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Identification and characterization of genes involved in *Bacillus cereus* biofilm formation

by

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SUMMARY

Identification and characterization of genes involved in *Bacillus cereus* biofilm formation

by

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Bacillus cereus is a Gram-positive, spore-forming bacterium that is frequently identified as the causative agent of food-borne diseases and is also implicated in food spoilage of especially dairy products. The capacity of *B. cereus* to form biofilms on different substrata is of great concern in the food industry. Not only does biofilm formation cause economic loss by equipment failure, but contamination of food products via biofilm cells also raises safety concerns. Bacterial biofilms have been defined as structured multicellular communities that form through a complex developmental process. In contrast to Gram-negative bacteria, biofilm formation by Gram-positive bacteria has only recently been examined. Relatively few genes have been identified that are required for these bacteria to form biofilms and little is known about how they coordinate biofilm formation. In order to contribute to the advancement of knowledge regarding the process of biofilm formation in Gram-positive bacteria, the aim of this investigation was essentially to identify and characterize genes involved in *B. cereus* biofilm formation.

To investigate, *B. cereus* ATCC 14579 was subjected to transposon mutagenesis with the Tn*917*-LTV1 transposon. Screening of a collection of 3 500 insertional mutants for the ability

to form biofilms at the solid-liquid-air interface of glass surfaces led to the identification of eight biofilm-impaired mutants. Each of the mutants contained a single transposon insertion, and no significant differences were observed in the planktonic growth rate between the *B. cereus* wild-type and biofilm-impaired mutant strains. The chromosomal transposon insertion in three of the mutants mapped to genes involved in purine biosynthesis (*purA*, *purC* and *purL*), while the transposon insertion in two other mutants mapped to the *ftsE* gene and to the promoter region of the *motA* gene, respectively. In one of the mutants the transposon was located in the intergenic region between two divergently transcribed genes, which encodes a murein hydrolase exporter and nucleoside hydrolase, respectively. In the final two biofilm-impaired mutants the transposon was respectively mapped to genes encoding a putative membrane spanning protein and a putative protein of unknown function. Results obtained by quantitative real-time PCR assays indicated that expression of each of the identified *B. cereus* ATCC 14579 genes, with the exception of the *motA* gene, was up-regulated in the biofilm population. In the case of *motA*, expression of the gene was down-regulated 3.2-fold in the biofilm population and results obtained during the course of this investigation indicated that motility, rather than the presence of flagella, is required for *B. cereus* biofilm formation. Although this result is in agreement with that reported previously for *B. subtilis*, none of the other genes identified in this investigation have previously been implicated in biofilm formation by Gram-positive bacteria.

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LIST OF ABBREVIATIONS

A	Absorbance
AI-2	autoinducer-2
bp	base pair
bla	β-lactamase gene
ca.	approximately
cat	chloramphenicol acetyltransferase gene
°C	degrees Celsius
cDNA	complementary DNA
cfp	cyan fluorescent protein gene
cm	centimetre
CP	crossing point
CsCl	cesium chloride
CSF	competence and sporulation factor
CTAB	hexadecyltrimethylammonium bromide
d	day
DEPC	diethyl pyrocarbonate
dH_2O	distilled water
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleoside-5'-triphosphate
EDTA	ethylenediaminetetra-acetic acid
e.g.	for example
erm	erythromycin resistance gene
EtOH	ethanol
Fig.	figure
gfp	green fluorescent protein gene
× *g*	centrifugal force
h	hour
IPTG	isopropyl β-D-thiogalactoside
i.e.	that is
kb	kilobase pairs

KOAc	potassium acetate
lacZ	β-galactosidase gene
LB	Luria-Bertani
l	litre
M	molar
mg	milligram
min	minute
ml	millilitre
mM	millimolar
Mut	mutant
ng	nanogram
nM	nanomolar
nm	nanometer
nt	nucleotide
ORF	open reading frame
PCR	polymerase chain reaction
PVC	polyvinylchloride
RNA	ribonucleic acid
RT	reverse transcriptase
s	second
SDS	sodium dodecyl sulphate
TE	Tris-EDTA
2-DE	two-dimensional electrophoresis
U	units
µg	microgram
µl	microlitre
µM	micromolar
UV	ultraviolet
v.	version
V	volts
v/v	volume per volume
w/v	weight per volume
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside
yfp	yellow fluorescent protein gene

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RESEARCH COMMUNICATIONS

Papers published:

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