

Seminario Regional Internacional. Comité Veterinario permanente del Cono Sur (CVP)


10 de septiembre de 2024

Loque Americana de las abejas

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- Las abejas melíferas además de producir miel, son esenciales para la polinización de cultivos
- Las tres cuartas partes de los cultivos mundiales, cuyo valor se estima en 160 billones de dólares, requieren polinización entomófila  Abeja: principal polinizador.
- Abejas: susceptibles a enfermedades, parásitos y predadores

Bacterias

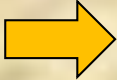
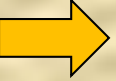

Hongos

Virus

Microsporidios



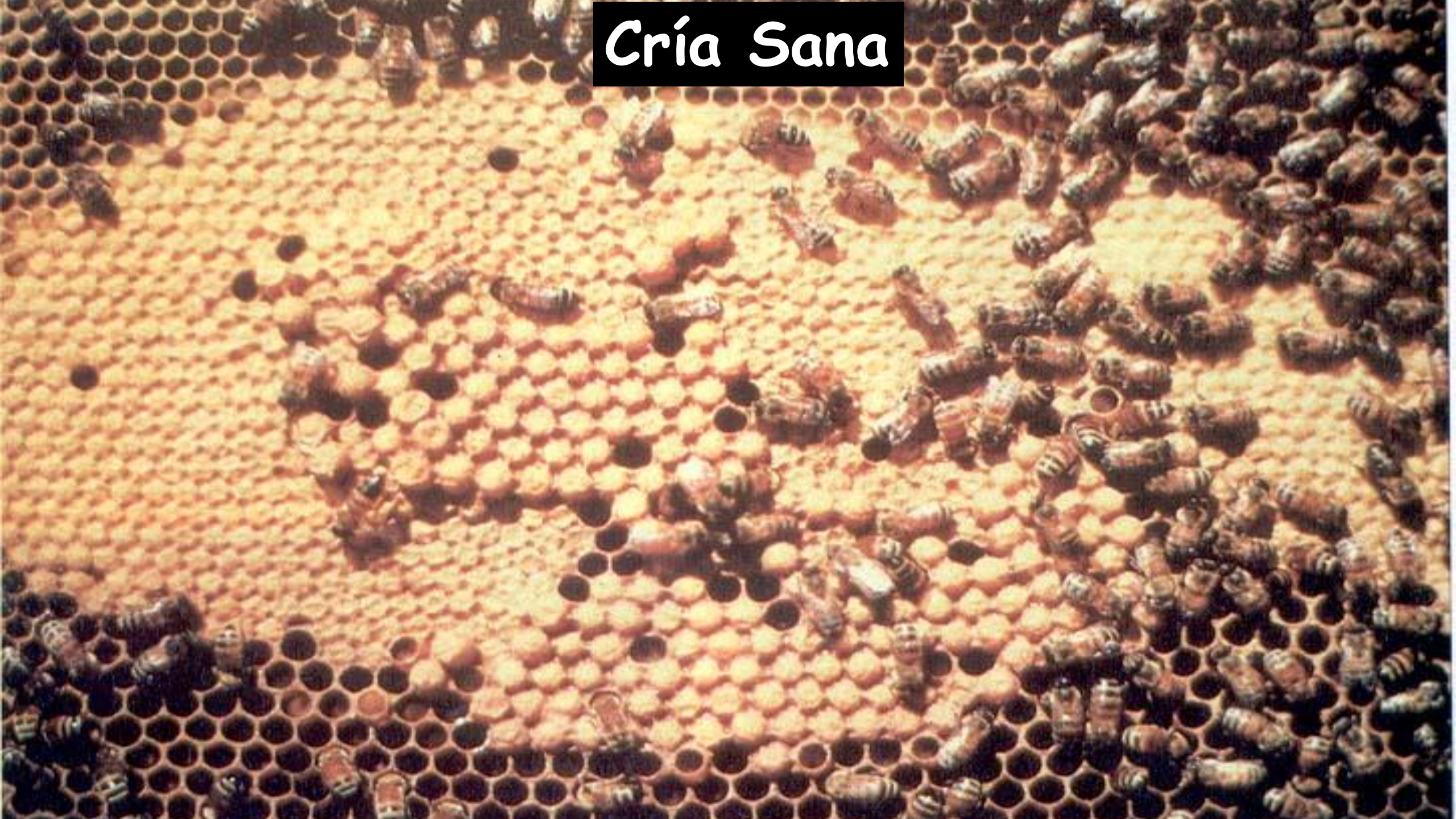
agentes causales de enfermedades

- Enfermedades bacterianas: Loque americana  *Paenibacillus larvae*
 (Larvas) Loque europea  *Melissococcus plutonius*
 Septicemias (Adultas)  *Serratia marcescens* - *Hafnia alvei*
Spiroplasma melliferum - *Ps. aeruginosa*

