Journal of Microbiology, Immunology and Infection (2017) xx, 1–10

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.e-jmii.com

Original Article

Phosphoenolpyruvate phosphotransferase system components positively regulate *Klebsiella* biofilm formation

Yu-Tze Horng [a], Chi-Jen Wang [a], Wen-Ting Chung [a], Huei-Jen Chao [b], Yih-Yuan Chen [c], Po-Chi Soo [a,*]

[a] *Department of Laboratory Medicine and Biotechnology, Tzu Chi University, College of Medicine, 701 Section 3, Zhongyang Road, Hualien 97004, Taiwan, ROC*
[b] *Department of Laboratory Medicine, Buddhist Tzu Chi General Hospital, Hualien, Taiwan, ROC*
[c] *Department of Biochemical Science and Technology, National Chiayi University, Chiayi 60004, Taiwan, ROC*

Received 4 October 2016; received in revised form 7 January 2017; accepted 24 January 2017
Available online ■ ■ ■

KEYWORDS
Klebsiella;
PTS;
Biofilm;
eDNA;
Polysaccharide

Abstract *Background/Purpose: Klebsiella pneumoniae* is one of the leading causes of device-related infections (DRIs), which are associated with attachment of bacteria to these devices to form a biofilm. The latter is composed of not only bacteria but also extracellular polymeric substances (EPSes) consisting of extracellular DNAs, polysaccharides, and other macromolecules. The phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) regulates diverse processes of bacterial physiology. In the genome of *K. pneumoniae* MGH 78578, we found an uncharacterized enzyme II complex homolog of PTS: KPN00353 (EIIA homolog), KPN00352 (EIIB homolog), and KPN00351 (EIIC homolog). The aim of this study was to characterize the potential physiological role of KPN00353, KPN00352, and KPN00351 in biofilm formation by *K. pneumoniae*.
Methods/Results: We constructed the PTS mutants and recombinant strains carrying the gene(s) of PTS. The recombinant *K. pneumoniae* strain overexpressing *KPN00353–KPN00352–KPN00351* produced more extracellular matrix than did the vector control according to transmission and scanning electron microscopy. Judging by quantification of biofilm formation, of extracellular DNA (eDNA), and of capsular polysaccharide, the recombinant strain overexpressing *KPN00353-KPN00352-KPN00351* produced more biofilm and capsular polysaccharide after overnight culture and more eDNA in the log phase as compared to the vector control.

* Corresponding author. Department of Laboratory Medicine and Biotechnology, College of Medicine, Tzu Chi University, No. 701, Section 3, Zhongyang Rd., Hualien 97004, Taiwan, ROC. Fax: +886 3 8571917.
E-mail address: pcsoo@mail.tcu.edu.tw (P.-C. Soo).

http://dx.doi.org/10.1016/j.jmii.2017.01.007

Conclusion: The genes, *KPN00353—KPN00352—KPN00351*, encode a putative enzyme II complex in PTS and positively regulate biofilm formation by enhancing production of eDNA and capsular polysaccharide in *K. pneumoniae*. Five proteins related to chaperones, to the citric acid cycle, and to quorum sensing are upregulated by the KPN00353—KPN00352—KPN00351 system.

Introduction

Klebsiella pneumoniae is not only a saprophyte microorganism in the environment but also a pathogen causing infections in the respiratory tract, urinary tract, soft tissues, and surgical wounds, even subsequent septicemia that primarily affects immunocompromised patients who are hospitalized.[1—3] Indwelling medical devices (such as an intravascular catheter, endotracheal tube, and urinary catheter) have greatly improved modern healthcare. Nonetheless, device-related infections (DRIs) are observed in patients with a device who were admitted to a hospital for a reason other than that infection, and such infections develop more than 48 h after admission to an intensive-care unit.[4] A cohort study indicated that the majority of DRIs in pediatric patients were caused by *K. pneumoniae* followed by staphylococcal biofilms.[5] In addition, it was reported that duodenoscopes contaminated with *K. pneumoniae* have resulted in several nosocomial outbreaks.[6] DRIs are associated with attachment of bacteria to these devices to form a biofilm; these infections substantially increase morbidity and mortality among patients.[4] Le Chevallier et al. reported that *K. pneumoniae* has the ability to grow on abiotic surfaces in 1988.[7] Then, Reid et al. found a bacterial biofilm caused by *K. pneumoniae* on the bladder epithelium.[8] In addition, it was reported that an oral biofilm formed by *K. pneumoniae* is associated with heart disease in the elderly.[9] Subsequently, several studies revealed biofilm formation by *K. pneumoniae* on indwelling medical devices.[10—12]

