

## D-Galactose as an autoinducer 2 inhibitor to control the biofilm formation of periodontopathogens

Eun-Ju Ryu<sup>1</sup>, Jaehyun Sim<sup>1</sup>, Jun Sim<sup>3</sup>,  
Julian Lee<sup>3</sup>, and Bong-Kyu Choi<sup>1,2\*</sup>

<sup>1</sup>Department of Oral Microbiology and Immunology, <sup>2</sup>Dental Research Institute, School of Dentistry, Seoul National University, Seoul 03080, Republic of Korea

<sup>3</sup>Department of Bioinformatics and Life Science, Soongsil University, Seoul 06978, Republic of Korea

(Received Jul 19, 2016 / Revised Aug 9, 2016 / Accepted Aug 9, 2016)

**Autoinducer 2 (AI-2) is a quorum sensing molecule to which bacteria respond to regulate various phenotypes, including virulence and biofilm formation. AI-2 plays an important role in the formation of a subgingival biofilm composed mostly of Gram-negative anaerobes, by which periodontitis is initiated. The aim of this study was to evaluate D-galactose as an inhibitor of AI-2 activity and thus of the biofilm formation of periodontopathogens. In a search for an AI-2 receptor of *Fusobacterium nucleatum*, D-galactose binding protein (Gbp, Gene ID FN1165) showed high sequence similarity with the ribose binding protein (RbsB), a known AI-2 receptor of *Aggregatibacter actinomycetemcomitans*. D-Galactose was evaluated for its inhibitory effect on the AI-2 activity of *Vibrio harveyi* BB152 and *F. nucleatum*, the major coaggregation bridge organism, which connects early colonizing commensals and late pathogenic colonizers in dental biofilms. The inhibitory effect of D-galactose on the biofilm formation of periodontopathogens was assessed by crystal violet staining and confocal laser scanning microscopy in the absence or presence of AI-2 and secreted molecules of *F. nucleatum*. D-Galactose significantly inhibited the AI-2 activity of *V. harveyi* and *F. nucleatum*. In addition, D-galactose markedly inhibited the biofilm formation of *F. nucleatum*, *Porphyromonas gingivalis*, and *Tannerella forsythia* induced by the AI-2 of *F. nucleatum* without affecting bacterial growth. Our results demonstrate that the Gbp may function as an AI-2 receptor and that galactose may be used for prevention of the biofilm formation of periodontopathogens by targeting AI-2 activity.**

**Keywords:** quorum sensing, periodontopathogens, biofilm, AI-2 inhibitor, D-galactose

### Introduction

Quorum sensing (QS) is mediated via small signaling molecules that are secreted from bacteria. QS signaling molecules above a threshold can affect various phenotypes, including bioluminescence, virulence, and biofilm formation (Taga and Bassler, 2003). Autoinducer-2 (AI-2) is a QS molecule secreted from both Gram-negative and Gram-positive bacteria (Rickard *et al.*, 2006). It is synthesized through pathways involving LuxS and released out of cells, and then it reenters bacteria through a transport or two-component system and acts on gene transcription (Schauder *et al.*, 2001; Xavier and Bassler, 2003). LuxP in *Vibrio harveyi*, LsrB in *Salmonella* Typhimurium, and RbsB in *Escherichia coli* are known to be an AI-2 transporter (LuxP) or AI-2 receptors (LsrB/RbsB).

Periodontitis is initiated by multispecies bacteria present in subgingival biofilms. Major periodontopathogens are highly proteolytic and induce immune and inflammatory responses, which result in tissue damage. AI-2 plays a critical role in the biofilm formation and virulence of periodontopathogens. AI-2 can thus be a good target for the control of periodontal infections, and AI-2 inhibitors are ideal for applications that inhibit oral biofilm formation (Kolenbrander *et al.*, 2010; Shao and Demuth, 2010; Jiang and Li, 2013). Two types of AI-2 receptors, LsrB and RbsB, have been reported in *Aggregatibacter actinomycetemcomitans* (James *et al.*, 2006; Shao *et al.*, 2007), but those in other periodontopathogens remain to be identified. AI-2 binds to LsrB and RbsB in the periplasm to transduce intracellular signaling. AI-2 and D-ribose bind competitively to the RbsB of *A. actinomycetemcomitans*, whereby D-ribose can inhibit AI-2-induced intracellular communication. *Fusobacterium nucleatum* plays a critical role in dental biofilm development, which leads to periodontitis by coaggregation with pathogenic bacteria species. Recently, furanone compound and D-ribose have been demonstrated to inhibit the biofilm formation of major periodontopathogens including *F. nucleatum*, *Porphyromonas gingivalis*, *Tannerella forsythia*, and *Treponema denticola* by inhibiting AI-2 (Jang *et al.*, 2013).