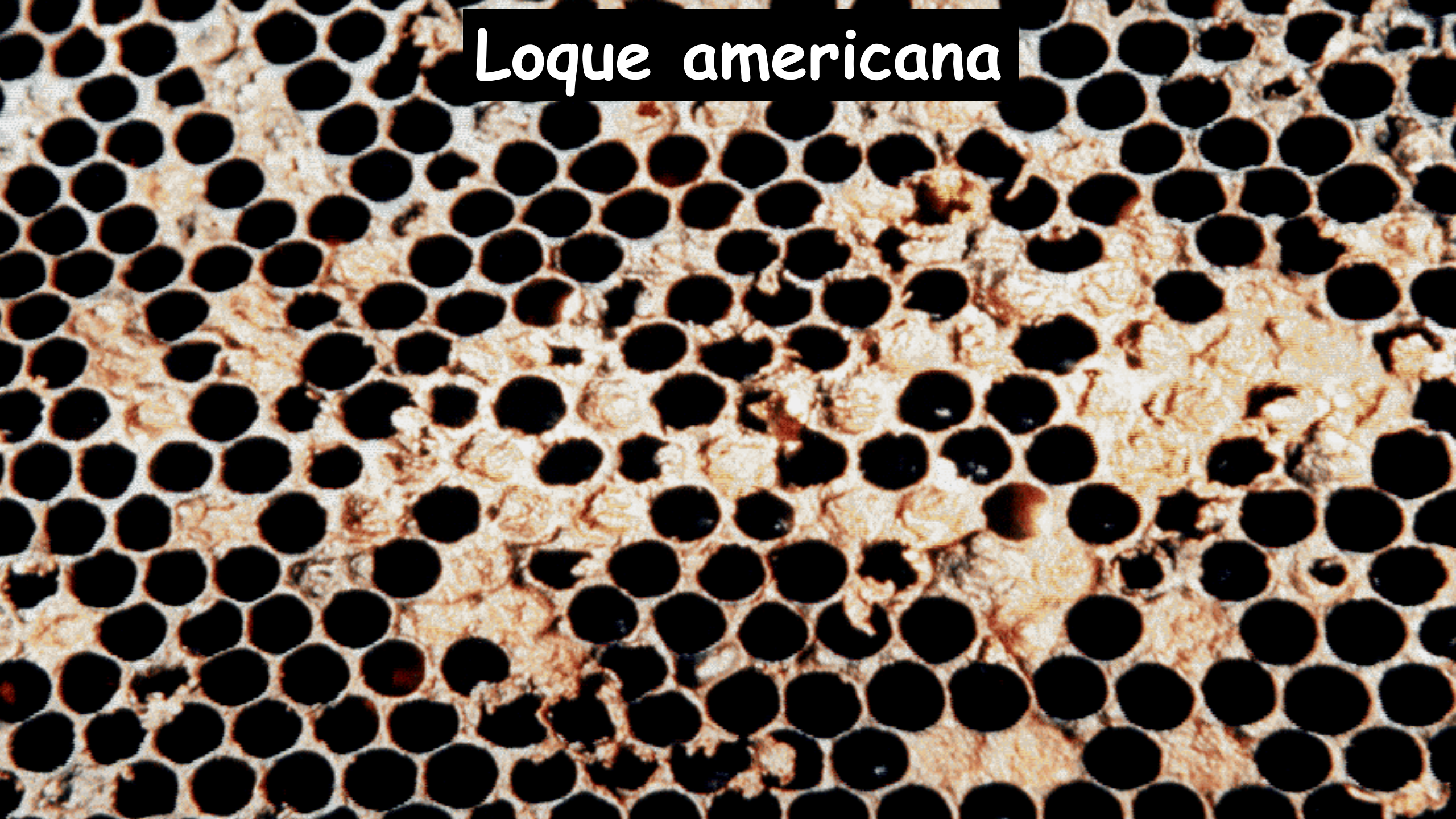
Loque americana - AFB -

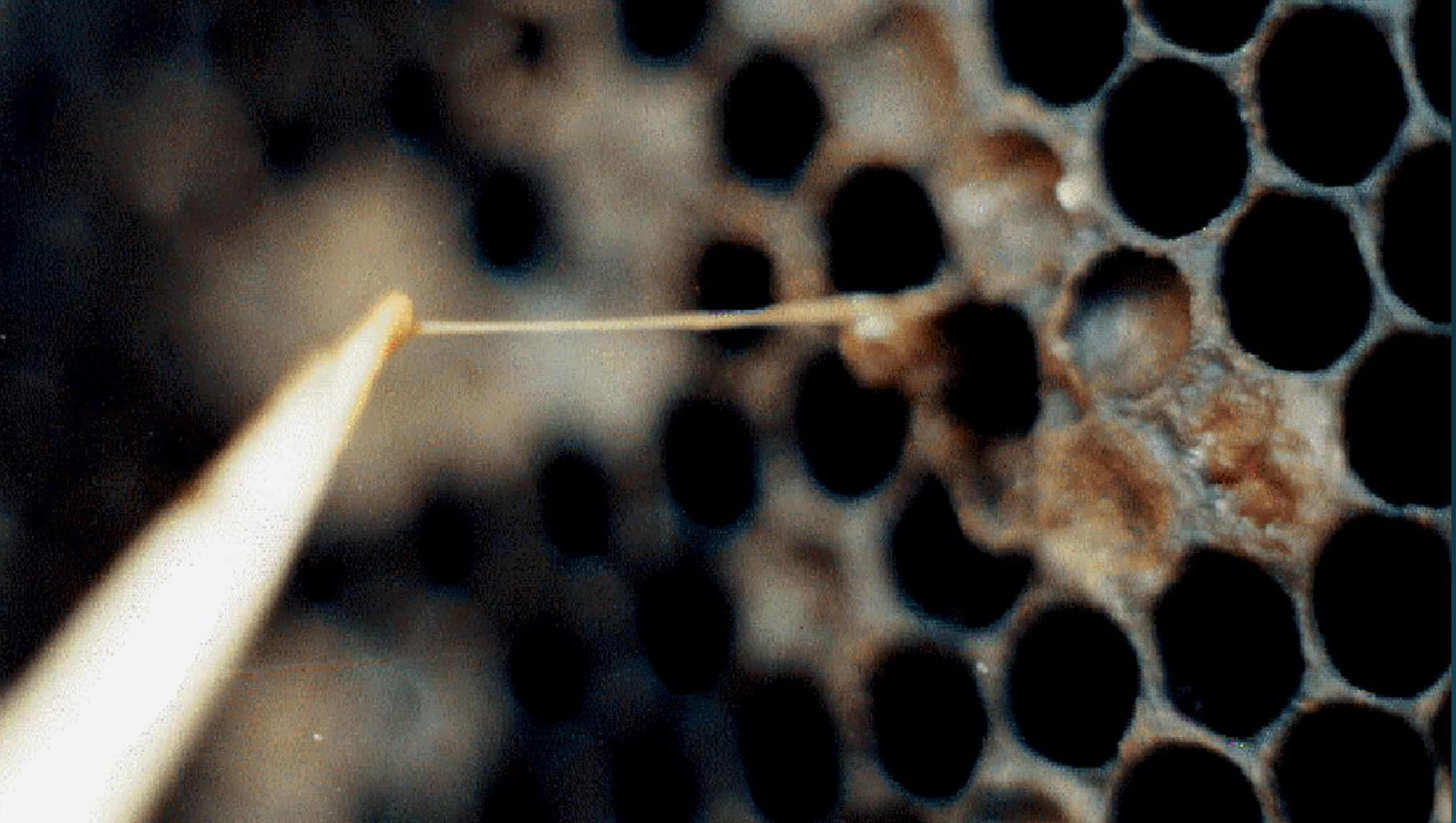
- Es la enfermedad más grave y peligrosa que afecta a las larvas de las abejas.
- Agente causal: *Paenibacillus larvae*-bacteria esporulada Gram (+)
- Es muy contagiosa, puede matar a una colmena y diseminarse a otras rápidamente.
- No existen brotes estacionales - Se manifiesta si hay cría.
- Esporas bacterianas viables por largos períodos y resistentes a condiciones adversas.
- Figura en la lista de enfermedades de la OMSA (WOAH-World organization for animal health).
- Tiene difusión mundial en todos los países productores de miel.

