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Research Article

Hericium erinaceus powder inhibits the growth of *Porphyromonas gingivalis*

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Abstract

Disruption of the oral microbiota may contribute to the pathogenesis of oral diseases such as gingivitis. It is desired to explore for a natural material that is safe for the body and specifically suppresses the growth of periodontopathic bacteria. *Hericium erinaceus*, which is consumed as food or used as a herbal medicine in East Asia, has been previously reported to inhibit bacterial growth. However, few studies addressed its effects on oral bacteria. Here we determined the effects of *H. Erinaceus* Powder (HEP) on the periodontal pathogenic bacterium *Porphyromonas gingivalis* and a nonpathogenic commensal bacterium *Streptococcus gordonii*. The antibacterial activity of HEP were evaluated by colony forming unit assay. As a result, HEP significantly inhibited the growth of *P. gingivalis* in a concentration-dependent manner, but in contrast, did not exhibit significant antibacterial activity against *S. gordonii*. *H. erinaceus* may selectively inhibit the growth of *P. gingivalis* and be useful for prevention of periodontal disease.

Abbreviations

ATCC: American Type Culture Collection; CFU: Colony Forming Unit; eTSB: Enriched Tryptic Soy Broth; HEP: *Hericium Erinaceus* Powder; *P. gingivalis*: *Porphyromonas gingivalis*; *S. gordonii*: *Streptococcus gordonii*

Introduction

Maintenance of the oral microbiota contributes to health. Homeostasis between resident oral microbes may be disrupted by external factors such as diet, smoking, and consumption of antibiotics [1]. Disruption of the oral microbiota may contribute to the pathogenesis of oral diseases such as gingivitis [2]. The periodontal bacterium *Porphyromonas gingivalis* causes gingivitis and periodontitis [3]. Streptococcal species, which dominate the bacterial population of the human oral cavity, comprise beneficial commensals such as *Streptococcus sanguinis*, *Streptococcus cristatus*, *Streptococcus mitis*, *Streptococcus oralis*, and *Streptococcus gordonii*. Commensal streptococci inhibit the growth of pathogenic species and maintain oral health [4]. For example, *S. gordonii* generates alkali from arginine in salivary and dietary substrates to raise the pH of an acidic cariogenic microenvironment [5]. Furthermore, *S. gordonii* generates H₂O₂, which suppresses the proliferation of pathogens such as *P. gingivalis* [6]. Cetylpyridinium chloride and chlorhexidine

inhibit the growth of beneficial as well as pathogenic oral bacteria [7]. Moreover, certain antibiotics may lead to an increase in the populations of antibiotic-resistant bacteria [8]. Inappropriate use of antibiotics may cause adverse effects such as allergic reactions and end-organ toxic effects [9]. Therefore, safe and specific antibiotics that inhibit the growth of oral bacterial pathogens are required. Certain mushrooms possess antimicrobial activity [10–13]. For example, *H. erinaceus*, known as Yamabushitake in Japan, has served since ancient times as food and natural medicine in East Asia. We found that *H. erinaceus* possesses strong antibacterial activity against *P. gingivalis*, consistent with the findings of others [14–16]. However, little information is available about the antibacterial potential of *H. erinaceus* against oral bacteria. Here we used a colony forming assay to determine the effects of *H. erinaceus* on oral bacteria.

Material and methods

Materials and bacterial strains

Fruit bodies of *H. erinaceus* cultivated by the Hokuto Corporation (Nagano, Japan) were freeze-dried and milled to prepare *H. Erinaceus* Powder (HEP). The bacterial strains *P. gingivalis* ATCC 33277 and *S. gordonii* ATCC 10558 were purchased from the American Type Culture Collection (ATCC: Manassas,

VA, USA) and cultured under anaerobic conditions at 37°C in enriched Tryptic Soy Broth (eTSB; Difco Laboratories, Detroit, MI, USA) containing 1 g/L yeast extract (BD, Franklin Lakes, NJ, USA) supplemented with 0.005 g/L hemin (Wako Pure Chemical industries, Ltd., Osaka, Japan) and 0.001 g/L menadione (Wako Pure Chemical industries, Ltd., Osaka, Japan).