In searching for AI-2 receptors in periodontopathogens and for AI-2 inhibitors, we investigated the galactose-binding protein with a molecular weight of 36 kDa (Gbp) of *F. nucleatum*, which showed high sequence similarity with the RbsB of *A. actinomycetemcomitans* and *E. coli*. A galactose-binding adhesin (30 kDa) of *F. nucleatum*'s surface has been demonstrated to be involved in coaggregation with other periodontopathogens, including *P. gingivalis* and *A. actinomycetemcomitans* (Shaniztki *et al.*, 1997; Rosen and Sela, 2006). However, the roles of *F. nucleatum* galactose binding protein as an AI-2 receptor and galactose as an AI-2 in-

\*For correspondence. E-mail: bongchoi@snu.ac.kr; Tel.: +82-2-740-8640; Fax: +82-2-743-0311  
Copyright © 2016, The Microbiological Society of Korea

hibitor have not been assessed.

In this study, we tested whether D-galactose can act as an AI-2 inhibitor by inhibiting AI-2 activity and the biofilm formation of periodontopathogens. We demonstrated that D-galactose showed high inhibitory activity against the AI-2 activity of *F. nucleatum* and *V. harveyi* and the biofilm formation of *F. nucleatum*, *P. gingivalis*, and *T. forsythia* in the presence of semi-purified AI-2 or secreted molecules of *F. nucleatum*.

## Materials and Methods

### Identification of RbsB-like proteins

To identify an AI-2 binding protein in *F. nucleatum*, we used LuxP of *V. harveyi*, LsrB of *S. Typhimurium*, LsrB and RbsB of *A. actinomycetemcomitans*, and RbsB of *E. coli* as query sequences to perform a BLAST search against protein sequences of *F. nucleatum*.

### Bacterial culture

*F. nucleatum* (ATCC 25586) and *P. gingivalis* (ATCC 33277) were cultured anaerobically (10% H<sub>2</sub>, 10% CO<sub>2</sub>, 80% N<sub>2</sub>) in brain heart infusion broth supplemented with hemin (10 µg/ml) and vitamin K (0.2 µg/ml) at 37°C. *T. forsythia* (ATCC 43037) was cultured anaerobically in new oral spirchete broth (ATCC medium 1494) supplemented with N-acetylmuramic acid (0.01 µg/ml) and vitamin K (0.2 µg/ml). AI-2 reporter strain *V. harveyi* BB170 (ATCC BAA-1117) and AI-2 producing strain *V. harveyi* BB152 (ATCC BAA-1119) were aerobically cultured in autoinducer bioassay (AB) medium, which consisted of 0.3 M sodium chloride, 0.05 M magnesium sulfate, 0.2% casamino acids, 10 mM potassium phosphate (pH 7.0), 1 mM L-arginine, and 2% glycerol at 30°C with shaking.

### Purification of *F. nucleatum* AI-2

The AI-2 of *F. nucleatum* was partially purified as described previously (Jang *et al.*, 2013). Briefly, bacteria from an overnight culture of *F. nucleatum* and *V. harveyi* BB152 were diluted 1:20 with fresh culture medium and cultured at 37°C and 30°C, respectively, until the late exponential phase (optical density at 660 nm = 0.7). The culture supernatants were collected by centrifugation at 10,000 × *g* at 4°C. After the culture supernatants were passed through 0.2-µm pore-size membrane filters (Sartorius Stedium Biotech), the filtrate was subsequently passed through a Centricon YM-3 3-kDa exclusion filter (Millipore) and then chromatographed on a C18 Sep-Pak reverse-phase column (Waters Co.) according to the manufacturer's instructions.

### Determination of AI-2-mediated bioluminescence and AI-2 inhibitory activity of D-galactose

*V. harveyi* BB170 was diluted to a concentration of 1 × 10<sup>6</sup> bacteria/ml in fresh AB medium. Then, the bacterial suspension was mixed with 10% (vol/vol) partially purified AI-2 of *F. nucleatum* or *V. harveyi* BB152 in the presence or absence of D-ribose and D-galactose, and incubated for 1–12 h

at 30°C under aerobic conditions. The bioluminescence was measured using a luminometer (GloMax-Multi Detection System, Promega).

### Effect of D-galactose on biofilm formation of periodontopathogens

Biofilm formation assays were performed by staining with crystal violet and confocal laser scanning microscopy as described previously (Jang *et al.*, 2013). Briefly, bacterial suspensions were added to 24-well plates with round glass slips (12-mm radii) in the presence of *F. nucleatum* AI-2 (10% vol/vol) and/or sugars at various concentrations and were incubated for 24–48 h under anaerobic conditions. The initial numbers of *F. nucleatum*, *P. gingivalis*, and *T. forsythia* were 2 × 10<sup>7</sup>/ml, 2 × 10<sup>8</sup>/ml, and 2 × 10<sup>8</sup>/ml, respectively. After the glass slips were washed three times with phosphate buffered saline (PBS), the biofilms that had formed on the glass slips were stained with 1% crystal violet for 10 min, washed three times with PBS, and destained with 1 ml of acetone–alcohol (20:80, vol/vol). The absorbance at 590 nm of the destaining solution containing crystal violet was measured using a microplate reader (Wallac Victor3 microtiter, PerkinElmer Life Sciences).

