

Protein Profile of *Prevotella intermedia* and *Porphyromonas gingivalis* Cell Isolated from Chronic Periodontitis Patient

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ABSTRACT

Prevotella intermedia and *Porphyromonas gingivalis* are two of the specific microorganisms that frequently occurs in chronic periodontitis. As protein plays an important role in bacteria's life and it's virulence factor, we tried to explore variation in *P. intermedia* and *P. gingivalis* cell protein profile which isolated from different periodontal pocket depth of chronic periodontitis patient. *P. intermedia* and *P. gingivalis* were identified using Polymerase Chain Reaction (PCR) technique, and it's protein profile were evaluated using Sodium Dodecyl Sulfate - Polyacrylamide Gel Electrophoresis (SDS-PAGE). SDS-PAGE result showed that there were significant difference between protein profile of *P. intermedia* cell which isolated from shallow and deep pocket with dominant proteins of molecular weight 200 kDa, 110 kDa, 40 kDa, and 25 kDa, however, relationship between pocket depth and *P. intermedia* cell protein profile could not be concluded because unrepresentative number of *P. intermedia* colonies. In the other hand the variation in *P. gingivalis* cell protein profile was not influenced by pocket depth with dominant protein called hemin binding protein (HbBp).

Key words : Chronic periodontitis, *Prevotella intermedia*, *Porphyromonas gingivalis*, protein profile, PCR, SDS-PAGE.

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INTRODUCTION

Chronic Periodontitis is the most frequent form of periodontitis occurred in Indonesia. Two of the specific microorganisms caused chronic periodontitis is *Prevotella intermedia* and *Porphyromonas gingivalis* which are dominant pathogen in chronic periodontitis.¹⁻⁷ Bacteria life and activity in gingiva sulcus and periodontal pocket won't be done without protein which is highly varied in bacteria cell. Protein arranged most of the bacteria cell and participate in each process within cell, but the difference of cell protein profile at different location hasn't clear.^{8,9} The purpose of this study is to analyze variation in *P. intermedia* and *P. gingivalis* cell protein profiles at shallow and deep periodontal pocket depth.

MATERIAL AND METHODS

Cultivation Method. First, subgingival plaque from periodontal pocket with depth less than 4 mm and more than 4 mm of 6 chronic periodontitis patients who visited our university hospital were homogenized in 1 ml Phosphate Buffer Saline (PBS) using a vortexer, then it were spreaded on Brucella Blood Agar (BBA) supplemented with vit K, and were incubated for 3 x 24 hours. On BBA we put metronidazole tablet to made a zone of Gram negative bacteria. Four black pigmented bacteria colonies around the zone from subgingival plaque isolate were then grown on BBA, and were incubated for 3 x 24 hours, so,

we had 48 colonies of black pigmented bacteria.

Identification of Bacteria Species.

Prevotella intermedia and *Porphyromonas gingivalis* were indentified using Gram Staining and Polymerase Chain Reaction (PCR) method. Gram staining were used to confirmed whether the black pigmented bacteria were rod shaped and red colored which proved that the colonies were Gram negative. PCR were used to identified bacteria species based on it's 16s rDNA location. This technique amplify a sequence of DNA to millions of copy using polymerase enzyme.^{10,11} Bacteria DNA were extracted using instagene matrix (Biorad), and PCR were performed used DNA thermal unit (Biorad) under the following conditions : 32 cycles of denaturation at 94°C for 30 seconds, annealing at 65°C for 30 seconds, and extension at 72°C for 2 minutes, with initial denaturation at 94°C for 3 minutes and final extension at 72°C for 5 minutes. PCR products were electrophoresed on 1,5% agarose gel, stained with ethidium bromide, and visualized under UV light using gel doc. The Primers used to detect *P. gingivalis* and *P. intermedia* are summarized in table 10.

Bacteria Cell Protein Extraction. After *P. intermedia* and *P. gingivalis* were identified, it's cell protein were extracted using Ultrasonic Homogenizer (OMNI International Inc.). *P. intermedia* and *P. gingivalis* were grown in Brucella broth first, and the medium were centrifuged at 5000 x g for 10 minutes. The

obtained pellet which were bacteria cells precipitate then washed with PBS for 2 times. Bacteria cells in PBS were sonicated in 1 minutes for 3 times, after that bacteria cells were centrifuged at 1300 x g for 20 minutes at 4°C to separate debris from sonicating. Debris will precipitate into pellet, supernatant then were centrifuged at 10.000 x g for 1 hour at 4°C. Pellet obtained from this centrifugation were outer membrane complex, which contain cell protein, this pellet were dissolved in 50 µl PBS.

