GILLESPIE.D, and SPIEGELMAN.S. 1965. A quantitative assay for DNA-RNA hybrids with DNA immobilised on a membrane filter. J. Mol. Biol. 12:829.

GILLIS.M, DE LEY.J, AND DECLEENE.M. 1970. The determination of molecular weights of bacterial genome DNA from renaturation rates. Eur. J. Biochem. 12:143.

HARDY.K. 1981. Bacterial plasmids. In: Cole and Knowles(eds): Aspects of microbiology. SERIES No 4 Thomas Nelson and Sons,Ltd. Walton-on-Thames. U.K.

HARWOOD.C.R. 1980. Plasmids. In: Goodfellow and Board (eds). Microbiological classification and identification, Academic Press, London, New York. pp 27.

HAYES.W. 1968. The genetics of bacteria and their viruses (2nd edition). Blackwell Scientific Publications, Oxford.

HOLDEMAN.L.V, MOORE.W.E.C, CHURN.P.J, and JOHNSON.J.L. 1982. *Bacteriodes oris* and *Bacteriodes buccae*, new species from human periodontitus and other human infections. Int.J. Syst. Bacteriol. 32:125.

HOPKINS.D.L. 1977. Diseases caused by leafhopper-borne Rickettsia-like bacteria. Ann. Rev. Phytopath. 17:277.

IGWEGBE.E.C.K. 1970. Studies on the nature and transmission of the causal agent of stubborn of citrus: Association of a mycoplasma-like organism with the disease. PhD. Thesis. Univ. of California, Riverside. U.S.A.

JOHNSON.J.L. 1973. Use of nucleic acid hybridization in the taxonomy of anaerobic bacteria. Int. J. Syst. Bacteriol. 23:308.

JOHNSON.J.L. 1978. Taxonomy of the *Bacteroides*. I. Deoxyribonucleic acid homologies among *Bacteroides fragilis* and other saccharolytic *Bacteroides* species. Int. J. Syst. Bacteriol. 28:245.

JOHNSON.J.L. 1980. Classification of anaerobic bacteria. In: Proc. Int. Symp. Anaerobes (Tokyo, Japan) Tokyo, pp 19.

JOHNSON.J.L. 1981. Genetic characterisation. In: Gerhardt et al (edt.). Manual of methods for general bacteriology, American Scciety for Microbiology, pp 450.

JOHNSON.J.L. 1984. Bacterial classification III. Bergey's manual of systematic bacteriology (9th edition). Williams and Wilkins.(eds). Baltimore.

JOHNSON.J.L. and AULT.A. 1978. Taxonomy of the *Bacteriodes*. II. Correlation of phenotypic characteristics with deoxyribonucleic acid homology groupings for *Bacteriodes fragilis* and other saccharolytic *Bacteriodes* species. Int.J. Syst. Bacteriol. 28:257.

JONES.A.L, WHITCOMB.R.F, WILLIAMSON.D.L, and COAN.M.E. 1976. Comparative growth and primary isolation of spiroplasmas in media based on insect tissue culture formulations. Phytopath. 67:738.

KADO.C.I, and HESKETT.M. 1970. Selective media for isolation of *Agrobacterium*, *Corynebacterium*, *Erwinia*, *Pseudomonas* and *Xanthomonas* Phytopath. 60:969.

KADO.C.I, and LIU.S.T. 1981. Rapid procedure for detection of large and small plasmids. J. Bact. 145:1365.

KAMPER.S, FRENCH.W, and DeKLOET.S. 1985. Genetic relationships of some fastidious Xylem-limited bacteria. Int. J. Syst. Bacteriol. 35:185.

KELLY.M.S. 1967. Physical and mapping properties of distant linkages between genetic markers of *Bacillus subtilis*. Mol. Gen. Genetics. 99:333.

KIRBY.K.S. 1968. Isolation of nucleic acid with phenolic solvents. Meth. Enzymol. 12(B):87.