The biofilms formed on the cover glasses were stained using the Live/Dead-BacLight bacterial viability kit (Invitrogen), observed using a confocal laser scanning microscope (Carl Zeiss LSM 700, Gena) at a magnification of 1,000 ×, and quantified by measuring the mean intensity values of green (SYTO9) and red (propidium iodide) fluorescence.

### Effects of co-culture of *F. nucleatum* and other periodontopathogens on biofilm formation using a transwell system

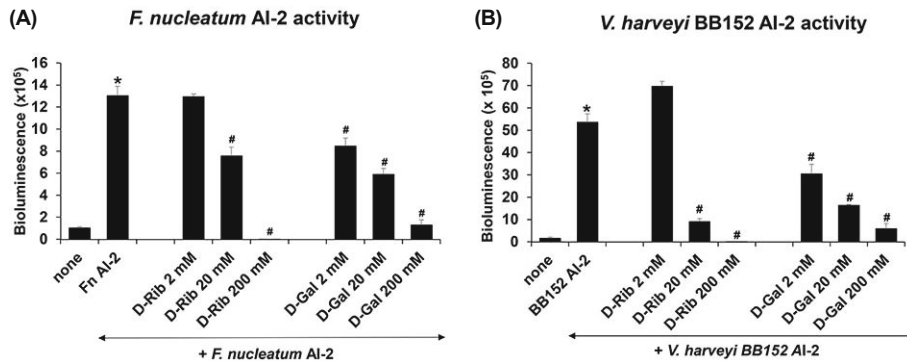
A two-compartment separated co-culture system was used to evaluate the effects of secreted molecules of *F. nucleatum* on the biofilm growth of *P. gingivalis* and *T. forsythia*. *P. gingivalis* (1 × 10<sup>7</sup> /ml) or *T. forsythia* (1 × 10<sup>7</sup> /ml) cells were suspended in coculture medium and placed in the bottom wells of transwell plates (BD Falcon™), and *F. nucleatum* (1 × 10<sup>7</sup> /ml) cells were placed in the upper wells, which were separated from the bottom wells by a membrane with a 0.4-µm pore size. The coculture medium was composed of one part of *F. nucleatum* culture medium and one part of the culture medium of the other partner bacterium. The bacterial growth for each species in the co-culture medium was confirmed to be indistinguishable from the growth observed in their own culture media. After anaerobic incubation at 37°C for 72 h, the biofilms that had formed on the glass slips in the bottom wells were stained with crystal violet and measured as described above.

### Effect of D-galactose on planktonic bacterial growth

*F. nucleatum* were grown anaerobically in the presence or absence of various concentrations of D-galactose and D-ribose. Bacterial growth was monitored for up to 48 h by measuring the absorbance at 600 nm using a spectrophotometer.

### Statistical analysis

Statistical analyses were performed using Student's *t*-test.



**Fig. 1.** Inhibitory effect of D-galactose on AI-2 activity. *V. harveyi* BB170 ( $1 \times 10^6$  bacteria/ml) was incubated for 6 h with 10% partially purified AI-2 of *F. nucleatum* (A) and *V. harveyi* BB152 (B) in the presence or absence of D-galactose (D-Gal) and D-ribose (D-Rib). The bioluminescence was measured using a luminometer. The experiments were performed three times in triplicates, and representative data are shown. \* $P < 0.05$  compared to the untreated control value; # $P < 0.05$  compared to the *F. nucleatum* AI-2 or *V. harveyi* AI-2-treated value.

Statistically significant differences between the control and AI-2 groups or between the AI-2 and sugar-treated groups were analyzed. A  $p$ -value less than 0.05 was considered statistically significant.

## Results

### Structural similarities of galactose-binding protein and RbsB

Because there have been no previously identified AI-2 receptors of *F. nucleatum*, we searched for a protein in *F. nucleatum* that had a sequence similarity with known AI-2 receptors in other bacteria. We found that the RbsB of *E. coli* and *A. actinomycetemcomitans* has a high level of sequence similarity with periplasmic D-galactose-binding protein (341 amino acid residues, Mw 36 kDa, Gbp) of *F. nucleatum* ATCC 25586 (gene ID FN1165 in EnsemblBacteria and KEGG), with  $e$ -values of  $5 \times 10^{-8}$  and  $2 \times 10^{-11}$ . No significant hits were found for the remaining AI-2 receptors. Significant amino acid sequence similarity between the RbsB and *F. nucleatum* Gbp suggests that these proteins share a common function. In particular, the result implies that although *F. nucleatum* Gbp is simply known as the galactose binding protein ATP-binding cassette (ABC) transporter in the periplasm, it may have a second function of being a quorum sensing AI-2 receptor. Therefore, we evaluated D-galactose for the competitive binding of AI-2 to Gbp and thus its feasi-

