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

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2015-02-05
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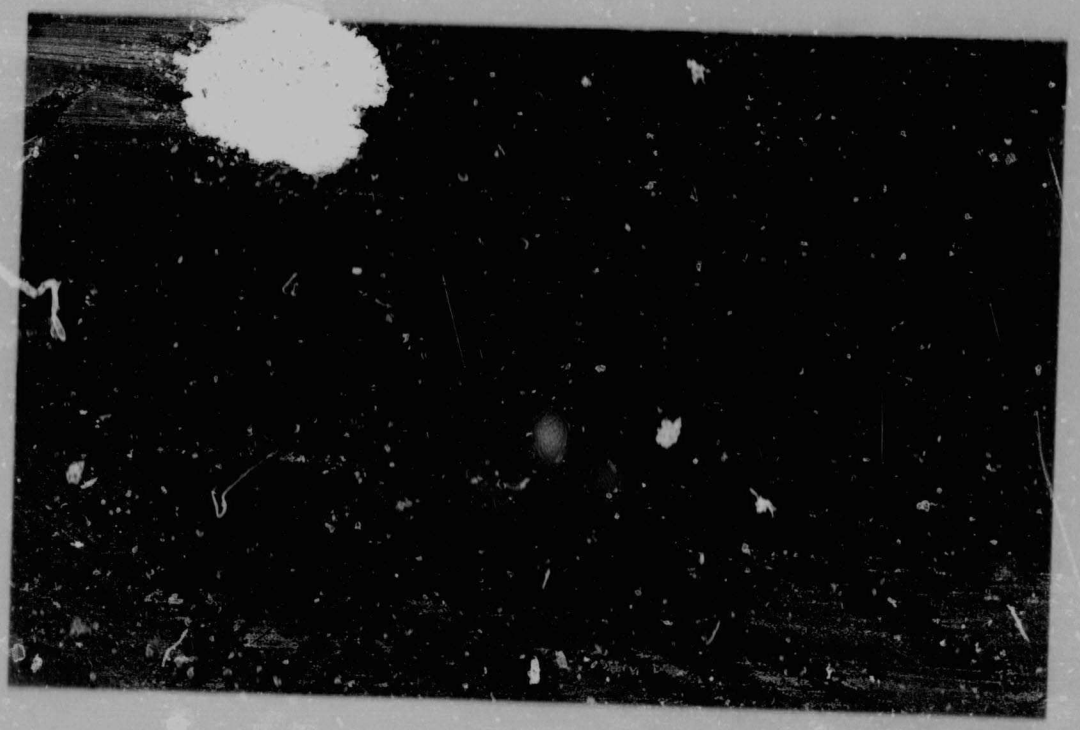


Figure 7 - Gram stain of code F-1.