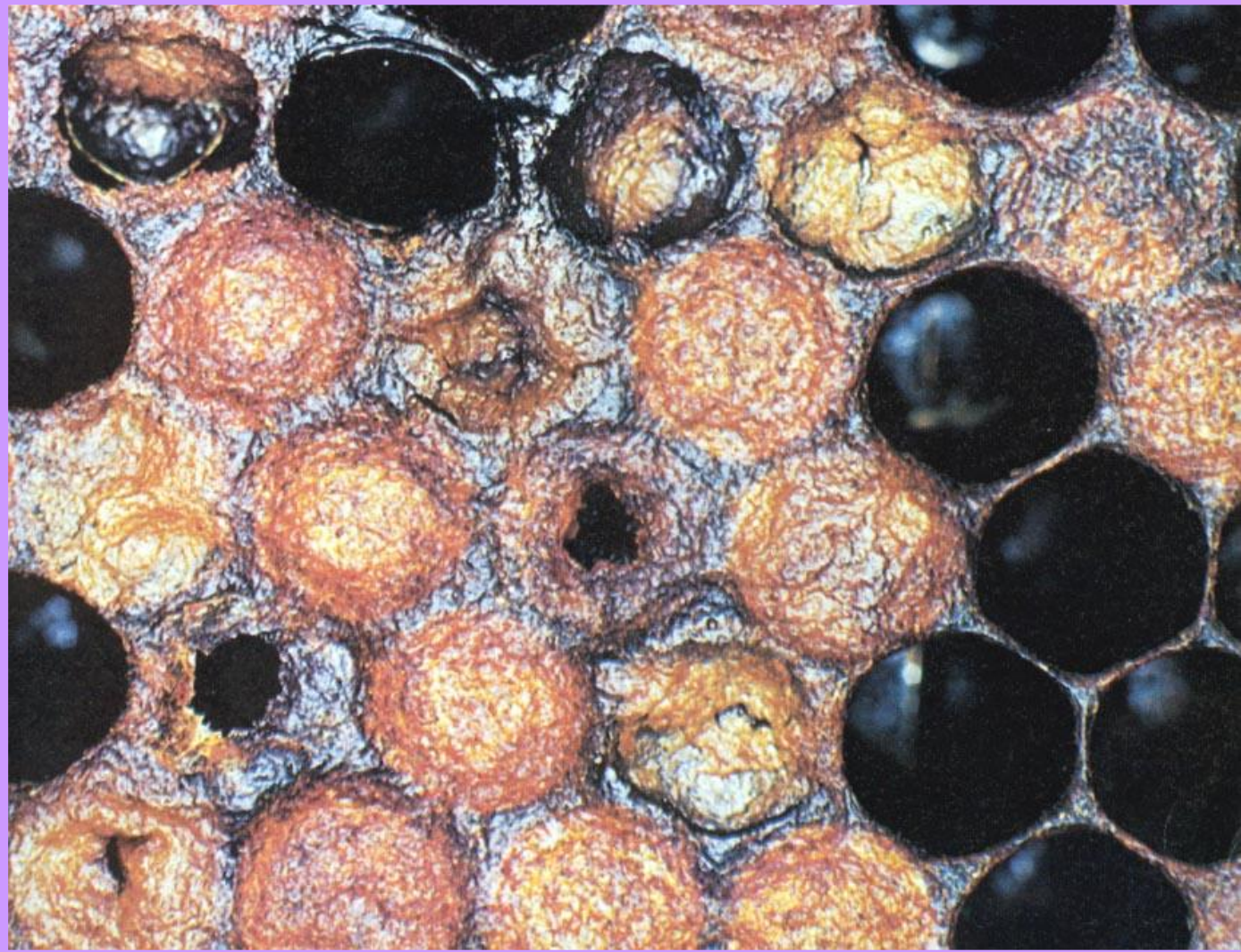
