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Original Article

# Growth behavior of eikenella corrodens and streptococcus gordonii in response to a short chain fatty acid metabolite-acetic acid

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## Abstract

**Aim:** Periodontal diseases are chronic, inflammatory and infectious diseases. Therefore, periodontal treatment aims to eliminate periodontopathogenic bacteria causing periodontal diseases. The aim of present study was to evaluate the effect of a bacterial end metabolite, acetic acid, on periodontopathogenic bacteria, Streptococcus gordonii and Eikenella corrodens.

**Material and Method:** In present research, Eikenella corrodens (ATCC® 23834™) and Streptococcus gordonii (NCTC 7870) were tested. Acetic acid was used in 5% concentration dissolved in distilled water. Negative control agent was distilled water and positive control agents were 0.012% chlorhexidine, penicillin, tetracycline and ciprofloxacin. The antibacterial efficacy of acetic acid against bacteria was tested via disc-diffusion method, MIC test and minimum bactericidal concentration tests.

**Results:** The inhibition zone of ciprofloxacin, penicillin, tetracycline, CHX and acetic acid against Eikenella corrodens and Streptococcus gordonii were 32 and 37 mm, 16 and 14 mm, 21 and 16 mm, 13 and 17 mm, and 14 and 11 mm respectively. Ciprofloxacin and penicillin inhibited bacterial growth in MIC and MBC tests against both bacteria. MIC tests of acetic acid and chlorhexidine against Eikenella corrodens revealed inhibitory effect at 7.81 µl/mL and 0.97 µl/mL concentrations, respectively. Against Streptococcus gordonii, MIC of acetic acid and chlorhexidine were 1.95 µl/mL and 3.90 µl/mL, respectively.

**Conclusion:** Acetic acid is a bacterial end product and has a daily consumption as vinegar. Due to the antibacterial efficacy against periodontopathogenic bacteria, it can be useful in adjunction to periodontal treatment. Further studies to evaluate clinical use of acetic acid as mouthwash, dentifrice, gel and/or irrigation agent are necessary.

**Keywords:** Acetic Acid; Oral Health; Periodontopathogenic Bacteria; Short Chain Fatty Acids.

## INTRODUCTION

Periodontal disease is chronic and infectious disease which cause soft tissue loss and bone destruction around teeth. Infectious character of the disease result from periodontopathogenic bacteria found in dental plaque (1). Bacteria initiate a series of inflammatory reaction and elimination of dental plaque usually provides an improvement (2).

Mechanical cleaning is generally enough for elimination of dental plaque. In addition,antibiotics can be necessary for certain forms of diseases such as aggressive periodontitis, necrotizing ulcerative gingivitis, chronic periodontitis which does not respond periodontal treatment or recurrence of the disease after treatment (3,4). Chemotherapeutic agents such as chlorhexidine mouth rinsing solution should alsobe recommended besides antibiotics in the post- operative care period. However, long-term use of antibacterial agents cause further problems likeantibiotic resistance, suppression of regular oral microbiota and superinfection with Candida spp (5). Therefore new approaches to prevent bacterial accumulation is necessary. Bacteria in dental plaque synthesize exopolysaccharide to adhere each other and tooth surfaces. This well-organized association of bacteria is called biofilm.

Biofilm is a survival mechanism responsible for metabolism, energy production and communication. Extracellular matrix structure in biofilm also protects inhabitant bacteria from environmental threads such as antibiotics and provides an advantage over planctonic bacteria (6).

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Eikenella corrodens is a Gram(-), slowly growing, facultative anaerobe that is frequently found in the mouth and upper respiratory tract of humans (7). E. corrodens belongs to native oral flora, but may also be an opportunistic pathogen. Scientific research reported that E. corrodens participates in the early stages of biofilm formation by specific co-aggregation with some Gram (+) and Gram (-) bacteria found in human periodontal pockets (8). Furthermore, E. corrodens mono-infection in germ-free rats resulted in periodontal disease with severe alveolar bone loss, indicating its role as aperiodontopathogenic bacteria (9). Streptococcus gordonii is a gram-positive, stationary, facultative anaerobe bacteria which is also one of commensal species of the human oral flora (10). S. gordonii; plays a central role in biofilm maturation by initiating biofilm formation and providing binding sites for subsequent colonizers such as Porphyromonas gingivalis (11).

