Biofilm Formation of *Enterococcus faecalis* in Kidney Stone Patients

การสร้างไบโอฟิล์มของเชื้อเอนเทอโรคอคคัส ฟีคาลิสในผู้ป่วยโรคนิ่วไต

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ABSTRACT

Kidney stone disease is associated with urinary tract infections (UTIs). The ability of biofilm formation may be an important factor in stone formation. The object of this study is to study biofilm formation of *Enterococcus faecalis* isolates in kidney stone patients. Seven *E. faecalis* were isolated from urine (5 isolates) and stone matrices (2 isolates) of kidney stone patients (4 cases). Chemical compositions of kidney stones were performed by using Fourier-transformed infrared spectroscopy. Biofilm formation was detected by light microscopy and confirmed by scanning electron microscopy. The results showed that all of them were calcium stones (3 calcium oxalate/calcium phosphate and 1 calcium oxalate/uric acid stones). *E. faecalis* in the urine and stones of these patients could form biofilm 60% (3/5) and 50% (1/2), respectively. This study indicated that biofilm formation by *E. faecalis* has high tendency in these kidney stone patients.

บทคัดย่อ

โรคนิ่วไตมีความสัมพันธ์กับการติดเชื้อในทางเดินปัสสาวะ ซึ่งการสร้างไบโอฟิล์มของเชื้อแบคทีเรียอาจเป็นปัจจัยสำคัญอย่างหนึ่งที่ส่งเสริมให้เกิดนิ่ว การศึกษานี้มีวัตถุประสงค์ เพื่อศึกษาความสามารถในการสร้างไบโอฟิล์มของเชื้อเอนเทอโรคอคคัส ฟีคาลิสในผู้ป่วยโรคนิ่วไต โดยเชื้อเอนเทอโรคอคคัส ฟีคาลิส จำนวน 7 ไอโซเลต แยกได้จากปัสสาวะ (5 ไอโซเลต) และก้อนนิ่ว (2 ไอโซเลต) ของผู้ป่วยโรคนิ่วไต จำนวน 4 ราย ซึ่งวิเคราะห์องค์ประกอบทางเคมีของก้อนนิ่วไตด้วยเครื่องฟูเรียร์ทรานสฟอร์มอินฟราเรดสเปกโทรสโกปี และศึกษาการสร้างไบโอฟิล์มของเชื้อเอนเทอโรคอคคัส ฟีคาลิสภายใต้กล้องจุลทรรศน์และยืนยันผลการสร้างไบโอฟิล์มด้วยกล้องจุลทรรศน์อิเล็กตรอนชนิดส่องกราด จากผลการศึกษาพบว่าก้อนนิ่วที่แยกได้จากผู้ป่วยทั้ง 4 ราย มีองค์ประกอบทางเคมีเป็นนิ่วชนิดแคลเซียม

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(แคลเซียมออกซาเลต/แคลเซียมฟอสเฟต 3 ราย และแคลเซียมออกซาเลต/กรดยูริก 1 ราย) ส่วนผลการสร้างไบโอฟิล์มของเชื้อเอนเทอโรคอคคัส ฟีคาลิสในปัสสาวะ และก้อนนิ่ว คิดเป็นร้อยละ 60 (3/5) และร้อยละ 50 (1/2) ตามลำดับ แสดงให้เห็นว่ามีการสร้างไบโอฟิล์มของเชื้อเอนเทอโรคอคคัส ฟีคาลิสค่อนข้างสูงในผู้ป่วยโรคนิ่วไตนี้

Key Words: Biofilm formation, Kidney stone, *Enterococcus faecalis*

คำสำคัญ: การสร้างไบโอฟิล์ม, โรคนิ่วไต, เชื้อเอนเทอโรคอคคัส ฟีคาลิส

Introduction

Previously, urinary tract infection (UTI) was not recognized as a cause of calcium stone. At present time, Tavichakorntrakool et al., 2012 found that the prevalence of UTI associated with kidney stone disease is high (36%) in the northeastern Thailand (Tavichakorntrakool et al., 2012). *Enterococcus faecalis* was a common pathogen found in urine and stone matrices of these stone formers. The next study by Chutipongtanate *et al*. showed that bacteria can directly promote calcium oxalate crystal growth and aggregation (Chutipongtanate et al., 2013). The bacterial biofilm formation may be an important factor which can cause the persistence in urinary tract and stone genesis. We therefore analyzed the biofilm formation of *E. faecalis* isolates in urine and stone metrices of kidney stone patients.