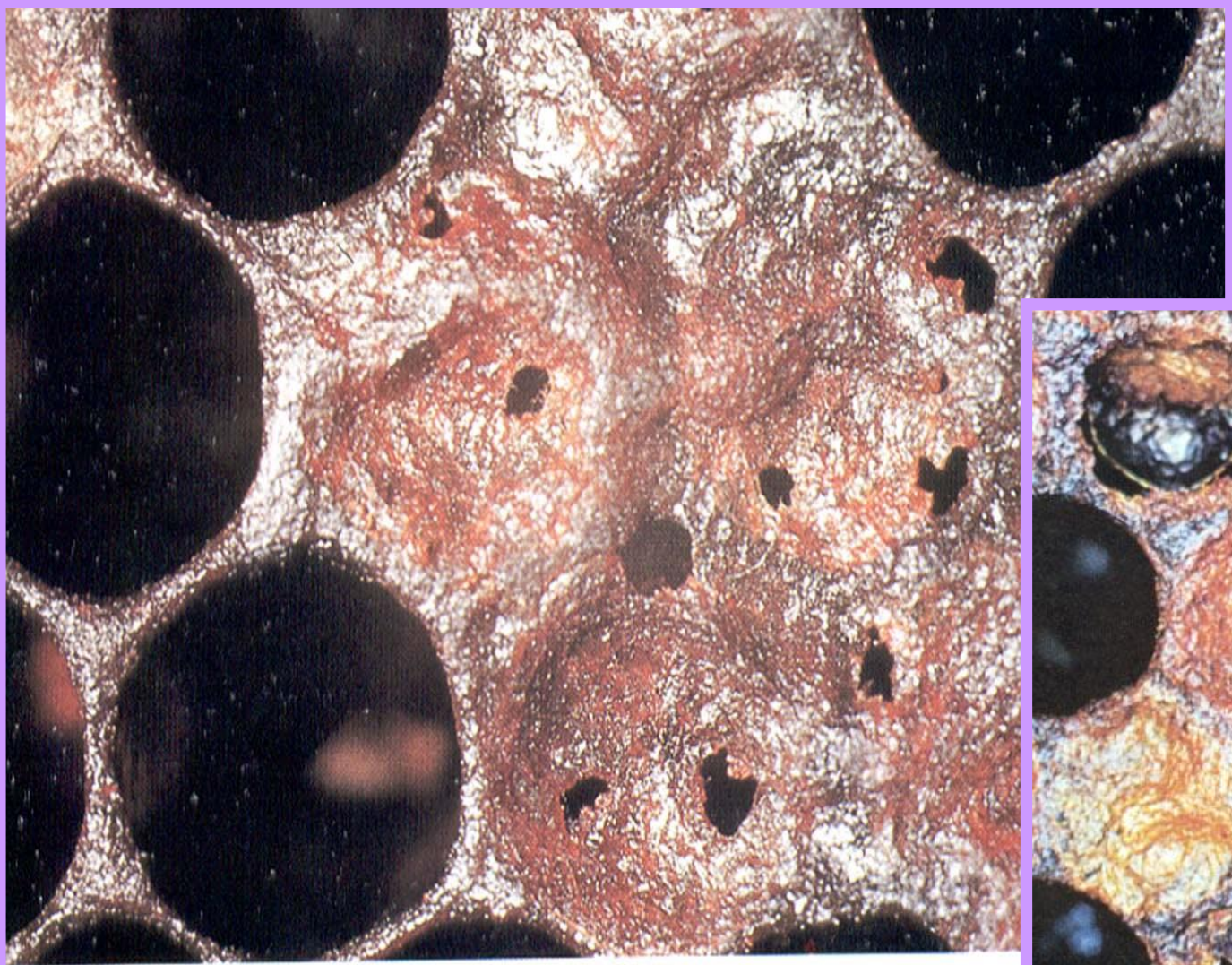
Cría Sana

