

EFFECT OF AMMONIUM HEXAFLUOROSILICATE ON INHIBITING GROWTH OF *VEILLONELLA PARVULA*

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ABSTRACT

Objectives: This study analyzes the effects of ammonium hexafluorosilicate (AHF) on *Veillonella parvula*.

Methods: In this *in vitro* study, solutions of NaF (25%, 50%, and 100%), AHF (25%, 50%, and 100%), and SDF (38%) were applied to solid medium cultures of *V. parvula* and *Streptococcus mutans*. The disc diffusion method was used for testing bacterial sensitivity.

Results: AHF was less effective than SDF but more effective than NaF for inhibiting growth of bacteria.

Conclusions: AHF could be effective for inhibiting the growth of *V. parvula* without the side effects of SDF.

Keywords: Arrested caries product, *Streptococcus mutans*, *Veillonella parvula*.

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INTRODUCTION

Reports from Basic Health Research (RISKESDAS) state that the prevalence of caries in Indonesia is 72.1% [1]. Dental caries continues to be a global health problem, affecting humans of all ages, particularly children where caries disease is on the rise [2]. The caries process can begin at an early age immediately after a tooth erupts in the oral cavity. Caries suffered by infants and children under 5 years old is defined as early childhood caries (ECC). The prevalence of ECC in the United States is 90%, while in Thailand and Indonesia it is 82.8% and 81.2%, respectively [3]. ECC is caused by prolonged use of baby bottles or breastfeeding. ECC typically occurs in a short period, involves several teeth at once, and causes many teeth to turn a white or yellowish [4]. Previous study found that parents usually do not maintain proper dietary habits for their children which may lead to the increased incidence of caries in childhood [5].

In Indonesia, fluoride has been used to prevent caries. While one fluoride preparation used is sodium fluoride (NaF), previous research has shown that the combination of fluoride and silver in the form of silver diamine fluoride (SDF) is more effective in preventing childhood caries in enamel and stopping the progression of disease to dentin. However, problems have arisen from the use of SDF, such as tooth discoloration and metal aftertaste [6]. Tooth discoloration caused by SDF can be avoided use of an ammonium hexafluorosilicate (AHF) preparation. However, there is no adequate research regarding the effect of AHF on oral bacteria. Therefore, this research will study the effect of AHF on inhibiting the growth of *Veillonella parvula*.

METHODS

The experiments in this research used an *in vitro* method for testing the effects of fluoride solutions on the growth of bacteria. The samples used were *V. parvula* (ATCC 10790) and *Streptococcus mutans* (ATCC 25175). All tools used were prepared and sterilized using an autoclave at 121°C for 15 minutes.

Seeding media preparation

Brain heart infusion (BHI) broth

To prepare the BHI broth, 37 g of BHI was prepared in an Erlenmeyer tube filled with 1000ml aquades. After the BHI completely dissolved, the Erlenmeyer tube was closed with cotton, and the entrance was covered with aluminum foil. The tube was then sterilized using an autoclave for 15 minutes. Finally, 4ml of vitamin K solution was added, and the tube was refrigerated at 4°C. To breed *V. parvula* in the prepared BHI broth medium, a reaction tube, an Eppendorf pipette, and disposable tips sized 1000 µL and 200 µL were sterilized and prepared. The *V. parvula* and *S. mutans* bacteria stock was then moved from an -80°C refrigerator into a cool box filled with ice. Next, 10 ml of BHI broth was moved into two reaction tubes using a micropipette and disposable tips sized 1000 µL. 10 µL each of *V. parvula* and *S. mutans* were taken using disposable tips sized 200 µL and poured into a reaction tube-containing BHI broth. The reaction tube was closed with cotton and sealed in a zipper bag filled with a gas pack. The tube was then incubated for 24 hrs at 37°C.

