J. Gen. Appl. Microbiol., **64**, 174–179 (2018)
doi 10.2323/jgam.2017.11.005

jGAM

Full Paper

Aciduricity and acid tolerance mechanisms of *Streptococcus anginosus*

(Received May 26, 2017; Accepted November 25, 2017; J-STAGE Advance publication date: April 17, 2018)

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Although *Streptococcus anginosus* constitutes a proportion of the normal flora of the gastrointestinal and genital tracts, and the oral cavity, it has been reported that *S. anginosus* infection could be closely associated with abscesses at various body sites, infective endocarditis, and upper gastrointestinal cancers. The colonization in an acidic environment due to the aciduricity of *S. anginosus* could be the etiology of the systemic infection of the bacteria. To elucidate the aciduricity and acid tolerance mechanisms of the microbe, we examined the viability and growth of *S. anginosus* under acidic conditions. The viabilities of *S. anginosus* NCTC 10713 and *Streptococcus mutans* ATCC 25175 at pH 4.0 showed as being markedly higher than those of *Streptococcus sanguinis* ATCC 10556, *Streptococcus gordonii* ATCC 10558, and *Streptococcus mitis* ATCC 49456; however, the viability was partially inhibited by dicyclohexylcarbodiimide, an H^+-ATPase inhibitor, suggesting that H^+-ATPase could play a role in the viability of *S. anginosus* under acidic conditions. In addition, *S. anginosus* NCTC 10713 could grow at pH 5.0 and showed a marked arginine deiminase (ADI) activity, unlike its Δ*arcA* mutant, deficient in the gene encoding ADI, and other streptococcal species, which indicated that ADI could also be associated with aciduricity. These results suggest that *S. anginosus* has significant aciduric properties, which can be attributed to these enzyme activities.

Key Words: acid tolerance; arginine deiminase; H^+-ATPase; *Streptococcus anginosus*

Introduction

Streptococcus anginosus, one of the oral viridans streptococci, is part of the normal flora found in the human oral cavity, gastrointestinal and genital tracts (Rahman et al., 2016; Whiley et al., 1992). The organism is generally considered to have a low pathogenicity; however, it can cause serious purulent abscesses at various body sites (Terzi et al., 2016), subacute infective endocarditis (Willcox, 1995), and upper gastrointestinal cancers (Sasaki, H. et al., 1995; Sasaki, M. et al., 2005; Shiga et al., 2001; Tateda et al., 2000). We have previously reported a novel bioactive *S. anginosus* antigen (SAA), which induced the expression of inducible nitric oxide synthase (iNOS), TNFα, IL-1β and IL-6 in murine peritoneal exudative cells (Sasaki, M. et al., 1995, 2001). Therefore, the overgrowth of *S. anginosus* in these organs could induce damage to these tissues by the continuous production of NO or due to the inflammatory cytokines, which could increase the risk of bacteremia, followed by infective endocarditis and abscesses. Further, iNOS upregulation has been detected in cancer tissues (Doi et al., 1999; Marrogi et al., 2000; Zhang et al., 1998) and could be a risk factor for carcinogenesis (Ambs et al., 1999; Giardiello et al., 1993; Iwasaki et al., 1997; Jadeski and Lala, 1999). Because the environments of these organs are generally mild acidic conditions (Fallingborg, 1999; Hassen et al., 2015; Khutoryanskiy, 2015; O'Hanlon et al., 2013), the aciduricity of *S. anginosus* can be a pathogenicity trait, allowing the microorganism to promote infection in an acidic condition followed by an aberrant infection at other body sites, or systemic infections (Mak et al., 2011; Shah et al., 2017; Wenzler et al., 2015).

The human gastric pathogen *Helicobactor pylori* is extremely well adapted to the highly acidic conditions of

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None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.

the stomach (Karita et al., 2003; Wiśniewska et al., 2002). The pronounced acid resistance of *H. pylori* is mainly due to the production of urease, an ammonia-producing enzyme (Scott et al., 2002; Wen et al., 2003). Bacterial H^+-ATPase is the enzyme to maintain intracellular pH near neutral through expelling protons across the cell membrane under low-pH conditions (Belli and Marquis, 1991; Bender et al., 1986; Hamilton and Buckley, 1991; Kobayashi, 1985). In addition, many less acid-tolerant bacteria present in dental plaque are able to catabolize arginine via the arginine deiminase (ADI) pathway. The major function of this pathway appears to be the protection of the organisms against acid damage through the production of a base compound (ammonia) from arginine (Barcelona-Andrés et al., 2002; Dong et al., 2002; Maghnouj et al., 1998; Winterhoff et al., 2002). Thereby, these enzymes protect cells from the bactericidal effects of acidic conditions.

The acid tolerance related to streptococcal pathogenicity, and the properties of the key enzymes, H^+-ATPase and ADI, of *S. mutans*, *S. pyogenes*, and *S. suis* have been considerably studied (Belli and Marquis, 1991; Cusumano and Caparon, 2015; Fulde et al., 2014); however, to the best of our knowledge, the aciduricity and the role of these enzymes of *S. anginosus* have not been investigated. Therefore, in this study, we investigated the H^+-ATPase and ADI activities of *S. anginosus* to elucidate the roles of these enzymes in the acid tolerance of the bacterium and its ability to grow in the acid environment.