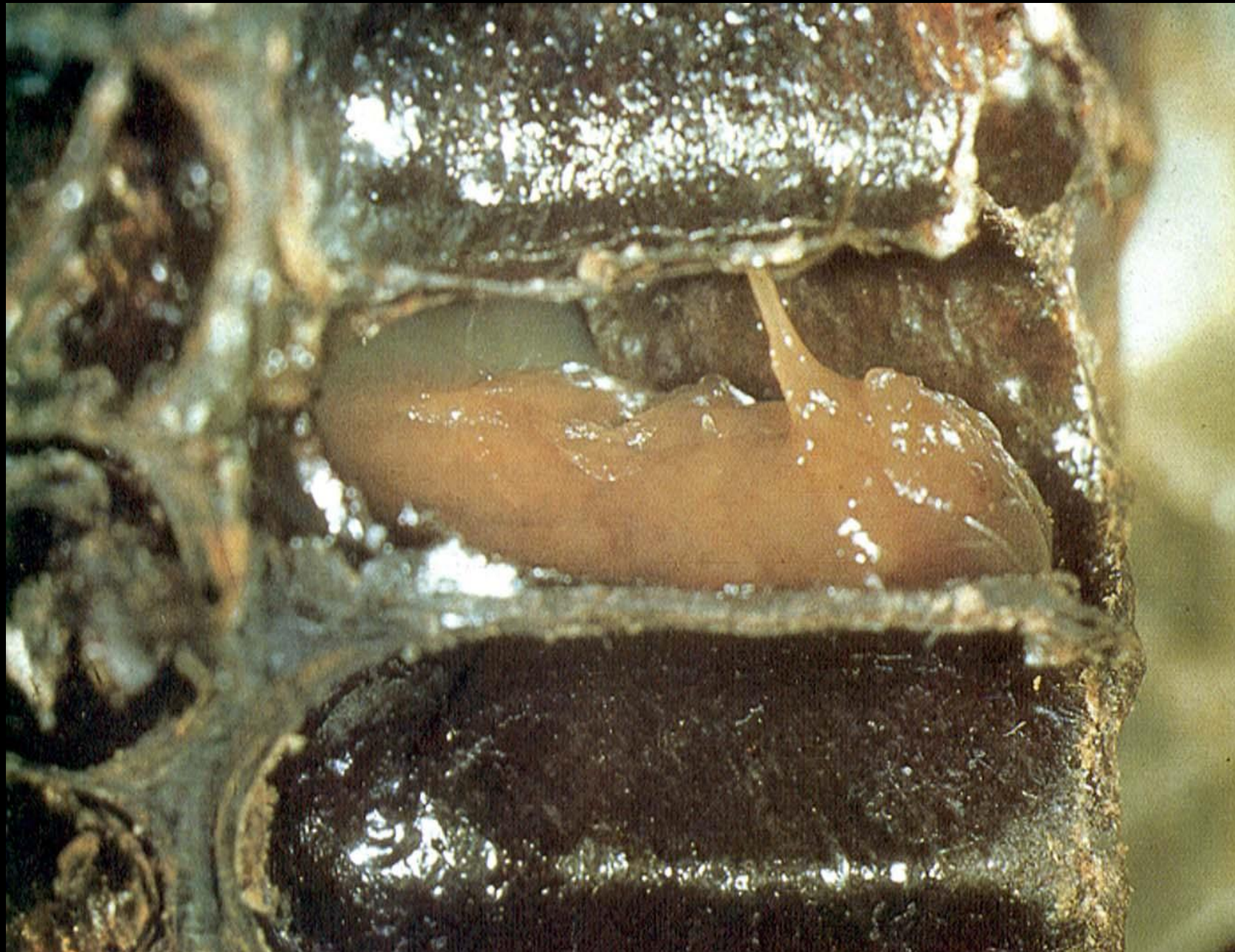


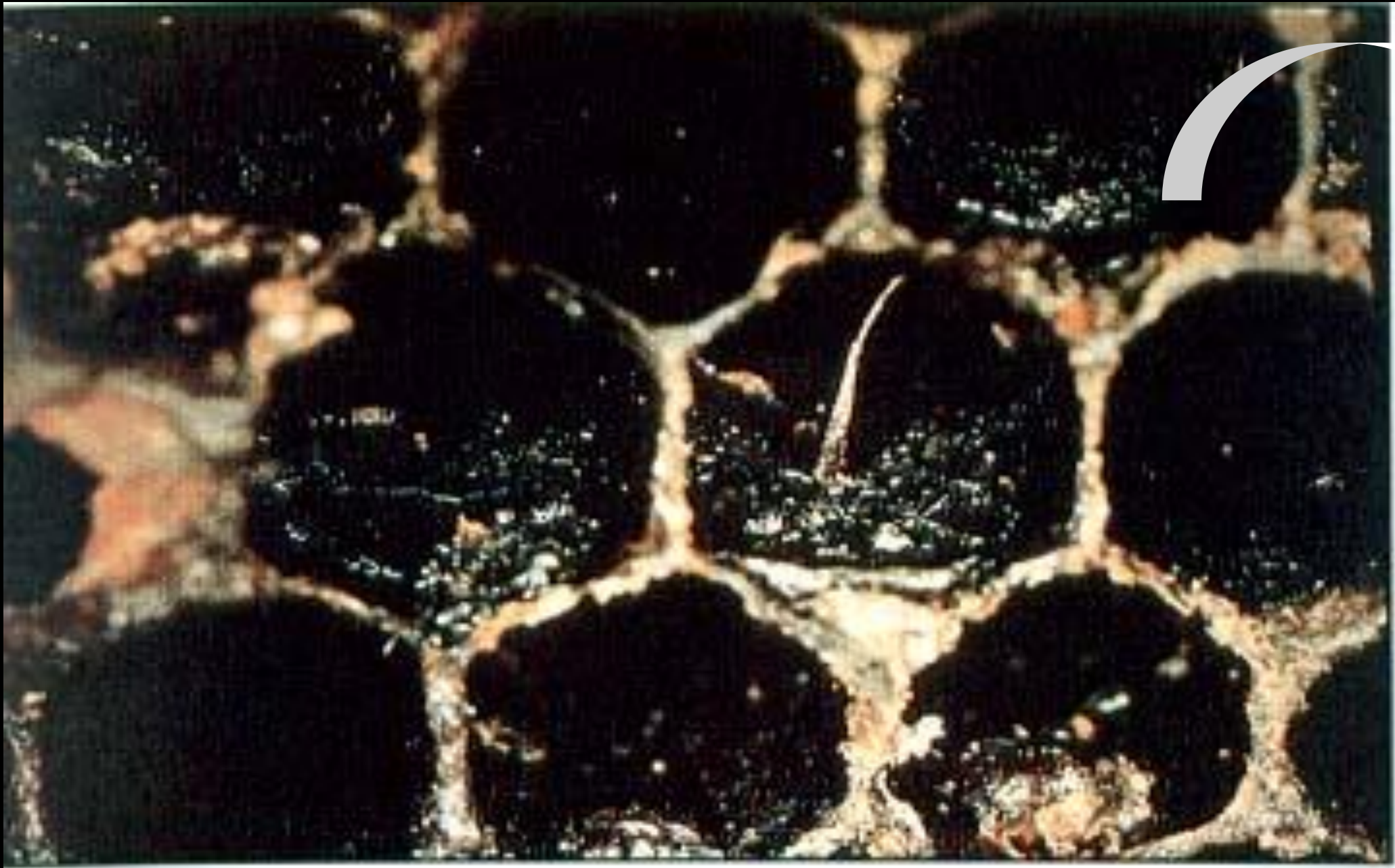