KLESIUS.P.H, and SCHUHARDT.V.T. 1968. Use of lysostaphin in the isolation of highly polymerized deoxyribonucleic acid, and in the taxonomy of aerobic *micrococcaceae*. J. Bact. 95:739.

KORNBERG.A. 1974. DNA synthesis. Freeman & Co. San Francisco.

KRYCH.V.A, JOHNSON.J.L,. and YOUSTEN.A.A. 1980. Deoxyribonucleic acid homologies among strains of *Bacillus sphericus*. Int.J.Syst.Bacteriol. 30:476.

LAFLECHE.D,. and BOVE.J. 1970. Mycoplasmes dans les agrumes atteints de greening, de stubborn ou de maladies similaires. Fruits. 25:455.

LARPENT.J.P, and LARPENT- GOURGOUD.M. 1970. Microbiologie practique. Hermann, Paris.

LEDERBERG J. 1952. Cell genetics and hereditary symbiosis. Physiol. Revs. 32:403.

LEDERBERG.J, CAVALLI.L.L, AND LEDERBERG.E.M. 1952. Sex compatibility in *E.Coli*. Genetics. 37:720.

LEHNINGER.A. 1975. Bioquimica. Ed. Omega, Barcelona.

LEVITT.J. 1974. Introduction to plant physiology (2nd edition). C.V Mosby Co; Saint Louis.

LAWN.R.M, FRITSCH.E.F, PARKER.R.L, BLAKE.G, AND MANIATIS.T. 1978. The isolation and characterisation of a linked δ and β globin gene from a cloned library of human DNA. Cell 15:1157.

MANDEL.M. 1969. New approaches to bacterial taxonomy. Ann. Rev. Microbiol. 23:239.

MANIATIS.T, FRITSCH.E.F, and SAMBROOK.J. 1982. Molecular cloning. A laboratory manual. Cold Spring Harbour Laboratory, Cold Spring Harbour, New York.

MARAMOROSCH.K. 1974. Mycoplasmas and ricketsias in relation to plant diseases. Ann. Rev. Microbiol. 18:301.

MARMUR.J. 1961. A procedure for the isolation of deoxyribonucleic acid from microorganisms. J. Mol. Biol. 3:208.

MARMUR.J, and DOTY.P. 1959. Heterogeneity in deoxyribonucleic acids I. Dependence on composition of the configuration stability of deoxyribonucleic acids. Nature. 183:1428.

MARMUR.J, and DOTY.P. 1962. Determination of the base composition of deoxyribonucleic acids from its thermal denaturation temperature. J. Mol. Biol. 5:109.

MARMUR.J, FALKOW.S, and MANDEL.M. 1963. New approaches to bacterial taxonomy. Ann. Rev. Microbiol. 17:329.

MARMUR.J, ROWND.R, FALKOW.S, BARON.L.S, SCHILDKRAUT.C, and DOTY.P 1961. The nature of intergeneric episomal infection. Proc. Natl. Acad. Sci. 47:972.

MAYS.T.D, HOLDEMAN.L.V, MOORE.W.E.C, ROGOSA.M, and JOHNSON.J.L. 1982. Taxonomy of the genus *Veillonella*. Int. J. Syst. Bacteriol. 32:28.

MACCLEAN.A ..ERHOLZER.P.C.J. 1965. Greening disease of sweet orange: evidenc.. . it is caused by a transmissible virus. S. A. J. Agric. Sci. 8:253.

MACCLEAN.A.P.D, and SCHWARZ.R.E. 1970. Mottle-leaf (Greening) disease of citrus. Phytophylactica. 3:6.

MCCONAUGHY.B.L, LAIRD.C.D, and MCCARTHY.B.J. 1969. Nucleic acid reassociation in formamide. Biochem. 8:3289.

MEYERS.J.A. 1976. Simple agarose gel electrophoresis method for the identification and characterisation of plasmid deoxyribonucleic acid. J. Bact. 117:1329.

MEYER.S.A, and SCHLEIFER.K.M. 1975. Rapid procedure for the approximate determination of the deoxyribonucleic acid base composition of *Micrococci*, *Staphilococci* and other bacteria. Int. J. Syst. Bacteriol. 25:363.