Materials and Methods

Bacterial strains and culture conditions. We used the following *S. anginosus* strains: *S. anginosus* NCTC 10713 (wild type); a Δ*arcA* mutant deficient in the gene encoding ADI, which was obtained by homologous recombination from *S. anginosus* NCTC 10713, designated as *S. anginosus* Δ*arcA*; and an *S. anginosus* from the dental plaque of a healthy volunteer, designated as *S. anginosus* DP. In addition, the strains isolated from humans of *Streptococcus mutans* ATCC 25175, *Streptococcus sanguinis* ATCC 10556, *Streptococcus gordonii* ATCC 10558, and *Streptococcus mitis* ATCC 49456 were also used. These bacteria were cultured anaerobically at 37°C and maintained in Todd Hewitt broth (THB; Difco Laboratories, Detroit, MI, USA).

Identification of arcA and preparation of an arcA-deficient mutant (ΔarcA). A partial sequence (1,150 bp) of the coding region of the *arcA* gene of *S. anginosus* NCTC 10713 was determined by direct sequencing of PCR products of genomic DNA, amplified with the S1 (5′-ATGTCTACACATCCAATTCATGTT-3′) and AS2 (5′-TTAGATTTCTTTCACGTTCGAATGGC-3′) primers. The sequence corresponded to well-conserved regions within the streptococcal ADI-encoding gene (*Streptococcus pyogenes*, accession number NC 011375, and *S. gordonii*, accession number NC 009785), which were obtained from GenBank by an Entrez cross-database search on the National Center for Biotechnology Information website. The unknown sequence of the 5′ coding region of *arcA* was determined by genome sequencing (ABI PRISM 310 Genetic Analyzer, Applied Biosystems, Foster City, CA, USA) using genomic DNA as a template and ASW (5′-CTGGCAATTCTGCTTTTTGG-3′; positions 82–101 bp) as an upstream sequencing primer. The complete nucleotide sequence (1,230 bp) of the *arcA* gene of *S. anginosus* NCTC 10713 was submitted to the DNA Data Bank of Japan database under the accession number AB818535.

To construct an *arcA*-deficient mutant strain, an 866 bp fragment (positions 81–946 bp in the coding sequence of *arcA*) was amplified by PCR from genomic DNA of *S. anginosus* NCTC 10713 using primers ArcF1 (5′-GGAAAACTTGATGCCGGATC-3′) and ArcR1 (5′-CGCCTTTTTCTTCTGCAA-3′). After blunt-end treatment of the PCR product using the Sure Clone Ligation kit (GE Healthcare Japan, Tokyo, Japan), the fragment was cloned into the *Sma*I site of pResEmBBN. The plasmid can be used as an integration vector for gene inactivation by a single crossover with the streptococcal chromosome because it cannot replicate in streptococcal species (Shibata et al., 2005). Erythromycin (300 μg/mL)-resistant colonies were selected following transformation of the recombinant plasmid into *Escherichia coli* strain XL-1 Blue (Agilent Technologies, Santa Clara, CA, USA). Then, *S. anginosus* NCTC 10713 was transformed with a purified resultant plasmid. The insertion of the vector was confirmed by PCR with primers ArcF1 and BBN-N (5′-GATTTGAGCGTCAGATTTCG-3′), which is an internal sequence of pResEmBBN, and the mutant phenotype was confirmed by an ADI enzyme activity test.

Bacterial cell viability and growth assay. Bacterial cells were harvested by a centrifugation at 4°C for 10 min, and washed three times with sterile phosphate-buffered saline (pH 7.4). The portion of the cells [10^6 colony-forming units (CFU)] were resuspended in 1 mL of THB, whose pH was adjusted to 4.0 or 7.5, with or without 10 μM of dicyclohexylcarbodiimide (DCCD; Kanto Chemical Co., Inc., Tokyo, Japan), an H^+-ATPase inhibitor. After incubation at 37°C for 1.5 h, the number of surviving bacteria was measured by counting the colony on Trypticase soy agar (Difco Laboratories). Results were expressed as the percentage of survival relative to the CFU count of the initial inoculum.

To measure cell growth at pH 5.0, streptococcal cultures were statically incubated overnight to the stationary phase in THB at 37°C. Ten microliters of the cultures (10^5 CFU) was inoculated into 1 mL of fresh THB (pH 7.5) or THB with pH adjusted to 5.0 with citric acid. The cultures were incubated at 37°C, and the optical density (OD_{600}) was monitored at 600 nm from 0 to 48 h of cultivation.

Enzyme activities. The H^+-ATPase assay was performed by the method of Takahashi and Yamada (Takahashi and Yamada, 1998). Briefly, cells were harvested by a centrifugation at 5,000 × *g* for 10 min at 4°C, washed three times with sterilized 75 mM Tris-HCl buffer (pH 7.0) containing 10 mM $MgSO_4$, and resuspended in the same buffer. The cells were permeabilized by mixing vigorously with a 0.01% (v/v) of toluene for 90 s at room temperature. After a centrifugation at 5,000 × *g* for 10 min at 4°C, the supernatant was removed, and the pellet was resuspended in the same buffer. The assay mixture con-

tained 5 mM ATP (Sigma-Aldrich, St. Louis, MO, USA), 10 mM $MgSO_4$, and 0.1 mg (dry weight) of permeabilized cells in 100 mM Tris-malate buffer (pH 5.5) with or without 10 μM DCCD. After incubation at 37°C for 1 h, the inorganic phosphate derived from ATP hydrolysis was measured using an ATPase/GTPase assay kit (BioAssay Systems, Hayward, CA, USA), according to the manufacturer's instructions.

