Cyclodextrin-mediated quorum quenching in the *Aliivibrio fischeri* bioluminescence model system – modulation of bacterial communication

Mónika Molnár [1], Zsófia Berkl [1], Imre Németh [1], Ildikó Fekete-Kertész [1], Rita Márton [1], Blanka Timár [1], Éva Fenyvesi [2], Lajos Szente [2]

[1] Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, Műegyetem rkp. 3. 1111 Budapest, Hungary

[2.] CycloLab Cyclodextrin R&D Laboratory Ltd., Illatos u. 7. Budapest, Hungary

E-mail: mmolnar@mail.bme.hu

SCIENTIFIC BACKGROUND

Quorum sensing (QS) is the process of bacterial communication based on the production, detection and response to low molecular weight compounds, called signal molecules (N-acyl-L-homoserine lactones, AHLs) or autoinducers (AIs). The QS mechanism allows bacteria to communicate, cooperate and to perceive the cell population density and respond to the information by controlling gene expression. A wide range of bacterial processes are known to be influenced by QS, including bioluminescence, toxin production, biofilm formation, virulence factor production etc. The disruption (or silencing) of QS has become research focus in the fields of health and environmental science for controlling undesired microbial activities [1, 2]. Cyclodextrin-mediated Quorum Quenching (QQ) is an innovative approach, the available information about their effects is very scarce [3].

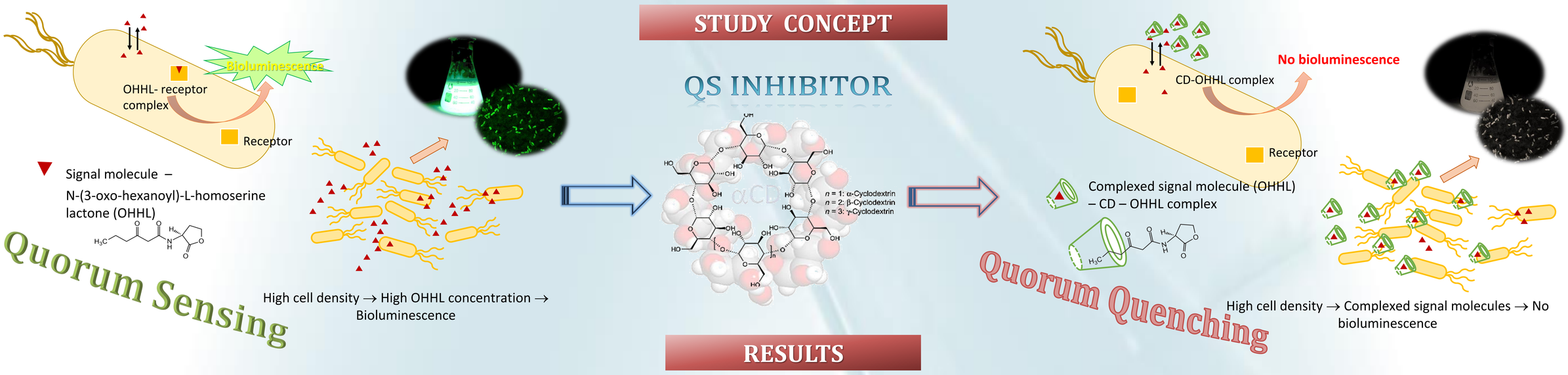
AIMS & HYPOTHESES

The α, β-CDs (ACDs, BCDs) accept the acyl chains of AHL forming AHL-CD inclusion complexes in aqueous media [3].

The main aims of this research are:

- to test the applicability of *Aliivibrio fischeri* bioluminescence assay as a high-throughput screening tool in assessing the efficiency of CD-mediated QQ,
- to investigate the concentration- and time-dependent quorum quenching (QQ) effect of cyclodextrins on the bioluminescence intensity,
- to study the effect of cavity size and substituents of CDs,
- to compare the QQ effectiveness of the different CD molecules by determining Effective Concentration (EC_{20}) and Minimum Inhibitory Concentration (MIC) values.

STUDY CONCEPT



RESULTS

Only the α- and ß-cyclodextrin and their derivatives were able to efficiently inhibit the bioluminescence of *A. fischeri* due to their cavity size. The highest inhibition (~64%) was achieved by 10 mM ACD after 120 minutes, and in the case of 0.625 mM ACD the inhibition was also significant after 120 minutes.