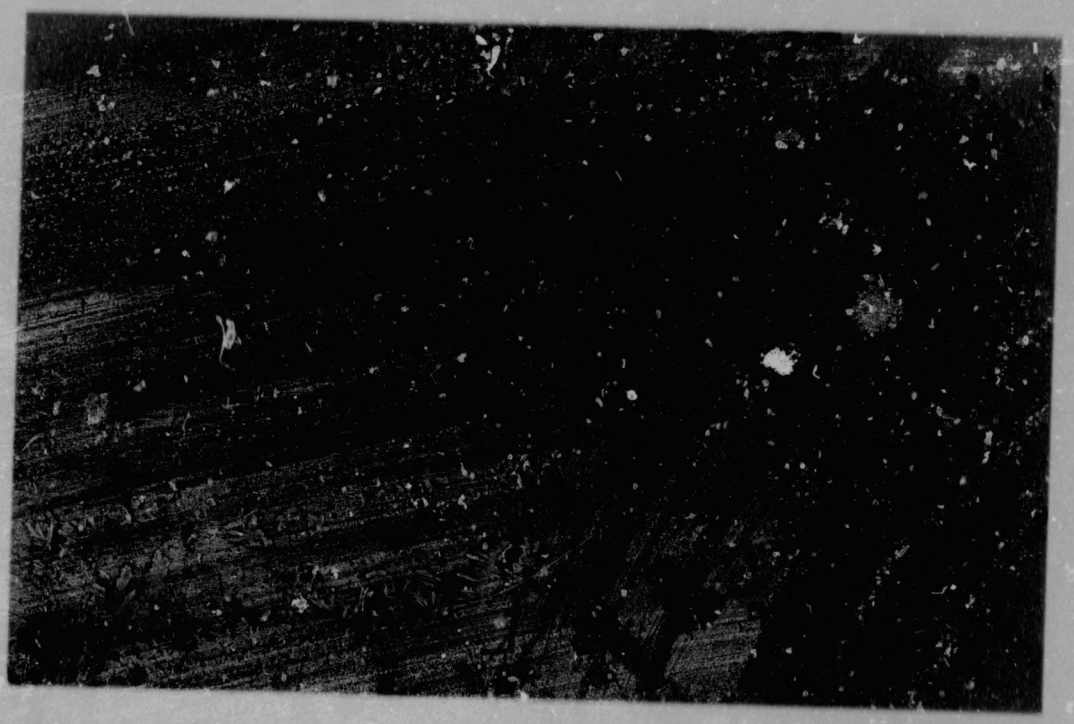


Figure 8 - Gram stain of code F-2.

3.3 ESTABLISHMENT OF THE PURIFICATION PROCEDURE FOR DNA

DNA from LC-1 was prepared following each of the methods mentioned in 2.6.

In order to determine the purity of the DNA preparation, the 260/280 nm ratios were determine in a 2200 VARIAN spectrophotometer, the results being 1,64 for the method of Marmur (1961), 1,48 for the method of Meyer and Schleifer (1975) and 1,40 for the method of Brenner et al (1969), as shown in tables 4 to 6.

Maniatis et al (1982), consider that a good DNA preparation must have a 260/280 nm ratio of 1,8, and a 260/240 nm ratio of 1,4. According to these criteria, the best DNA preparation appeared to be the one prepared following the method of Marmur (1961).

Since the optimal OD values determined by Maniatis et al (1982) as representative of pure DNA were still not obtained, the modifications described in 2.6 to this method were introduced and this method was subsequently used to prepare all DNA samples utilized in this work. With this technique, the 260/280 and 260/240 nm ratios obtained for LC-1 were of 1,8 and 1,49 respectively, as shown in table 9, thus suggesting a high degree of DNA purity with no evidence of protein contamination.

3.4 DNA MOL % C+G STUDIES OF PUTAT E GREENING ISOLATES

3.4.1 ULTRAVIOLET SPECTROPHOTOMETRY

The absorption of the DNA of the various isolates from greening infected citrus, NC-1, GC-1 and code 01 together with LC-1, was recorded at a range of wavelength from 240 to 280 nm. The ratios for 260/280 and 260/240 nm being respectively 1,82 and 1,47 for NC-1, 1,8 and 1,52 for GC-1, and 1,84 and 1,5 for code 01, suggest all samples to be pure (tables 10 to 12). The absorption spectrum of DNA of *Micrococcus lysodeikticus* (Sigma,MO.USA) and *Escherichia coli* K-12 were recorded as controls (tables 7 and 8). The results, being 1,85 and 1,41 for *Micrococcus lysodeikticus*, and 1,8 and 1,57 for *Escherichia coli*.

Each absorbance reading shown is the average absorption of three independently preparated DNA samples at the same wavelength.

All DNA absorption profiles appeared to be smooth (Figure 9), with no "humps" and bearing a maximum absorbance at about 260 nm as expected from the literature (Lehninger, 1975). This indicates a high quality, protein-free, DNA preparations.

Utilising the mol% C+G content found for each of the different ratios 240/280, 240/275, and 245/270 nm, a mol% C+G content was established for each organism as the average of the three values this being 50 for *Escherichia coli*, 59,9 for LC-1, 60,1 for NC-1, 60,5 for code 01 and 58,4 for GC-1, as shown in tables 8 to 12.

WAV.	240	245	250	255	260	265	270	275	280
ABS.	0,257	0,260	0,273	0,290	0,294	0,286	0,267	0,241	0,210

RATIOS	260/280	260/240
VALUES	1,40	1,02

Table 4 - Absorbance ratios of LC-1 DNA prepared following the method of Brenner et al (1969).

WAV.	240	245	250	255	260	265	270	275	280
ABS.	0,257	0,261	0,284	0,312	0,320	0,317	0,288	0,249	0,216

RATIOS	260/280	260/240
VALUES	1,48	1,24

Table 5 - Absorbance ratios of LC-1 DNA prepared following the method of Meyer and Schleifler (1975).