The ADI activity was measured by monitoring the citrulline production from arginine by the method of Degnan et al. (1998). Briefly, bacteria were grown in THB at 37°C to an OD_{600} of 1.0, then harvested by centrifugation at 5,000 × *g* for 10 min, washed once with Tris-HCl buffer (pH 7.0), and resuspended in 1/10 of the original volume of the same buffer. The cells were disrupted by sonication for five times at a frequency of 20 kHz with a power density of 50 W for 1 min on ice, and soluble fractions were recovered after a centrifugation at 5,000 × *g* for 10 min. After dialysis of the fraction against distilled water, the protein contents were determined by a protein assay (Bio-Rad, Hercules, CA, USA) based on the method of Bradford (1976). The assay mixture containing 10 mM L-arginine and 10 mM $MgCl_2$ in 100 mM Tris-maleate buffer (pH 6.5) was incubated at 37°C. The reaction was started by adding the cell lysate and was terminated after 2 h by adding a mixture of H_3PO_4 and H_2SO_4 (3:1, v/v). Citrulline could be quantified by colorimetric determination of its reaction product using diacetyl-monoxime according to the method of Oginsky (1957).

Effect of arginine metabolism on the growth and pH of culture media of S. anginosus NCTC 10713 and S. anginosus ΔarcA. *S. anginosus* NCTC 10713 and *S. anginosus* Δ*arcA* were cultured at 37°C in THB (pH 7.5) with or without 10 mM L-arginine hydrochloride. The OD_{600} and pH of the culture media were measured at 0–48 h after inoculation.

Statistical analysis. Results are expressed as means ± standard deviation. Statistical differences were determined using a Student's *t*-test.

Results

Cell viabilities in acidic conditions and H⁺-ATPase activities of streptococcal species

The viabilities of the *S. anginosus* strains and streptococcal species used in this study after cultivation at 37°C for 1.5 h under acidic conditions at the lethal pH 4.0 are shown in Fig. 1a. The survival rates of *S. anginosus* NCTC 10713, *S. anginosus* DP, and *S. mutans* ATCC 25175 were 73.3%, 74.8%, and 64.7%, respectively, while those of the other streptococcal species ranged from 20.8% to 29.2%. Among the five oral streptococcal species, *S. anginosus* NCTC 10713, *S. anginosus* DP and *S. mutans* ATCC 25175 showed a markedly higher viability under acidic conditions. All viabilities were decreased by the addition of DCCD under the culture at pH 4.0; however, the depressions were not recognized at pH 7.5 (Fig. 1b). Furthermore, *S. anginosus* showed an H^+-ATPase activity at pH 4.0, which was inhibited by DCCD, similar to that in the other streptococcal species (Fig. 1c).

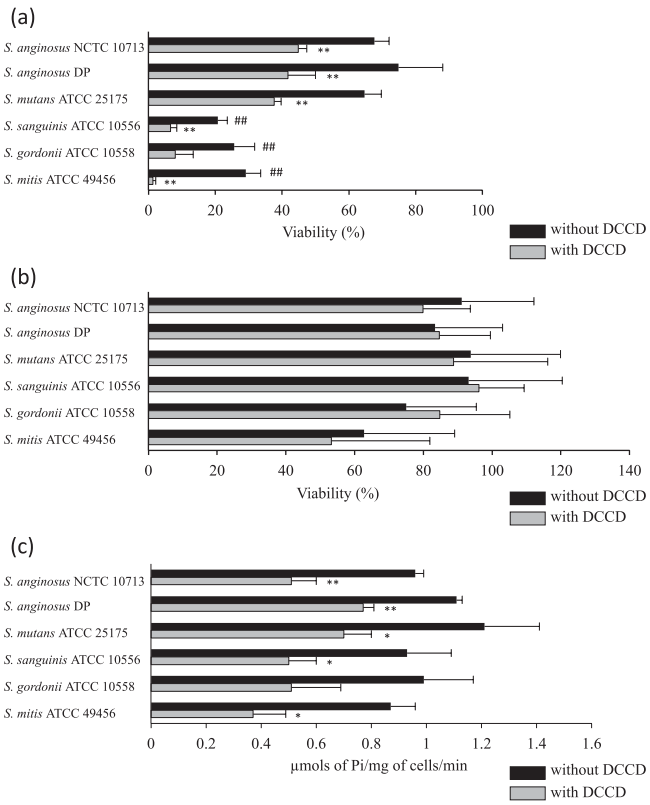

Fig. 1. Viabilities under acidic conditions (a), (b) and ATPase activity (c) of streptococcal species.