MILNE.D.L, MOLL.J.M, VAN VUUREN.S.P, and BREDELL.G. 1978. Prevention and cure of greening disease in citrus. Citrus. H. 2,3:1. Dept. Agric. Tech. Services, Pretoria.

MITSUHASHI.S, HARADA.K, and HASHIMOTO.H. 1960. Multiple resistance of bacteria and transmission of drug resistance to other strains by mixed cultivation. Japanese. J. Exp. Med. 30:179.

MOLL.J. 1974. Citrus greening disease. PhD. Thesis. Univ. of Natal, Pietermaritzburg, South Africa.

MOLL.J. 1979. Report on the 8th I.O.C.V. conference and subsequent phytopathological discussions in Australia. Citrus and Subtropical Fruit Research Institute, Nelspruit.

MOLL.J, and MARTIN.M.M. 1973. Electron microscope evidence that citrus phsylla (Trioza Erytreae) is a vector of greening disease in South Africa. Phytophylactica. 5:41.

MOLL.J, and MARTIN.MM. 1974. Comparison of the organism causing the greening disease with several plant pathogenic gram- negative bacteria, ricketsia-like organisms and mycoplasma-like organisms. I.N.S.E.R.M. 33:89.

MOLL.J, and VAN VUUREN. S.P. 1977. Greening disease in Africa. Proc. Int. Soc. Citriculture. 3:903.

MONTOYA.A.L, CHILTON.M.D, GORDON.M.F, SCIAK.D, and NESTER.E.W. 1977. Octopine and nopaline metabolism in *agrobacterium tumefaciens* and crown gall tumour cells: role of plasmid genes. J. Bacteriol. 129:101.

NYGAARD.A.D, and HALL.B.D. 1963. A method for detection of RNA-DNA complexes. Biochem. Biophys. Res. Comm. 12:98.

OBERHOLZER.P.C.J. 1965. Oorsig oor ondervindinge met vergroening. Report of colloquium on greening disease, Nelspruit. pp 9.

PARKER.R.C, and SEED.B. 1980. Two-dimensional agarose gel electrophoresis "sea plaque" agarose dimension. Meth. Enzymol. 65:358.

RIGBY.P.W, DIECKMAN.M, RHODES.C, and BERG.P. 1977. Labelling deoxyribonucleic acid to high specific activity in vitro by nick translation with DNA polymerase I. J. Mol. Biol. 113:237.

ROBERTS.R. 1982. Restriction and modification enzymes and their recognition sequences. Nucleic Acid Res. 10:117.

ROLFE.R, and MESELSON.M. 1959. Relative homogeneity of microbial DNA. Pro. Natl. Acad. Sci. 45:1039.

ROSETTI.V, MELLO.O.F, NAMEKATA.T, and CONTI.E.D. 1969. A new type of decline on citrus trees in Brazil. In: Proc. 5th I.O.C.V. Univ. of Florida Press, Gainesville.

SAGLIO.P, LAFLECHE.D, BONISOL.C, and BOVE.J. 1971. Isolement, culture et observation au microscope electronique de structures de type mycoplasme associees a la maladie du stubborn des agrumes et leur comparaison avec les structures observees dans le cas de la maladie du greening des agrumes. Physiol. veg. 9:569.

SCHILDKRAUT.C.L, MARMUR.J, and DOTY.P. 1962. Determination of the base composition of deoxyribonucleic acid from its buoyant density in CsCl. J. Mol. Biol. 4:430

SCHNEIDER.H. 1968. Anatomy of greening disease sweet orange shoots. Phytopath. 58:1155.

SELDIN.L, and DUBNAU.D. 1985. Deoxyribonucleic acid homology among *Bacillus polymyxa*,*Bacillus macerans*, *Bacillus azotofixans*, and other Nitrogen fixing *Bacillus* strains. Int. J. Syst. Bacteriol. 35(2):151.

SIBARA.M. 1982. Characterisation of a microorganism isolated from citrus with "greening" disease. MSc. Thesis. Univ. of the Witwatersrand, Johannesburg.