WAV.	240	245	250	255	260	265	270	275	280
ABS.	0,677	0,768	0,827	0,864	0,872	0,863	0,822	0,715	0,531

RATIOS	260/280	260/240
VALUES	1,64	1,25

Table 6 - Absorbance ratios of LC-1 DNA prepared following the method of Marmur (1961).

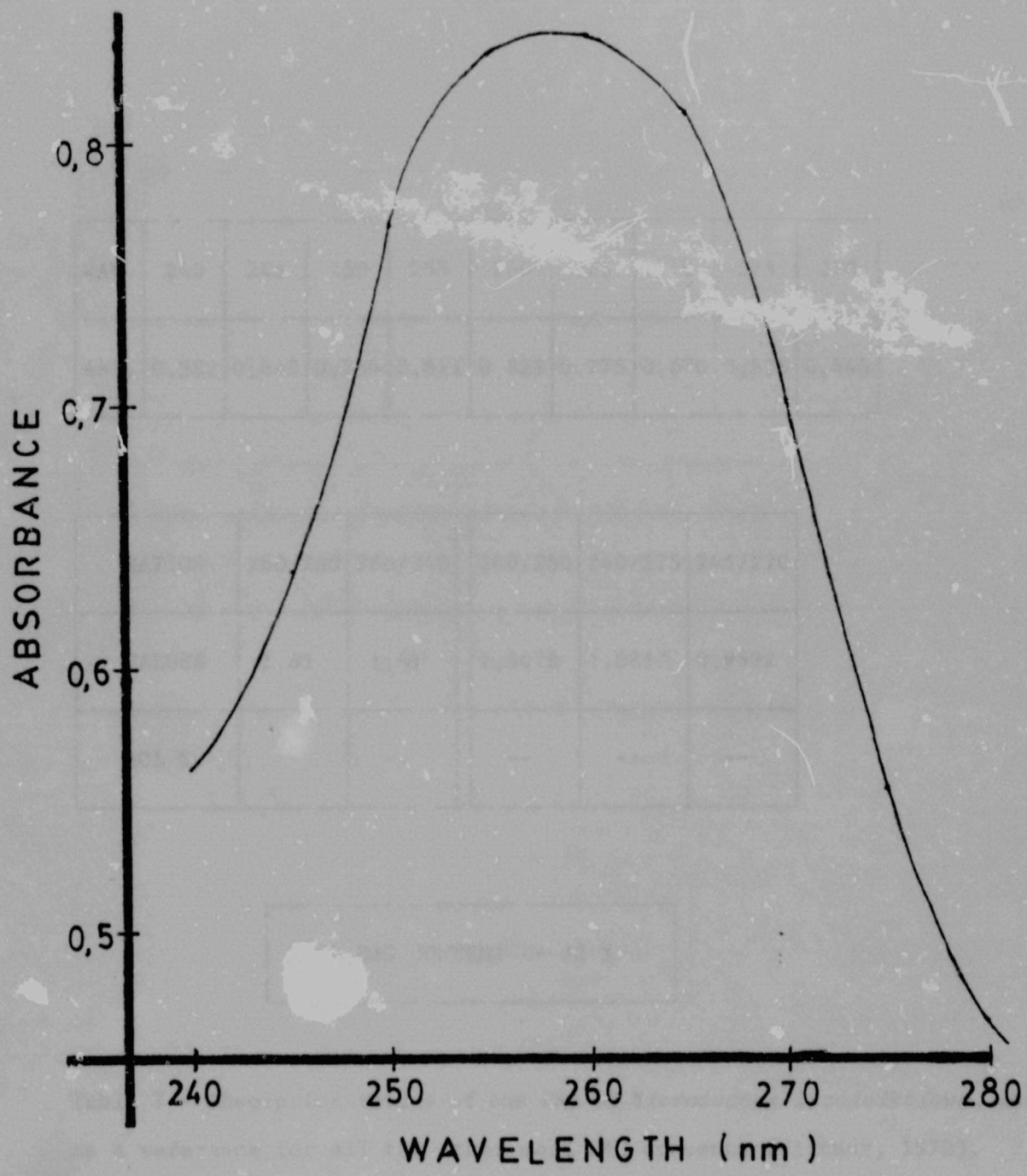


Figure 9 - Absorbance profile of DNA of LC-1 at different wavelength.