Sodium Dodecyl Sulfate Polyacrilamide Gel Electrophoresis (SDS-PAGE). The extracted cell protein were then separated by its molecular weight using SDS-PAGE. SDS-PAGE was performed according to the method of Laemmli, using 12,5% gels. The protein sample were mixed with native sample buffer (Bio-rad). Electrophoresis was performed in two phases, phase 1 at 100 V, 80 mA, 30 W, for 30 minutes, and phase 2 at 200 V, 80 mA, 30 W, for 1,5 hour. After completion of SDS-PAGE, the gels were stained with Coomassie Blue for 1 hour. The Gels were destained and fixed with 100% ethanol and 100% acetic acid for 30 minutes. Molecular weight of protein bands in the gels were determined by comparison to molecular weight standart using Pre-stained Protein Markers – Broad Range (Nacalai Tesque). In this research, we also analyze the protein profile of *P. gingivalis* ATCC 33277 as *P. gingivalis* standard and then it was compared with protein profile of wild strain *P. gingivalis*.

RESULTS

From 48 colonies of black pigmented bacteria, 37 colonies were identified as *P. gingivalis*, 3 colonies as *P. intermedia*, and 8 others colonies were unknown bacteria.

We found that the protein profile of wild strain *P. gingivalis* and *P. intermedia* that isolated from periodontal pocket depth less than 4 mm and more than 4 mm of chronic periodontitis patients, were highly varied. The protein profile of *P. gingivalis* and *P. intermedia* as shown in figure 1 until figure 9.

Proteins that were expressed by wild strain *P. intermedia* in their protein profile are proteins with molecule weight approximately 200 kDa, 110 kDa, 88 kDa, 60 kDa, 55 kDa, 42 kDa, 40 kDa, 35 kDa, 29 kDa, 25 kDa, and 21 kDa. In this research, proteins 200 kDa, 110 kDa, 40 kDa, and 25 kDa were found in protein profile of all *P. intermedia* that have been isolated from pocket depth less than 4 mm and more than 4 mm.

P. gingivalis expressed about 28 protein with different molecule weight in their protein profile as shown in table 2 until table 8. The main proteins of *P. gingivalis* protein profile that was found in this research are protein with molecule weight approximately 9.5 kDa, 19 kDa, 32 kDa, 35 kDa, 40 kDa, 41 kDa, 48 kDa, 50 kDa, 67 kDa, 88 kDa, 95 kDa, 105 kDa, and 190 kDa. In those protein profile, there were no proteins specific to differ *P. gingivalis* that were isolated from pocket depth less than 4 mm or more than 4 mm.

DISCUSSIONS

In this study, the type of proteins that were found in *P. intermedia* and *P. gingivalis* protein profile will be discussed. Su-min *et al.* reported that protein with molecule weight approximately 60 kDa is hemoglobin binding protein in *P. intermedia*. This protein was found in *P. intermedia* protein profile in this research.¹² According Matsushita *et al.* 55 kDa protein in *P. intermedia* is fimbrial protein as one of virulence factors to induce blood for release cytokine.¹³ The other virulence factor of *P. intermedia* that was found in this research is protein with molecule weight approximately 42 kDa. This protein can bind glycoprotein in extracellular matrix of host tissues for adhesion and invasion of *P. intermedia* to epithelial cell. Yu *et al.* reported that 29 kDa protein is a broken protein from AdPb. This protein was also found in protein profile *P. intermedia* in this research.¹⁴ According Kalfas *et al.* 21 kDa, 25 kDa, and 35 kDa protein are broken protein from laminin binding protein from 62 kDa protein. Those protein are used by *P. intermedia* for invasion and act as virulence factors.¹⁵ Cookson *et al.* reported that 21 kDa protein is one of specific protein in *P. intermedia* but in this research, 21 kDa protein was only found in *P. intermedia* protein profile from pocket depth 10 mm, so the theory of Cookson *et al.* is not valid for this research.¹⁶ Proteins that were found in *P. intermedia* protein profile are included as virulence factors and used by *P. intermedia* for fulfill their needs.