SQARAMELLA.V. 1972. Enzymatic oligomerization of bacteriophage P22 DNA and of linear Simian virus 40 DNA. Proc. Natl. Acad. Sci. 69:3389.

SMITH.H.O, and BIRNSTIEL.M.L. 1976. A simple method for DNA restriction site mapping. Nucleic Acid Res. 3:2387.

SMITH.M.G. 1967. Isolation of high molecular weight DNA from normal and phage-infected*E.Coli*. Meth. Enzymol. 12(A):545.

SOBER.H.A. (ed) 1970. Handbook of biochemistry, selected data for molecular biology (2nd ed). The Chemical Rubber Co. Cleveland.

STACKEBRANDT.E, and KANDLER.O. 1979. Taxonomy of the genus *Cellulomonas* based on phenotypic characters and deoxyribonucleic acid-deoxyribonucleic acid homology, and proposal of seven neotype strains. Int. J. Syst. Bacteriol. 29(4):273.

STANIER.R.Y, DOUDOROFF.M, and ADELBERG.E.A. 1972. The microbial world. Prentice-Hall Inc. Englewoods Cliffs, N.Y.

STRICKBERGER.M.W, 1968. Genetics. The MacMillan Company, N.Y.

SUEOKA.N, MARMUR.J, and DOTY.P. 1959. Heterogeneity in deoxyribonucleic. II. Dependence of the density of deoxyribonucleic acids on guanine-cytosine content. Nature. 183:1429.

TAKAHASHI.S, and NAGANO.Y. 1984. Rapid procedure for the isolation of plasmid DNA and applications to epidemiology. J. Clin. Microbiol. 20:608.

TOOZE.J. 1973. The molecular biology of tumour viruses. Cold Spring Harbor Laboratory.

ULITZUR.S. 1972. Rapid determination of DNA base composition by ultra-violet spectroscopy. Biochem. Biophys. Acta. 272:1.

VAN VUUREN.S.P. 1977. Determination of optical concentration and pH of tetracycline hidrochloride for trunk injection of greening-infected citrus trees. Phytophylactica. 9:77.

WATANABE.T. 1963. Infectious heredity of multiple drug resistance in bacteria. Bacteriol. Revs. 27:87.

WATSON.J.D, and CRICK.F.C. 1953. Molecular structure of nucleic acids. A structure for deoxyribonucleic acids. Nature. 171:737.

WETMUR.J.G. 1976. Hybridisation and renaturation kinetics of nucleic acids. Ann. Revs. Biophys. Bioeng. 5:337.

WETMUR.J.G, and DAVIDSON.N. 1968. Kinetics of renaturation of DNA. J. Mol. Biol. 31:370.

WILKINS.M.H.F. 1956. Physical studies of the molecular structure of deoxyribonucleic acid and nucleoprotein. Cold Spring Harbor Symp. 21:75.

APPENDIX 1

ISOLATION BUFFERS

- *523 MEDIUM* - (grams per liter)

SUCROSE.....10

YEAST EXTRACT.....4

CASEIN HYDROLYSATE.....8

$MgSO_4 \cdot 7H_2O$.....0,3

$K_2H\ PO_4$.....2

The pH was adjusted to 6,9. When plates were needed,

AGAR.....15 g/l

- *MIG MEDIUM* -

INORGANIC SALTS (grams per liter)

KH_2PO_4.....0,200

Na_2HPO_4.....0,250

$MgSO_4 \cdot 7H_2O$.....0,050

NaCl.....0,675

$NaHCO_3$.....0,020

$CaCl_2 \cdot 2H_2O$.....0,450

$MgCl_2 \cdot 2H_2O$.....0,125

NH_4Cl	0,030
$CuSO_4 \cdot 5H_2O$	0,005
$MnSO_4 \cdot 4H_2O$	0,025

ORGANIC ACIDS	(grams per liter)
α-KETOGLUTARIC ACID	0,800
FUMARIC ACID	0,650
MALIC ACID	0,[illegible]0
SUCCINIC ACID	0,750