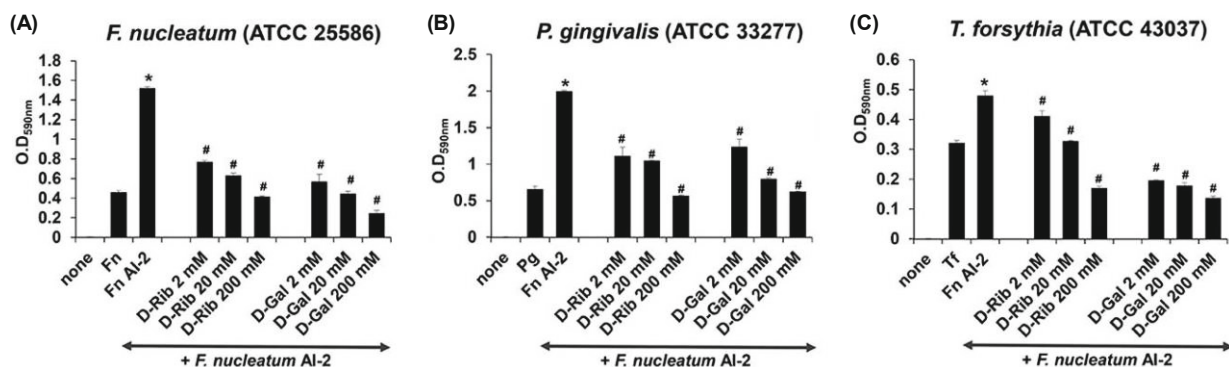
bility as a quorum sensing inhibitor for further analysis.

### Inhibitory effect of D-galactose on AI-2 activity

In a previous report, we demonstrated that D-ribose inhibited the AI-2 activity of *V. harveyi* BB152, an AI-2 producing strain, and that of *F. nucleatum* (Jang et al., 2013). In this study, we evaluated the inhibitory effect of D-galactose on AI-2 activity and compared it with that of D-ribose. As shown in Fig. 1, D-galactose significantly inhibited the AI-2 activity of *V. harveyi* BB152 and *F. nucleatum* in a dose-dependent manner. The inhibitory activity of D-galactose against the AI-2 of *F. nucleatum* and *V. harveyi* was comparable to that of D-ribose at 20 and 200 mM. However, the AI-2 inhibitory activity of D-galactose at 2 mM was superior to that of D-ribose. D-Ribose did not show an inhibitory effect at 2 mM.

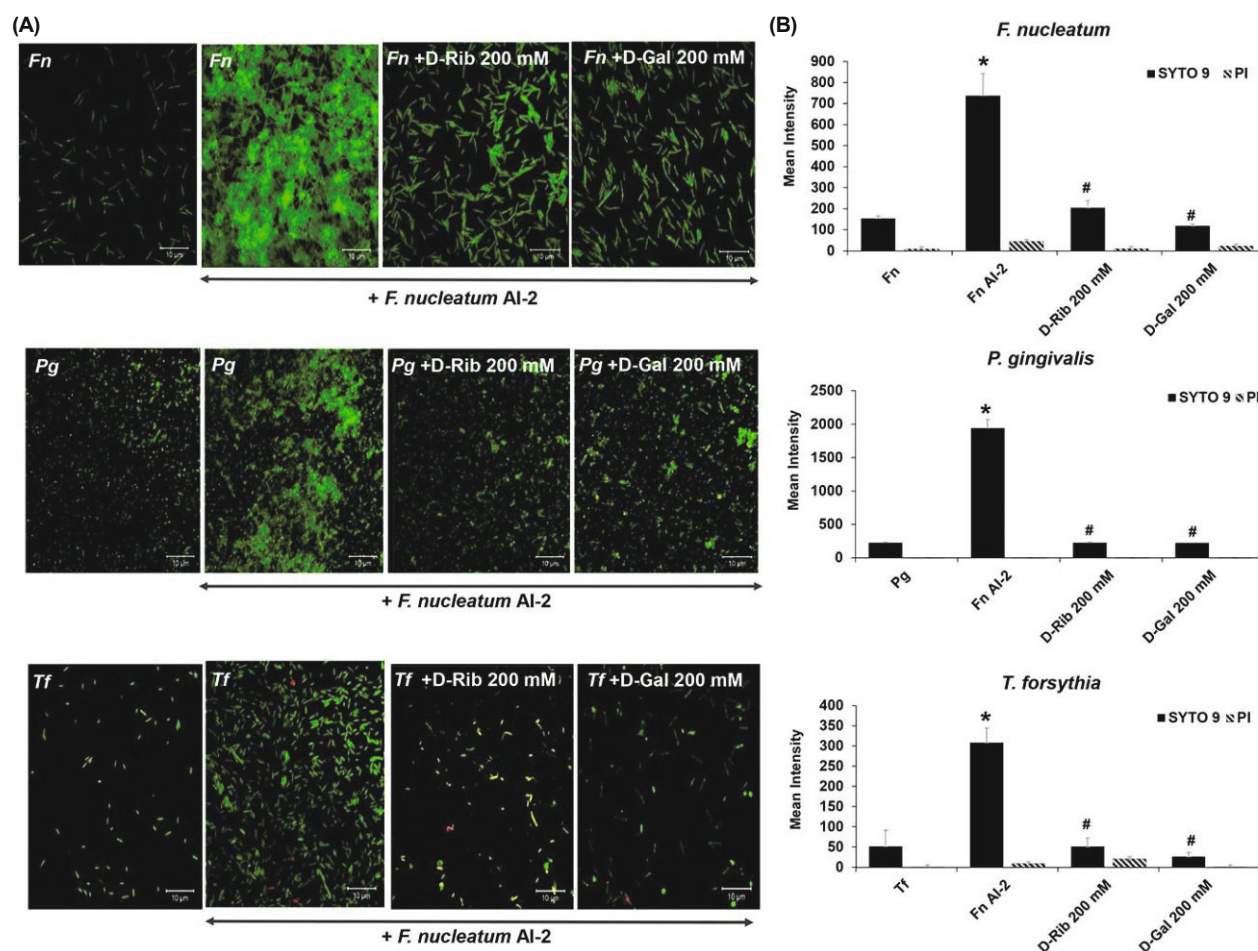
### Inhibitory effect of D-galactose on biofilm formation of periodontopathogens induced by *F. nucleatum* AI-2