Materials and Methods

Ethics statement

This study was reviewed and approved by the Institutional Ethical Committee at Khon Kaen University (Ethical number; HE 521177).

Bacterial isolation and identification

The total of 7 *E. faecalis* isolates (5 from catheterized urine and 2 from stone matrices) was studied. All bacterial isolates were identified by standard biochemical tests (Garrity GM et al., 2005). Moreover, the species of *E. faecalis* isolates were confirmed by PCR method modified from (Deasy et al., 2000; Jackson et al., 2004).

Analysis of chemical compositions of stones

Analysis of chemical compositions of stone was done by using stone powder derived from the stone sections that was left after bacterial culture. Chemical analysis was performed using Fourier-transformed infrared spectroscopy as described previously (Sriboonlue et al., 1993).

Analysis of biofilm formation

Biofilm formation of all *E. faecalis* isolates were analyzed by light microscopy (LM) and also confirmed by scanning electron microscopy (SEM). *E. faecalis* ATCC 29212 (Duggan et al., 2007) was used as positive control strains.

Sample preparation for light microscopy and Scanning electron microscopy

Bacterial isolates were grown in tryptic soy broth (Oxoid; UK) at 37°C for 24 hrs. Then bacterial cultures were adjusted to 0.5 McFarland. Biofilm formations were performed by using the 6-well culture polystyrene plates (Nunc®, Denmark) with cover glasses.

Light microscopic examination; biofilm formations were assessed by inoculated 100 µL of the bacterial suspension in each well plate which contained in 5000 µL of tryptic soy broth (Oxoid; UK). The culture plates were incubated overnight at 37°C without shaking. After 24 hrs, the wells were washed three times with phosphate buffer saline. The bacterial cells were then fixed by using 10% (v/v) formalin for 10 min and stained with 1% crystal violet for 5 min, washed and air-dried. The biofilm formation of bacterial cells were examined under a 400X of light microscopy (Nikon ECLIPSE 80i microscope) modified from (Zalewska-Piatek et al., 2009).

Scanning electron microscopic examination; biofilm formations were assessed by sub-culturing 20 µL of bacterial suspension into each well plate containing 800 µL of tryptic soy broth (Oxoid; UK) with cover glasses and incubated at 37°C for 48 hrs without shaking. Then all wells were rinsed once time with sterile water and fixed overnight with 4% formaldehyde. The fixed samples were then dehydrated with 25, 50, 75 and 96% ethanol for 20 min each at room temperature and finally air-dried. The cover glasses were removed from wells and observed under scanning electron microscopy (LEO SEM 1450VP microscope) modified from (Salo et al., 2009).

Statistical analysis

The data were presented in percentage.

Results

All chemical compositions of stones were calcium (3 calcium oxalate/calcium phosphate and 1 calcium oxalate/uric acid stones). All *E. faecalis* isolates were evaluated the biofilm formation. Based on biofilm formation, 7 bacterial isolates were divided into two groups; biofilm-formation (BF) and non-biofilm formation (NBF) groups. BF morphology of *E. faecalis* was shown in loose clusters of cells in Figure 1A and 2A.

Whereas, NBF morphology of *E. faecalis* was observed no cell aggregation as shown in Figure 1B and 2B respectively. The testing of biofilm formation by light microscopy was corresponded to scanning electron microscopy. *E. faecalis* in the urine and stones of these patients could form biofilm 60% (3/5) and 50% (1/2), respectively.

