Quorum quenching effect of cyclodextrins on the pyocyanin and pyoverdin production *of Pseudomonas aeruginosa*

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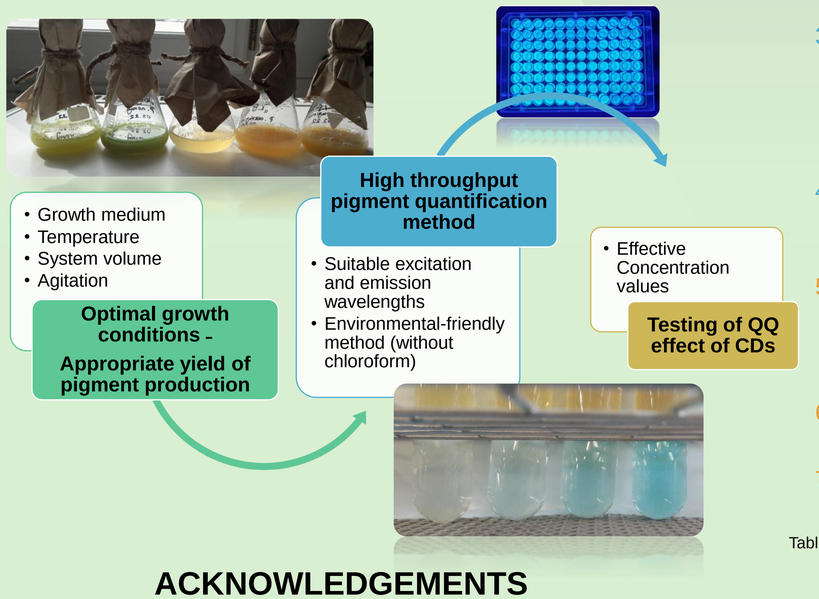
INTRODUCTION AND PURPOSE

Due to the misuse of antibiotics, there is an urgent need for antimicrobial agents able to control efficiently bacterial infectious diseases. In *Pseudomonas aeruginosa*, diverse virulence determinants and secondary metabolites are regulated via the action of a hierarchical quorum sensing (QS) system, which involves the production, detection and response to low molecular weight compounds, called signal molecules or autoinducers. The regulation, moreover the disruption of the QS system in bacteria opens new potentials in efficiently overcoming the problem of antibiotic resistance. The QS process can be altered by different mechanisms such as reducing the activity of the receptor protein or autoinducer synthase, inhibiting the production of QS signal molecules, degrading autoinducers and mimicking the signal molecules primarily by using synthetic compounds as analogues of signal molecules. The molecules responsible for inhibition of autoinducer-induced QS systems are called quorum-sensing inhibitors (QSIs). Ikeda et al. [2002] succesfully controlled autoinducer activities, hence quorum sensing in *P. aeruginosa* without inhibiting bacterial growth by adding cyclodextrin (CD) to the bacterial culture medium. This effect is based on inclusion complex formation of autoinducers with CD. Morohoshi et al. [2013] demonstrated that modified CDs show specific binding ability to autoinducers in order to improve their quorum quenching (QQ) activity in *P. aeruginosa* through the formation of spatial conformations.

OBJECTIVES

The overall objective of our research was ***(1)*** to investigate the QQ effect of different CD molecules and their derivatives in the *P. aeruginosa* model system based on pyoverdin and pyocyanin pigment production, ***(2)*** to develop a small volume high throughput test system with quick response for the detection of QQ effect, taking into consideration the high price of CD molecule products. ***(3)*** In the case of pyocyanin pigment quantification to eliminate the step of chloroform extraction in pyocyanin pigment determination due to environmental and health issues.



ACKNOWLEDGEMENTS

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MATERIALS AND METHODS

1. The yield of pigment production both for pyoverdin and pyocianin was studied under different growth conditions where the tested influential factors were temperature (30°C and 37°C), contact time (24, 48, 72 h), growth medium (King A, King B, LB and LB+2% glucose), agitation (no or 160 rpm) and test volume (200 µL or 30 mL).
2. In both cases a preliminary experiment was carried out applying the following excitation and emission wavelength pairs [400 ex; 480 em], [400 ex; 520 em] and [485 ex; 520 em] when measuring a five member two-fold dilution series of cell suspensions containing pyoverdin or pyocyanin pigments in the presence of cells and in the cell-free supernatant in order to select the most feasible detection parameters.
3. The inhibitory effect of α- and ß-CD (ACD, BCD), randomly-methylated α- and ß-CD (RAMEA, RAMEB), quaternary ammonium α-CD (QAACD), (2-hydroxypropyl)-α- and ß-CD (HPACD, HPBCD), sulfobutyl ether ß-CD (SBEBCD) and α- and ß-CD polymers (ACDPS, BCDPS) on the pyoverdin production of *P. aeruginosa* (DSM 117) was tested within the 0.195−12.5 mM concentration range in a small volume (200 µL) model system. The effect of ACD and BCD on the production of pyocyanin was also tested in the 1−10 mM concentration range in increased volume test systems (30 mL) with exponentially growing bacterial cultures.

