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

Hortelano, Gonzalo

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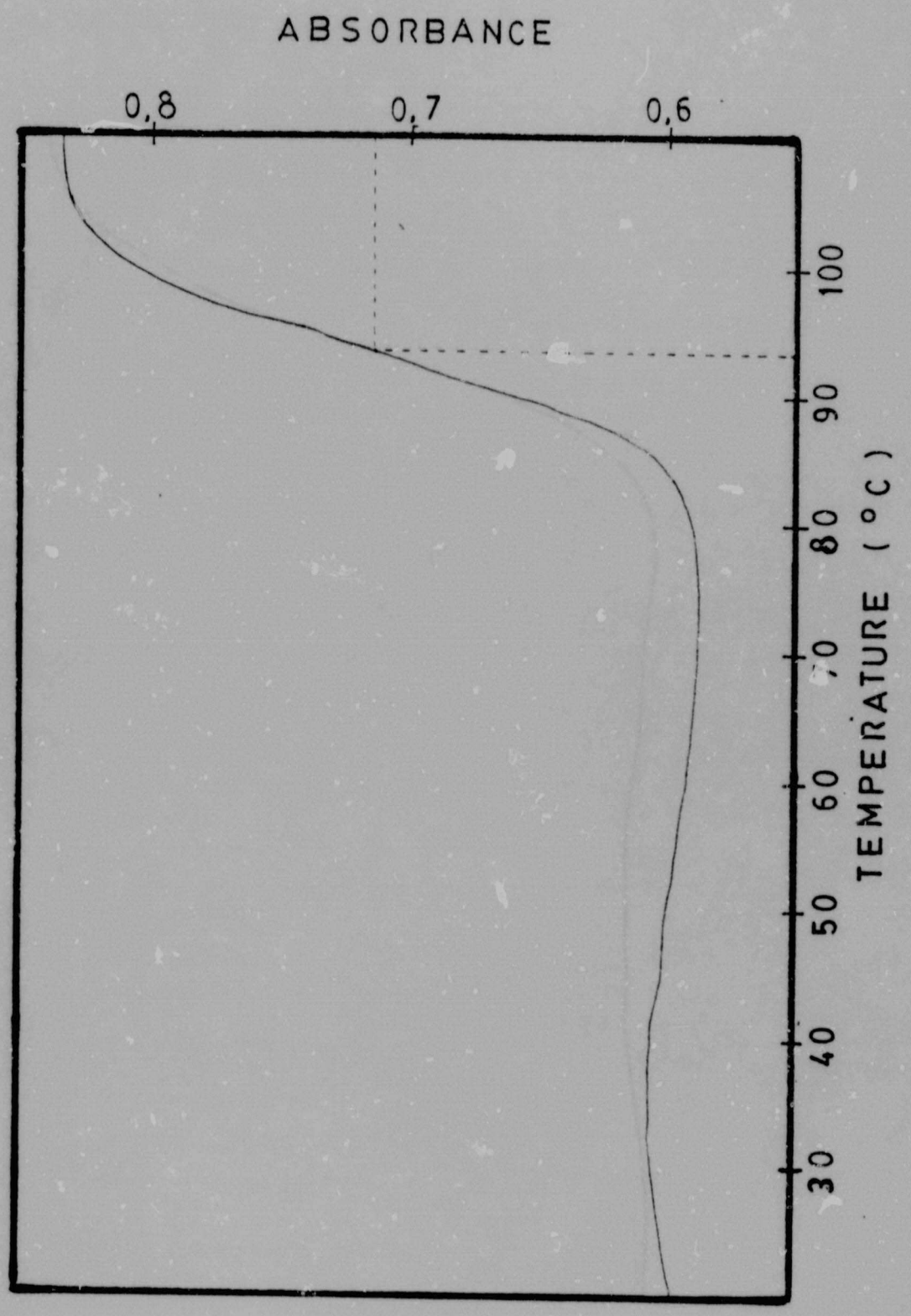


Figure 12 - Thermal denaturation profile of the DNA of LC-1.

