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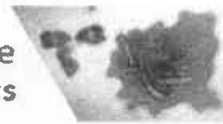
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Development of a dry, stable and inhalable acyl-homoserine-lactone-acylase powder formulation for the treatment of pulmonary *Pseudomonas aeruginosa* infections

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ABSTRACT

In the lungs of cystic fibrosis (CF) patients, *Pseudomonas aeruginosa* commonly causes chronic infections. It has been shown that the *P. aeruginosa* quorum sensing (QS) system controls the expression of virulence factors during invasion and infection to host cells. PvdQ is an acyl-homoserine lactone (AHL) acylase able to degrade the signal molecule of *P. aeruginosa* QS. The role of PvdQ in inhibiting the QS and its successive virulence determinants has been established in *in vitro* as well as in *in vivo*, the latter in a *Caenorabditis elegans* model. For the treatment of pulmonary *P. aeruginosa* infections, we propose that PvdQ can be best administered directly to the lungs of the patients as a dry powder because this is expected to give specific advantages in delivery as compared to nebulizing. Therefore in this study we investigated the production of a PvdQ powder by spray-freeze drying using mannitol, trehalose and inulin as excipient. The activity of PvdQ in the powder was determined immediately after production and after subsequent storage during 4 weeks at 20 °C and 55 °C. We found that the enzymatic activity of PvdQ is fully maintained during spray-freeze drying using mannitol, trehalose or inulin as excipient. However, mannitol was not able to stabilize the protein during storage, while PvdQ incorporated in trehalose or inulin was fully stabilized even during storage at 55 °C for at least 4 weeks. The poor stabilizing capacities of mannitol during storage could be related to its crystalline nature while the excellent stabilizing capacities of trehalose and inulin during storage could be related to their amorphous nature. The trehalose and inulin-based particles consisted of porous spheres with a volume average aerodynamical diameter of ~1.8 μm implying that they are suitable for pulmonary delivery. This is the first study in which an AHL-degrading enzyme is processed into spray-freeze-dried powder suitable for inhalation.

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1. Introduction

Pseudomonas aeruginosa is an opportunistic pathogen to humans, that becomes virulent in many hospital-acquired contaminations, such as in urinary tract, surgical wound, pneumonia and bloodstream infections. Patients with immunosuppression, cystic fibrosis (CF), chemotherapy and trauma have an increased risk for the infection (Jones et al., 2010; Lai et al., 2003).

Cell-to-cell signaling is an essential prerequisite for the establishment of *P. aeruginosa* infections (Donabedian, 2003; Van Delden and Iglewski, 1998). During invasion and infection, this bacterium switches on a subset of genes important for virulence to its host cells. Many of those virulence factors, such as rhamnoli-

pid (Jensen et al., 2007), are produced under the control of quorum sensing (QS) signaling molecules (Bjarnsholt et al., 2010; Defoirdt et al., 2010; Nadal Jimenez et al., 2012; Van Delden and Iglewski, 1998). *P. aeruginosa* possesses a complex QS system with at least three signal molecules, N-3-oxododecanoyl-L-homoserine lactone (3-oxo-C₁₂-HSL), N-butyryl-L-homoserine lactone (C₄-HSL) and 2-heptyl-3-hydroxy-4-quinolone (PQS) (Williams and Camara, 2009). Several studies reported that, these signal molecules (Erickson et al., 2002; Favre-Bonte et al., 2002; Singh et al., 2000) and also mRNA of the auto-inducer synthase gene *lasI* (Erickson et al., 2002) can be detected at elevated levels in sputum samples of CF patients' lungs. The signal molecules not only induce virulence, but they can also cause themselves inflammatory responses (Mayer et al., 2011; Zhu et al., 2008). These facts suggest that a suppression of the *P. aeruginosa* QS system might reduce expression of the virulence factors in the lung tissue of CF patients.

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