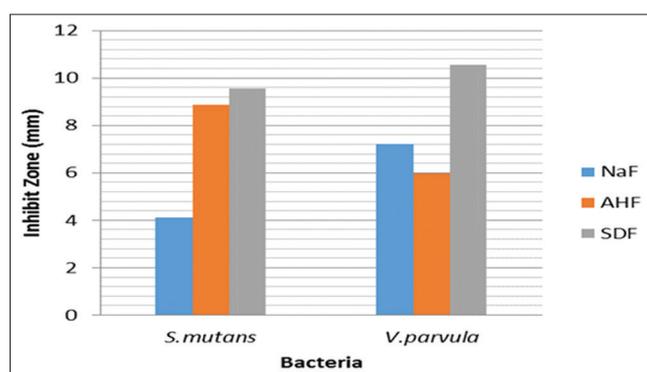
BHI agar

To prepare the BHI agar, 37 g of BHI powder and 13 g of Bacto agar were used. The BHI and agar were dissolved in an Erlenmeyer tube-containing 1000 ml of aquades. After the solution was completely dissolved, the Erlenmeyer tube was closed with cotton, and the top was covered with aluminum foil. The tube was sterilized using an autoclave and chilled until 50°C. Then, 20 ml of BHI agar solution was poured into a petri dish and set aside until it hardened. It was refrigerated at 4°C. To breed *V. parvula* in the agar medium, the following items were prepared: A sterilized micropipette; the BHI agar medium; inoculating loops; and *V. parvula* and *S. mutans* bacteria. A Bunsen burner was used to maintain the sterility of the working environment. The neck of the tube that contained the bacteria was heated to prevent contamination. Then, the inoculating loops was heated in the Bunsen flame until it began to smolder. The heated hose was then chilled in agar. Once cooled, the hose was used to make a single etching in the BHI agar with the bacteria. Finally, the agar medium that contained the bacteria was incubated at 37°C for 24 hrs in anaerobic conditions.

Table 1: Inhibit zone diameter (mm) of AHF, NaF, and SDF for *S. mutans* and *V. parvula* breed

Solution	<i>S. mutans</i>	<i>V. parvula</i>
NaF 25%	4.65	4.05
NaF 50%	3.5	7.85
NaF 100%	4.25	9.8
Mean NaF	4.13	7.23
AHF 25%	8.05	3.9
AHF 50%	10.95	4.55
AHF 100%	7.6	9.45
Mean AHF	8.86	5.96
Mean SDF 38%	9.55	10.56

AHF: Ammonium hexafluorosilicate, NaF: Natrium fluoride,
S. mutans: *Streptococcus mutans*, *V. parvula*: *Veillonella parvula*

**Fig. 1: Mean inhibit zone diameter around coated solution disk**

Creating AHF and NaF test material in concentrations of 100%, 50%, and 25%

To create the AHF and NaF solutions, 1 ml of AHF and NaF were poured inside of two Eppendorf tubes to get a 100% solution of each. Next, 0.5 ml of each solution was poured into two Eppendorf tubes, and 0.5 ml of aquabides was added to each to make a 50% solution. Then, 0.25 ml of each solution and 0.75 ml of aquabides was added to two Eppendorf tubes to get a concentration of 25%. Finally, 0.03 ml of each SDF, AHF, and NaF solutions (100%, 50%, and 25%) was spread on a blank disk. A blank disk diffusion test was performed by pouring *V. parvula* and *S. mutans* into a petri dish-containing BHI agar and then placing the prepared disk-containing 0.03 ml of test material inside the petri dish containing the bacteria breed. The petri dish was then incubated in anaerobic conditions at 37°C for 24 hrs.

Observation

The test media was placed in a colony counter and then observed in the monitor. Zone counting mode in the colony counter monitor was activated. The inhibit zone diameter was found using the device's scan button. The same procedure was repeated for all test media.

RESULTS

Table 1 shows that the inhibit diameter zone values were greater than zero, which means that an inhibit zone was formed in all test materials and for all solution concentrations. Fig. 1 also shows mean inhibit zone diameter around coated solution disk. The results of this research also show that the SDF inhibition zone had a relatively high value.

DISCUSSION

The results shows that the inhibit diameter zone values were greater than zero, which means that an inhibit zone was formed in all test materials and for all solution concentrations. These values were

the result of fluoride-containing test materials that inhibited the colonization of bacteria. As previous research has stated, fluoride solutions influence the remineralization process, and affect bacteria and plaque growth [7,8]. Table 1 also shows that the mean inhibit zone diameter value in AHF for *V. parvula* was greater than for *S. mutans* while the mean inhibit zone diameter value in SDF for *S. mutans* was greater than for *V. parvula*.

Based on these results, AHF more effectively inhibits *S. mutans* growth, while SDF more effectively inhibits the growth of *V. parvula*. These results, which demonstrate that silver has a better ability to inhibit bacteria than does silica, is supported by previous research that explains that the tolerance of Gram-positive bacteria to silver is higher than Gram-negative bacteria. This is due to structural differences because Gram-positive bacteria have more peptidoglycan layers in its membrane cell than gram-negative bacteria [9,10].

The results of this research also show that the NaF inhibition zone had a relatively low value. The active ingredient of NaF is fluoride which has a diminished ability to kill bacteria without another active ingredient such as silica in AHF or silver in SDF. Again, this result is supported by previous research which showed that fluoride ion alone has less effectivity than fluoride combined with another material [11,12].

CONCLUSION

This research concludes that AHF inhibits *V. parvula* growth effectively and that the most optimal inhibition was achieved in a 100% concentration of AHF. Further studies and research are suggested to examine the efficacy of AHF application beyond single-celled bacteria, and also to examine whether AHF could be used in place of SDF without the side effects.

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