The AHL-complexing ability of the most efficient ACD was proved in the presence of varying amounts of artificially added N-(3-oxo-hexanoyl)-L-homoserine lactone (OHHL) signal molecule → ACD proportionally reduced the increased bioluminescence induced by OHHL.

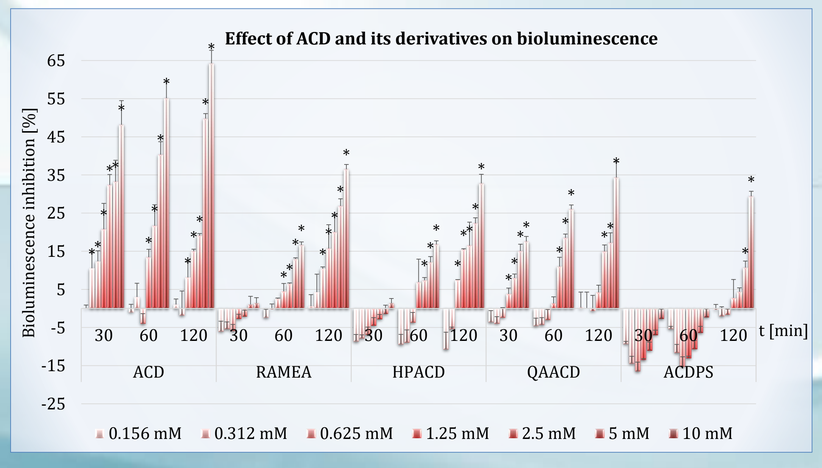


Fig. 1 Effect of ACD and its derivatives on bioluminescence
Significant inhibition compared to control is marked by asterisk (*)

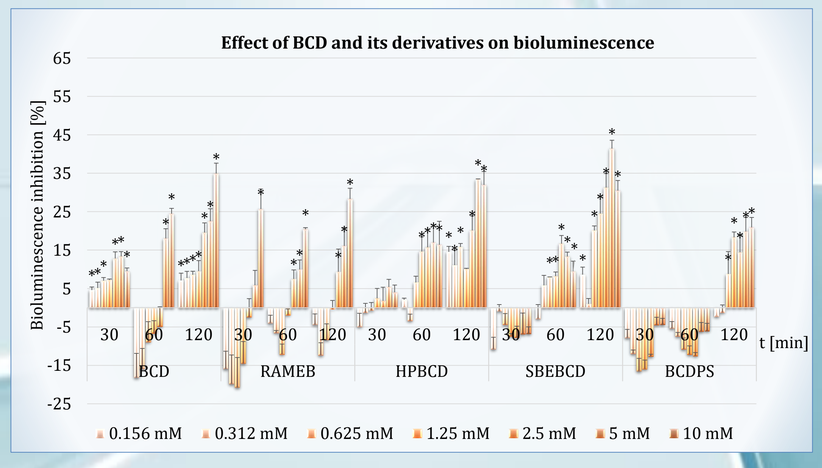


Fig. 2 Effect of BCD and its derivatives on bioluminescence
Significant inhibition compared to control is marked by asterisk (*)

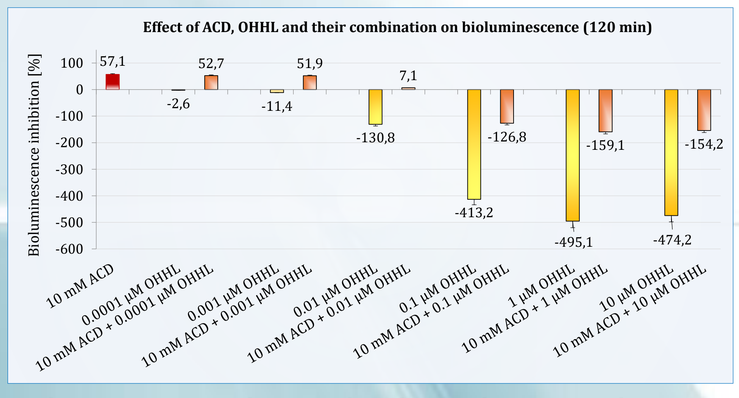


Fig. 3 The compensation of ACD-mediated QQ with OHHL supplementation

Table 1 Effective concentrations causing 20% inhibition of bioluminescence (EC_{20}) and the Minimum Inhibitory Cconcentration* (MIC)

