


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.


WILKINS, M. H. F. 1956. Physical studies of the molecular structure of
APPENDIX 1

ISOLATION BUFFERS

- 523 MEDIUM -

SUCROSE ................................10
YEAST EXTRACT ..........................4
CASEIN HYDROLYSATE ...................8
MgSO₄ . 7H₂O ..........................0,3
K₂H PO₄ ................................2

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

AGAR ....................................15 g/l

- MIG MEDIUM -

INORGANIC SALTS  

<table>
<thead>
<tr>
<th>Salt</th>
<th>Concentration (grams per liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>KH₂PO₄</td>
<td>0,200</td>
</tr>
<tr>
<td>Na₂HPO₄</td>
<td>0,250</td>
</tr>
<tr>
<td>MgSO₄ . 7H₂O</td>
<td>0,050</td>
</tr>
<tr>
<td>NaCl</td>
<td>0,675</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>0,020</td>
</tr>
<tr>
<td>CaCl₂ . 2H₂O</td>
<td>0,45</td>
</tr>
<tr>
<td>MgCl₂ . 2H₂O</td>
<td>0,125</td>
</tr>
</tbody>
</table>
NH₄Cl.............................................0,030
CuSO₄·5H₂O.................................0,005
MnSO₄·4H₂O.................................0,025

ORGANIC ACIDS (grams per liter)

ACETOACETIC ACID............................0,800
FUMARIC ACID...............................0,650
MALIC ACID.....................................0,50
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 for use at room temperature.

- 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 = 1 TO 3000
40 NEXT X
50 INPUT"PLEASE, GIVE ME THE VALUE OF YOUR REFERENCE DNA";DA
60 INPUT"PLEASE, GIVE ME THE VALUE OF YOUR CONTROL DNA";DB
70 CLS
80 PRINT"ENTER YOUR DATA"
90 FOR X = 1 TO 3000:NEXT X
100 PRINT:PRINT:PRINT
110 PRINT"VALUES OF YOUR REFERENCE DNA?"
120 PRINT:PRINT
130 INPUT"YOUR ABSORBANCE AT 240 nm";AA
140 GOSUB 2000
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";AB
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"; A
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"; A
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"; A
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/FB
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 RAT10";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
Author  Hortelano Gonzalo
Name of thesis Genetic Study Of A Bacterium Isolated From "greening" Infected Citrus.  1986

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