WAV.	240	245	250	255	260	265	270	275	280
ABS.	0,582	0,643	0,734	0,811	0,825	0,775	0,670	0,538	0,445

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,85	1,41	1,3078	1,0817	0,9592
MOL %	--	--	--	--	--

MOL% C+G CONTENT - 72 %

Table 7 - Absorption ratios of the DNA of *Micrococcus lysodeikticus*, used as a reference for all the other mol% C+G contents (Ulitzur, 1972).

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,533	0,616	0,723	0,813	0,842	0,815	0,718	0,559	0,467

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,80	1,57	1,1413	0,9534	0,8579
MOL %	--	--	50,09	49,72	50,44

MOL% C+G CONTENT - 50,08 %
STANDARD DEVIATION - 0,29

Table 8 - Absorbance ratios of DNA of *Escherichia coli*.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,562	0,636	0,769	0,833	0,838	0,811	0,700	0,555	0,466

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,80	1,49	1,2060	1,0122	0,9085
MOL %	--	--	58,60	59,93	61,21

MOL% C+G CONTENT - 59,91 %
Standard deviation - 1,06

Table 9 - Absorbance ratios of DNA of LC-1.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,563	0,625	0,757	0,823	0,833	0,795	0,692	0,561	0,457

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,82	1,47	1,2319	1,0035	0,9031
MOL %	--	--	62,01	58,42	60,06

MOL% C+G CONTENT - 60,16 %
Standard deviation - 1,46

Table 10 - Absorption ratios of DNA of NC-1.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,546	0,595	0,739	0,822	0,831	0,783	0,664	0,536	0,461

RATIOS	60/280	60/240	40/280	40/275	40/270
VALUES	1,80	1,52	1,1843	1,0186	0,8960
MOL %	--	--	55,75	61,04	58,55

MOL% C+G CONTENT - 58,44 %

Standard deviation - 2,16

Table 11 - Absorbance ratios of DNA of GC-1.

WAV.	240	245	250	255	260	265	270	275	280
VAL.	0,545	0,606	0,732	0,809	0,818	0,778	0,668	0,540	0,444

RATIOS	260/280	260/240	240/280	240/275	245/270
VALUES	1,84	1,50	1,2274	1,0092	0,9071
MOL %	--	--	61,64	59,41	60,91

MOL% C+G CONTENT - 60,58 %

Standard deviation - 0,85

Table 12 - Absorbance ratios of DNA of code 01.

3.4.2 THERMAL DENATURATION TEMPERATURE

First, the DNA samples prepared as described in 2.6, being the same preparation tested for purity in 3.3.1, were precipitated in two volumes of cold ethanol (100%) and resuspended in 1 x SSC (Appendix 2) for three days before subsequent experimentation. A total of 30 µg of pure DNA were used for each experiment, corresponding to an absorbance of 0,6 at 260 nm.

In order to avoid the evaporation of the solvent, a thin layer of oil was located on top of the DNA sample, inside the quartz cuvette of all samples tested.

The denaturation profile appeared to be smooth with a sharp increasing pattern (Figures 10 to 14). From this, the DNA sample was found to be RNA-free, which would have interfered with the calculations.

The increasing percent in absorption due to the denaturation was of of the order of 39% which indicated a good DNA preparation (Lehninger, 1975).

The Tm value of the DNA of various putative greening causing microorganisms, was established as 59 for LC-1, 60 for NC-1 and 59,5 for code 01. DNA from *Micrococcus lysodeikticus* (Sigma, MO. USA) was tested as a control for the thermal denaturation technique itself, and DNA from *Escherichia coli* K-12 was used as a control for the DNA purification technique. The experiments yielded values of 71,2 for *Micrococcus lysodeikticus*, and 50,4 for *Escherichia coli* (Table13).

When determining the Tm value of *Micrococcus lysodeikticus* which was known to have a high mol% C+G content (72%), the concentration of the solvent was changed to 0,01 SSC (Appendix 2), thus allowing the DNA to denature

at a lower temperature. This experiment was performed in the same manner as the other thermal denaturation experiments, but the equation employed to determine the mol% C+G content from the Tm value was different, namely (Sibara, 1982),

$$\text{MOL\% C+G CONTENT} = 2{,}64 \times (\text{Tm} - 59{,}6)$$

	A	B	C	D	E
Absorbance at 260 nm of native DNA	0,614	0,604	0,596	0,606	0,607
Absorbance at 260 of denatured DNA	0,854	0,803	0,834	0,837	0,842
Increase (%)	39,08	32,94	39,93	38,11	38,94
Absorbance at 260 of mid-increase	0,734	0,703	0,715	0,721	0,723
Melting point (°C)	98,5	90	93,5	93,9	93,7
MOL% C+G CONTENT					
(found)	71,2	50,4	59	60	59,5
(literature)	72	50,0	--	--	--

A - *Micrococcus*

B - *Escherichia*

C - LC-1

D - NC-1

E - code 01

Table 13 - Results from the thermal denaturation experiments.