(a), (b) Cells (10^6 CFU) were inoculated into 1 mL of THB at pH 4.0 (a) or at pH 7.5 (b) and incubated at 37°C for 1.5 h, with or without 1 mM DCCD. The viable number of surviving bacteria was measured by counting the colony on Trypticase soy agar, followed by cultivation at 37°C for 24 h. (c) The assay mixture contained 5 mM ATP, 10 mM $MgSO_4$, and 0.1 mg (dry weight) of permeabilized cells in 100 mM Tris-malatel buffer (pH 5.5) with or without 1 mM DCCD. After incubation at 37°C for 1 h, the inorganic phosphate derived from ATP hydrolysis was measured by an ATPase/GTPase assay. Values and error bars represent means and SD from three independent experiments performed in triplicate. Statistically significant differences are indicated. *, without DCCD versus with DCCD; #, *S. anginosus* NCTC 10713 versus the other *Streptococcus* species; 2 symbols, $P < 0.01$; 1 symbol, $P < 0.05$.

Growth of streptococci under acidic conditions

To examine the growth of streptococcal species under acidic conditions, bacteria were cultured in THB at the initial pH values of 5.0 and 7.5. *S. anginosus* NCTC 10713 and *S. anginosus* DP were able to grow at both pH 5.0 and 7.5. In contrast, the growth of *S. mutans* ATCC 25175 was partially depressed and that of the other streptococci was strongly depressed or delayed at pH 5.0 (Fig. 2).

Effect of arcA deletion on ADI activity and the growth under acidic conditions

The ADI activities of *S. anginosus* NCTC 10713, *S. anginosus* DP, *S. anginosus* Δ*arcA* and other streptococcal species used in this study are shown in Fig. 3a. Marked ADI activities were observed in *S. anginosus* NCTC 10713 and *S. anginosus* DP but not in the *S. anginosus* Δ*arcA* and the other streptococci. Furthermore, the growth of the *S. anginosus* Δ*arcA* was partially depressed under the acidic condition (pH 5.0) (Fig. 3b).

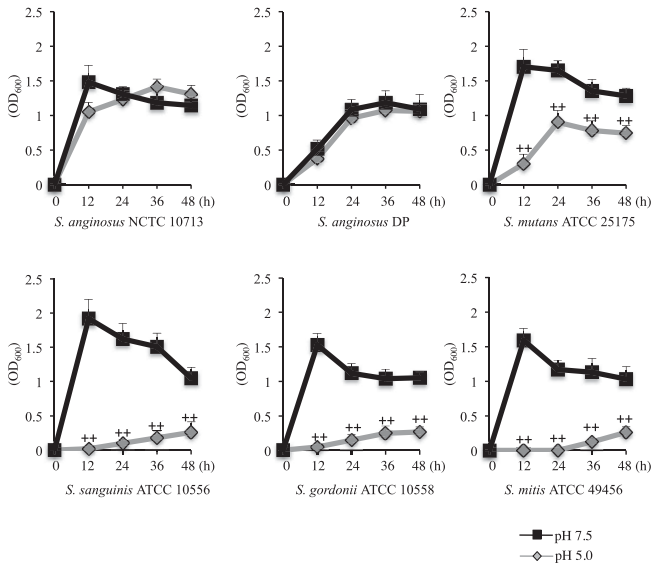

Fig. 2. Growth of streptococci under acidic conditions.

THB at pH 5.0 or 7.5 was inoculated with 10^5 CFU of streptococcal cells and incubated anaerobically at 37°C for 48 h. The cultures were periodically monitored for their OD_{600} values from 0 to 48 h after inoculation. Values and error bars represent means and SD from three independent experiments performed in triplicate. Statistically significant differences are indicated. *, pH 7.5 versus pH 5.0; 2 symbols, $P < 0.01$.

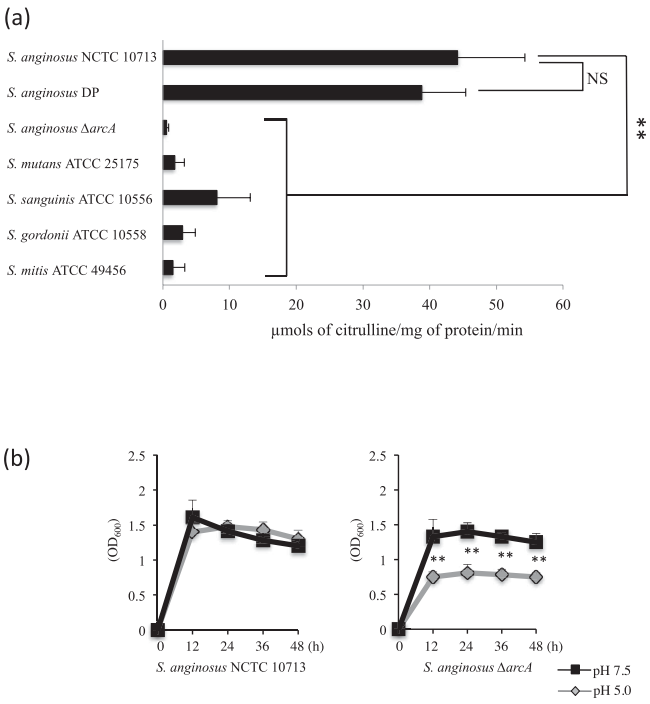

Fig. 3. ADI activity (a) of *S. anginosus* strains and *Streptococcus* species, and growth under acidic conditions (b) of *S. anginosus* NCTC 10713 and *S. anginosus* Δ*arcA*.

