Ain Shams University
Faculty of science

Molecular studies on *Spiroplasma citri* for the development of sensitive and specific genotyping assay.

Thesis Submitted for the partial fulfillment of Master Degree of Science (M.Sc.) in Biochemistry

By

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B. Sc. Biochemistry, (2008)

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Biochemistry Department
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A Thesis

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آية (١١٣) سورة النساء

Declaration

I declare that this thesis has been composed and the work recorded here has been done by me.

I have not been submitted for any other degree at thesis or any other university.

Wessam Hassan Abd El Monem

Dedication

I would like to dedicate this work with all my deepest love and appreciation to every member of my faithful family, father, mother and my sisters Dina and Shereen, for their endless love, support and encouragement and for all my friends and, those from whom I have learned, whatever and whenever they are

Wessam Hassan Abd El Monem

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Wessam Hassan

Contents

Abstract

Stubborn disease of citrus is one of the main causes of quality deterioration of citrus fruits in Egypt. The early detection and the molecular characterization of the causal agent (*Spiroplasma citri)* are vital for revealing its real distribution and for management. Citrus included sweet orange (*C. sinensis*) and mandarin (*C.reticulate Planc*o) showing typical symptoms of stubborn disease were detected from different fields located at Qualubia, Ismailia and Kafr El-Sheikh governorates in Egypt. The detected *S. citri* from diseased samples were cultured in the artificial C-3G liquid medium and the color gradually changed from red to yellow, an indication of the presence of *Spiroplasma* in the cultured samples. The *Spiroplasma citri,* when growing on low-agar medium, forms a fried-egg, fuzzy colonies with occasional surrounding satellite colonies due to the ability of the *Spiroplasma* cells to move through the agar matrix. DNA extracted from symptomatic samples were used as a template for amplification of products of 675 bp using primer pair Spiralin- f / Spiralin- r by PCR. Furthermore the spiralin gene was cloned, sequenced and the obtained isolate was characterized molecularly by sequence analysis showing close relationship with Egyptian isolates, Iran isolate and France isolate.

Keywords: *Spiroplasma citri*, Stubborn Disease, C-3G Media, PCR, Spiralin Gene, Sequence Analysis.

List of Abbreviations

%	Percentage
°C	Degree Celsius
µ	Micro
µg	Microgram
µl	Microliter
µm	Micrometer
µM	Micromole
3’	Three prime *end*
5’	Five prime *end*
A	adenosine
ABC	ATP binding cassette
Arp	Adhesion related protein
AS	Aggregation substance
BLAST Tool	Basic Local Alignment Search
bp	Base pair
C	cytidine
$CaCl_2$	Calcium Chloride
Ccc	Covalently closed circular
CDS	Coding sequence
CI	California isolate
CIE	Crossed immunoelectrophoresis
CPPC	*Caribbean Plant Protection Commission*
CSD	Citrus Stubborn Disease
CSS	Corn Stunt Spiroplasma
CTAB	Cetyl Trimethyl Ammonium Bromide
DAPI	4',6-diamidino-2-phenylindole
DAS-ELISA	Double Antibody Sandwich- Enzyme Linked Immunosorbent Assay
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide Triphosphate
EDTA	Ethylene Diamine Tetra Acetic acid.

EPPO	European Plant Protection Organization
EtOH	Ethanol
FAO	Food and Agriculture Organization
FGE	Fold Gene Expression
Fig	Figure
g	Gram
G	guanosine
ha	Hectare
HCl	Hydrochloridic acid
IAPSC	Inter-African Phytosanitary Council
IC-PCR	Immune-Capture Polymerase Chain Reaction
INRA	French National Institute for Agricultural Research
Kbp	Kilobasepare
KDa	kilodalton
KH_2PO_4	Potassium Dideuterophosphate
l	Litre
LB	Lysogeny Broth
M	Molar
mg	Milligram
mg/ml	Milligram per Milliliter
$MgCl_2$	Magnesium Chloride
MI	Morocco isloate
Min	Minute
ml	Millilitre
MLO	Mycoplasma Like Organism mm
mM	Mill molar
NaCl	Sodium Chlorid
oc	Open circular
OD	Optical density
pCF10	Conjugative plasmid belongs to a family of pheromone-inducible plasmidsin the genus *Enterococcus*
PCR	Polymerase Chain Reaction
pSci	*Spiroplasma citri* plasmids
pSku	*Spiroplasma kunkelii* plasmids
PTS	Phosphotransferase

RAPD	Random Amplified Polymorphic DNA
RF	Replicative form
RNA	Ribonucleic acid
rRNA	Ribosomal RNA
SCARP	*Spiroplasma citri* adhesion related protein
Sec.	Second sp.
SPV	Spiroplasma virus
SSCP	Single Strand Confirmation Polymorphism
T	Thymidine
Tab.	Table
TAE	Tris- Acetate- EDTA
Taq	*Thermophilus aquaticus*
Tris	(Hydroxymethyl) amino methane
Tris-EDTA	Tris hydroxymethyl aminoethane-ethylene diamine tetracetic acid
UFU	*colony-forming units*
UNDP	United Nations Development Programme
USDA	United States Department of Agriculture
UV	Ultraviolet
V	Volt
V/V	Volume per Volume
Vol.	Volume
Xgal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside