Towards the development of a *Pseudomonas aeruginosa* DSM1707 biofilm specific expression system for producing alkaline protease

by

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SUMMARY

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In the vast majority of ecosystems, microbial cells grow in association with surfaces, which ultimately leads to the formation of biofilms. The transition to surface-attached growth is associated with diverse changes in gene expression that are necessary to develop the complex architectures and unique physiological properties of a mature biofilm. The potential for altered gene expression at surfaces and in biofilm cells, together with their ability to retain a metabolically active cell population, may have many applications in biotechnology. One such application is the development of a biofilm-specific expression system. Towards the development of such a system, the aim of this investigation was to evaluate different approaches whereby the expression level of an extracellular alkaline protease of *Pseudomonas aeruginosa* could be increased. *P. aeruginosa* was chosen as a model organism for these studies as it readily forms biofilms and several genes and promoters that are actively up-regulated in biofilm cells have been identified.

To facilitate studies regarding optimization of extracellular expression of the alkaline protease, an isogenic alkaline protease-deficient *P. aeruginosa* DSM1707 strain was constructed by homologous recombination via a double crossover event using an allelic exchange vector containing a null *aprAI*::Gm^R allele. The resultant strain, DSMap0, was subsequently used to determine whether the level of alkaline protease activity could be

increased by making use of different promoters, gene dosages and culturing conditions. Thus, the constitutive *lac* promoter as well as two different attachment-inducible promoters, *algD* and 703, were cloned upstream of the alkaline protease-encoding structural gene in both a pseudomonad-specific integration vector and a high-copy-number vector. Following the introduction of the various recombinant plasmid DNA constructs into both the parental and isogenic alkaline protease-deficient *P. aeruginosa* strains, the respective strains were grown as planktonic or as biofilm cultures using glasswool as a substratum for attachment. The dialyzed cell-free culture supernatant of each culture was used to assay the extracellular proteolytic activity by means of azocasein hydrolysis assays.

The results obtained during the course of these investigations indicated that the use of different promoters or an increased gene dosage did not result in increased levels of extracellular protease activity in plaktonic- or biofilm-grown *P. aeruginosa* cultures. Various factors that may account for the lack of up-regulation in extracellular alkaline protease activity are discussed. These could include, amongst other, the inhibition of the alkaline protease by an intracellular inhibitor. These insights should facilitate the design of alternative strategies whereby these limiting factors can be effectively addressed and overcome.

LIST OF ABBREVIATIONS

ATP	adenosine triphosphate
APR	alkaline protease
bp	base pair
BSA	bovine serum albumin
$CoCl_2$	cobalt chloride
CTAB	hexadecyltrimethylammonium bromide
Da	Dalton
d	day
ddH_2O	double distilled water
dNTP	deoxyribonucleic-5'-triphosphate
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
dNTP	deoxynucleoside triphosphate
DTT	dithiothreitol
°C	degrees Celsius
EtOH	ethanol
EDTA	ethylenediaminetetra-acetic acid
Fig.	figure
g	gram
$\times g$	centrifugal force
µg	microgram
h	hour
IPTG	isopropyl β-D-thiogalactoside
kb	kilobase pairs
KCl	potassium chloride
kDa	kilodalton
l	litre
µl	microlitre
MCS	multiple cloning site
ml	milliliter
min	minute
$MgCl_2$	magnesium chloride

M	molar
mM	millimolar
MWCO	molecular weight cut off
NaCl	sodium chloride
NaI	sodium iodide
NaOH	sodium hydroxide
nm	nanometer
nt	nucleotide
OD	optical density
ORF	open reading frame
PCR	polymerase chain reaction
PEG	polyethylene glycol
PIA	*Pseudomonas* isolation agar
pmol	picomole
rpm	revolutions per minute
s	second
SDS	sodium dodecyl sulphate
TAE	Tris-acetic acid-EDTA
TLCK	N-α-ρ-tosyl-L-lysine chloromethyl ketone
TCA	trichloroacetic acid
Tm	DNA dissociation temperature
TSS	transformation and storage solution
U	unit
UHQ	ultra high quality
UV	ultra violet
v/v	volume per volume
w/v	weight per volume

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