Long fimbria (FimA) of P. gingivalis is linked to glyceraldehyde-3-phosphate dehydrogenase, which is located on the surface of S. gordonii (12). In addition, short fimbria (Mfa) of P. gingivalis engages streptococcal SspA / B (antigen I / II) adhesins through approximately 80 amino acid binding epitopes (13). Therefore, interaction between S. gordonii and P. gingivalis is believed to play an important role in the development of bacterial populations associated with the onset and progression of severe periodontal disease forms (14).

Supporting the role of S. gordonii in biofilm formation, it was demonstrated that high amount of S. gordonii in the dental plaque has been associated with periodontal inflammation (15). S. gordonii is metabolically compatible with bacteria identified as definite periodontopathogens, such as A.actinomycetemcomitans (16), F.nucleatum and P. gingivalis (17, 18). Besides, S. gordonii, has recently been proposed as a member of a specific group of bacteria called "Helper Pathogens Facilitating the Formation of Periodontal Disease" (19).

Most of the bacteria in oral cavity can synthesize bacteriocins (20), quorum sensing molecules (unique communication mechanism of bacteria) (21), and metabolic end-products (22) to gain an advantage in a competitive environment like oral cavity. Some of these metabolites are short chain fatty acids (SCFAs) like acetic acid, butyric acid, propionic acid. Recently, SCFAs were suggested to have a role in competitive and/or mutualistic interactions and bacterial communication (21, 23).

SCFAs could even take a part in quorum sensing. Most of the bacteria responsible for dental caries and periodontal diseases can produce these SCFAs. However, the role of SCFAs in periodontontal diseases development and on periodontopathogenic bacteria needs to be clarified (24).

Acetic acid is a bacterial metabolite which has a wide range use in daily life. SCFAs and the possible role in dental biofilm is a relatively popular topic with an increasing interest. Therefore, the aim of present study was to evaluate the growth behavior of Streptococcus gordonii and Eikenella corrodens in response to acetic acid.

## MATERIAL and METHODS

This study was carried out under the supervision of Prof. Dr Isa KARAMAN at Microbiotechnology Laboratory of Gaziosmanpaşa University Faculty of Engineering and Natural Sciences, Department of Bioengineering. This laboratory complies with international laboratory standards. Acetic acid (Sigma) was used as test material. Penicillin, ciprofloxacin, tetracycline, metronidazole and chlorhexidine (CHX) were used as positive controls and distilled water was used as negative control. All solutions except CHX were prepared as 5% dilutions of each material in distilled water. 0.012% CHX was used. Antibacterial efficacy of acetic acid was test via Kirby- Bauer (Disc-diffusion) method and minimum inhibitory concentration and minimum bactericidal concentrations were also determined.

Disc-diffusion method (25)
The bacterial species and strains used in this study were E.corrodens (ATCC 23834) and S.gordonii (NCTC 7870). The antimicrobial activity was determined with the disc-diffusion method.Firstly, nutrient agar (NA) was prepared and 108 CFU/mL of bacteria was added to 100 mL NA solution. Then, bacteria were inoculated to the petri dish containing Mueller-Hinton agar (MHA) medium which does not include any indicator or inhibitor. 38.0 g/L MHA was sterilized by autoclave (121°C, 15 min). After cooling to 45-50 °C 5% de-fibrinated sheep blood was added. 20 mL of blood-enriched MHA was poured to sterile petri dishes. The blank discs (6 mm diameter, Oxoid) were impregnated with 20 mL of test compound dissolved in distilled water (105 µg/disc) and placed on the inoculated agar.

The inoculated plates were incubated at aerobic conditions with 36°C for 24 h. After incubation, the growth inhibition zones were measured via a millimetric scale. The procedure was repeated thrice and the arithmetic mean of three measurements were recorded as one inhibition zone. The results were shown in Table 1 and Table 2.

