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学位論文要旨
Dissertation Abstract

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論文題名 Title of Doctoral Dissertation	*Porphyromonas gulae* proteases influence not only bacterial growth, coaggregation, and hemagglutination but also the maintenance of human protein （*Porphyromonas gulae*のタンパク分解酵素は細菌の増殖、共凝集、赤血球凝集だけでなく、ヒトタンパクの維持にも影響をおよぼす）

論文内容の要旨（2000 字程度）Dissertation Abstract (approx. 800 words)

Porphyromonas gulae, previously known as the animal biotype of the human periodontal pathogen *P. gingivalis*, is Gram-negative, anaerobic, rod-shaped, asaccharolytic, black-pigmented, non-motile, non-spore-forming, and non-motile. *P. gulae* organisms have been isolated from the gingival sulcus of various animal species, including bear, brushtail, possum, dog, cat, coyote, kangaroo, monkey, ovine, wallaby, and wolf. Interestingly, it has also detected in human gingival tissues from healthy and diseased sites. Although recent studies have reported that *P. gulae* possesses a wide variety of virulence factors, such as including fimbriae, LPS, and proteases, the proteases have yet to be well clarified in details. The present study aimed to clarify the characters of the proteases from varieties of *P. gulae* strains available at this time. Based on biochemical and functional characters similar to the factors from *P. gingivalis*, enzyme roles, hemagglutination, and degradation of host proteins were clarified in this study.

***P. gulae* strains possess trypsin protease-like activity:** *P. gulae* exhibits several virulence characteristics similar to those of the human periodontal pathogen *P. gingivalis*. However, the proteolytic enzyme activities of *P. gulae* strains have not been fully elucidated. All of the examined *P. gulae* strains as well as the *P. gingivalis* ATCC 33277 strain consistently produced alkaline phosphatase and showed trypsin activity, while no other enzyme activities were detected in any of the strains tested. Moreover, protease activity was found in both cell extracts and supernatants, with negligible differences among the examined strains. Protease inhibitors, including antipain (cysteine protease inhibitor), phenylmethylsulfonyl fluoride (PMSF; serine proteinase inhibitor), tosyllysine chloromethyl ketone (TLCK; serine endopeptidase specific inhibitor) and leupeptin (serine protease inhibitor as bacterial metabolite, a cathepsin B inhibitor, a calpain inhibitor and a trypsin inhibitor), diminished *P. gulae* proteolytic activity up to 50%.

Hemagglutination activity: *P. gulae* and *P. gingivalis* reportedly possess protease-related and hemagglutinin genes. However, the hemagglutination ability of *P. gulae* has yet to be investigated. The present findings showed distinct hemagglutination activity in *P. gulae* ATCC 51700, and found that protease inhibitors, such as antipain, PMSF, TLCK, and leupeptin, failed to cause agglutination of mouse erythrocytes. These results suggest that *P. gulae* proteases may contribute to the hemagglutination.

***P. gulae* growth:** Previous reports noted that *P. gingivalis* growth mediated by gingipains was reported to increase in chemically defined medium (CDM). After inoculation in CDM for the present assays, *P. gula*e ATCC 51700 was found to be in clearly in the stationary phase from 144 h. Furthermore, antipain, PMSF, TLCK, and leupeptin inhibited the growth of *P. gulae* ATCC 51700, suggesting that *P. gulae* proteases may be essential for bacterial growth.

Coaggregation reaction of *P. gulae* with *A. viscosus*: Coaggregation of *A. viscosus* with *P. gingivalis* has been previously reported. *P. gulae* ATCC 51700 was found to coaggregate with *A. viscosus* ATCC 15987, while inhibition of *P. gulae* proteases using protease inhibitors significantly abrogated that activity of the bacterium. These data suggest that coaggregation reactions between *P. gulae* ATCC 51700 and *A. viscosus* ATCC 15987 are regulated via the activity of *P. gulae* proteases.

Morphological changes and inhibition of proliferation of human cells by *P. gulae*: *P. gulae* ATCC 51700 caused rounding and detachment of human gingival carcinoma Ca9-22. The nature of morphological changes is reportedly linked to the proliferation rate of host cells infected with microorganisms. *P. gulae* ATCC 51700 inhibited proliferation of Ca9-22 cells in both multiplicity of infection (MOI) at 500. Pretreatments with antipain, PMSF, TLCK, and leupeptin prevented inhibition of Ca9-22 proliferation by *P. gulae* ATCC 51700, suggesting that *P. gulae* proteases cause morphological changes in Ca9-22 cells, leading to inhibition of their proliferation.

Degradation of human proteins by *P. gulae*: Previous studies have reported that focal contact and adherence junction components, including E-cadherin, β-catenin, focal adhesion kinase (FAK), and paxillin, were associated with epithelial morphology. Following *P. gulae* ATCC 51700 infection at an MOI of 500, E-cadherin, β-catenin, FAK and paxillin also shown to be cleaved at 6 hours. To further evaluate the role of *P. gulae* proteases, *P. gulae* ATCC 51700 was preincubated with several protease inhibitors prior to bacterial infection. Cleavage of focal contact and adherence proteins by *P. gulae* ATCC 51700 was diminished by antipain, PMSF, TLCK, and leupeptin. Furthermore, degradation of recombinant human proteins, γ-globulin and fibrinogen, was observed within 6 hours. Antipain, TLCK, and leupeptin were prevented the cleavage of γ-globulin and fibrinogen, while PMSF did not.

Conclusion: *P. gulae* proteases would be a crucial virulence factors factor for bacterial colonization, such as hemagglutination and coaggregation, and bacterial growth, as well as host defense and cell contact and adherence destruction.