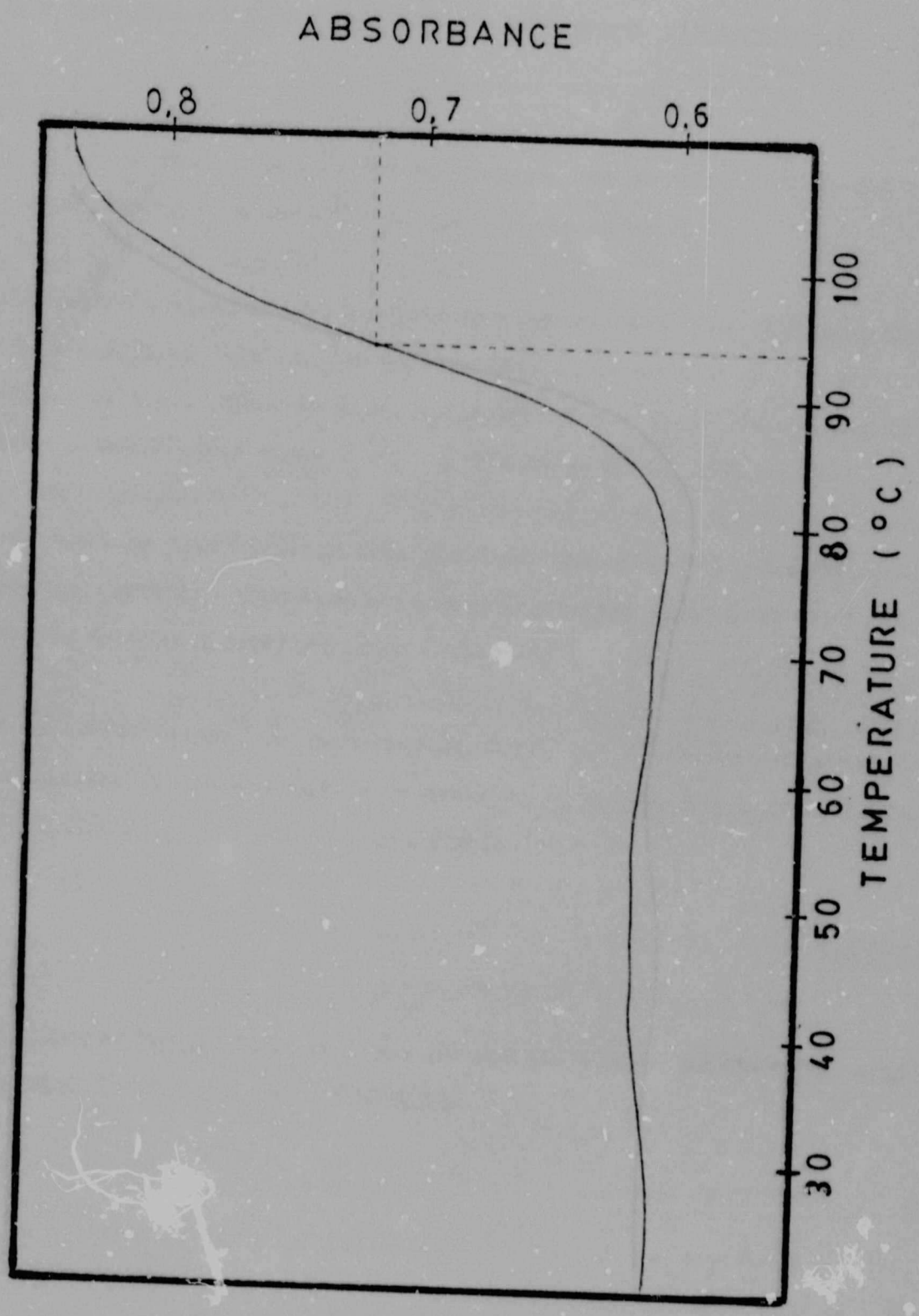


Figure 13 - Thermal denaturation profile of the DNA of NC-1.