Loque americana











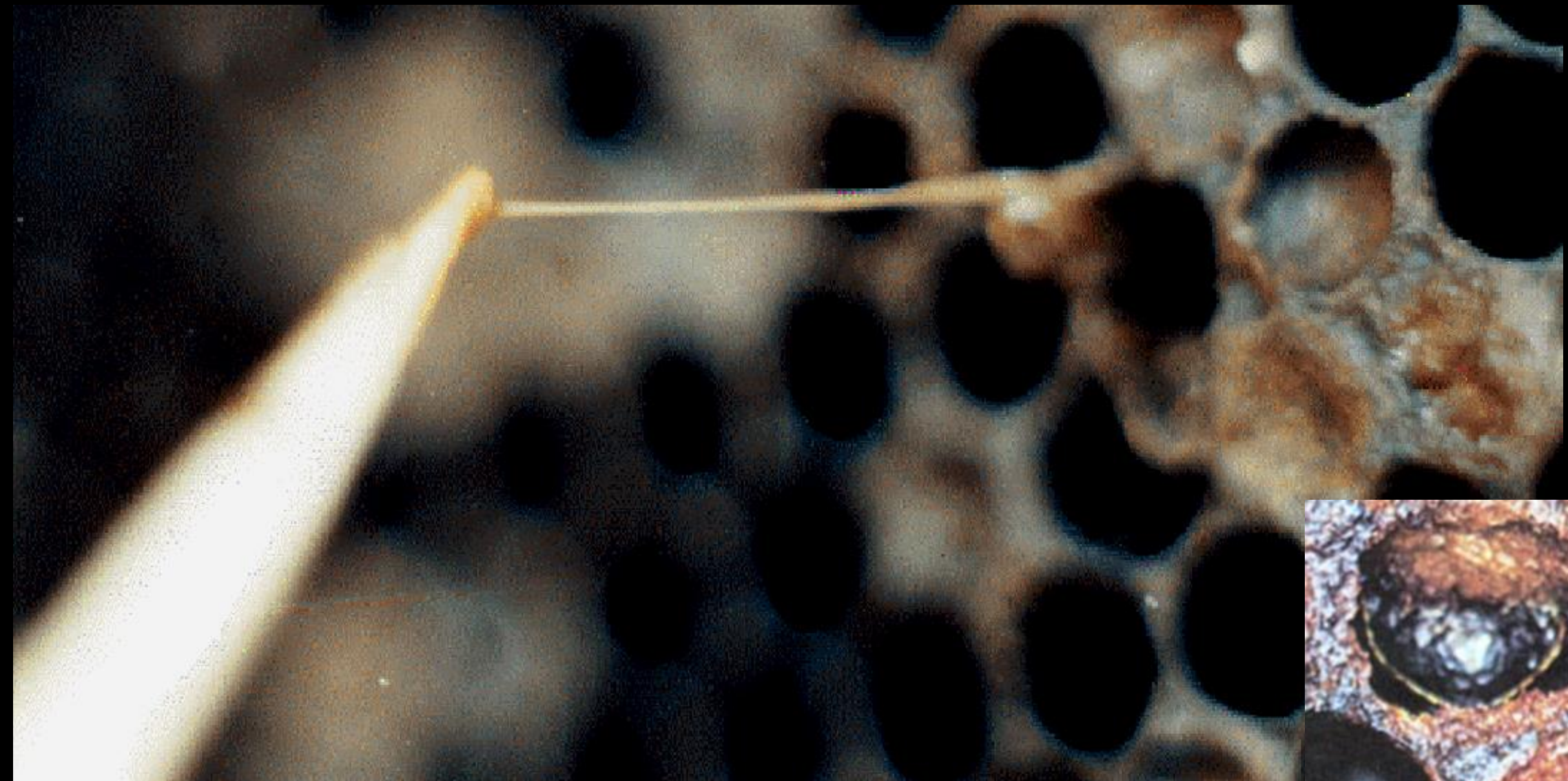