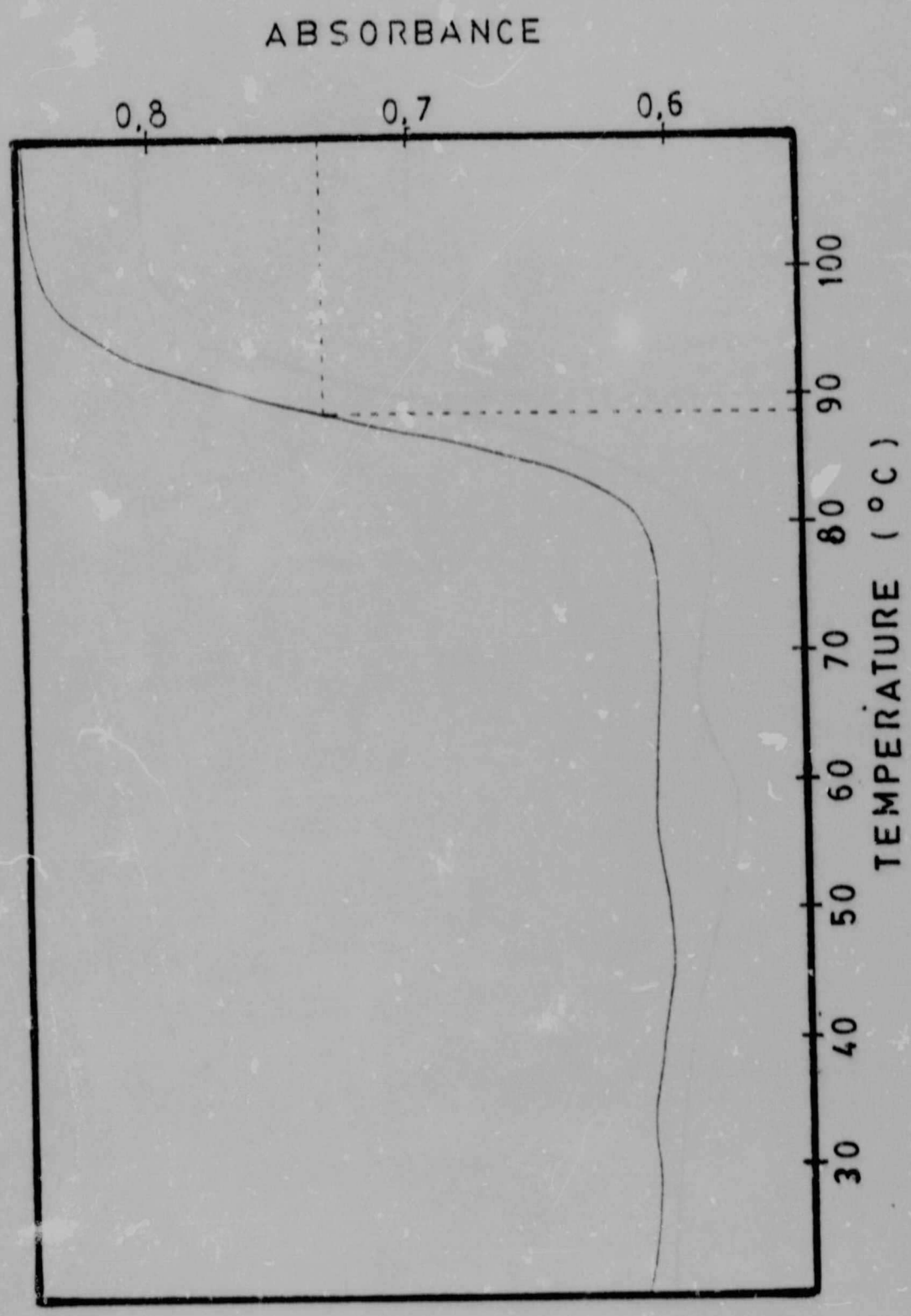


Figure 10 - Thermal denaturation profile of the DNA of *Micrococcus lysodeikticus*.