(a) Cells were disrupted by sonication, and soluble fractions (cell lysates) were recovered. The assay mixture contained 10 mM L-arginine and 10 mM $MgCl_2$ in 100 mM Tris-maleate buffer (pH 6.5). The reaction was started by the addition of the cell lysate. After 1 h of incubation at 37°C, aliquots of the reaction mixture were taken and assayed for the citrulline produced from arginine. Statistically significant differences are indicated. *, *S. anginosus* NCTC 10713 versus the other *Streptococcus* species; 2 symbols, $P < 0.01$. (b) The cultures were periodically monitored by measuring OD_{600} values from 0 to 48 h after inoculation. Values and error bars represent means and SD from three independent experiments performed in triplicate. Statistically significant differences are indicated. *, pH 7.5 versus pH 5.0; 2 symbols, $P < 0.01$.; NS, not significant.

Effect of arginine metabolism on the growth and pH of the culture media of S. anginosus NCTC 10713 and S. anginosus ΔarcA

The growth of *S. anginosus* NCTC 10713 (Fig. 4a), but not that of the *S. anginosus* Δ*arcA* (Fig. 4b), was increased by the addition of 0.5% L-arginine to the culture medium. The reduced pH of the *S. anginosus* NCTC 10713 culture medium was restored after 6 h when the medium contained L-arginine (Fig. 4c). *S. anginosus* NCTC 10713 could increase the pH of the culture medium by producing an alkali by arginine metabolism via the ADI pathway, which could result in the induction of *S. anginosus* growth.

Discussion

An acidic environment is one of the most common stresses encountered by bacteria in infected tissues (Cotter and Hill, 2003); therefore, bacteria are equipped with various acid tolerance mechanisms to grow even in acidic environments for the establishment of infection. Bacterial H^+-ATPase is the enzyme that actively expels protons out of the cell (Kobayashi, 1985), and ADI catalyzes the conversion of arginine to ornithine, ammonia, and CO_2, with the concomitant production of ATP (Zeng et al., 2006). Although acid resistance mechanisms of numerous streptococcal species associated with the establishment of infection has been clarified (Belli and Marquis, 1991; Cusumano and Caparon, 2015; Fulde et al., 2014; Liu and Burne, 2009; Xu et al., 2016), this remains unknown regarding *S. anginosus*. Therefore, in this paper, we investigated the two major acid tolerance mechanisms of *S. anginosus* by which the H^+-ATPase and ADI activities contribute to the viability and growth under low pH environments. Among the five streptococcal species used in this study, *S. anginosus* NCTC 10713, *S. anginosus* DP, and *S. mutans* ATCC 25175 showed markedly higher viabilities under acidic conditions. Furthermore, the tolerance of *S. anginosus* NCTC 10713 and *S. anginosus* DP to the killing by lethal acidification decreased significantly by DCCD only at pH 4.0, but not at pH 7.5. Datta and Benjamin indicated that DCCD inhibits the survival of *Listeria monocytogenes* at pH 3.0 but had no effect on the survival at pH 7.3, suggesting that the H^+-ATPase may be more essential for survival under acidic conditions (Datta and Benjamin, 1997). Our data also demonstrated that H^+-ATPase of *S. anginosus* could be the key player for acid tolerance, especially under low-pH induced lethality.

Xu et al. suggested that ADI in *Streptococcus equi* spp. *zooepidemicus* contributes to environmental adaptability via ammonia synthesis to reduce pH stress (Xu et al., 2016). Furthermore, Cusumano and Caparon suggested that the upregulation of *S. pyogenes* ADI activity promoted growth in a mouse model with soft tissue infection and in human blood (Cusumano and Caparon, 2015). *S. anginosus* NCTC 10713 and *S. anginosus* DP showed significant ADI activity and growth under acidic conditions (pH 5.0),

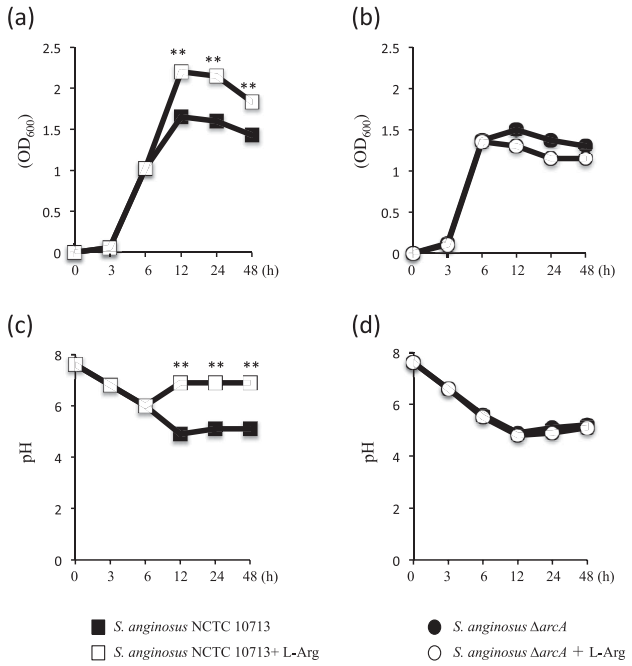

Fig. 4. Effect of L-arginine metabolism on the growth of *S. anginosus*.

S. anginosus strains were grown in THB medium with or without 0.5% L-arginine. The growth of *S. anginosus* was evaluated by measurement of OD_{600} (a), (b). The changes in pH values of the culture media (c), (d) were periodically monitored from 0 h to 48 h. The symbols indicate: *S. anginosus* NCTC 10713 cultured without (closed square) or with 0.5% L-arginine (open square); the *S. anginosus* Δ*arcA* mutant cultured without (closed circle) or with 0.5% L-arginine (open circle). Values and error bars represent means and SD from three independent experiments performed in triplicate. Statistically significant differences are indicated. *, the culture with L-arginine versus without L-arginine; 2 symbols, $P < 0.01$.

which were significantly suppressed in other streptococcal species used in this study, including the Δ*arcA* mutant of *S. anginosus* Δ*arcA*. In our study, we used only one strain of bacterial species except for *S. anginosus*, but several streptococcal strains should be considered for bacterial diversity in future studies. Further, the growth of *S. anginosus* NCTC 10713, but not that of the *S. anginosus* Δ*arcA*, was induced by the exogenous supplementation of L-arginine. Besides, the decreased pH of the culture medium containing L-arginine was increased up to pH 7.0 upon growth of *S. anginosus* NCTC 10713, which could neutralize the culture medium by metabolizing the substrate to produce an alkali. These properties of *S. anginosus*, which is able to adapt and not only grow but also upregulate its growth under acidic conditions, could be attributed, at least in part, to acid tolerance mechanisms. Furthermore, the cell viability of *S. anginosus* Δ*arcA* is observed to be similar to that of *S. anginosus* NCTC 10713 with DCCD at pH 4.0 (data not shown), suggesting that ADI plays a critical role in growth, rather than survival, under acidic conditions.

In conclusion, *S. anginosus* showed significant aciduric properties similar to that of *S. mutans* or *S. pyogenes* which may be attributed to its H^+-ATPase and/or ADI activities. It has been suggested that the acid tolerance of *S. mutans*, *S. pyogenes*, or *S. suis* have contributions to virulence during an infection (Ajami et al., 2015; Cusumano and Caparon, 2015; Fulde et al., 2014; Nemoto et al., 2008). Therefore, the acid tolerance exhibited by *S. anginosus* may facilitate the infection of the oral cavity or gastrointestinal organs by the microorganism, which can lead to chronic inflammation, and, consequently, give rise to infective endocarditis, and abscesses at various body sites.

Acknowledgments

This work was supported, in part, by a Grant-in-Aid for Scientific Research (23659861) and (25462871) from the Ministry of Education, Science, Sports and Culture, Japan.

Conflicts of interest

None of the authors of this manuscript has any financial or personal relationship with other people or organizations that could inappropriately influence their work.

References

Ajami, B., Abolfathi, G., Mahmoudi, E., and Mohammadzadeh, Z. (2015) Evaluation of salivary *Streptococcus mutans* and dental caries in children with heart diseases. *J. Dent. Res. Clin. Dent. Prospects*, **9**, 105–108.

Ambs, S., Hussain, S. P., Marrogi, A. J., and Harris, C. C. (1999) Cancer-prone oxyradical overload disease. *IARC Sci. Publ.*, **150**, 295–302.

Barcelona-Andrés, B., Marina, A., and Rubio, V. (2002) Gene structure, organization, expression, and potential regulatory mechanisms of arginine catabolism in *Enterococcus faecalis*. *J. Bacteriol.*, **184**, 6289–6300.

Belli, W. A. and Marquis, R. E. (1991) Adaptation of *Streptococcus mutans* and *Enterococcus hirae* to acid stress in continuous culture. *Appl. Environ. Microbiol.*, **57**, 1134–1138.

Bender, G. R., Sutton, S. V., and Marquis, R. E. (1986) Acid tolerance, proton permeabilities, and membrane ATPases of oral streptococci. *Infect. Immun.*, **53**, 331–338.

Bradford, M. M. (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.*, **72**, 248–254.

Cotter, P. D. and Hill, C. (2003) Surviving the acid test: responses of Gram-positive bacteria to low pH. *Microbial. Mol. Biol. Rev.*, **67**, 429–453.

Cusumano, Z. T. and Caparon, M. G. (2015) Citrulline protects *Streptococcus pyogenes* from acid stress using the arginine deiminase pathway and the F1Fo-ATPase. *J. Biochem.*, **197**, 1288–1296.

Datta, A. and Benjamin, M. M. (1997) Factors controlling acid tolerance of Listeria monocytogenes: effect of nisin and other ionophores. *Appl. Environ. Microbiol.*, **62**, 4123–4126.

Degnan, B. A., Palmer, J. M., Robson, T., Jones, C. E., Fischer, M. et al. (1998) Inhibition of human peripheral blood mononuclear cell proliferation by *Streptococcus pyogenes* cell extract is associated with arginine deiminase activity. *Infect. Immun.*, **66**, 3050–3058.