Next, we tested whether D-galactose inhibited the *F. nucleatum* AI-2-induced biofilm formation of three species of periodontopathogens. As semi-purified *F. nucleatum* AI-2 induced the biofilm formation of periodontopathogens (Jang et al., 2013), we assessed the inhibitory effects of D-galactose and D-ribose on the biofilm formation of major periodontopathogens in the presence of *F. nucleatum* AI-2 or secreted



**Fig. 2.** Inhibitory effect of D-galactose on the biofilm formation of periodontopathogens. (A) *F. nucleatum* (Fn,  $2 \times 10^7$ /ml), (B) *P. gingivalis* (Pg,  $2 \times 10^8$ /ml), and (C) *T. forsythia* (Tf,  $2 \times 10^8$ /ml) were cultured with 10% partially purified *F. nucleatum* AI-2 in the presence of D-galactose (D-Gal) or D-ribose (D-Rib) for 48 h under anaerobic conditions at 37°C. Biofilm formation on glass slips was assessed by crystal violet staining. \* $P < 0.05$  compared to the untreated control value; # $P < 0.05$  compared to the biofilm formation of the *F. nucleatum* AI-2-treated value in the absence of the quorum sensing inhibitors.

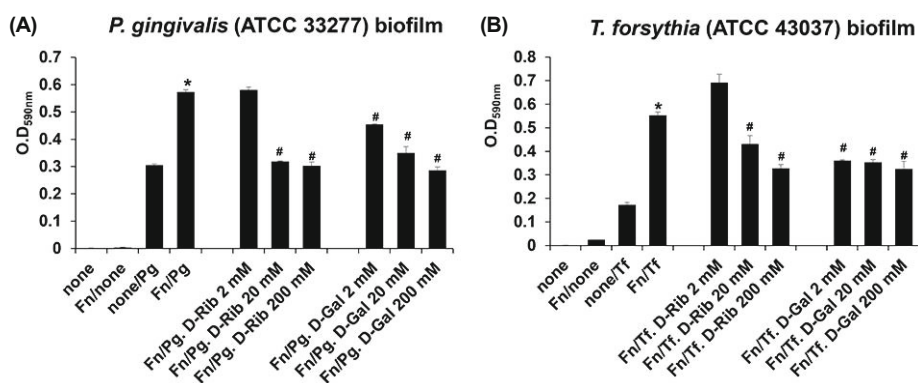




**Fig. 3.** Confocal images showing the inhibitory effect of D-galactose on the biofilm formation of periodontopathogens. *F. nucleatum* (Fn,  $2 \times 10^7$ /ml), *P. gingivalis* (Pg,  $2 \times 10^8$ /ml), and *T. forsythia* (Tf,  $2 \times 10^8$ /ml) were cultured with 10% partially purified *F. nucleatum* AI-2 in the presence of D-galactose (D-Gal) or D-ribose (D-Rib) for 48 h under anaerobic conditions at 37°C. The biofilms formed on cover glasses were stained using a Live/Dead-BacLight bacterial viability kit and observed using a confocal laser scanning microscope at a magnification of 1,000 $\times$  (A) and quantified by measuring the mean intensity values of green (SYTO 9) and red (propidium iodide) fluorescence (B). \* $P < 0.05$  compared to the untreated control value; # $P < 0.05$  compared to the biofilm formation of the *F. nucleatum* AI-2-treated value in the absence of the quorum sensing inhibitors.

molecules of this bacterium by crystal violet staining and confocal microscopy. As shown in Fig. 2, D-galactose inhibited the biofilm formation of *F. nucleatum*, *P. gingivalis*, and *T. forsythia* in the presence of *F. nucleatum* AI-2 in a dose-dependent manner when measured by crystal violet staining.

Its inhibitory effect was comparable to or better than that of D-ribose at the same concentrations. Although D-ribose did not inhibit AI-2 activity at 2 mM during 6 h of treatment (Fig. 1), a biofilm formed over 48 h was inhibited by D-ribose at this concentration (Fig. 2).



