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Genetic study of a bacterium isolated from "greening" infected citrus

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```
2110 AF=1+AF
2120 RETURN
2200 AH=AG+AH
2210 AI=1+AI
2220 RETURN
2300 AK=AK+AJ
2310 AL=1+AL
2320 RETURN
2400 AN=AM+AN
2410 AO=1+AO
2420 RETURN
2500 AQ=AP+AQ
2510 AR=1+AR
2520 RETURN
3000 END
```

APPENDIX 4

ELECTROPHORESIS BUFFERS

- *TRIS-EDTA* -

TRIS-Cl....(pH 7,6).....10 mM

EDTA.....(pH 8).....1 mM

Adju .o pH 7,6

- *TRIS-BORATE-EDTA* -

TRIS BASE.....54 g/l

BORIC ACID.....27,5 g/l

EDTA (0,5 M, pH 8).....20 ml

- *FYCOLL* -

BROMOPHENOL BLU 0,25%

XYLENE CYANOL.. .....0,25%

FYCOLL TYPE 40 (in water).....25%

Store at room temperature.

APPENDIX 5

INCUBATION BUFFERS FOR THE RESTRICTION ENDONUCLEASES

- *ECO RI* -

TRIS-HCl.....100 mM

NaCl.....50 mM

$MgCl_2$.....10 mM

Adjusted to pH 7,5

- *HIND III* -

TRIS-HCl.....10 mM

NaCl.....50 mM

$MgCl_2$.....10 mM

DITHIOERYTHRITOL.....14 MM

Adjusted to pH 7,6

- *BGL II* -

GLYCINE-NaOH.....20 mM

$MgCl_2$.....10 mM

MERCAPTOETHANOL.....7 mM

Adjusted to pH 9,5

APPENDIX 6

HYBRIDIZATION BUFFERS

- SODIUM PHOSPHATE BUFFER -

Na_2HPO_4....(ph 8,9).....1 M

NaH_2PO_4....(pH 3,9).....1 M

Adjusted to pH 6,5

- DEIONIZING FORMAMIDE -

Mix 50 ml of formamide and 5 g of mixed-bed, ion-exchange resin (i.g.Bio-Rad AG 501-X8, 20-50 mash).Stir for 30 minutes at room temperature. Filter twice through a Whatman no 1 filter paper (Whatman, USA). Dispense into 1 ml aliquots and store at -20°C.

DENHARDTS SOLUTION (50 x) (grams)

FICOLL.....10

POLYVINYLPYRKOLIDONE.....10

BSA (Pentax fraction V).....10

H_2O.....up to 1l