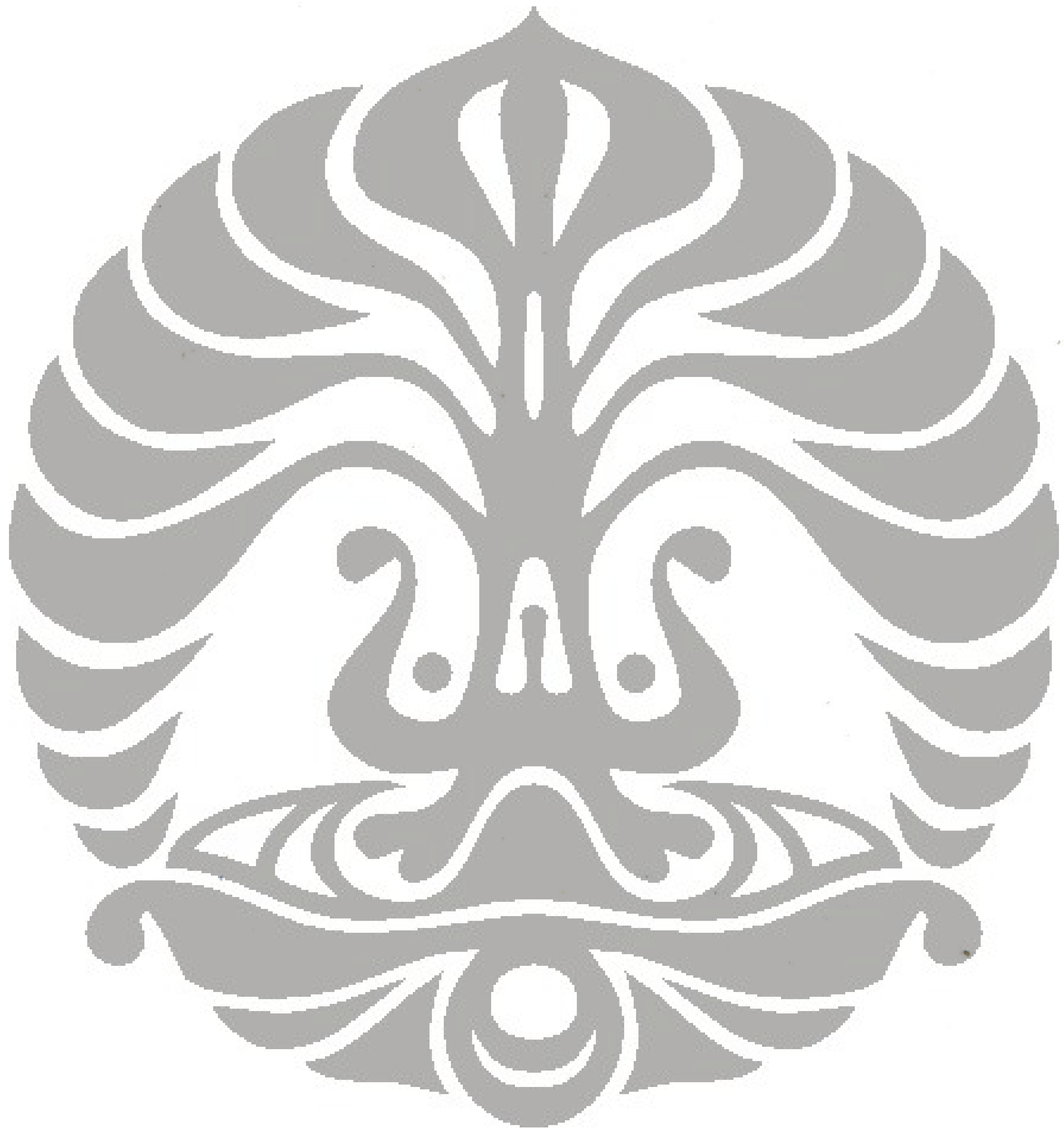
In *P. gingivalis* protein profile, there are so many proteins are found. First, hemin binding protein (HbBP) that expressed in 19 kDa protein (Smalley *et al.* and Fujimura *et al.*), 32 kDa (Smalley *et al.*), and 35 kDa (Yasuko *et al.* and Kakuda *et al.*). HbBP 19 kDa is a major protein in all *P. gingivalis* from clinical isolated in this research. HbBP is found in outer membrane cell *P. gingivalis* and its function is to bind hemoglobin from gingival cell. Smalley *et al.* reported that HbBP 19 kDa is a modification form from 32 kDa hemin-binding protein because of heating process.¹⁷⁻¹⁹ In this research, 32 kDa protein was found in one colonies *P. gingivalis* protein profile and *P. gingivalis* ATCC 33277, so referred to Smalley *et al.* research, protein band with molecule weight approximately 31-32 kDa is hemin binding protein too.