**Table 1. The mean value of S. gordonii inhibition zone, MIC and MBC values. X: Not detected**

| | | Streptococcus gordonii | |
|---|---|---|---|
| Agents | Inhibition zone | MIC value | MBC value |
| Penicillin | 16 mm | MIC was not detected in 50-0.0243 dilutions | x |
| Tetracycline | 16 mm | MIC was not detected in 50-0.0243 dilutions | x |
| Ciprofloxacin 0.12% | 37 mm | MIC was not detected in 50-0.0243 dilutions | x |
| Chlorhexidine 0.12% | 17 mm | 3.905 µg/ml | 31.25 µg/ml |
| Acetic acid 5% | 11 mm | 1.9525 µg/ml | 125 µg/ml |

MIC Values of test materials against E. corrodens and S. gordonii were determined with a micro-well dilution method. Tryptic soy broth (TSB) was used in MIC tests. TSB; 20 gr tryptone, 5 gr soytone, 5 gr NaCl, 950 ml distilled water were mixed to form a 30 gr/L solution. And then sterilized with autoclave (121°C,15 min). After cooling to 47°C, 5.0µg/mL hemin and 0.5 µg/mL vitamin K1 were added and gently mixed. The inoculum of microorganisms were prepared using 12 h broth cultures, and suspensions were adjusted to 0.5 McFarland standard turbidity. Acetic acid and the positive control agents dissolved in distilled water were first diluted to the highest concentration tested (1000 mg/ml), and then serial twofold dilutions were made (concentration range 7.8–1000 mg/ml) in sterile 10-ml test tubes containing TSB. 96-Well plates were prepared by dispensing 95 ml of TSB and 5 ml of the inoculums into each well. Then, 100 ml of solutions were added. Wells containing 195 ml of TSB without compound and 5 ml of the inoculums were used as negative control. The final volume in each well was 200 ml. The 96-well plates were incubated at 368 for 24 h. The assay was performed in triplicate. MBC tests.

**Table 2. The mean value of E. corrodens inhibition zone, MIC and MBC values. X: Not detected**

| | | Streptococcus gordonii | |
|---|---|---|---|
| Agents | Inhibition zone | MIC value | MBC value |
| Penicillin | 14 mm | MIC was not detected in 50-0.0243 dilutions | x |
| Tetracycline | 21 mm | 0.390 µg/ml | 0.390 µg/ml |
| Ciprofloxacin | 32 mm | MIC was not detected in 50-0.0243 dilutions | x |
| Chlorhexidine | 13 mm | 0.97625 µl/ml | 0.97625 µl/ml |
| Acetic acid 5% | 14 mm | 0.97625 µl/ml | 500 µg/ml |
| MIC tests | | | |

Samples were taken from MIC test tubes and inoculated on petri dishes containing MHA. The lowest concentration inhibiting bacterial growth was recorded as MBC.

## RESULTS

In the tests of S. gordonii, the most effective antibiotic was ciprofloxacin with an inhibition zone of 37 mm. The inhibition zones of penicillin and tetracycline were 16 mm.CHX provided a slightly better inhibition zone as 17 mm. Regarding MIC and MBC results, antibiotics inhibited bacterial growth even in the lowest concentration in both MIC and MBC tests. However, CHX was not strong as the antibiotics with a 3.90µg/mL MIC concentration and 31.25 µg/mL MBC concentration. Acetic acid caused a narrow inhibition zone compared to CHX. In addition, MIC value of acetic acid was two-fold lower than CHX while MBC value was four-fold higher.

In the tests of E. corrodens, similar with S. gordonii, the most effective antibiotic was ciprofloxacin with an inhibition zone of 32 mm. The second effective antibiotic was tetracycline with an inhibition zone of 21 mm and lastly the inhibition zone of penicillin was 14 mm. In terms of MIC and MBC results, penicillin and ciprofloxacin inhibited bacterial growth even in the lowest concentration in both MIC and MBC tests. However, tetracycline inhibited bacterial growth in 0.39 µg/mL concentration in both MIC and MBC tests. CHX was not strong as the antibiotics with an inhibition zone of 13 mm, and MIC and MBC concentrations of 0.97 µg/mL. Acetic acid caused a slightly wider inhibition zone compared to CHX as 14 mm. But Mic and MBC values were much higher. The results of S. gordonii and E. corrodens were shown in table 1 and 2, respectively.

## DISCUSSION

Present study evaluated behaviors of S. gordonii and E. corrodens in response to acetic acid. Results demonstrated that acetic acid inhibited bacterial growth and had a strong antibacterial efficacy equivalent to CHX, observed in disc-diffusion method.