**Fig. 4.** Inhibitory effect of D-galactose on the biofilm formation of *P. gingivalis* and *T. forsythia* by secreted molecules of *F. nucleatum* using a transwell system. Using a transwell system, *F. nucleatum* ( $1 \times 10^7$ /ml) was cultured in the upper well, while (A) *P. gingivalis* ( $1 \times 10^7$ /ml) or (B) *T. forsythia* ( $1 \times 10^7$ /ml) was cultured in the bottom well in the presence of D-ribose (D-Rib) or D-galactose (D-Gal) for 72 h under anaerobic conditions at 37°C. Biofilm formation on glass slips was assessed by crystal violet staining. \* $P < 0.05$  compared to the untreated control value; # $P < 0.05$  compared to the biofilm formation in the absence of the quorum sensing inhibitors.

The biofilm formed was also visualized by confocal image analysis. As shown in Fig. 3, D-galactose markedly reduced the biofilm formation of *F. nucleatum*, *P. gingivalis*, and *T. forsythia* in the presence of *F. nucleatum* AI-2. Quantitative analyses of the fluorescences of the biofilms revealed that D-galactose significantly reduced the biofilm formation of *F. nucleatum*, *P. gingivalis*, and *T. forsythia*. D-Galactose reduced the fluorescence of the biofilms to a similar degree as that observed with D-ribose at 200 mM.

Next, we tested the inhibitory effects of D-galactose on the biofilm formation of periodontopathogens in a transwell culture system, where secreted molecules including AI-2 of *F. nucleatum* in the upper well could affect the biofilm formation of periodontopathogens in the bottom well without physical contact between the bacterial pairs. As shown in Fig. 4, secreted molecules from *F. nucleatum* in the upper well significantly increased the biofilm formation of *P. gingivalis* and *T. forsythia* in the bottom well, and D-galactose significantly inhibited their biofilm formation at all of the concentrations used, as measured by crystal violet staining.

#### Effect of D-galactose on the growth of *F. nucleatum*

As *F. nucleatum* plays the central role in the biofilm formation of periodontopathogens, we tested whether the inhibitory effect of D-galactose and D-ribose on biofilm formation could be attributed to the growth inhibition of *F. nucleatum*. As shown in Fig. 5, D-galactose did not affect the planktonic bacterial growth for 48 h incubation at the concentrations of 2 and 20 mM, while the enhanced growth was observed at 48 h of incubation with 200 mM of D-galactose. D-Ribose did not affect the planktonic bacterial growth for 48 h incubation at the concentrations of 2 and 20 mM, but reduced growth was observed at 24 h incubation with 200 mM D-ribose.

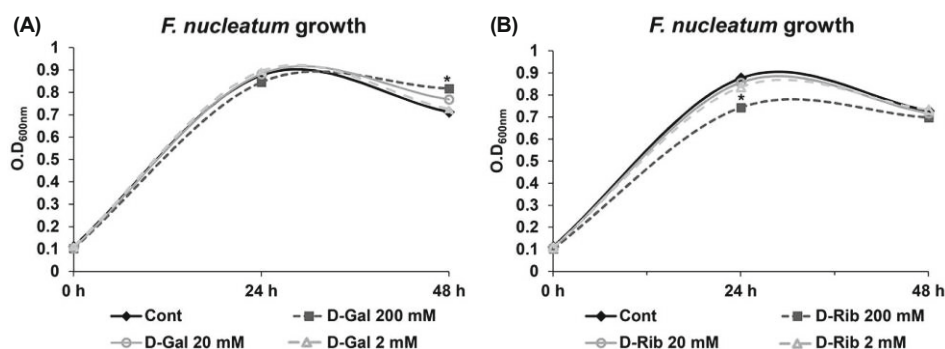
#### Discussion

This study demonstrated that D-galactose can be used as an AI-2 inhibitor to prevent the biofilm growth of periodontopathogens, possibly by binding to the Gbp. As QS enhances biofilm formation and virulence expression, it has emerged as a potential target against bacterial infection (Jiang and Li, 2013; Sepahi et al., 2015; Zhu et al., 2015). Periodontopathogens including *F. nucleatum*, *P. gingivalis*, *Prevotella intermedia*, and *A. actinomycetemcomitans* produce AI-2 (Frias et

al., 2001; Novak et al., 2010). AI-2 of *F. nucleatum* has been demonstrated to induce biofilm formation and surface adhesins of the so-called 'red complex': *P. gingivalis*, *T. denticola*, and *T. forsythia* (Socransky et al., 1998; Jang et al., 2013). As AI-2 plays an important role in the biofilm formation of periodontopathogens (Novak et al., 2010; Shao and Demuth, 2010), the antagonists of AI-2 receptors can be good candidates to inhibit biofilm formation.