A biofilm is a complicated community of bacterial cells embedded in a self-produced extracellular polymeric substances (EPS) matrix. EPS are a complex high-molecular-weight mixture of polymers excreted by bacteria, resulting from bacterial-cell lysis, and adsorbed organic matter from the environment. EPS keep bacteria attached to the surface and together in a three-dimensional (3D) matrix. The components of EPS, mainly polysaccharides, proteins, nucleic acids, and lipids, strongly affect the stability of a biofilm.[13] The capsule and lipopolysaccharide (LPS) are reported to be involved in *K. pneumoniae* biofilm formation. LPS is required for initial adhesion to the surface of glass or polyvinylchloride, whereas the capsular polysaccharide is involved in the appropriate initial coverage of a substratum and construction of mature biofilm architecture.[14] In addition, extracellular DNA (eDNA) is reported to be involved in adhesion at the early stages of biofilm formation and serves as a structural component of mature bacterial aggregates.[15,16]

The phosphoenolpyruvate (PEP):carbohydrate phosphotransferase system (PTS) regulates diverse processes of bacterial physiology in response to the availability of carbohydrates in the environment via translocation of carbohydrates across the cell membrane.[17—19] The PTS is a phosphorylation cascade composed of a membrane-spanning protein and cytosolic proteins that sequentially transfer phosphate from PEP to the substrate, carbohydrates. Enzyme I (EI) and histidine phosphocarrier protein (HPr) are the general cytoplasmic PTS proteins involved in the transfer of all PTS carbohydrates in most bacteria, whereas substrate specificity depends on the enzyme II (EII) complex. Bacteria contain several EII complexes: for example, *Escherichia coli* contains at least 15 different EII complexes. In most of PTS, the EII complex consists of two soluble proteins/domains (EIIA, EIIB) and one membrane-bound protein/domain (EIIC). In the mannose-type PTS, the EII complex also contains membrane-bound EIID. EIIC and EIID facilitate the translocation of a substrate across the cell membrane. To phosphorylate the carbohydrate in most cases of translocation by PTS, the phosphoryl transfer chain begins with autophosphorylation of EI while using PEP as a substrate to provide a phosphoryl group. Subsequently, the phosphoryl group is transferred from EI to HPr. The phosphorylated HPr phosphorylates one of the carbohydrate-specific EIIAs, which then passes the phosphoryl group to its cognate, EIIB. Finally, in most of PTS, phosphorylated EIIB transfers the phosphoryl group to the carbohydrate bound to the cognate EIIC. In addition to sugar transport, the bacterial PTS has been reported to be involved in the regulation of utilization of a nitrogen source, in potassium uptake, and carbohydrate metabolic pathways, such as carbohydrate catabolism repression (CCR) and inducer exclusion. In addition to phosphorylation, components of a PTS may perform their regulatory function via protein—protein interactions.[17,19] In the *K. pneumoniae* MGH 78578 genome sequence, we found an uncharacterized enzyme II complex homolog: KPN00353 (EIIA homolog), KPN00352 (EIIB homolog), and KPN00351 (EIIC homolog). The aim of this study was to characterize the potential physiological role of KPN00353, KPN00352, and KPN00351 in biofilm formation by *K. pneumoniae*.

Methods

Bacterial strains and growth conditions

The bacterial strains and plasmids used in this study are listed in Table 1. The growth conditions are described in Supplementary Material.

Please cite this article in press as: Horng Y-T, et al., Phosphoenolpyruvate phosphotransferase system components positively regulate *Klebsiella* biofilm formation, Journal of Microbiology, Immunology and Infection (2017), http://dx.doi.org/10.1016/j.jmii.2017.01.007