Doi, C., Noguchi, Y., Marat, D., Saito, A., Fukuzawa, K. et al. (1999) Expression of nitric oxide synthase in gastric cancer. *Cancer Lett.*, **144**, 161–167.

Dong, Y., Chen, Y. Y., Snyder, J. A., and Burne, R. A. (2002) Isolation and molecular analysis of the gene cluster for the arginine deiminase system from *Streptococcus gordonii* DL1. *Appl. Environ. Microbiol.*, **68**, 5549–5553.

Fallingborg, J. (1999) Intraluminal pH of the human gastrointestinal tract. *Dan. Med. Bull.*, **46**, 183–196.

Fulde, M., Willenborg, J., Huber, C., Hitzmann, A., Willms, D. et al. (2014) The arginine-ornithine antiporter ArcD contributes to biological fitness of *Streptococcus suis*. *Front. Cell. Infect. Microbiol.*, **4**, 1–10.

Giardiello, F. M., Offerhaus, G. J., Lee, D. H., Krush, A. J., Tersmette, A. C. et al. (1993) Increased risk of thyroid and pancreatic carcinoma in familial adenomatous polyposis. *Gut*, **34**, 1394–1396.

Hamilton, I. R. and Buckley, N. D. (1991) Adaptation by *Streptococcus mutans* to acid tolerance. *Oral Microbiol. Immunol.*, **6**, 65–71.

Hassan, H., Lingström, P., and Carlén, A. (2015) Plaque pH in caries-free and caries-active young individuals before and after frequent rinses with sucrose and urea solution. *Caries Res.*, **49**, 18–25.

Iwasaki, T., Higashiyama, M., Kuriyama, K., Sasaki, A., Mukai, M. et al. (1997) N^G-Nitro-L-arginine methyl ester inhibits bone metastasis after modified intracardiac injection of human breast cancer cells in a nude mouse model. *Jpn. J. Cancer Res.*, **88**, 861–866.

Jadeski, L. C. and Lala, P. K. (1999) Nitric oxide synthase inhibition by N(G)-nitro-L-arginine methyl ester inhibits tumor-induced angiogenesis in mammary tumors. *Am. J. Pathol.*, **155**, 1381–1390.

Karita, M., Matsumoto, S., and Kamei, T. (2003) The size of *cagA* based on repeat sequence has the responsibility of the location of *Helicobacter pylori* in the gastric mucus and the degree of gastric mucosal inflammation. *Microbiol. Immunol.*, **47**, 619–630.

Khutoryanskiy, V. V. (2015) Supramolecular materials: Longer and safer gastric residence. *Nat. Mater.*, **14**, 963–964.

Kobayashi, H. (1985) A proton-translocating ATPase regulates pH of the bacterial cytoplasm. *J. Biol. Chem.*, **260**, 72–76.

Liu, Y. and Burne, R. A. (2009) Multiple two-component systems modulate alkali generation in *Streptococcus gordonii* in response to environmental stresses. *J. Bacteriol.*, **191**, 7353–7362.

Maghnouj, A., de Sousa Cabral, T. F., Stalon, V., and Vander, W. C. (1998) The *arcABDC* gene cluster, encoding the arginine deiminase pathway of *Bacillus licheniformis*, and its activation by the arginine repressor argR. *J. Bacteriol.*, **180**, 6468–6475.

Mak, G. S., Milliken, J. C., and Saremi, F. (2011) Multisite infective endocarditis with mural vegetations in the right atrium and right ventricle. *Circulation*, **123**, 457–458.

Marrogi, A., Pass, H. I., Khan, M., Metheny-Barlow, L. J., Harris, C. C. et al. (2000) Human mesothelioma samples overexpress both cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (NOS2): in vitro anti proliferative effects of a COX-2 inhibitor. *Cancer Res.*, **60**, 3696–3700.

Nemoto, H., Nakano, K., Nomura, R., and Ooshima, T. (2008) Molecular characterization of *Streptococcus mutans* strains isolated from the heart valve of an infective endocarditis patient. *J. Med. Microbiol.*, **57**, 891–895.

Oginsky, E. L. (1957) Isolation and determination of arginine and citrulline. *Methods Enzymol.*, **3**, 639–643.

O'Hanlon, D. E., Moench, T. R., and Cone, R. A. (2013) Vaginal pH and microbicidal lactic acid when *Lactobacilli dominante* the microbiota. *PLoS ONE*, **8**, e80074.

Rahman, M., Nguyen, S. V., McCullor, K. A., King, C. J., Jorgensen, J. H. et al. (2016) Comparative genome analysis of the daptomycin-resistant *Streptococcus anginosus* strain J4206 associated with breakthrough bacteremia. *Genome Biol. Evol.*, **8**, 3446–3459.

Sasaki, H., Igaki H., Ishizuka, T., Kogoma, Y., Sugimura, T. et al. (1995) Presence of *Streptococcus* DNA sequence in surgical specimens of gastric cancer. *Jpn. J. Cancer Res.*, **86**, 791–794.

