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Direct effect of periodontitis-associated bacteria on the glucose metabolic activity of host cells.

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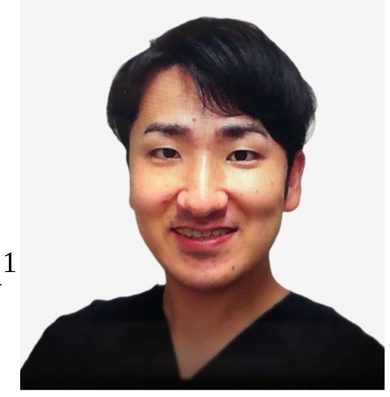


INTRODUCTION

Periodontitis causes the destruction of periodontal tissues and subsequent tooth loss. The pathogenesis of periodontitis has been investigated by many previous studies; however, the onset of periodontitis, especially the direct effect of periodontitis-associated bacteria on periodontal tissues is still unclear.
On the other hands, the glucose metabolic activity is a basic and essential function for host cells, as it provides energy, such as ATP, and components necessary for cell survival. Therefore, **in this study, we investigated the effects of culture supernatants of representative periodontitis-associated bacteria, *Porphyromonas gingivalis* , *Prevotella intermedia* and *Fusobacterium nucleatum* on host cells by measuring their glucose metabolic activity.**

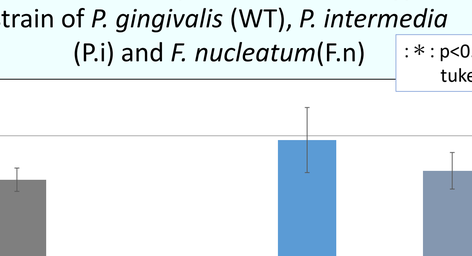
MATERIALS & METHODS

Preparation of bacterial culture supernatants

***Porphyromonas gingivalis* ATCC33277 (Wild type, WT)**
***Porphyromonas gingivalis* KDP136**
(gingipain-defective mutant, DM)
***Prevotella intermedia* ATCC25611 (P.i)**
***Fusobacterium nucleatum* ATCC25586 (F.n)**

Culture Conditions
Modified BM medium
Anaerobic condition
37°c

Harvested at the log phase of growth.
Culture supernatants were used in this study.

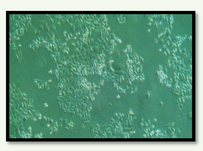


Preparation of host cells

HaCaT (human skin keratinocyte cell line)

Culture Conditions
D-MEM medium with FBS and antibiotics
37°C
with 5 % CO^2

Harvested at 80% confluence period.



Monitoring of glucose metabolic activity of host cells by pH-Stat system

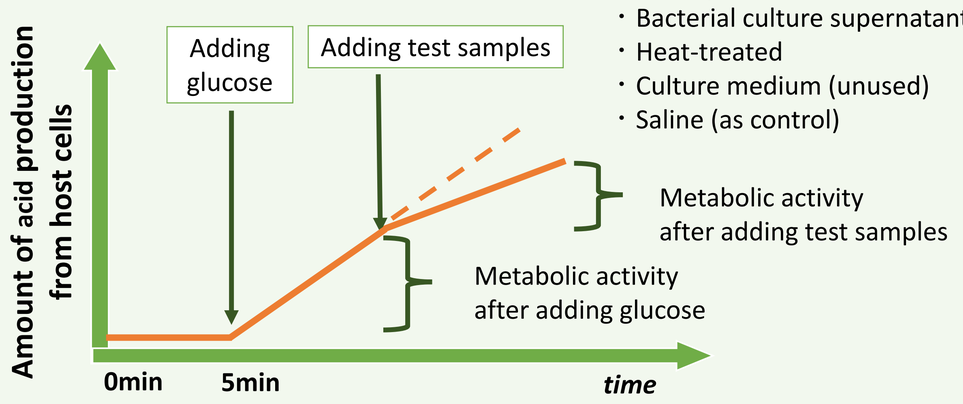
Glucose → Cell → Acids

Glucose is taken up into host cells and metabolized to acids such as lactic and carbonic acids. These acids are released from cells.

Monitoring acid production with pH-stat system

This system can evaluate the metabolic activity of cells, by monitoring the acid production due to their glucose metabolism in real-time.

The test samples were added during glucose metabolism by host cells.



Test samples
- Bacterial culture supernatant
- Heat-treated
- Culture medium (unused)
- Saline (as control)

Measurement of organic acids

Organic acids in bacterial culture supernatants were analyzed by HPLC.
The effects of the detected organic acids on the glucose metabolic activity of cells were also evaluated.