	*EC_{20} and MIC values [mM]**									
	ACD	RAMEA	HPACD	QAACD	ACDPS	BCD	RAMEB	HPBCD	SBEBCD	BCDPS
EC_{20}	2.300	3.200	3.500	6.000	7.390	3.240	6.790	4.080	7.390	10.000
MIC	0.625	0.625	0.625	2.500	2.500	0.156	2.500	0.156	0.625	0.625

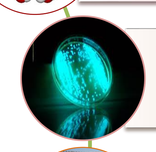
* EC_{20} and MIC values based on bioluminescence measured at 120 min
MIC- the lowest concentration of the tested CDs significantly inhibiting bioluminescence

Table 2 Correlation factors between CD-concentrations and bioluminescence inhibition

	Correlation factors (r^2) *Red marked correlations are significant at p <0.05000*									
	ACD	RAMEA	HPACD	QAACD	ACDPS	BCD	RAMEB	HPBCD	SBEBCD	BCDPS
LUM 30 min	0.768	0.397	0.375	0.798	0.157	0.359	0.333	0.317	0.167	0.074
LUM 60 min	**0.917**	0.873	0.660	0.888	0.121	0.635	0.584	0.358	0.175	0.003
LUM 120 min	**0.921**	0.815	0.753	**0.922**	**0.963**	0.865	0.599	0.526	0.434	0.541

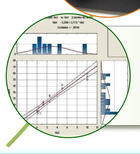
MATERIALS & METHODS

Native α-, ß- and γ-cyclodextrins (ACD, BCD and GCD), 2-hydroxypropyl (HPACD, HPBCD), random-methylated (RAMEA, RAMEB, RAMEG), trimethylaminopropyl (QAACD), sulfobutyl ether (SBEBCD) derivatives and their epichlorohydrin-crosslinked polymers (ACDP and BCDP) were tested at 0.156–10 mM concentration range.



The applied bacterium strain *Aliivibrio fischeri* (NRRL B-111 77) was cultured and maintained in the laboratory under axenic circumstances. Overnight culture was applied for the tests.. Bioluminescence and cell viability based on tetrazolium reduction were the tested endpoints.

The bioluminescence inhibition test (according to modified ISO 11348-3), the optical density measurement and the tetrazolium reduction assay were carried out by high-throughput microtiter plate method with different exposure times (30 min, 60 min, 120 min).



Statistical analysis of variance (ANOVA) by STATISTICA 13.1® software was used for identifying significant effects ($p<0.05$). In case of significance the Minimum Inhibitory Concentration (MIC) values were determined using Dunnett's test ($\alpha = 0.05$).

CONCLUSIONS

- ✓ The applicability of the bioluminescence model system as a high-throughput screening tool for characterizing the influence of additives on *Aliivibrio fischeri* bacterial communication was proved.
- ✓ It was demonstrated that the α- and ß-cyclodextrins can influence efficiently the QS regulated bioluminescence without considerably inhibiting reproduction of *Aliivibrio fischeri*. (Fig. 1-2)
- ✓ The native ACD resulted the highest QS-inhibition indicating the highest affinity for complexation of the signal molecules. (Fig. 1)
- ✓ Strong correlation was found between ACD concentration and bioluminescence inhibition. (Table 2)
- ✓ Based on the statistically determined EC_{20} values the efficiency order of the cyclodextrins is the following:
  GCD, RAMEG, BCDPS < ACDPS, SBEBCD < RAMEB < QAACD < HPBCD < HPACD < BCD < RAMEA < ACD
- ✓ Although the efficiency of BCD and HPBCD did not reach the level of ACD, these CDs significantly inhibited bioluminescence already at 0.156 mM concentration.
- ✓ Experiments with the co-administration of ACD and OHHL signal confirmed the OHHL-ACD complexation responsible for the observed QS suppression. (Fig. 3)

ACKNOWLEDGEMENT

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REFERENCES

[1] V.C. Kalia, H.J. Purohit, Critical Reviews in Microbiology, 37, 121–140, 2011
[2] W. Zhang, C. Li, Frontiers in Microbiology, 6, 1535, 2016
[3] C. Okano, E. Nasuno, K. Iimura, N. Kato, Journal of Applied Polymer Science, 133, 43198, 2016