The effect of cyclodextrins on the biofilm formation of *Pseudomonas aeruginosa* – Modulation of quorum sensing

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INTRODUCTION

The misuse of antibiotics is recognized to result development of antibiotic-resistant bacterial strains, therefore there is an increasing need for the development of antimicrobial agents with novel or unexplored properties to be able to efficiently control bacterial infectious diseases. Amongst several infectious mechanisms of bacteria, biofilm formation is thought to determine 65–80% of all microbial infections [1]. *Pseudomonas aeruginosa* is an opportunistic pathogen with the ability to form biofilm, hence causing acute and chronic infections [2]. One of the most relevant regulation systems of *P. aeruginosa* associated with biofilm formation is the quorum sensing (QS) mechanism [3].

OBJECTIVES

- To investigate the applicability and the optimal parameters of the microtiter plate assay as a potential tool for studying the early stages of biofilm formation.
- To study the potential concentration- and time-dependent quorum quenching (QQ) ability of different cyclodextrins (CDs), which may interfere with the control mechanisms of biofilm formation by *P. aeruginosa.*
- To determine the Minimum Inhibitory Concentrations (MIC) of CDs → the lowest concentration of the tested cyclodextrins significantly inhibiting biofilm formation under defined test conditions.

GROWING A BIOFILM → STAINING THE BIOFILM → QUANTIFYING THE BIOFILM

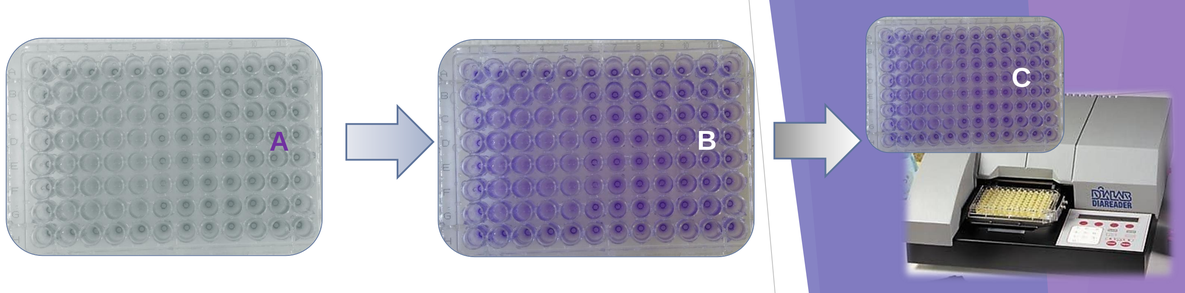

Fig. 1 Schematic O'Toole's modified method on biofilms in a microtiter plate. Biofilm formation in 96 well microtiter plate during the incubation and washing (A), treatment with crystal violet and washing (B), suspending of the dyed biofilm in acetic acid and its transfer to a new microtiter plate, absorbance measurement at 544 nm with DIALAB EL800 microplate reader (C).

MATERIALS & METHODS

The α-, ß- and γ-CD (ACD, BCD, GCD), randomly-methylated α- and ß-CD (RAMEA, RAMEB), quaternary ammonium α- and ß-CD (QAACD, QABCD), (2-hydroxypropyl)-α- and ß-CD (HPACD, HPBCD), sulfobutyl ether ß-CD (SBEBCD) and α-, ß- and γ-CD polymers (ACDPS, BCDPS, GCDPS) were tested at 0.098−12.5 mM concentration range based on O'Toole's modified method [4]. (Fig. 1)
Influence of cyclodextrins was tested on bacterial culture grown both in the biofilm and in planktonic form. (Fig. 1 and Fig. 5)

RESULTS

Our results indicated clearly that, ACD, RAMEA, QAACD, RAMEB and HPBCD negatively affected biofilm formation. Especially significant inhibitory effect (60–70%) was found for ACD, RAMEA and RAMEB at 6.25 mM concentration (Fig. 2 and Fig. 3). These derivatives presented concentration-dependent inhibition; the inhibitory effect was already detectable after 6 hours and it kept on growing significantly for 72 hours. GCD presented slight (~10–20%) non concentration-dependent inhibition.