AMINOACIDS	(grams per liter)
L - ALANINE	0,500
L - ARGININE	0,250
L - ASPARRAGINE	0,150
L - ASPARTIC ACID	0,350
L - CYSTEINE	0,400
L - GLUTAMIC ACID	0,450
L - HISTIDINE	0,300
L - LEUCINE	0,350
L - METHIONINE	0,350
L - LYSINE	0,500
L - PHENYLALANINE	0,250
L - PROLINE	0,200
L - TRYPTOPHAN	0,400
L - TYROSINE	0,500
L - VALINE	0,350

CARBOHYDRATES	(grams per liter)
SUCROSE	16,5
SORBITOL	23,5

OTHER COMPONENTS	(grams per liter)
TRYPTONE	4,00
BRAIN HEART INFUSION	3,00
PEPTONE	6,00
YEAST EXTRACT	5,00
CALF SERUM	16,5%

PREPARATION OF MIG

To prepare this medium, the following method was followed: The aminoacids, together with the organic salts were dissolved in 200 ml of distilled water, with 0,1 N HCl. Separately, the inorganic salts were dissolved in 200 ml adding NaOH. Once both mixtures were dissolved, they were combined into one 1,5 l flask, adjusted to pH 6,5, and filter sterilized through 0,22 µm pore size millipore filters (Millipore).

The carbohydrates and the other compounds were dissolved in 400 ml of distilled water, adjusted to pH 7,3 and autoclaved for 20 minutes at 120°C.

Finally, all compounds were mixed together, and the foetal calf serum added, using the same size millipore filters.