Discussion and Conclusion

In the past, UTI was not considered as a cause of calcium oxalate stone (unlike UTI with urease-producing bacteria that can cause struvite stone) (Miano et al., 2007). However, Venkatesan and coworkers (Venkatesan et al., 2011) showed that *Escherichia coli* (non urease-producing bacteria) could aggravate calcium oxalate encrustation on the surface of polyurethane film (the same material that used in urinary stents). In addition, our results showed that all of four stones belonged to mixed calcium oxalate stones (3 calcium oxalate/calcium phosphate and 1 calcium oxalate/uric acid). This result suggested that *E. faecalis* (non urease-producing bacteria) may produce some important factors involved in calcium stone genesis.

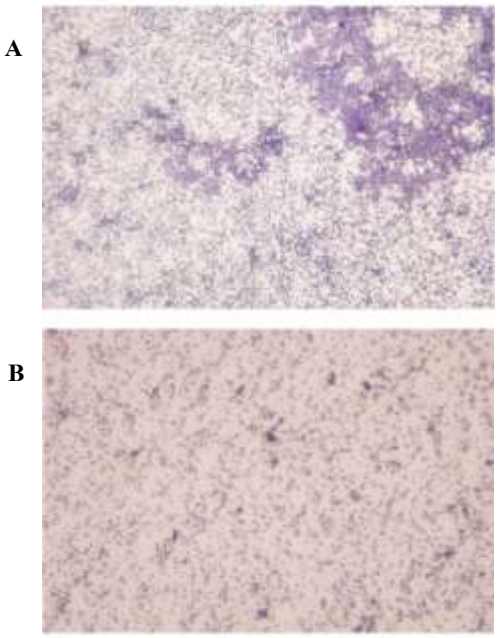

Figure 1 Two representative figures showing differentially expressed biofilm productions between biofilm-producing (A) and nonbiofilm-producing *E. faecalis* (B) by light microscopy (×400).

Recently, Chutipongtanate *et al*. founded that *E. coli* or *Streptococcus pneumoniae* could promote the growth and aggregation of calcium oxalate crystal (Chutipongtanate et al., 2013). Venkatesan and coworkers (Venkatesan et al., 2011) also suggested that biofilm created by *E. coli* might be responsible for calcium oxalate encrustation. In the present study, all *E. faecalis isolates* in the urine (60%) and stone matrices (50%) could form biofilm. This study indicated that biofilm formation by *E. faecalis* has high tendency in these kidney stone patients.

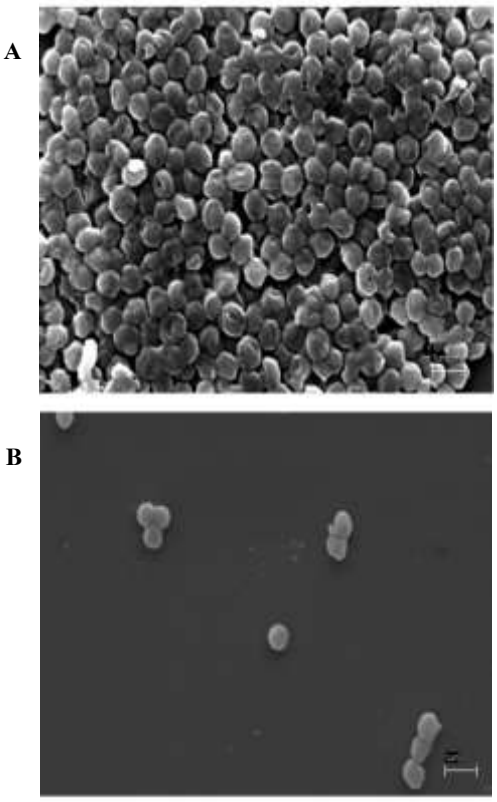

Figure 2 Two representative figures showing differentially expressed biofilm productions between biofilm-producing (A) and non-biofilm-producing *E. faecalis* (B) by scanning electron microscopy (×7000).

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