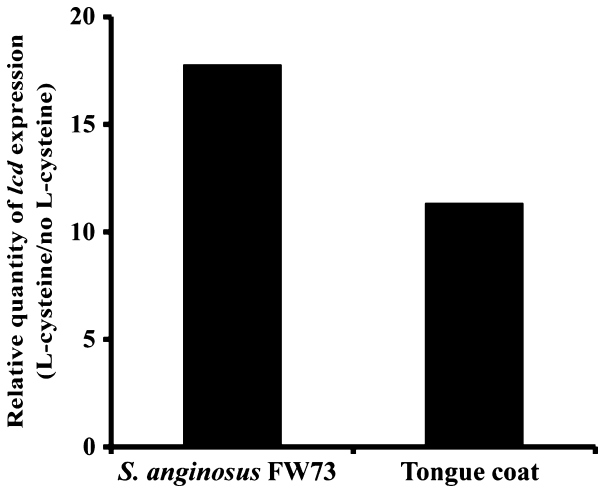

Figure 1 Quantitative reverse transcription–polymerase chain reaction analysis of *Streptococcus anginosus lcd* gene expression. Expression of *lcd* transcripts was assessed before and after stimulation with 100 mM L-cysteine. Data are expressed as the mean of four replicates.

L-cysteine (Mann–Whitney *U*-test, $P < 0.05$ for day 1, $P < 0.01$ for day 2; Fig. 2B). Data relating to abscess formation at day 2 are shown in Fig. 3. In day 2, *S. anginosus* with L-cysteine showed ulcerative lesions on the abscess, whereas *S. anginosus* without L-cysteine showed no ulcerative lesion. Similar results were observed for tongue coat (Fig. 3).

DISCUSSION

Several studies have defined the relationship between *S. anginosus* and odontogenic abscess formation (Yoshida *et al.*, 2002, 2008; Okayama *et al.*, 2005; Robertson & Smith, 2009). However, none of these studies tested the role of hydrogen sulfide production. Hydrogen sulfide has been identified as a cause of inflammation. Its production by gut bacteria is a pathogenic factor in Crohn's disease, which is characterized by mucosal inflammatory lesions that bear similar features to those that occur in periodontal disease (Sigusch, 2004). Elsewhere, hydrogen sulfide produced by oral bacteria promotes the release of the proinflammatory cytokine interleukin-8 from gingival and oral epithelial cells (Chen *et al.*, 2010).

Previous investigations reported that *S. anginosus* is the oral bacterium that is primarily responsible for the production of hydrogen sulfide from L-cysteine through the action of the enzyme L-cysteine desulfhydrase (βC-S lyase) (Yoshida *et al.*, 2002, 2008; Yoshida *et al.*, 2009). *Streptococcus anginosus* is the member of the anginosus group that is typically linked to periapical abscesses (Fisher & Russell, 1993). Therefore, we investigated the relationship between *S. anginosus*-associated abscess formation in mice and the *S. anginosus lcd* gene expression in abscess fluids to clarify the etiological role of *S. anginosus* βC-S lyase in odontogenic abscess formation.

Initially, we designed primers to specifically analyze *S. anginosus lcd* gene expression in oral specimens (with *gyrB* as an internal control). Expression of the *lcd* gene was normalized to *gyrB* gene expression. The suitability of *gyrB* as a housekeeping gene was confirmed by L-cysteine stimulation (data not shown). βC-S lyase in *S. anginosus*, *S. constellatus* and *S. intermedius* has an extremely high capacity to produce hydrogen sulfide compared with other streptococci, including *S. gordonii*, *S. oralis*, *S. mutans*,

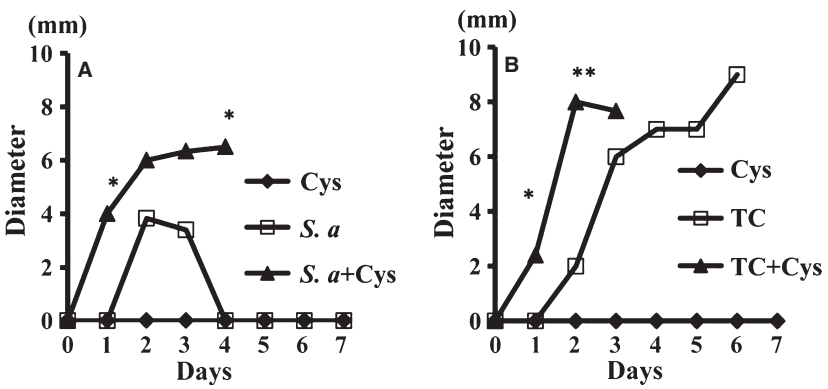

Figure 2 Diameters of the abscesses formed after injection of 0.1 ml *Streptococcus anginosus* [A, 1.0×10^9 colony-forming units (CFU) ml^{-1}] or tongue plaque (B, 2.8×10^8 CFU ml^{-1}). Data are expressed as the mean ($n = 5$). Cys, 100 mM L-cysteine, *S. a*, *S. anginosus*, TC, tongue coat, $^*P < 0.05$, $^{**}P < 0.01$ (Mann–Whitney *U*-test).