Shimadzu Prominence LC-20A

Treatment of bacterial culture supernatants

For removing the effect by proteins in supernatant, bacterial culture supernatants were treated described below. The effects of treated supernatants were also evaluated.

Heating treatment
Incubation at 80°C for 15min
→ Proteins such as enzymes were inactivated.

RESULTS

Figure 1 Effect of the supernatant of wild type strain of *P. gingivalis* (WT), *P. intermedia* (P.i) and *F. nucleatum*(F.n)

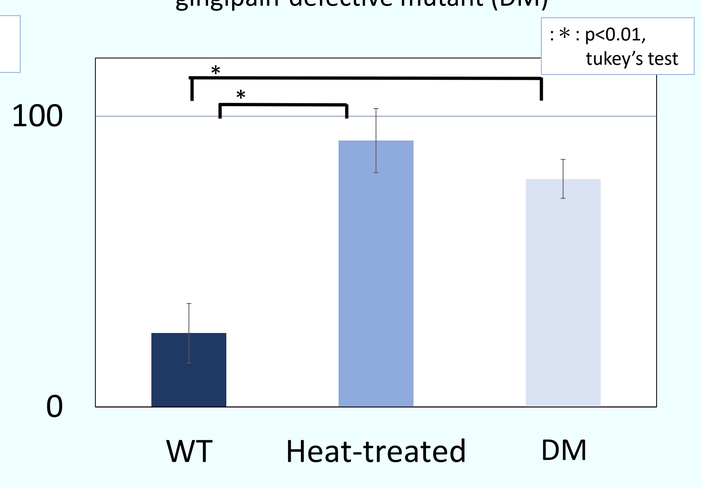
Culture supernatants of the wild type strain of *P. gingivalis* inhibited the glucose metabolic activity of host cells by about 60%.
On the other hand, those of *P. intermedia* showed no effect.

How to calculate relative metabolic activity

Relative metabolic activity
=(after adding test samples) / (after adding glucose)

When the metabolic activity after adding saline (control) was defined as 100%.

Figure 2 Effect of Heat-treated culture supernatants and gingipain-defective mutant (DM)

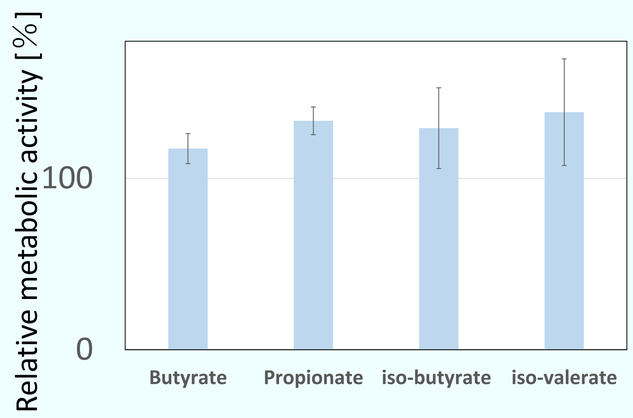


Inhibitory effect of culture supernatants of the wild type strain of *P. gingivalis* was abolished by heat treatment.
Culture supernatants of gingipain defective mutant did not inhibit the glucose metabolic activity.

Table Mainly detected organic acids in supernatants

Organic Acids	Concentration (mM)
Butyrate	14.9
Propionate	2.0
Iso-butyrate	11.4
Iso-valerate	6.7

Figure 3 Effect of mainly detected organic acids in supernatants



(The concentration of these organic acid was 10mM)

These organic acids was detected mainly in supernatant of the wild type strain of *P. gingivalis*. Then, we assessed the effect of these organic acids.

However, these organic acids did not inhibit the glucose metabolic activity.

DISCUSSION & CONCLUSION

In this study, it was demonstrated that culture supernatants of the wild type strain of *Porphyromonas gingivalis* (WT) directly inhibited the glucose metabolic activity of host cells. However, the inhibitory effect was abolished by heat treatment of culture supernatants. Furthermore, culture supernatants of gingipain-defective mutant (DM) had no effect on the glucose metabolic activity of host cells. On the other hands, organic acids detected in bacterial culture supernatants of *P. gingivalis* WT did not affect the glucose metabolic activity.
These results suggest that *Porphyromonas gingivalis* (WT)-derived gingipains and/or gingipain-associated proteins directly inhibit the glucose metabolism of host cells.

The authors declare that there is no conflict of interest.

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