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Identification of differentially expressed proteins in the bacterial biofilm

Chi-Dug Kang · Jeom-il Choi*

Department of Biochemistry, School of Medicine,
*Department of Periodontics, School of Dentistry, Pusan National University

I. Intoduction

Bacterial biofilm is covered by protective surface glycoproteins with convection, metabolic, oxygen, and nutrient flow circulating that renders resistance to antimicrobial agents or phagocytes.[1] Periodontal disease is primarily an inflammatory disease initiated by infection of mixed bacterial coaggregates composed of complex biofilm mass.[2,3] Among complex oral microbial biofilms, *P. gingivalis* is a major pathogenic organism that causes destructive periodontal diseases4. However, majority of microbiological and immunological studies on this organism have been made on the bacteria particularly grown as a planktonic culture.[4-6] Only a few approaches have been performed on *P. gingivalis* biofilm with respect to growth characteristics and antimicrobial resistance.[3,7]

For more comprehensive understanding of virulence mechanisms and survival strategies that *P. gingivalis* biofilm utilizes, tools for bacterial genomics, proteomics and immunology may have to be constitutively practiced. While scarcity for identifying differentially expressed gene(s) or protein(s) in *P. gingivalis* biofilm may warranty the focused research in the area, elucidating the mechanisms by which *P. gingivalis* biofilm evade and perturb the host response could also be of potential value in understanding antimicrobial resistance and consequently designing the effective chemotherapeutic agents.

The aim of the present study was to identify the differentially expressed outer membrane proteins of *Porphyromonas gingivalis*, a principal periodontal pathogen, when it

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Corresponding author : Jeom-il Choi, Department of Periodontics, School of Dentistry, Pusan National Uni- versity, 1-10, Ami-Dong, Seo-Ku, Pusan, Korea, E-mail : jrapa@pusan.ac.kr

was grown either as a biofilm or as a planktonic culture.

II. Materials and Methods

1. Growth condition of *P. gingivalis* biofilm

Growth of *P. gingivalis* biofilm. *P. gingivalis* 381 was grown in Tryptic soy broth (Difco, MI) supplemented with 5 micrograms /ml hemin and 0.5 micrograms/ml Vitamin K in anaerobic chamber(COY, MI). Overnight-grown bacteria was inoculated onto 6-well cell culture plate coated with either polystyrene. Outer membrane proteins was isolated from pure *P. gingivalis* biofilm grown on tissue culture plates, and subject to SDS -PAGE for a comparison of electrophoretic profiles with *P. gingivalis* grown in planktonic cultures.

2. Isolation and purification of outer membrane proteins(OMP) from *P. gingivalis*

Bacterial cells were harvested and washed 3 times with phosphate-buffered saline (PBS). Cocktail of 2mM TLCK+2mM PMSF +2mM benzamidine were added to PBS and cells were subject to French cell disruption. Cell disruption was confirmed by phase contrast microscopy. The fraction was centrifuged at 9500×g for 30 min and supernatants were collected. The fraction was ultracentrifuged at 45,000×g for 90min. Protein concentration was determined by Lowry method.

3. Isoelectric focusing

For proteomic analysis, 100 μ g of lysates solubilized in 260 μ l 2D sample buffer(7 M urea, 2 M thiourea, 4% CHAPS and 40 mM Tris) were loaded onto 13 cm pH 3-10 L IPG strips(Amersham Parmacia Biotech AB, Piscataway, USA), which was rehydrated in buffer containing 7 M urea, 2 M thiourea, 2% (w/v) CHAPS(3-[(3-cholamidopropyl) dimethylamino]-1-propanesulfonate), 100mM DTT(dithiothreitol), 0.5% IPG buffer and a trace of bromophenol blue. Isoelectric focusing(IEF) was performed using IPGphor(Amersham Pharmacia Biotech AB, Piscataway, USA). Following IEF, the IPG strips were equilibrated for 5 min in buffer containing 50mM Tris-HCl(pH 8.8), 6mM urea, 30% glycerol, 2% SDS, 2% DTT and trace bromophenol blue. Strips were then mounted on 12% SDS-PAGE gels and electrophoresed at 50 mA per gel(SE 600, Hoe- fer Parmacia Biotech Inc, USA). 2D gels were stained with silver.