APPENDIX 2

DNA PURIFICATION BUFFERS

- *SALINE SODIUM CITRATE (SSC)* -

NaCl.....0,15 M

TRISODIUM CITRATE.....15 mM

Adjusted to pH 7,0

- *SALINE-EDTA* -

NaCl.....0,15 M

EDTA.....0,10 M

Adjusted to pH 9,0

- *ACETATE-EDTA* -

SODIUM ACETATE.....3 M

EDTA.....1 mM

Adjusted to pH 7,0

APPENDIX 3

PROGRAMME IN BASIC FOR THE MOL% C+G CALCULATIONS

```
10 CLS
20 PRINT"PROGRAMME FOR MOL% C+G ANALYSIS"
30 FOR X = TO 1 to 3000
40 NEXT X
50 INPUT"PLEASE, GIVE ME THE VALUE OF YOUR REFERENCE DNA";DA
70 INPUT"PLEASE, GIVE ME THE VALUE OF YOUR CONTROL DNA";DB
80 CLS
90 PRINT"ENTER YOUR DATA"
100 FOR X = 1 TO 3000:NEXT X
110 PRINT:PRINT:PRINT
120 PRINT"VALUES OF YOUR REFERENCE DNA?"
130 PRINT:PRINT
140 INPUT"YOUR ABSORBANCE AT 240 nm";AA
150 GOSUB 2000
160 INPUT"DO YOU HAVE ANY OTHER VALUE AT 240 nm";A$
170 IF A$ ="Y" THEN GOTO 140
180 IF A$ < > "N" THEN GOTO 160
190 PRINT:PRINT
200 INPUT"YOUR ABSORBANCE AT 245 nm";AD
210 GOSUB 2100
220 INPUT"DO YOU HAVE ANY OTHER VALUE AT 245 nm";A$
230 IF A$ = "Y" THEN GOTO 200
240 IF A$ < > "N" THEN GOTO 220
250 PRINT:PRINT
260 INPUT"YOUR ABSORBANCE AT 260 nm ";AG
270 GOSUB 2200
```

```
280 INPUT"DO YOU HAVE ANY OTHER VALUE AT 260 nm?";A$
290 IF A$ ="Y" THEN GOTO 260
300 IF A$ < > "N" THEN GOTO 280
310 PRINT:PRINT
320 INPUT"YOUR ABSORBANCE AT 270 nm";AJ
330 GOSUB 2300
340 INPUT"DO YOU HAVE ANY OTHER VALUE AT 270 nm?";A$
350 IF A$="Y" THEN GOTO 320
360 IF A$ < > "N" THEN GOTO 340
370 PRINT:PRINT
380 INPUT"YOUR ABSORBANCE AT 275 nm";AM
390 GOSUB 2400
400 INPUT"DO YOU HAVE ANY OTHER VALUE AT 275 nm?";A$
410 IF A$ ="Y" THEN GOTO 380
420 IF A$ < > "N" THEN GOTO 400
430 PRINT:PRINT
440 INPUT"YOUR ABSORBANCE AT 280 nm";AP
450 GOSUB 2500
460 INPUT"DO YOU HAVE ANY OTHER VALUE AT 280 nm?";A$
470 IF A$ ="Y" THEN GOTO 440
480 IF A$ < > "N" THEN GOTO 460
490 PRINT:PRINT
500 AS= AB/AC
510 AT=AE/AF
520 AU=AH/AI
530 AV=AK/AL
540 AX=AN/AO
550 AY=AQ/AR
560 YA=AS/AY
570 YB=AS/AX
580 YC=AT/AV
590 YD=AU/AY
```

```
600 YE=AU/AS
610 ZA=ZA+1
620 ON ZA GOTO 630, 720, 810
630 BA=YA:AB=0:AC=0
640 BB=YB:AF=0:AE=0
650 BC=YC:AH=0:AI=0
660 BD=YD:AK=0:AL=0
670 BE=YE:AN=0:AO=0
680 AQ=0:AR=0
690 CLS
700 PRINT"VALUES FOR YOUR CONTROL DNA"
710 GOTO 130
720 CA=YA:AB=0:AC=0
730 CB=YB:AF=0:AE=0
740 CC=YC:AH=0:AI=0
750 CD=YD:AK=0:AL=0
760 CE=YE:AN=0:AO=0
770 CLS
780 PRINT "VALUES FOR YOUR SAMPLE DNA"
810 EA= (0,0076*DA)-BA+YA /0,0076
820 EB= (0,00576*DA)-BB+YB /0,00576
830 EC= (0,0047*DA)-BC+YC /0,0047
840 ED= (0,0076*DA)+CA-BA)/0,0076
850 EE= (0,00576*DA)+CB-BB)/0,00576
860 EF= (0,0047*DA)+CB-BC)/0,0047
870 EM=(EA+EB+EC)/3
880 EN=(ED+EF+EE)/3
890 FA=ABS(EA-EM)
900 FB=ABS(EB-EM)
910 FC=ABS(EC-EM)
920 FD=ABS(ED-EM)
930 FE=ABS(EE-EM)
```

```
940 FF=ABS(EF-EM)
950 GA=SQR (FA+FB+FC)/2
960 GB=SQR (FD+FE+FF)/2
962 HA=ABS(DB-EN)
965 HB=HA*100/DB
970 CLS
980 PRINT"RESULTS"
990 FOR X=1 TO 3000
1000 NEXT X
1010 PRINT:PRINT:PRINT
1020 PRINT"          ";"CONTROL","SAMPLE","REFERENCE"
1030 PRINT:PRINT
1040 PRINT"PURITY OF DNA"
1050 PRINT
1060 PRINT"260/280 RATIO";CD,YD,BD
1070 PRINT:PRINT
1080 PRINT"260/240 RATIO";CE,YE,BE
1090 PRINT:PRINT
1100 PRINT"MOL% VALUE";EN,EM
1110 PRINT
1120 PRINT"STANDARD DEVIATION";GB,GA
1130 PRINT
1140 PRINT"ERROR FROM REAL VALUE";HB;"%"
1150 PRINT:PRINT:PRINT:PRINT
1160 INPUT"DO YOU WANT TO COMPARE ANY OTHER VALUES";P$
1170 IF P$ ="Y" THEN GOTO 10
1180 IF P$ < > "N" THEN GOTO 1160
1190 END
2000 AB=
2010 AC=1+
2020 RETURN
2100 AE=AD+AE
```