Filter through a disposable Nalgene filter. Dispense into 25 ml aliquots and store at -20°C.

- BOVINE SERUM ALBUMIN (BSA) -

BSA (Pentax fraction V).....1g

H_2O.....10 ml

Dispense into aliquots and store at -20°C.

PREHYBRIDIZATION SOLUTION

DEIONIZED FORMAMIDE.....50 ml (50%)

NaCl.....4,37 g

SODIUM CITRATE.....2,2 g

DENHARDT (20 x)..... .25 ml

SODIUM PHOSPHATE BUFFER..(pH 6,5).....5 ml

HERRING SPERM DNA..(5 mg/ml).....1,5 ml

H_2C .....up to 100 ml

- HYBRIDIZATION SOLUTION -

DEIONIZIDED FORMAMIDE.....50 ml

NaCl.....4,37 g

SODIUM CITRATE.....2,2 g

DENHARDT (20 x).....25 ml

SODIUM PHOSPHATE BUFFER..(pH 6,5).....5 ml

HERRING SPERM DNA..(5 mg/ml).....1,5 ml

DEXTRAN SULPHATE (50% sol.).....20 ml

H_2O.....up to 100 ml

- *POSTHYBRIDIZATION* -

DEIONIZED FORMAMIDE.....75 ml

NaCl.....4,37 g

SODIUM CITRATE.....2,2 g

DENHARDT (20 X).....25 ml

SODIUM PHSPHATE BUFFER..(pH 6,5).....5 ml

H_2O.....up to 100 ml

APPENDIX 7

NICK TRANSLATION REACTION MIXTURE

DNA	0,5 µg
DNA ENZYMES	5 µl
NUCLEOTIDE SOLUTION	10 µl
H_2O	15 µl
RADIOACTIVE NUCLEOTIDE	2 µg

APPENDIX 8

SCINTILLATION SOLUTION

POPOP.....0,1 g/l

PPO.....5 g/l

POPOP - 1'4-bis-2-(5-Phenyloxazolyl-benzene)

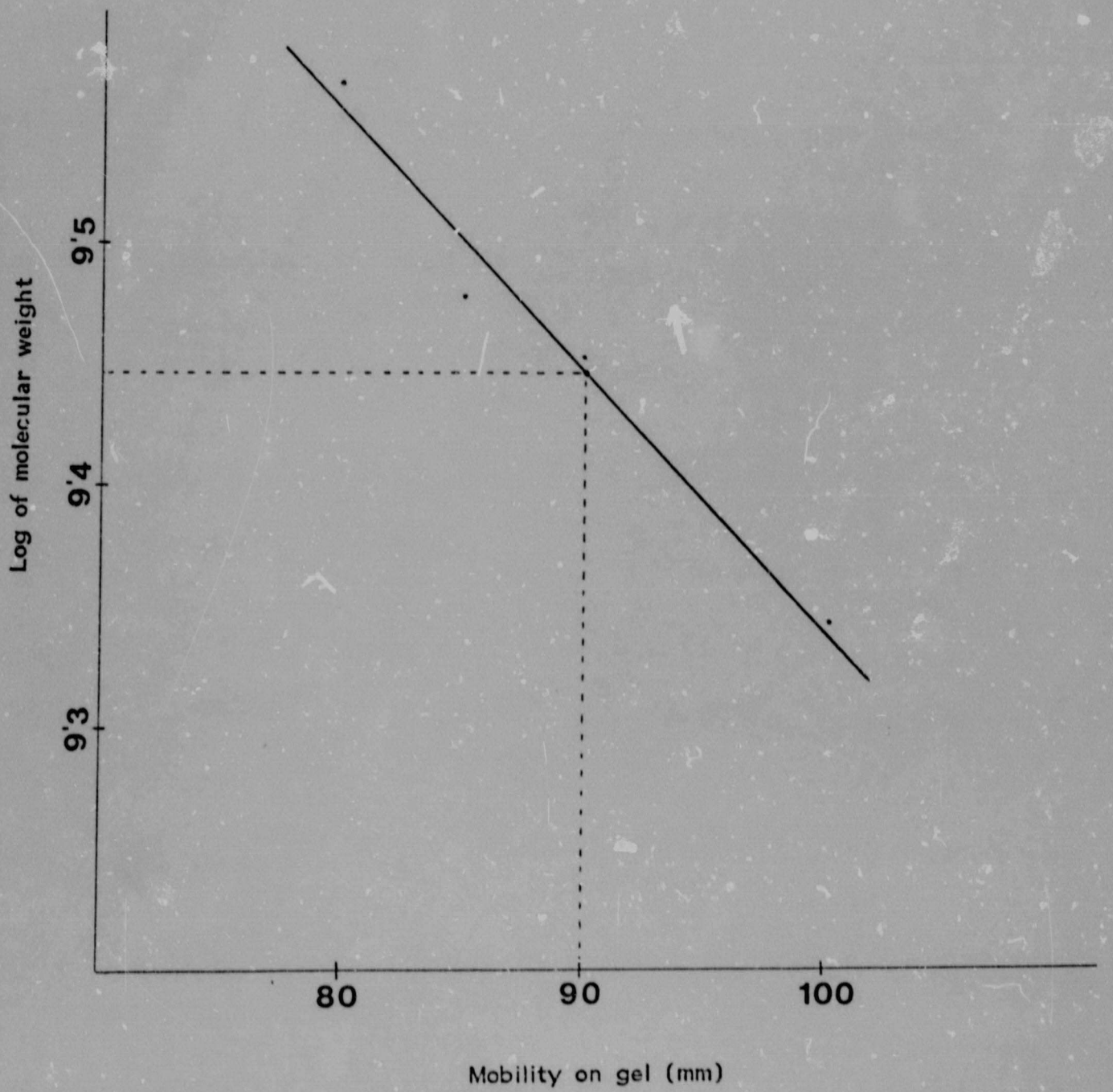
PPO - 2-5-Diphenyloxazole

APPENDIX 9

STANDARD MOLECULAR WEIGHT DNA MARKERS

MICROORGANISM	MOLECULAR WEIGHT
Agrobacterium tumefaciens	$3{,}65 \times 10^9$ daltons
Bacillus subtilis	$3{,}0 \times 10^9$ daltons
Calf thymus	5×10^6 daltons
Erwinia herbicola	$2{,}83 \times 10^9$ daltons
Escherichia coli	$2{,}2 \times 10^9$ daltons

STANDARD CURVE FOR THE MOLECULAR WEIGHT ESTIMATION OF DNA
USING BACTERIAL DNA MARKERS



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