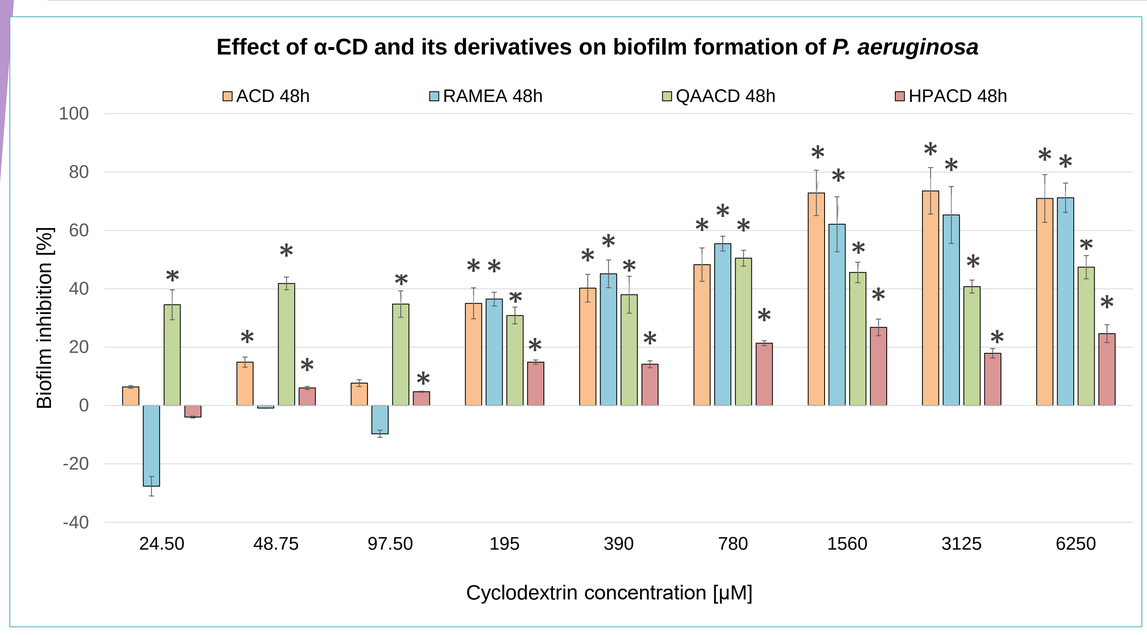

Fig. 2 Effect of α-CD and its derivatives on biofilm formation after 48 hours incubation

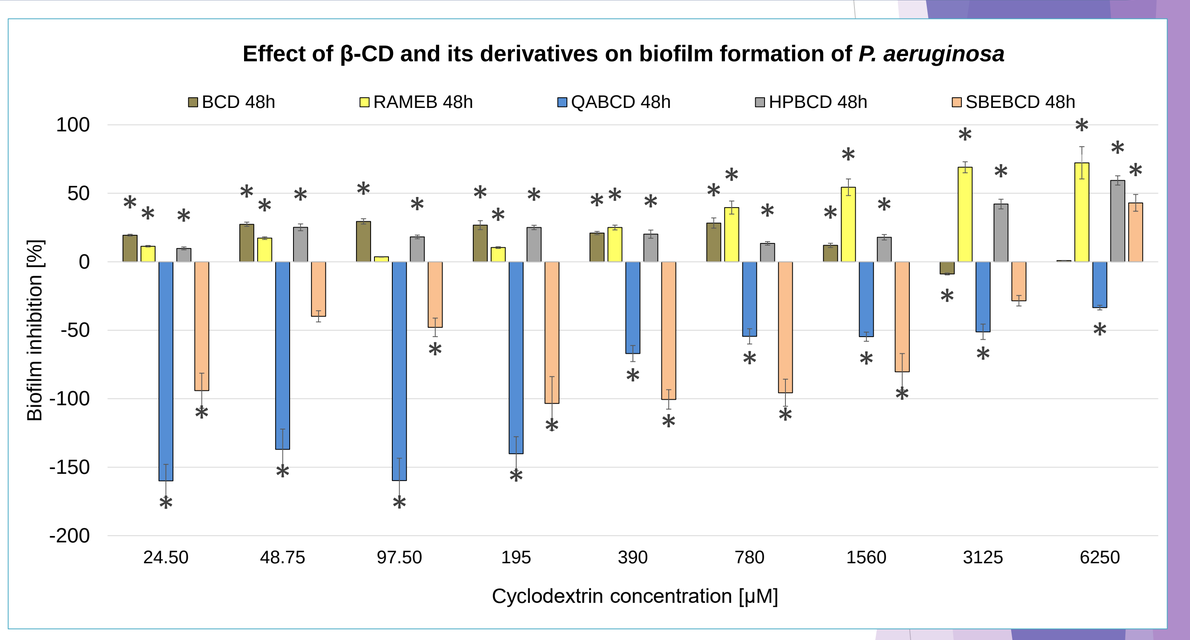

Fig. 3 Effect of β-CD and its derivatives on biofilm formation after 48 hours incubation

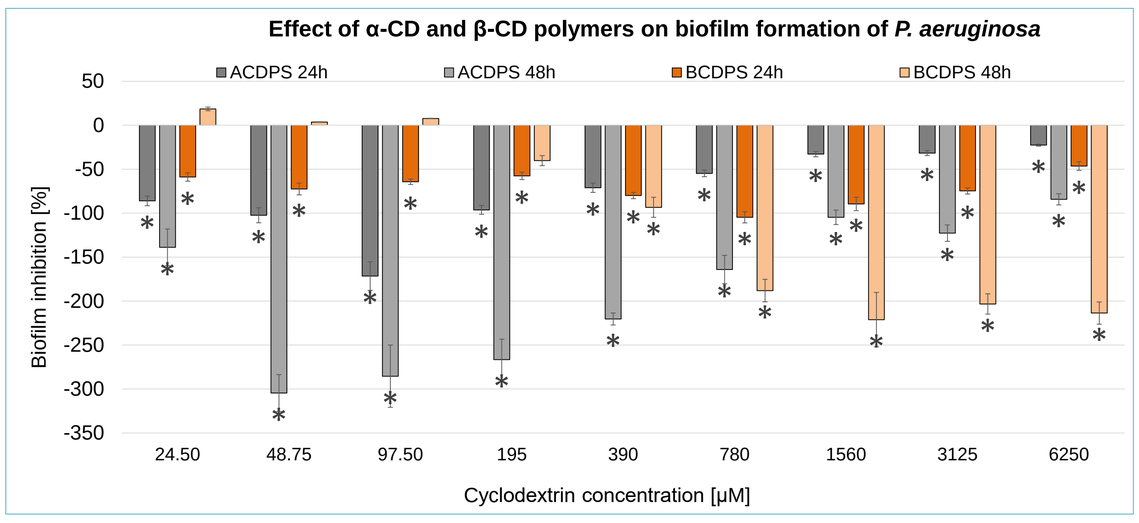

Fig. 4 Effect of α-CD and β-CD polymers on biofilm formation after 24 and 48 hours incubation

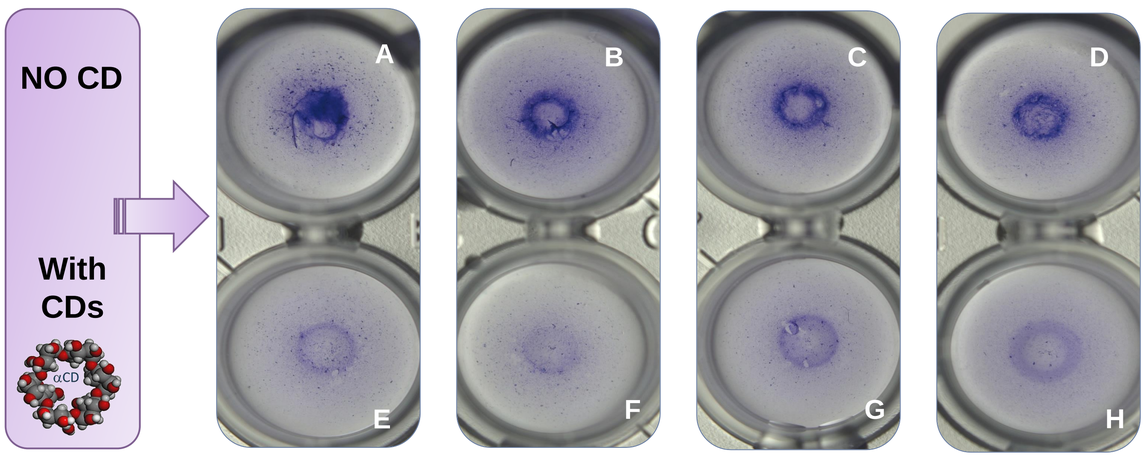

Fig. 5 Light microscopy images of *P. aeruginosa* biofilm in microtiter plate after 48 hours incubation, in presence of distilled water (A, B, C, D), 24.5 μM α-CD (E), 48.75 μM α-CD (F), 390 μM α-CD (G) and 6250 μM α-CD (H).

Table 1 MIC values of CDs displaying significant inhibition on biofilm formation

Contact time	MIC – Minimum Inhibitory Concentration [μM]						
	ACD	RAMEA	QAACD	HPACD	RAMEB	HPBCD	SBEBCD
24 h	780	195	1560	195	25	25	6250
48 h	49	195	25	49	25	25	6250

CONCLUSIONS

✓The applicability of the microtiter plate biofilm formation assay as a high-throughput screening tool for characterizing the influence of additives on biofilm formation was demonstrated.
The optimal parameters: Luria Bertani Broth, 37 ºC, 6 replicate wells, 48 h incubation time.

✓ACD, RAMEA, QAACD and RAMEB displayed the highest inhibitory activity on the biofilm formation→ possible QS-inhibitors against biofilm formation of *Pseudomonas aeruginosa.*

✓The polymers (ACDPS, BCDPS) noticeably stimulated the growth of biofilm. Presumably, these polymers do not complex the QS signal molecules but might serve as valuable nutrient source for the embedded bacteria. (Fig. 4)

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