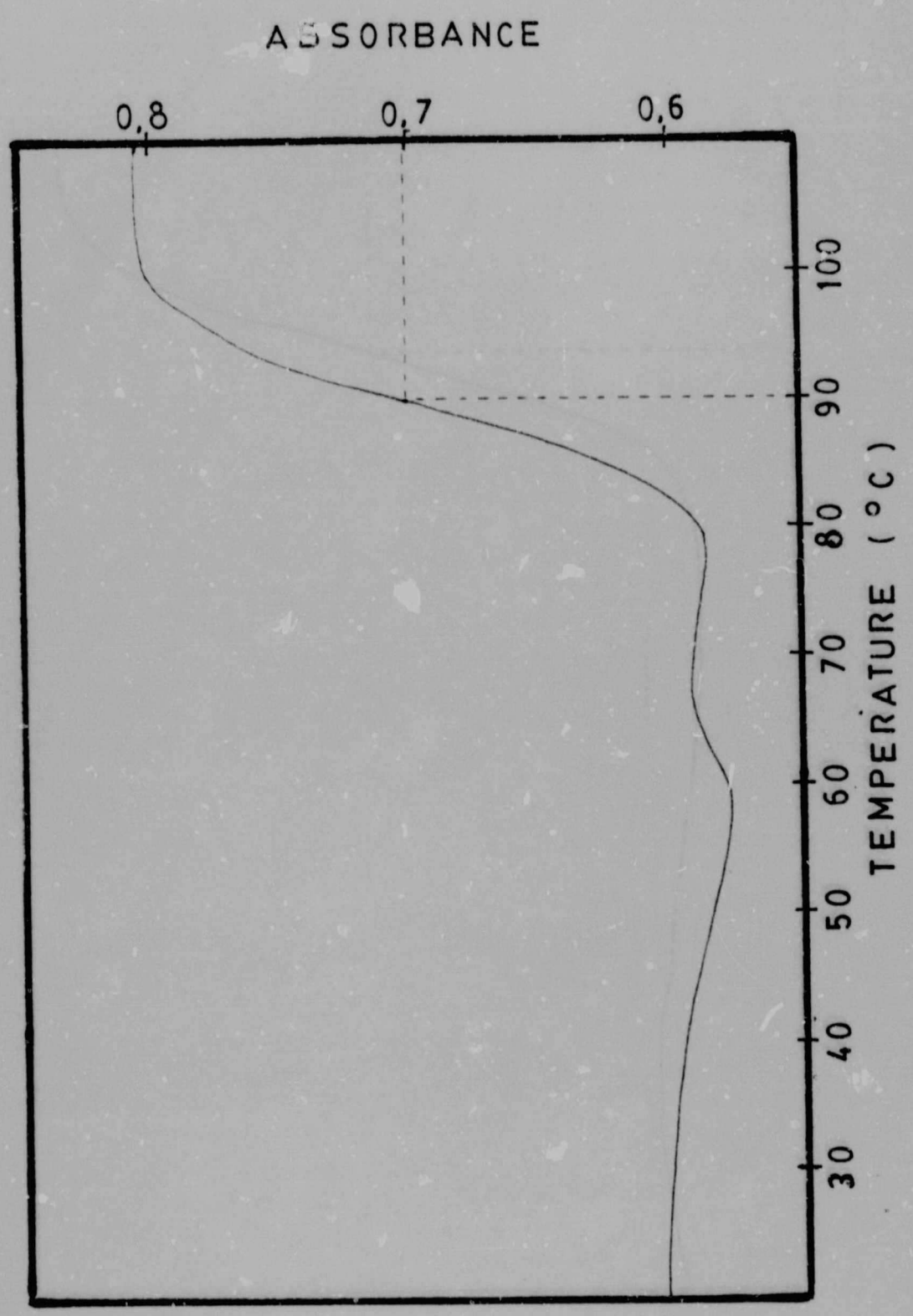


Figure 11 - Thermal denaturation profile of the DNA of *Escherichia coli*.

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Name of thesis Genetic Study Of A Bacterium Isolated From "greening" Infected Citrus. 1986

PUBLISHER:

University of the Witwatersrand, Johannesburg
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