AI-2 receptors or transporters have been reported in several species of bacteria: LsrB and LsrR in *E. coli* and *S. typhimurium*; AgrC in *S. aureus*; TlpB in *H. pylori*; and LuxP/Q in *Vibrio* species (Guo et al., 2013). Although periodontopathogens are known to produce AI-2, AI-2 receptors have been reported only in *A. actinomycetemcomitans* (James et al., 2006; Shao et al., 2007). *A. actinomycetemcomitans* expresses two periplasmic proteins, RbsB and LsrB, which can interact with AI-2. *F. nucleatum* connects early colonizers and late pathogenic colonizers including *P. gingivalis* and *T. forsythia*, which are highly proteolytic and are present in subgingival biofilms adjacent to pocket epithelium (Socransky and Haffajee, 2005; Kolenbrander et al., 2010). Thus, the identification of an AI-2 receptor of *F. nucleatum* and the blocking of that receptor would be a way to prevent the development of a biofilm composed of periodontal pathogens. *F. nucleatum* Gbp with the predicted molecular weight of approximately 36 kDa, which shows a high structural similarity to the RbsB of *A. actinomycetemcomitans* and *E. coli*, seems to be an AI-2 receptor, because D-galactose inhibited the AI-2 activity of *F. nucleatum* and *V. harveyi*.

A galactose-binding adhesin (30 kDa) in the outer membrane of *F. nucleatum* PK1594 has been reported to coaggregate with periodontopathogens, including *P. gingivalis* and *A. actinomycetemcomitans*, and to mediate hemagglutination (Shanitzki et al., 1997; Weiss et al., 2000; Rosen and Sela, 2006; Rosen et al., 2008). It has also been shown to mediate the interaction of *F. nucleatum* with epithelial cells, fibroblasts, lymphocytes, and erythrocytes. These interactions were inhibited by D-galactose. However, it is not likely that the galactose-binding adhesin refers to the Gbp identified in our study. In addition to having a different molecular weight, the subcellular locations of these two molecules are also different, so that this galactose-binding adhesin is in the bacterial surface (Shanitzki et al., 1997), while the Gbp is predicted to localize in the periplasm. According to the sequence homology, the Gbp is the galactose/glucose ABC transporter. *F. nucleatum* can utilize sugars and amino acids as carbon and energy sources, whereby amino acids are the preferred energy



**Fig. 5.** Effect of D-galactose on planktonic bacterial growth. *F. nucleatum* was grown in brain-heart infusion medium for 48 h under anaerobic conditions at 37°C in the presence of D-galactose (D-gal) or D-ribose (D-rib). Bacterial growth was monitored by measuring the absorbance at 600 nm using a spectrophotometer. \* $P < 0.05$  compared to the untreated control value

source (Kapatral *et al.*, 2002). *F. nucleatum* takes up galactose by a galactose/glucose ABC transporter, which is a member of the pentose/hexose sugar-binding protein family of the type I periplasmic binding protein superfamily in various bacterial species. The ABC transporter is involved in chemotaxis towards galactose and glucose, and in active transport of the sugars. *F. nucleatum* possesses genes encoding the enzymes involved in galactose utilization (Kapatral *et al.*, 2002). Galactose is converted to glucose-6-phosphate which can enter into a pathway of glycolysis. In our study, D-galactose did not affect the bacterial growth at the concentrations used for the experiments, suggesting its role as an AI-2 inhibitor rather than energy source. *F. nucleatum* is known to use sugars including glucose, galactose, and fructose for synthesis of intracellular polymers which can be utilized to survive under conditions of amino acid deprivation (Bolstad *et al.*, 1996).

The inhibition of biofilm formation by D-galactose could be solely attributed to coaggregation inhibition by the competitive binding of galactose to the galactose-binding adhesin rather than the inhibition of AI-2 activity. However, D-galactose inhibited the AI-2 activity of *F. nucleatum* and *V. harveyi* as indicated by bioluminescence assay, indicating its function as an AI-2 inhibitor. In addition, semi-purified AI-2 or secreted molecules including AI-2 of *F. nucleatum* increased the biofilm formation of periodontopathogens, which was significantly reduced by D-galactose. These results indicate that D-galactose inhibits the biofilm formation of periodontopathogens by competitive binding to the Gbp with AI-2. In addition to the direct effect of D-galactose on the Gbp, it is possible that D-galactose may inhibit other adhesins of periodontopathogens induced by AI-2, as do other AI-2 inhibitors including D-ribose and furanone compound, resulting in the further inhibition of biofilm formation (Jang *et al.*, 2013). However, this hypothesis remains to be investigated. In the future, construction of a *F. nucleatum* mutant strain defective in the Gbp may allow us to verify the role of the Gbp in the QS. In conclusion, the Gbp of *F. nucleatum* may be an AI-2 receptor, and D-galactose is a good candidate to inhibit biofilm formation of periodontopathogens effectively, thus contributing to the control of periodontitis.

## Acknowledgements

This study was supported by a grant (A120757) from the Korea Healthcare Technology R&D, Ministry of Health and Welfare, Republic of Korea.