Sasaki, M., Ohara-Nemoto, Y., Tajika, S., and Kaneko, M. (1995) Induction of inflammatory cytokine and cyclooxygenase-2 mRNA expression by secreted substances from oral streptococci. *Dent. J. Iwate Med. Univ.*, **20**, 284–290.

Sasaki, M., Ohara-Nemoto, Y., Tajika, S., Kobayashi, M., Yamaura, C. et al. (2001) Antigenic characterisation of a novel *Streptococcus anginosus* antigen that induces nitric oxide synthesis by murine peritoneal exudate cells. *J. Med. Microbiol.*, **50**, 952–958.

Sasaki, M., Yamaura, C., Ohara-Nemoto, Y., Tajika, S., Kodama, Y. et al. (2005) *Streptococcus anginosus* infection in oral cancer and its infection route. *Oral Dis.*, **11**, 151–156.

Scott, D. R., Marcus, E. A., Weeks, D. L., and Sachs, G. (2002) Mechanisms of acid resistance due to the urease system of *Helicobacter pylori*. *Gastroenterology*, **123**, 187–195.

Shah, M. S., DeSantis, T. Z., Weinmaier, T., McMurdie, P. J., Cope, J. L. et al. (2017) Leveraging sequence-based faecal microbial community survey data to identify a composite biomarker for colorectal cancer. *Gut*, doi:10.1136/gutjnl-2016-313189.

Shibata, Y., Kawada, M., Nakano, Y., Toyoshima, K., and Yamashita, Y. (2005) Identification and characterization of an autolysin-encoding gene of *Streptococcus mutans*. *Infect. Immun.*, **73**, 3512–3520.

Shiga, K., Tateda, M., Saijo, S., Hori, T., Sato, I. et al. (2001) Presence of *Streptococcus* infection in extra-oropharyngeal head and neck squamous cell carcinoma and its implication in carcinogenesis. *Oncol. Rep.*, **8**, 245–248.

Takahashi, N. and Yamada, T. (1998) Acid-induced acid tolerance and acidogenicity of non-mutans streptococci. *Oral Microbiol. Immunol.*, **14**, 43–48.

Tateda, M., Shiga, K., Saijo, S., Sone, M., Hori, T. et al. (2000) *Streptococcus anginosus* in head and neck squamous cell carcinoma: implication in carcinogenesis. *Int. J. Mol. Med.*, **6**, 699–703.

Terzi, H. A., Demiray, T., Koroglu, M., Cakmak, G., Ciftci, I. H. et al. (2016) Intra-abdominal abscess and primary peritonitis caused by *Streptococcus anginosus*. *Jundishapur J. Microbiol.*, **9**, e33863.

Wen, Y., Marcus, E. A., Matrubutham, U., Gleeson, M. A., Scott, D. R. et al. (2003) Acid-adaptive genes of *Helicobacter pylori*. *Infect. Immun.*, **71**, 5921–5939.

Wenzler, E., Chandrasekaran, V., Salvador, P., Anwar, M., Pancholi, P. et al. (2015) Clinical and microbiological outcomes in patients with *Streptococcus anginosus* group bacteraemia identified through use of a rapid microarray assay. *J. Med. Microbiol.*, **64**, 1369–1374.

Whiley, R. A., Beighton, D., Winstanley, T. G., Fraser, H. Y., and Hardie, J. M. (1992) *Streptococcus intermedius*, *Streptococcus constellatus*, and *Streptococcus anginosus* (the *Streptococcus milleri* group): association with different body sites and clinical infections. *J. Clin. Microbiol.*, **30**, 243–244.

Willcox, M. D. (1995) Potential pathogenic properties of members of the "*Streptococcus milleri*" group in relation to the production of endocarditis and abscesses. *J. Med. Microbiol.*, **43**, 405–410.

Winterhoff, N., Goethe, R., Gruening, P., Rohde, M., Kalisz, H. et al. (2002) Identification and characterization of two temperature-induced surface-associated proteins of *Streptococcus suis* with high homologies to members of the arginine deiminase system of *Streptococcus pyogenes*. *J. Bacteriol.*, **184**, 6768–6776.

Wiśniewska, M., Nilsson, H. O., Bąk-Romaniszyn, L., Rechciński, T., Bielański, W. et al. (2002) Detection of specific *Helicobacter pylori* DNA and antigens in stool samples in dyspeptic patients and healthy subjects. *Microbiol. Immunol.*, **46**, 657–665.

Xu, B., Yang, X., Zhang, P., Ma, Z., Lin, H. et al. (2016) The arginine deiminase system facilitates environmental adaptability of *Streptococcus equi* ssp. *zooepidemicus* through pH adjustment. *Res. Microbiol.*, **167**, 403–412.

Zeng, L., Dong, Y., and Burne, R. A. (2006) Characterization of cis-acting sites controlling arginine deiminase gene expression in *Streptococcus gordonii*. *J. Bacteriol.*, **188**, 941–949.

Zhang, X. J., Thompson, J. H., Mannick, E. E., Correa, P., and Miller, M. J. (1998) Localization of inducible nitric oxide synthase mRNA in inflamed gastrointestinal mucosa by *in situ* reverse transcriptase-polymerase chain reaction. *Nitric Oxide*, **2**, 187–192.