Colony Forming Unit (CFU) assay

After two passages from thawing of frozen stocks of *P. gingivalis* and *S. gordonii*, the bacterial cells were diluted 1/20 (v/v) with eTSB medium, and 500 µL of the bacterial suspensions and 500 µL of HEP suspensions (0.25–4 mg/mL) were poured into a 24-well plate and incubated at 37°C for 3, 6, 9, and 24 h. Sterile water was used as a control instead of the HEP suspension. After incubation with HEP, bacteria were harvested and serially diluted in degassed phosphate-buffered saline (pH 7.4). *P. gingivalis* was plated on Trypto-Soya agar (Nissui, Tokyo, Japan) containing 5% (v/v) defibrinated sheep blood (Nippon Bio test laboratories INC., Saitama, Japan). *S. gordonii* was plated on Trypto-Soya agar without sheep blood. Plates were incubated under anaerobic conditions at 37°C, and the colonies were counted.

Statistical analysis

Data were expressed as the means ± standard deviation (S.D.). All statistical analysis were performed using EZR

(v1.42) [17]. Data were analyzed using one-way analysis of variance (ANOVA) with Dunnett's test, and $p < 0.05$ indicates a significant difference.

Results

Figure 1 shows the CFU/mL of *P. gingivalis* and *S. gordonii* cultured with or without 4 mg/mL of HEP for 3, 6, 9, and 24 h. The growth of *P. gingivalis* was almost completely inhibited by 4 mg/mL of HEP after 6, 9, and 24 h. In contrast, in the absence of HEP, the CFUs increased approximately from 10^5 to 10^8 cells in 24 h (Figure 1-A). The peak number (9 h) of CFUs of the *S. gordonii* culture was not affected by HEP, although the growth rate seemed to be decreased from 3 h to 6 h in the presence of HEP (Figure 1-B). We next determined the growth rates of *P. gingivalis* and *S. gordonii* as a function of HEP concentration after 9-h incubation. The numbers of viable *P. gingivalis* were as follows: $5.4 \pm 2.0 \times 10^7$ CFU/mL (control), $4.5 \pm 1.3 \times 10^7$ CFU/mL (HEP 0.25 mg/mL), $2.0 \pm 0.9 \times 10^7$ CFU/mL (HEP 0.5 mg/mL), $6.6 \pm 1.3 \times 10^6$ CFU/mL (HEP 1 mg/mL), $2.0 \pm 1.0 \times 10^5$ CFU/mL (HEP 2 mg/mL), and $6.5 \pm 3.4 \times 10^4$ CFU/mL (HEP 4 mg/mL) (Figure 2-A). HEP, from 0.5 mg/mL to 4 mg/mL, significantly inhibited the growth of *P. gingivalis*. The numbers of *S. gordonii* were as follows: $1.5 \pm 0.5 \times 10^9$ CFU/mL (control), $1.9 \pm 0.3 \times 10^9$ CFU/mL (HEP 0.25 mg/mL), $1.3 \pm 0.2 \times 10^9$ CFU/mL (HEP 0.5 mg/mL), $1.7 \pm 0.2 \times 10^9$ CFU/mL (HEP 1 mg/mL), $1.8 \pm 0.6 \times 10^9$ CFU/mL (HEP 2 mg/mL), and $1.9 \pm 0.4 \times 10^9$ CFU/mL (HEP 4 mg/mL) (Figure 2-B). The growth of *S. gordonii* was

