REPORT

(RESEARCH AWARD OF ORAL SCIENCES)

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Title: The role of extracellular DNA in pyocyanin production from *Pseudomonas aeruginosa*

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Extracellular DNA (eDNA) has been recognized as pivotal biofilm component in various Gram negative and Gram positive bacterial species. In *Pseudomonas aeruginosa,* an opportunistic human pathogen, eDNA is essential for both biofilm formation and its structural stability (Das and Mike, 2012). Brinkmann *et al.* (2004) reported that eDNA whitin biofilms originate from both bacteria and host cells. *In vivo* studies showed that eDNA concentration has been detected in high level in human lung even under normal physiological condition (100-200 μg/ml) and at infection sites such as in cystic fibrosis patients reaches as high as 4 mg/ml (Potter *et al.* 1969).

P. aeruginosa uses several virulence factors to establish chronic respiratory infection in bronchiectasis, chronic obstructive pulmonary diseases and cystic fibrosis patients. One of its toxins, pyocyanin, is a redox-active pigment that required for full virulence in animal models and has been detected in patient`s airways secretion. Pyocyanin produced from *P. aeruginosa* has been known as a virulence factor playing a major role in chronic lung infection. Previous study demonstrated that pyocyanin promotes DNA release and then increases eDNA concentration in *P. aeruginosa*. Recently, we reported that eDNA increases biofilm mass of *Streptococcus intermedius* and rigidity of this biofilm structure (*J. Appl. Microbiol.* 2013). Since *P. aeruginosa* and *S. intermedius* are often isolated together from cystic fibrosis patients,

we assume that eDNA may also have a role on pyocyanin production from *P. aeruginosa* and may also influence cell host responses.

Althouth Das and Manefield (2012) demonstrated that pyocyanin promotes DNA release and then increases eDNA concentration in *P. aeruginosa* populations and several reports also suggest the important function of eDNA as a component of biofilm structure in wide range of bacteria, however so far, no data has been published the effect of eDNA on pyocyanin production from *P. aeruginosa.*

In this study, using two method of pyocyanin assay, I confirmed that extracellular DNA significantly increased pyocyanin production from *P. aeruginosa* and

Das and Manefield reported that 10-50 μM of purified pyocyanin promotes 1-10 μg/ml DNA release in *P. aeruginosa* culture supernatant.[3)] I found that in the concentration of 1-10 μg/ml of eDNA, *P. aeruginosa* produced pyocyanin in range of 10-50 μM. Based on this result, we suggest that eDNA and pyocyanin influence each other to form a continuous cycle to maintain the its high concentration in infection sites. Furthermore, it might be one of pathogenic strategy of *P. aeruginosa* in causing infection.

Now I am planning to continue this research to investigate the effect of eDNA on human cell which may important to develop a new strategy to fight against infections.

This research has been presented at the international scientific meeting organized by Universitas Gadjah Mada held on Yogyakarta, Indonesia, February 28th - March 1st, 2014.

Tokushima, March 2014

Applicant,

(Asikin Nur)

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Asikin Nur, Katsuhiko Hirota, Hiromichi Yumoto, Kouji Hirao, Dali Liu, Kanako Takahashi, Keiji Murakami, Takashi Matsuo, Rong Shu, Yoichiro Miyake: Effects of extracellular DNA and DNA-binding protein on the development of a Streptococcus intermedius biofilm. J. Appl. Microbiol. 115: 260-270, 2013.