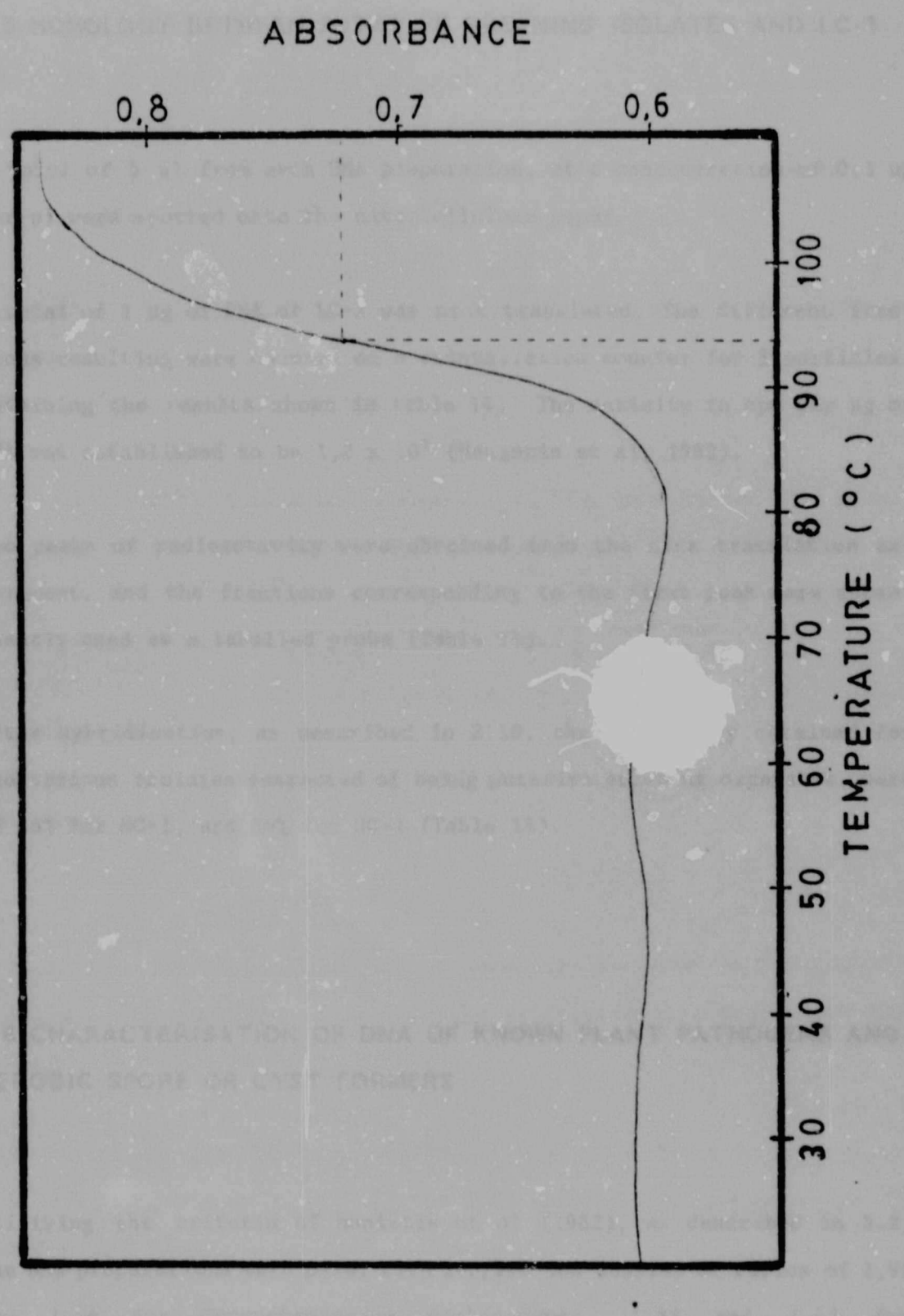


Figure 14 - Thermal denaturation profile of the DNA of code 01.