III. Results

2-demensional electrophoresis

To analyze the differentially expressed outer membrane proteins between broth and biofilm culture condition, two-dimensional gel electrophoresis was performed. Proteins marked with arrows and circle(Figure 1-right panel) were highly expressed in *P. gingivalis* OMP grown in broth culture, whereas proteins marked with arrows(Figure 1-left panel) were highly expressed in those grown in biofilm culture, compared with each other.

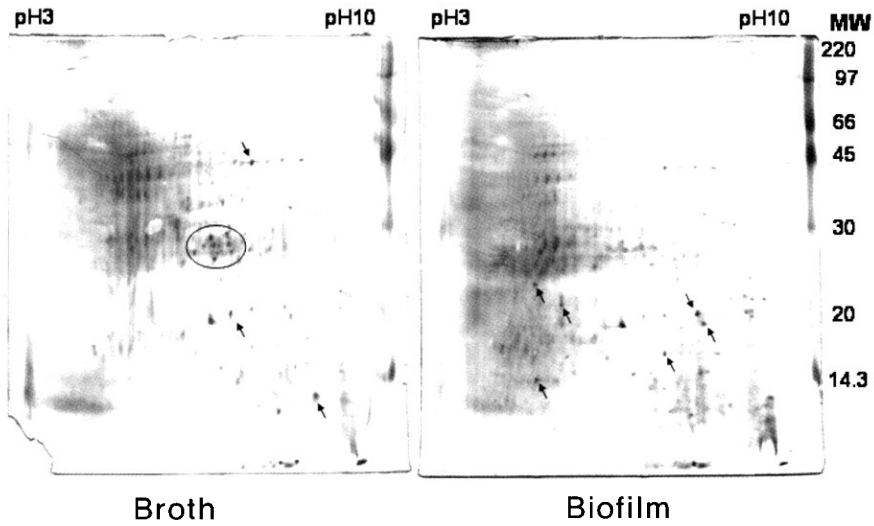

Figure 1. Analysis of Porphyromonas gingivalis OMP grown in broth and biofilm culture using two-dimensional polyacrylamide gel electrophoresis. The first-dimensional isoelectric focusing was done using linear gradient pH 3-10 IPG strip. Second dimension separation was performed with 12% SDS-PAGE.

IV. Discussion

In two-dimensional electrophoresis, more than 100 polypeptides were detected in both culture conditions. Some proteins were remarkably downregulated and upregulated in biofilm culture condition compared with broth culture condition. These proteins are necessary to be identified with mass spectrometry techniques to understand the strategy of bacteria to survive against the antibacterial tissue components or chemotherapeutic agents. To delineate the sequences and characteristic features of these specific proteins that were differentially regulated in the bacterial biofilm formation, further analysis may be needed using MALDI-TOF analysis.

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- 국문초록 -

세균성 바이오필름-특이 발현 단백질의 규명

강치덕 · 최점일*

부산대학교 의과대학 생화학교실,
*부산대학교 치과대학 치주과학교실

본 연구는 치주낭에 biofilm형태로 부착되어 질환을 유발시키고 항생제 및 항균제에 저항을 일으키는 세균 독성요소를 규명하기 위해 시행된 기초연구이다. 치주질환의 주 병원균의 하나인 *Porphyromonas gingivalis* 381 biofilm의 세포외막에 특이하게 발현되는 단백질을 규명하기 위한 기초적인 자료를 얻기 위해 시행하였다. *Porphyromonas gingivalis* 381을 통상적인 세균 배양용 broth를 사용하여 혐기성 세균 배양기로 24시간 배양한 것을 대조군으로 하고, tissue culture plate를 이용하여 혐기성 배양조건 하에서24시간동안 biofilm을 형성하여 실험군으로 설정하였다. 세균을 수획하여 세포외막을 분리하고 isotonic isoelectric focusing을 시행한 결과 주로 약 20-30 kilodaltons에 해당하는 수종의 세균세포막 단백질이biofilm으로 배양한 세균에서 더 상승적으로 발현됨이 관찰되었고, 상이한 수종의 단백질도 planktonic culture broth로 배양한 세균에서 다 상승적으로 발현됨을 관찰할 수 있었다. 이것은 세균의 배양조건과 환경에 따라 그 외막 단백질이 서로 다르게 발현됨을 입증하는 기초적인 자료로서 향후 단백질의 동정과 성격을 규명하는 근간 실험으로 추진할 계획이다.

key words : 바이오필름, differential expression, *Porphyromonas gingivalis*