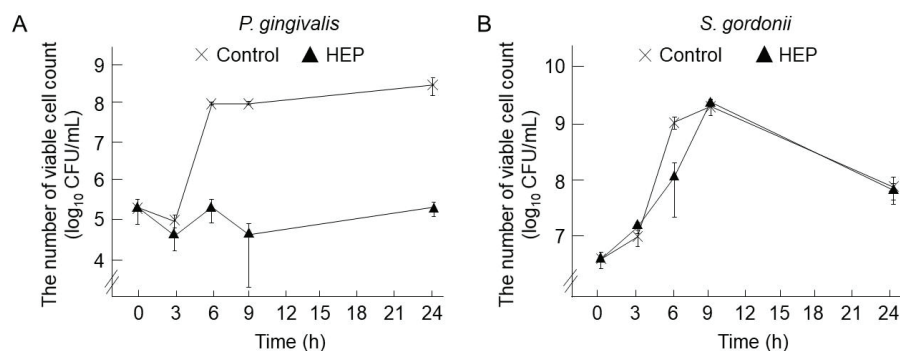


Figure 1: Growth rates of *P. gingivalis* (A) and *S. gordonii* (B) cultured with or without 4 mg/ml of HEP for 3, 6, 9, and 24 h at 37°C under anaerobic conditions. Each value represents the mean ± S.D. (n=4).

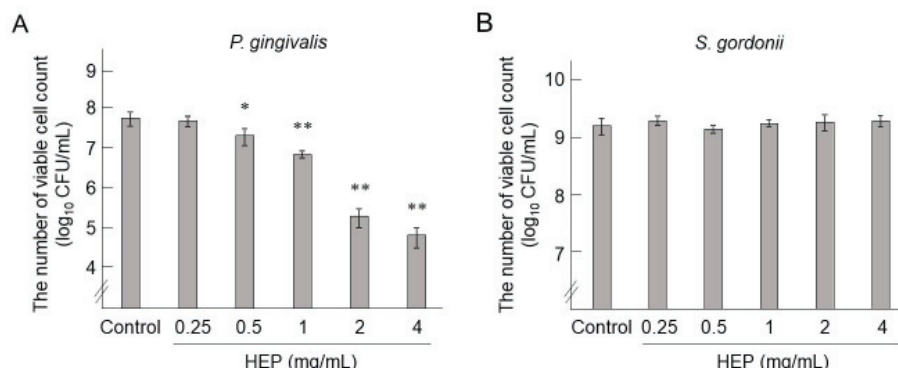


Figure 2: Numbers of viable *P. gingivalis* (A) and *S. gordonii* (B) cultured in the presence of various concentrations of HEP (0.25–4 mg/mL) for 9 h. at 37°C under anaerobic conditions. Each value represents the mean ± S.D. (n=4). * $p < 0.01$, ** $p < 0.001$ vs Control (Dunnett's test).



not inhibited by HEP at concentrations ranging from 0.25 mg/mL to 4 mg/mL.

Discussion

These results suggest HEP inhibits the growth of *P. gingivalis*, but not that of *S. gordonii*. Streptococci include many beneficial commensal species, which are associated with the homeostasis of the human oral cavity [4]. Furthermore, *S. gordonii* mitigates the effects of *P. gingivalis* on the host's inflammatory response and the epithelial-mesenchymal transition through regulation of signalling in host epithelial cells [18]. Periodontitis participates in an increased systematic pro-inflammatory state and associated with several systemic diseases, such as diabetes, atherosclerosis, myocardial infarction, stroke and cognitive impairment [19,20]. Therefore, it is thought that maintenance of oral microbiota is very important for health and longevity. Indeed, significant correlation between severe periodontitis and all-cause and cardiovascular mortality was reported in previous study [21,22]. Therefore, the selective antibacterial activity of HEP will likely contribute to preventing periodontal disease and lowering the risk for systemic diseases.

In this study, there is no data to explain the cause of a selective antibacterial effect of HEP on *P. gingivalis*. On the other hand, it is known that many antibacterial drugs show different strengths in Gram-positive and Gram-negative bacteria [23]. Although further research is needed, we speculate that one possible cause may be the difference between the anti-bacterial effect of HEP on gram-negative bacterium (*P. gingivalis*) and that on gram-positive bacterium (*S. gordonii*). Aqueous and methanol extracts, and the lipid-soluble erinacines of *H. erinaceus* exhibit antibacterial activity [14-16]. Thus, *H. erinaceus* may comprise multiple active compounds. Further investigations are required to identify the active compounds produced by *H. erinaceus* and to determine the mechanisms underlying their antibacterial activities. As for the safety of *H. erinaceus*, it has been eaten in East Asia since ancient times, and no adverse effects have been reported in a human clinical trial [24].

In conclusion, our data indicate that *H. erinaceus* may prevent periodontal disease without causing serious adverse effects.

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