3.5 HOMOLOGY BETWEEN PUTATIVE GREENING ISOLATES AND LC-1

A total of 5 µl from each DNA preparation, at a concentration of 0,1 µg per µl were spotted onto the nitrocellulose paper.

A total of 1 µg of DNA of LC-1 was nick translated. The different fractions resulting were counted on a scintillation counter for β particles, obtaining the results shown in table 14. The activity in cpm per µg of DNA was established to be $1,2 \times 10^7$ (Maniatis et al, 1982).

Two peaks of radioactivity were obtained from the nick translation experiment, and the fractions corresponding to the first peak were subsequently used as a labelled probe (Table 14).

After hybridisation, as described in 2.10, the % homology obtained for the various isolates suspected of being putative greening organisms, were of 85% for NC-1, and 86% for GC-1 (Table 15).

3.6 CHARACTERISATION OF DNA OF KNOWN PLANT PATHOGENS AND AEROBIC SPORE OR CYST FORMERS

Utilising the criteria of Maniatis et al (1982), as described in 3.2, the DNA preparations were pure, with 260/280 and 260/240 nm ratios of 1,92 and 1,56 for *Corynebacterium michiganense*, 1,83 and 1,43 for *Corynebacterium insidiosum*, 1,7 and 1,42 for *Erwinia herbicola*, 1,98 and 1,65 for *Erwinia carotovora*, 1,69 and 1,4 for *Pseudomonas phaseolicola*, 1,87 and 1,47 for *Xanthomonas vesicatoria*, 1,83 and 1,51 for *Azotobacter*

vinelandii, and 1,9 and 1,71 for *Bacillus subtilis*, as shown in tables 16 to 23.

From the OD values (Tables 16 to 23), the relevant ratios to enable the determination of the mol% C+G content, were calculated.

In addition, the absorption values recorded for *Micrococcus lysodeikticus* (Sigma,MO. USA) were used as a reference, following the method of Ulitzur (1972) as in section 2.7 (Table 7).

The mol% C+G content values obtained for all bacteria tested fall within the range of the values reported in the literature (De Ley, 1967, Table 24).

3.7 HOMOLOGY OF LC-1 WITH PLANT PATHOGENS AND AEROBIC CYST OR SPORE FORMERS

A total of 1 µg of LC-1 DNA was prepared as described in 2.6 and nick translated as described in 2.11.4. The activity of the pooled labelled DNA was $2,2 \times 10^7$ cpm per µg of DNA.

DNA of each bacteria (0,5 µg) was immobilised onto nitrocellulose paper, as described previously in 3.5, and hybridisation was performed as described in 2.11.5.

The results indicated little homology between LC-1 and all the organisms tested (Table 25).

FRACTIONS	COUNTS PER MINUTE
1	4700
2	13475
3	951120
4	1020865
5	343625
6	526390
7	1297610
8	1527910

Table 14 - Counts per minute of the different fractions obtained from the nick translation experiment.

MICROORGANISMS	cpm	% HOMOLOGY to LC-1
LC-1	1796	100
NC-1	1524	85
GC-1	1550	86

Table 15 - Homology value of LC-1 and other suspected putative greening organisms.

WAV.	240	245	250	255	260	265	270	275	280
VAL	0,565	0,662	0,828	0,881	0,882	0,816	0,690	0,542	0,459

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,92	1,56	1,2309	1,0424	0,9594
MOL %	--	--	61,8	65,1	72,0

MOL% C+G CONTENT - 66,3 %
Standard deviation - 4,2

Table 16 - Absorbance ratios of the DNA of *Corynebacterium michiganense*.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0.550	0,621	0,733	0,785	0,791	0,741	0,630	0,492	0,418

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,89	1,43	1,3157	1,1178	0,9857
MOL %	--	--	73,03	78,26	77,63