Protein with molecule weight 40 kDa was found in some wild strain *P. gingivalis* from patient's periodontal pocket. This protein was called 40-kDa outer membrane protein (40-kDa OMP). Some research by Nonaka *et al.* and Tsurumi *et al.* conclude that 40-kDa OMP is one of important aggregation factor on cell surface of *P. gingivalis*. Nonaka *et al.* and Tsurumi *et al.* used recombinant of 40-kDa OMP (r40-kDa OMP) in their research, since antibodies against the r40-kDa OMP have potent ability to kill *P. gingivalis* cells by complement activation and opsonization, r40-kDa OMP has been subject of considerable interest as a possible periodontitis vaccine candidate.^{20, 21}

Fimbriae is a very important virulence factor in adhesion of *P. gingivalis* to oral cavity such as to teeth, oral mucosa, and to others bacterial in oral cavity, also can induce the host response. Cell surfaces of *P. gingivalis* have two type of fimbriae, they are major fimbriae that expressed in 41 kDa protein (FimA, fimbrillin), was coded by *fimA* gene and minor fimbriae from 67 kDa protein that was coded by *mfa1* gene.²² Some *P. gingivalis* colonies that were isolated from periodontal pocket in this research also expressed band protein with molecule weight approximately 41 kDa and 67 kDa, so referred to Lin *et al.* experiment, those proteins are major and minor fimbriae in *P. gingivalis*. Major fimbriae helps *P. gingivalis* for intracellular invasion with trigger peripheral macrofag and human neutrofil to produce too much cytokine inflammation such as IL-1, IL-6, and TNF- α . *P. gingivalis* used minor fimbriae for microcolonies formation and maturation of *P. gingivalis* biofilm. Reynolds *et al.* also reported that 28 kDa protein is a specific fimbrial protein that usually appears in molecule weight approximately 28-31 kDa. So, protein bands with molecule weight approximately 28-29 kDa in this research were suspected as specific fimbrial binding protein. Beside that, protein band with molecule weight 53 kDa in *P. gingivalis* ATCC 33277 protein profile, was suspected as secondary fimbriae protein that has been investigate by Arai *et al.* in *P. gingivalis* strain 381 and Yoonsuk Park *et al.* in *P. gingivalis* ATCC 33277.²³⁻²⁵

There are two kind of proteinase that are produced by *P. gingivalis*, they are *Arg-gingipain* (HRgpA, RgpB) and *Lys-gingipain* (KGP). Both of them can destruct the host tissues. *Lys-gingipain* was expressed in 105 kDa and 48 kDa proteins (Neil *et al.*) also 190 kDa protein (Sato *et al.*). KGP of *P. gingivalis* has an ability to bind human hemoglobin for support their life and growth. There are two type of *Arg-gingipain* and Sugawara *et al.* reported that HRgpA expressed in 95 kDa protein and RgpB as 50 kDa protein. All of this type gingipains were expressed in some *P. gingivalis* colonies protein profile in this research. Gingipains can increase vascular permeability with kallikrein or kinin activation pathway, disregulation of plasma clot formation, activation of complemen component, and modification of neutrofil function to cause an onset inflammation.²⁶⁻²⁸

Protein bands with molecule weight approximately 9.5 kDa and 87-88 kDa have been found in this research. Those proteins have not been reported in previous investigation. So, those proteins are become the unknown protein yet. The major proteins that were found in this research included as outer membrane proteins and act as major virulence factors of *P. gingivalis* that have some function such as invasion to host tissues, binding hemin protein, and degradation of host tissues. In this research, there is protein polymorphism in *P. gingivalis* protein profile. The same proteins can appear or expressed as



protein with different molecule weight in different strain *P. gingivalis* but in one strain sometimes protein with the same function can expressed as two or more protein with different molecule mass.

There is no significance difference between protein profile of *P. gingivalis* that were isolated from periodontal pocket depth less than 4 mm and more than 4 mm. The variation of *P. gingivalis* protein profile in this research was suspected because there are variation strains of *P. gingivalis*. So, this strains variation that influence the protein profile variation of *P. gingivalis*, not the pocket depth. In profile protein *P. intermedia*, there are difference proteins expressed between *P. intermedia* from pocket depth less than 4 mm and more than 4 mm but this research can't prove the relationship between pocket depth and protein profile variation in *P. intermedia* because the amount of *P. intermedia* are not representative in this research. The dominant proteins in *P. gingivalis* profile are hemin binding protein, fimbriae protein, and gingipains. In *P. intermedia*, there are 200 kDa, 110 kDa, 40 kDa, and 25 kDa protein as the dominant proteins, but the type and function of those protein are unknown.

ACKNOWLEDGEMENT

We'd like to thank drg. Ariadna A. Djais, M. Biomed, PhD. and drg. Boy M. Bachtiar, MS, PhD. as our supervisor and funder to complete this research, and drg. Endang W. Bachtiar, M.

Biomed, PhD. as the manager of Laboratory of Oral Biology, Faculty of Dentistry, University of Indonesia who kindly let us to use the laboratory and it's facility.

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