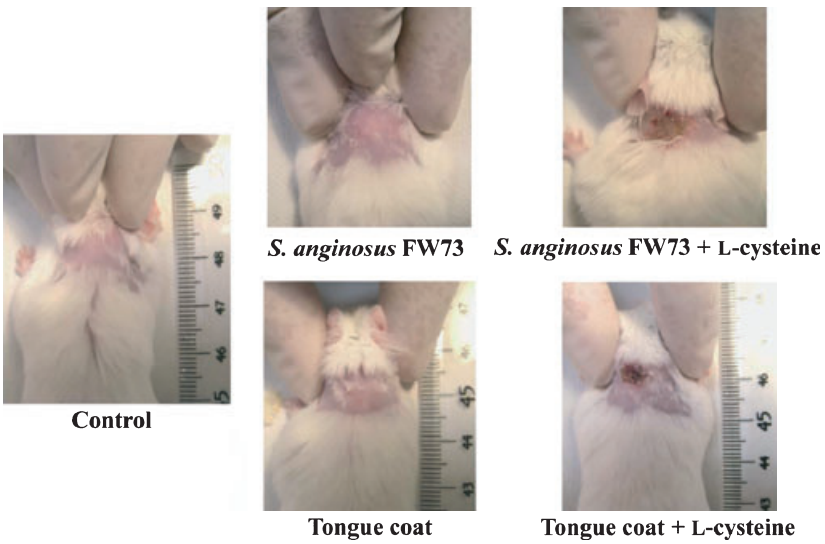

Figure 3 Abscess formation in BALB/c mice challenged through subcutaneous dorsal injection of 0.1 ml bacterial suspension [Day 2, 1.0×10^9 colony-forming units (CFU) ml^{-1} for *Streptococcus anginosus* and 2.8×10^8 CFU ml^{-1} for tongue coat] containing/not containing 100 mM L-cysteine.

S. salivarius and *S. sobrinus* (Yoshida *et al.*, 2008). Using these primers, we were able to evaluate the *lcd* gene expression specific to *S. anginosus* in tongue coat and abscess fluid.

Using these primers, we evaluated *lcd* gene expression in pus from mouse abscesses. This is the first report to evaluate the expression of a specific gene in pus from abscesses. In this assay, we could assume that various genes would be expressed, including, but not limited to, *S. anginosus* and mouse genes. Therefore, primer-specific primers for the *lcd* and *gyrB* genes of *S. anginosus* were required. Expression of *lcd* from pus was >15-fold higher in mice injected with *S. anginosus* and L-cysteine than in mice exposed to L-cysteine alone. Previously, Yoshida *et al.* (2002) reported that hydrogen sulfide or the combination of L-cysteine and L-cysteine desulfhydrase triggered the release of hemoglobin from red blood cells and the production of modified forms of hemoglobin. These modified types of hemoglobin, predominantly sulfhemoglobin and methemoglobin, are unable to carry oxygen. We hypothesize that the generation of sulfhemoglobin and methemoglobin induces local anemia because of a reduction in the oxygen supply. This, in turn, may induce tissue necrosis. Host immune systems recognize the necrotized tissue as being foreign and activate an immune response. As a result, local inflammation, periodontitis and odontogenic abscess formation are induced.

A mouse virulence assay showed that abscess growth is faster in mice injected with both *S. anginosus* FW73 and L-cysteine than in animals exposed to *S. anginosus* alone. This indicates that L-cysteine accelerates *S. anginosus*-induced abscess formation. Treatment with tongue coat yielded similar results. In this study, we employed a mouse virulence assay using tongue coat to elucidate *S. anginosus lcd* gene expression levels in tongue coat for comparison with *S. anginosus lcd* gene expression in culture. Other bacteria might produce hydrogen sulfide via L-cysteine in the tongue coat. Therefore, comparisons of the level of *S. anginosus lcd* gene expression in tongue coat and the diameter of the abscess are meaningless.

Hydrogen sulfide is produced in hosts in a manner that causes desulfhydration of L-cysteine, cystathionine β-synthetase and cystathionine γ-lyase. Therefore, hydrogen sulfide can be released from bacteria or as a result of host metabolism. However, although it remains to be determined whether bacteria release larger amounts of hydrogen sulfide than do host cells, no abscesses formed in BALB/c mice injected with L-cysteine alone (as a control), suggesting that host cell production of hydrogen sulfide was negligible in the mouse virulence assays.

In conclusion, we found *S. anginosus lcd* gene expression to be upregulated in mouse abscesses, leading us to conclude that hydrogen sulfide may be a virulence factor for abscess formation. Further stud-

ies are required to test this hypothesis. Knocking out the *lcd* gene may help to clarify its role in *S. anginosus*-associated abscess formation. We failed, in the present study, to generate *lcd* knockout bacterial strains because of difficulties in transforming *S. anginosus*. In addition, the use of βC-S lyase inhibitors to test whether the inhibitors block abscess formation would be useful in clarifying the role of this enzyme in odontogenic abscess formation. Previously, several candidates for *in vitro* inhibitors on βC-S lyase of *S. constellatus* were reported (Yoshida *et al.*, 2008). However, we cannot apply these inhibitors for mouse and human because of their toxicities. Our attempts to overcome these problems continue.