MOL% C+G CONTENT - 76,3 %

Standard deviation - 2,33

Table 17 - Absorbance ratios of the DNA of *Corynebacterium insidiosum*.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,589	0,631	0,744	0,818	0,837	0,807	0,712	0,599	0,490

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,70	1,42	1,2020	0,9833	0,8862
MOL %	--	--	58,0	54,91	56,46

MOL% C+G CONTENT - 56,5 %
Standard deviation - 1,29

Table 18 - Absorbance ratios of the DNA of *Erwinia herbicola*.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,500	0,594	0,736	0,817	0,828	0,793	0,688	0,514	0,418

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,98	1,65	1,1961	0,9727	0,8633
MOL %	--	--	57,3	53,0	51,5

MOL% C+G CONTENT - 53,9 %
Standard deviation - 2,41

Table 19 - Absorbance ratios of DNA of *Erwinia carotovora*.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,500	0,594	0,736	0,817	0,828	0,793	0,688	0,514	0,418

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,98	1,65	1,1961	0,9727	0,8633
MOL %	--	--	57,3	53,0	51,5

MOL% C+G CONTENT - 53,9 %
Standard deviation - 2,41

Table 19 - Absorbance ratios of DNA of *Erwinia carotovora*.

WAV.	240	245	250	255	260	265	270	275	280
VAL	0,605	0,665	0,774	0,838	0,851	0,826	0,737	0,593	0,501

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,69	1,40	1,2075	1,0202	0,9023
MOL %	--	--	58,8	61,32	59,8

MOL% C+G CONTENT - 60,0 %

Standard deviation - 1,03

Table 20 - Absorbance ratios of DNA of *Pseudomonas phaseolicola*.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,553	0,612	0,726	0,789	0,813	0,763	0,661	0,539	0,434

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,87	1,47	1,2741	1,0259	0,9285
MOL %	--	--	67,5	62,3	65,4

MOL% C+G CONTENT - 64,9 %
Standard deviation - 2,14

Table 21 - Absorbance ratios of DNA of *Xanthomonas vesicatoria*

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,534	0,597	0,748	0,806	0,811	0,765	0,653	0,526	0,443

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,83	1,51	1,2054	1,0152	0,9142
MOL %	--	--	58,5	60,4	62,4

MOL% C+G CONTENT - 60,4 %
Standard deviation - 1,59

Table 22 - Absorbance ratios of DNA of *Azotobacter vinelandii*.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,464	0,541	0,659	0,757	0,795	0,756	0,656	0,511	0,418

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,90	1,71	1.1100	0,9080	0,8246
MOL %	--	--	45,9	41,8	43,3

MOL% C+G CONTENT - 43,7 %
Standard deviation - 1,70

Table 23 - Absorbance ratios of DNA of *Bacillus subtilis*.

MICROORGANISM	MOL% LITERATURE	MOL% FOUND
C. insidiosum	78,1	76,3
C. michiganense	67,3-70,7	66,3
E. herbicola	53,6-57,66	56,5
P. phaseolicola	58,9-59,5	60
X. vesicatoria	66,4	64,9
A. vinelandii	60	60,4
B. subtilis	43	43,7
E. carotovora	50-53,8	53,9

Table 24 - Comparison of the mol% C+G content of bacteria found with the values reported in the literature.

MICROORGANISMS	cpm	% HOMOLOGY
LC-1	4720	100
C. michiganense	590	12,5
C. insidiosum	681	14,4
E. carotovora	473	10
P. phaseolicola	1419	30,2
A. vinelandii	584	12,4
B. subtilis	741	15,7
X. vesicatoria	813	17,3

Table 25 - Percent homology results between LC-1, known phytopathogenic bacteria, and other bacteria.

3.8 HOMOLOGY BETWEEN LC-1 AND CITRUS LEAVES ISOLATES

The different DNA samples were spotted onto nitrocellulose paper with 0,5 µg of DNA per spot, as described in 3.5.