## References

- Bolstad, A.I., Jensen, H.B., and Bakken, V. 1996. Taxonomy, biology, and periodontal aspects of *Fusobacterium nucleatum*. *Clin. Microbiol. Rev.* **9**, 55–71.
- Frias, J., Olle, E., and Alsina, M. 2001. Periodontal pathogens produce quorum sensing signal molecules. *Infect. Immun.* **69**, 3431–3434.
- Guo, M., Gamby, S., Zheng, Y., and Sintim H.O. 2013. Small molecule inhibitors of AI-2 signaling in bacteria: state-of-the-art and future perspectives for anti-quorum sensing agents. *Int. J. Mol. Sci.* **14**, 17694–17728.
- James, D.E., Shao, H., Lamont, R.J., and Demuth, D.R. 2006. *Actinobacillus actinomycetemcomitans* ribose binding protein RbsB interacts with cognate and heterologous autoinducer-2 signals. *Infect. Immun.* **74**, 4021–4029.
- Jang, Y.J., Choi, Y.J., Lee, S.H., Jun, H.K., and Choi, B.K. 2013. Autoinducer 2 of *Fusobacterium nucleatum* as a target molecule to inhibit biofilm formation of periodontopathogens. *Arch. Oral Biol.* **58**, 17–27.
- Jiang, T. and Li, M. 2013. Quorum sensing inhibitors: a patent review. *Expert. Opin. Ther. Pat.* **23**, 867–894.
- Kapatral, V., Anderson, I., Ivanova, N., Reznik, G., Los, T., Lykidis, A., Bhattacharyya, A., Bartman, A., Gardner, W., Grechkin, G., *et al.* 2002. Genome sequence and analysis of the oral bacterium *Fusobacterium nucleatum* strain ATCC 25586. *J. Bacteriol.* **184**, 2005–2018.
- Kolenbrander, P.E., Palmer, R.J.Jr., Periasamy, S., and Jakubovics, N.S. 2010. Oral multispecies biofilm development and the key role of cell-cell distance. *Nat. Rev. Microbiol.* **8**, 471–480.
- Novak, E.A., Shao, H., Daep, C.A., and Demuth, D.R. 2010. Autoinducer-2 and QseC control biofilm formation and *in vivo* virulence of *Aggregatibacter actinomycetemcomitans*. *Infect. Immun.* **78**, 2919–2926.
- Rickard, A.H., Palmer, R.J.Jr., Blehert, D.S., Campagna, S.R., Semmelhack, M.F., Eglund, P.G., Bassler, B.L., and Kolenbrander, P.E. 2006. Autoinducer 2: a concentration-dependent signal for mutualistic bacterial biofilm growth. *Mol. Microbiol.* **60**, 1446–1456.
- Rosen, G. and Sela, M.N. 2006. Coaggregation of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* PK 1594 is mediated by capsular polysaccharide and lipopolysaccharide. *FEMS Microbiol. Lett.* **256**, 304–310.
- Schauder, S., Shokat, K., Surette, M.G., and Bassler, B.B. 2001. The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. *Mol. Microbiol.* **41**, 463–476.
- Sepahi, E., Tarighi, S., Ahmadi, F.S., and Bagheri, A. 2015. Inhibition of quorum sensing in *Pseudomonas aeruginosa* by two herbal essential oils from *Apiaceae* family. *J. Microbiol.* **53**, 176–180.
- Shaniztki, B., Hurwitz, D., Smorodinsky, N., Ganeshkumar, N., and Weiss, E.I. 1997. Identification of a *Fusobacterium nucleatum* PK1594 galactose binding adhesin which mediates coaggregation with periopathogenic bacteria and hemagglutination. *Infect. Immun.* **65**, 5231–5237.
- Shao, H. and Demuth, D.R. 2010. Quorum sensing regulation of biofilm growth and gene expression by oral bacteria and periodontal pathogens. *Periodontol.* **2000** **52**, 53–67.
- Shao, H., James, D., Lamont, R.J., and Demuth, D.R. 2007. Differential interaction of *Aggregatibacter (Actinobacillus) actinomycetemcomitans* LsrB and RbsB proteins with autoinducer 2. *J. Bacteriol.* **189**, 5559–5565.
- Socransky, S.S. and Haffajee, A.D. 2005. Periodontal microbial ecology. *Periodontol.* **2000** **38**, 135–187.
- Socransky, S.S., Haffajee, A.D., Cugini, M.A., Smith, C.Jr., and Kent, R.L. 1998. Microbial complexes in subgingival plaque. *J. Clin. Periodontol.* **25**, 134–144.
- Taga, M.E. and Bassler, B.L. 2003. Chemical communication among bacteria. *Proc. Natl. Acad. Sci. USA* **100** Suppl 2, 14549–14554.
- Weiss, E.I., Shaniztki, B., Dotan, M., Ganeshkumar, N., Kolenbrander, P.E., and Metzger, Z. 2000. Attachment of *Fusobacterium nucleatum* PK1594 to mammalian cells and its coaggregation with periodontopathogenic bacteria are mediated by the same galactose-binding adhesin. *Oral Microbiol. Immunol.* **15**, 371–377.
- Xavier, K.B. and Bassler, B.L. 2003. LuxS quorum sensing: more than just a numbers game. *Curr. Opin. Microbiol.* **6**, 191–197.
- Zhu, J., Huang, X., Zhang, F., Feng, L., and Li, J. 2015. Inhibition of quorum sensing, biofilm, and spoilage potential in *Shewanella baltica* by green tea polyphenols. *J. Microbiol.* **53**, 829–836.