Most of the bacterial species in oral cavity are anaerobic and/or facultative anaerobic bacteria (26). These bacteria provide their energy from phosphorylation at substrate level and produce metabolic end products such as long, medium and short chain fatty acids (27). These byproducts inhibit other bacterial cells and even host defense mechanism to survive (28,29). Therefore, application of these compounds as irrigation and/or mouthwash might help preventing biofilm formation and/or disrupting formed biofilm structure. Thus, SCFAs might be beneficial as an adjunctive agent to periodontal therapy.

Recently, studies evaluating the role of SCFAs in periodontal diseases, oral microbiota and bone metabolism are rapidly increasing. Lu et al. have shown that acetic acid, propionic acid, butyric acid levels were elevated in gingival crevicular fluid of generalized aggressive periodontitis patients. They also reported that increased levels of these SCFAs were associated with periodontal infection (30). Saito et al. has suggested that acetoacetate stimulated osteoblastic activity and alkaline phosphatase levels while b-hydroxybutyrate suppressed osteoblastic activity (31). In contrast, another recent study found that butyrate increased bone collagen, alkaline phosphatase and ostegenic differentiation (32). Provenzano et al. also demonstrated that SCFAs have significant roles in pathogenesis of apical periodontitis (33).

Gingival fibroblasts are key cells in periodontal tissue healing. Periodontal pathogens produce butyrate and recently, butyrate was shown to participate in periodontitis development by inhibiting gingival fibroblast cell growth. Butyrate toxicity against human fibroblasts was attributed to increased reactive oxygen species produced by pathogenic bacteria (34). Other than butyrate, acetic acid and succinic acid were also found to be related to gingival inflammation (35).

Present study evaluated the effect of one of the SCFAs, acetic acid, on two different pathogenic bacteria. Streptococcus gordonii is particularly important in biofilm formation. S. gordonii can synthesize quorum sensing molecules and provide an adhesion surface for Porphyromonas gingivalis which is one of the most importantperiodontopathogenic bacteria.

Therefore, inhibiting biofilm properties of S. gordonii might decrease risk of periodontal disease development. E. corrodens is one of the organisms which was found to be related to periodontal infection. E. corrodens is a gram negative facultative anaerobic microorganism which belongs to green complex described by Socransky et al.(36,37). E. corrodens is usually isolated from periodontal lesions. Studies reported that E. corrodens is associated with deep periodontal pockets and bacterial counts decreased after successful periodontal treatment (9).

S. gordonii can produce several acids including lactic acid and acetic acid (38). In addition, a recent research reported that S. gordonii was very sensitive to SCFAs (24). The results of present study demonstrated that ciprofloxacin is the strongest antibiotic followed by penicillin and tetracycline. However, CHX showed more efficacy against S. gordonii than penicillin and tetracycline.

Furthermore, acetic acid inhibited S. gordonii cell growth but not higher than tested antibiotics or CHX. All tested antibiotics strongly inhibited S. gordonii by preventing bacterial growth even in the lowest MIC and MBC concentration. Apart from inhibition zone, MIC value of acetic acid was two folds lower than CHX. In terms of E. corrodens, ciprofloxacin provided the widest inhibition zone, 32 mm, followed by tetracycline, 21 mm, and penicillin, 14 mm. As observed in S. gordonii, ciprofloxacin and penicillin did not allow E. corrodens growth in MIC and MBC tests even in the lowest concentrations. This results showed that the efficacy of ciprofloxacin and penicillin against E. corrodens was higher than tetracycline which prevented bacterial growth in MIC and MBC concentrations higher than 0.39 µg/mL (39, 40).

In addition, acetic acid demonstrated higher efficacy than CHX but equal to penicillin. Inhibition zone of acetic acid against E. corrodens was greater than S. gordonii while MIC and MBC values were much lower. Other than being an end-metabolite, acetic acid is a daily used product as vinegar. Therefore, daily consumption might provide additional antibacterial effect on periodontal therapy.

## CONCLUSION

Biofilm is a complex, well-organized structure which protects bacteria against environmental threads. However, bacterial metabolites produced within biofilm such as SCFAs can inhibit other bacterial strains and disrupt biofilm. Acetic acid as an end metabolite and a daily consumed product, can be used against bacteria and be beneficial in periodontal therapy without any serious side effects.

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