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The role of phospholipids in the growth and development of *Streptomyces*

by

KHANUNGKAN KLANBUT

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Dedication

To my Mum and Dad, my family and my beloved husband Songkran Pintarin

without your love and support this thesis would not have been written.

To my sponsor and special to my supervisor Dr.Paul R. Herron for their supports

and encouragements

Declaration

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination, which has led to the award of the degree.

Khanungkan Klanbut

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Abbreviations

Standard Units

°C - Degress Celsius
g – gram
k – kilo
L – litre
M – Molar
ml – Milliliters
mM – Millimolar
µg – Micrograms
µl – Microliters
µm – Micrometres
ng - Nanograms
pmol – picamolar
Psi – pounds per square inch
V – volt
W – Watt
σ-sigma
µ-micro
α-alpha
Δ-mutant
~-more or less
β-beta
λ-lambda

DNA Bases

A - Adenine
C – Cytosine
G – Guanine
T – Thymine
U – Uracil

Textual abbreviations

am – apramycin
atc – Anhydrotetracyline
amp – amplicillin
BLAST – Basic local alignment tool
bp – base pairs
C – concentration
CL – cardiolipin
Cls – cardiolipin synthase
DNA – Deoxyribonucleic acid
DNase – Deoxyribonulease
cDNA – complementary DNA
dNTP – dinucleoside 5'-triphosphate
EDTA – Ethylendiaminotetraacetic acid
Etbr – ethidium bromide
G/C – guanine/cytosine content
gs – pre-germinated spores
HCl – hydrochloric acid
Hyg – hygromycin
kb – kilobase
km – Kanamycin
L – litre
LB – Lennox Broth
$MgCl_2$ – magnesium chloride
mL – millilitre
mM – millimolar
MS – Mannitol Soya
MYM – Mannitol salt agar
NaCl – sodium chloride
NAO – 10 nonyl acridine orange
NaOH – sodium hydroxide
OD – optical density
PCR – Polymerase chain reaction
RT-PCR – Reverse transcriptase- Polymerase chain reaction
PA – phosphatidic acid

PG – phosphotidylglycerol
PE– phosphatidyl ethanolamine
pH – Potential of Hydrogen
PI – phosphatidyl Inositol.
PL – phospholipid
PS – phosphatidyl serine
RNA – ribonucleic acid
rpm – revolutions per minute
RNase – Ribonuclease
SDS – sodium deodecyl sulphate
SDW – sterile distilled water
sp – species
TAE – Tris-acetate-EDTA
TES – tris (hydroxymethyl) methyl-2-aminoethanesulphonic acid buffer
tet_r – tetracycline
TLC – thin-layer chromatography
Tm – melting temperature
Tris – Trishydroxymethylaminomethane
tst_r – thiostrepton
UV – ultra violet light
V – volume
v/v – volume to volume ratio
w/w – weight to weight ratio
w/v – weight to volume ratio
YEME – Yeast Extract-Malt Extract Medium

Abstract

Whilst much is known about the development of the complex bacterium *Streptomyces coelicolor*, the role of membrane heterogeneity in this process has not been investigated. Six genes are thought to be responsible for phospholipid biosynthesis in *S. coelicolor*, *SCO1389, SCO1527, SCO5628, SCO5753, SCO6467* and *SCO6468*. *SCO1389* is predicted to encode a cardiolipin synthase that is an essential gene in this organism. When this gene was placed under the control of an inducible promoter, the strain developed poorly on agar, unless supplemented with the inducer of the promoter. We demonstrated that expression of *SCO1389* and phospolipids became dependent on addition of the inducer to this strain. In order to determine if phospholipids play a role in growth and morphogenesis in *S. coelicolor*, mRNA from plate grown-cultures was extracted at different developmental stages and analyzed by RT-PCR to detect expression of the phospholipid biosynthetic genes. No amplification products were detected in the absence of mRNA confirming that amplification products are mRNA dependent. Semi-quantitative analysis showed differential expression of these genes at different harvesting times. We also investigated the effect of glucose concentration in both solid and liquid grown YEME cultures in order to discover if this important nutrient source played a role in the determination of the PL content of the membrane. Further studies were carried out on the involvement of PLs in the development of *S. coelicolor* in strains that carried mutations in *bldA* and *whiD*. This effect was more pronounced in *S. coelicolor*Δ*bldA* and

*S. coelicolor*Δ*whiD.* We also investigated the involvement of PLs during development in *Streptomyces venezuelae* as this organism is able to undergo sporulation in liquid culture and therefore offered the opportunity to isolate large amounts of PLs at different stages of submerged sporulation. Taken together our results demonstrate that there are some changes that take place in the PL profile of streptomycetes during development, but changes in cardiolipin levels appeared to show the greatest fluctuations.