A total of 1 µg of LC-1 DNA was nick translated, the activity being of $1,2 \times 10^7$ cpm per µg of DNA.

Once the hybridisation was ended, the radioactivity remaining on the nitrocellulose paper was determined and the percent homology values established (Table 26).

As can be seen from table 41, the microorganism code 01 bear a high degree of homology with LC-1. The others have low homologies and, therefore, appeared not to be related to LC-1.

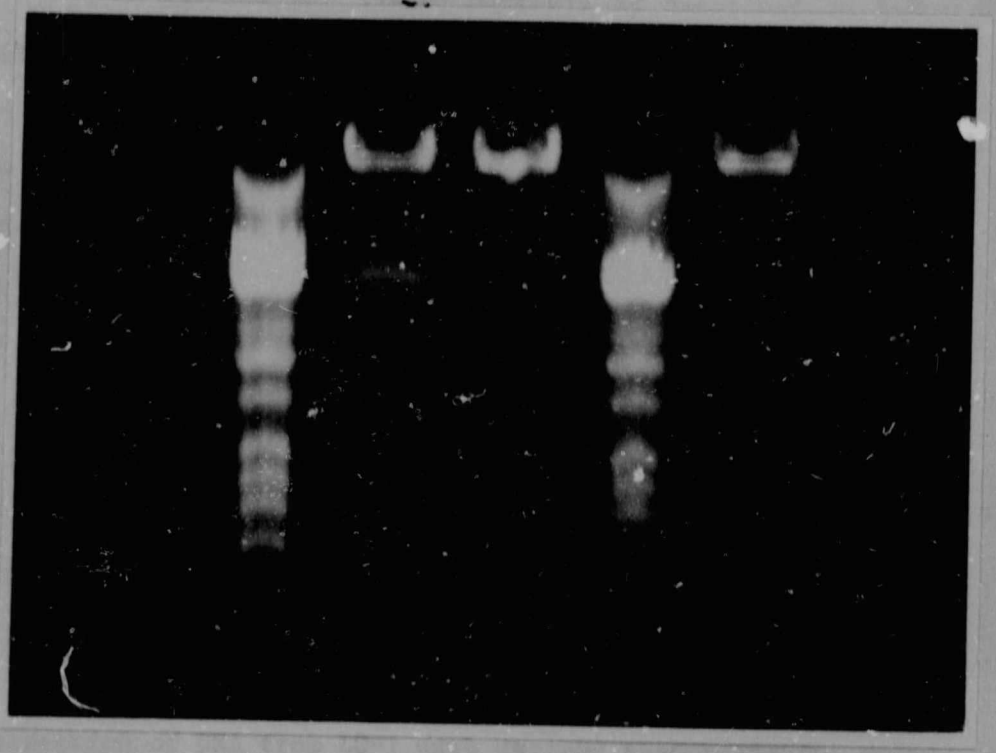
3.9 FURTHER CHARACTERISATION OF LC-1 DNA

3.9.1 EXISTENCE OF PLASMIDS

A plasmid extraction from the total DNA of LC-1, prepared as described in 2.6, was attempted. The two techniques as described in 2.8 were used. Whereas no plasmids were found on CsCl density gradient (data not shown) a band with an estimated molecular weight of $1,5 \times 10^6$ was obtained which could possibly be a plasmid.

MICROORGANISMS	cpm	%HOMOLOGY
LC-1	1796	100
code 4	696	38,7
code 7	1024	57
code 9	1063	59,2
code 05	866	48,2
code F-1	774	43,1
code F-2	490	27,2
code 01	1555	86,6

Table 26 - Percent homology between LC-1 and the microorganisms isolated from citrus leaves.



Lane 1 - Lambda DNA marker II (2µg)

Lane 2 - LC-1 DNA (5 µg)

Lane 3 - NC-1 DNA (5 µg)

Lane 4 - Lambda DNA marker II (2 µg)

Figure 15 - Agarose gel run with total DNA for plasmid extraction.

Author Hortelano Gonzalo
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