CONCLUSION

1. In the case of pyoverdin production, King B growth medium resulted in the best selectivity and yield at 37°C in small volume test system (200 µL) without agitation after 24 h (not presented on diagrams).
2. In the case of pyocyanin production, in small volume test system (200 µL) pyocyanin was not produced, therefore using a small volume test system proved to be a dead end. In the large volume test systems (30 mL) King A medium resulted in the best selectivity and yield after 72 h, at 160 rpm and 30°C. (not presented on diagrams).
3. Both in the case of pyoverdin and pyocyanin quantification the use of [485 ex; 520 em] nm wavelength pairs resulted in the best linear fit for the tested dilution series. Based on the R^2 values of the linear fitting formula, the presence of the cells in the cell suspensions did not disturb the quantification of pigment molecules compared to the cell-free supernatant (Fig. 1 and 2).
4. The conventional method using chloroform can be simplified by eliminating the step of chloroform extraction of pyocyanin pigment molecules resulting in a more environmental- and health-friendly method (Fig. 2)
5. ACD and its derivatives resulted in higher inhibition of pyoverdin production than BCD and its derivatives in the tested concentration range (0.195−12.5 mM), which may be explained by the different diameter of the cavity size of α- and ß-CD molecules, serving as complexing host compounds to autoinducers of *P. aeruginosa* QS system (Fig. 3).
6. Based on the Effective Concentration (EC_{20}) values, RAMEA and QAACD were the most effective causing significant decrease of pyoverdin production (Table 1).
7. The characteristics of the inhibition phenomenon of α- and ß-CD molecules proved to be similar in the case of pyoverdin and pyocyanin pigment production (Fig 3 and 4).

Table 1: Effective Concentration values of α-CD and its derivatives in the *P. aeruginosa* pyoverdin production model system

[mM]	EC_{20}	EC_{50}
ACD	4.24	11.44
RAMEA	2.49	10.04
HPACD	4.05	17.45
QAACD	1.63	4.64

Table 2: Effective Concentration values of α-CD in the *P. aeruginosa* pyocyanin production model system

[mM]	EC_{20}	EC_{50}
ACD	3.80	> 10

RESULTS

QUANTIFICATION METHODS OF PYOVERDIN AND PYOCYANIN PIGMENTS

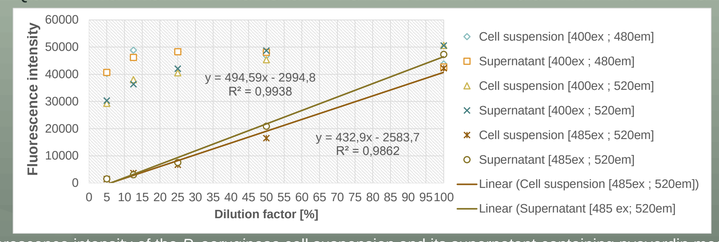

Fig.1: Fluorescence intensity of the *P. aeruginosa* cell suspension and its supernatant containing pyoverdin measured at different excitation and emission wavelength combinations

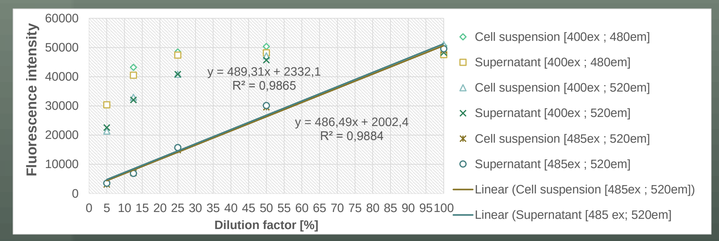

Fig.2: Fluorescence intensity of the *P. aeruginosa* cell suspension and its supernatant containing pyocyanin measured at different excitation and emission wavelength combinations

QS STUDIES ON PYOVERDIN PIGMENT PRODUCTION IN SMALL VOLUME TEST SYSTEM

Fig. 3: Modulation of pyoverdin production by α- and β-CD and their derivatives in the *P. aeruginosa* model system. Significant inhibition and stimulation compared to control is marked by asterisk (*)

QS STUDIES ON PYOCYANIN PIGMENT PRODUCTION IN AN INCREASED VOLUME TEST SYSTEM

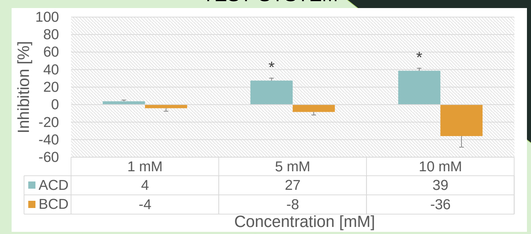

Fig. 4: Modulation of pyocyanin production by α- and β-CD in the *P. aeruginosa* model system. Significant inhibition and stimulation compared to control is marked by asterisk (*)