ACKNOWLEDGEMENT

This study was supported in part by Grants-in-Aid (C) 22592341 (A.Y.), (B) 20390537 (T.T.) and (B) 22390403 (T.A.) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

REFERENCES

Bateman, N.T., Eykyn, S.J. and Phillips, I. (1975) Pyogenic liver abscess caused by *Streptococcus milleri*. *Lancet* **1**: 657–659.

Beauchamp, R.O. Jr, Bus, J.S., Popp, J.A., Boreiko, C.J. and Andjelkovich, D.A. (1984) A critical review of the literature on hydrogen sulfide toxicity. *Crit Rev Toxicol* **13**: 25–97.

Carlsson, J., Larsen, J.T. and Edlund, M.B. (1993) *Peptostreptococcus micros* has a uniquely high capacity to form hydrogen sulfide from glutathione. *Oral Microbiol Immunol* **8**: 42–45.

Chen, W., Kajiya, M., Giro, G. *et al.* (2010) Bacteria-derived hydrogen sulfide promotes IL-8 production from epithelial cells. *Biomed Biophys Res Commun* **391**: 645–650.

Chu, L., Ebersole, J.L., Kurzban, G.P. and Holt, S.C. (1999) A 46-kDa L-cysteine desulfhydrase from *Treponema denticola*: biochemical and biophysical characterization. *Clin Infect Dis* **28**: 442–450.

Fisher, L.E. and Russell, R.R. (1993) The isolation and characterization of milleri group streptococci from dental periapical abscesses. *J Dent Res* **72**: 1191–1193.

Guarneros, G. and Ortega, M.V. (1970) Cysteine desulfhydrase activities of *Salmonella typhimurium* and *Escherichia coli*. *Biochim Biophys Acta* **198**: 132–142.

Hori, H., Takabayashi, K., Orvis, L., Carson, D.A. and Nobori, T. (1996) Gene cloning and characterization of *Pseudomonas putida* L-methionine-α-deamino-γ-mercaptomethane-lyase. *Cancer Res* **56**: 2116–2122.

Kurzban, G.P., Chu, L., Ebersole, J.L. and Holt, S.C. (1999) Sulfhemoglobin formation in human erythrocytes by cystalysin, an L-cysteine desulfhydrase from *Treponema denticola*. *Oral Microbiol Immunol* **14**: 153–164.

Legrand, J.C., Alewaeters, A., Leenaerts, L., Gilbert, P., Labbe, M. and Glupczynski, Y. (1989) *Gardnerella vaginalis* bacteremia from pulmonary abscess in a male alcohol abuser. *J Clin Microbiol* **27**: 1132–1134.

Livak, K.J. and Schmittgen, T.D. (2001) Analysis of relative gene expression data using real-time quantitative PCR and the $2^{-\Delta\Delta C}_{T}$ method. *Methods* **25**: 402–408.

de Louvois, J. (1980) Bacteriological examination of pus from abscesses of the central nervous system. *J Clin Pathol* **33**: 66–71.

Okayama, H., Nagata, E., Ito, H.-O., Oho, T. and Inoue, M. (2005) Experimental abscess formation caused by human dental plaque. *Microbiol Immunol* **49**: 399–405.

Persson, S., Edlund, M.B., Claesson, R. and Carlsson, J. (1990) The formation of hydrogen sulfide and methyl mercaptan by oral bacteria. *Oral Microbiol Immunol* **5**: 195–201.

Robertson, D. and Smith, A.J. (2009) The microbiology of the acute dental abscess. *J Med Microbiol* **58**: 155–162.

Ruoff, K.L. (1988) *Streptococcus anginosus* ("*Streptococcus milleri*"): the unrecognized pathogen. *Clin Microbiol Rev* **1**: 102–108.

Sigusch, B.W. (2004) Periodontitis as manifestation of Crohn's disease in primary dentition: a case report. *J Dent Child (Chic)* **71**: 193–196.

Yoshida, Y., Nakano, Y., Amano, A. *et al.* (2002) *lcd* from *Streptococcus anginosus* encodes a C-S lyase with α,β-elimination activity that degrades L-cysteine. *Microbiology* **148**: 3961–3970.

Yoshida, Y., Ito, S., Sasaki, T. *et al.* (2008) Molecular enzymatic characterization of βC-S lyase in *Streptococcus constellatus*. *Oral Microbiol Immunol* **23**: 245–253.

Yoshida, A., Yoshimura, M., Ohara, N. *et al.* (2009) Hydrogen sulfide production from cysteine and homocysteine by periodontal and oral bacteria. *J Periodontol* **80**: 1845–1851.