Loque Europea

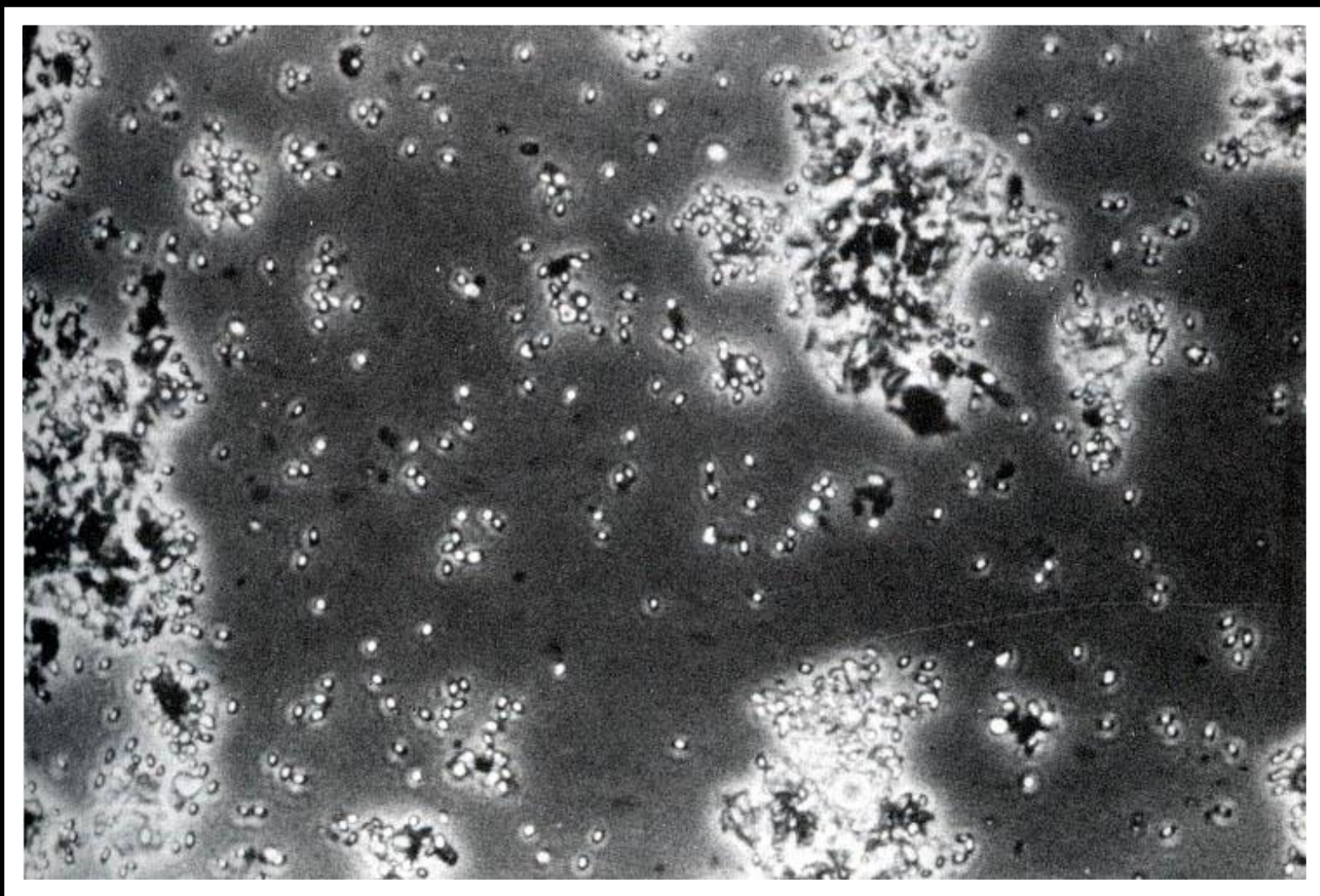




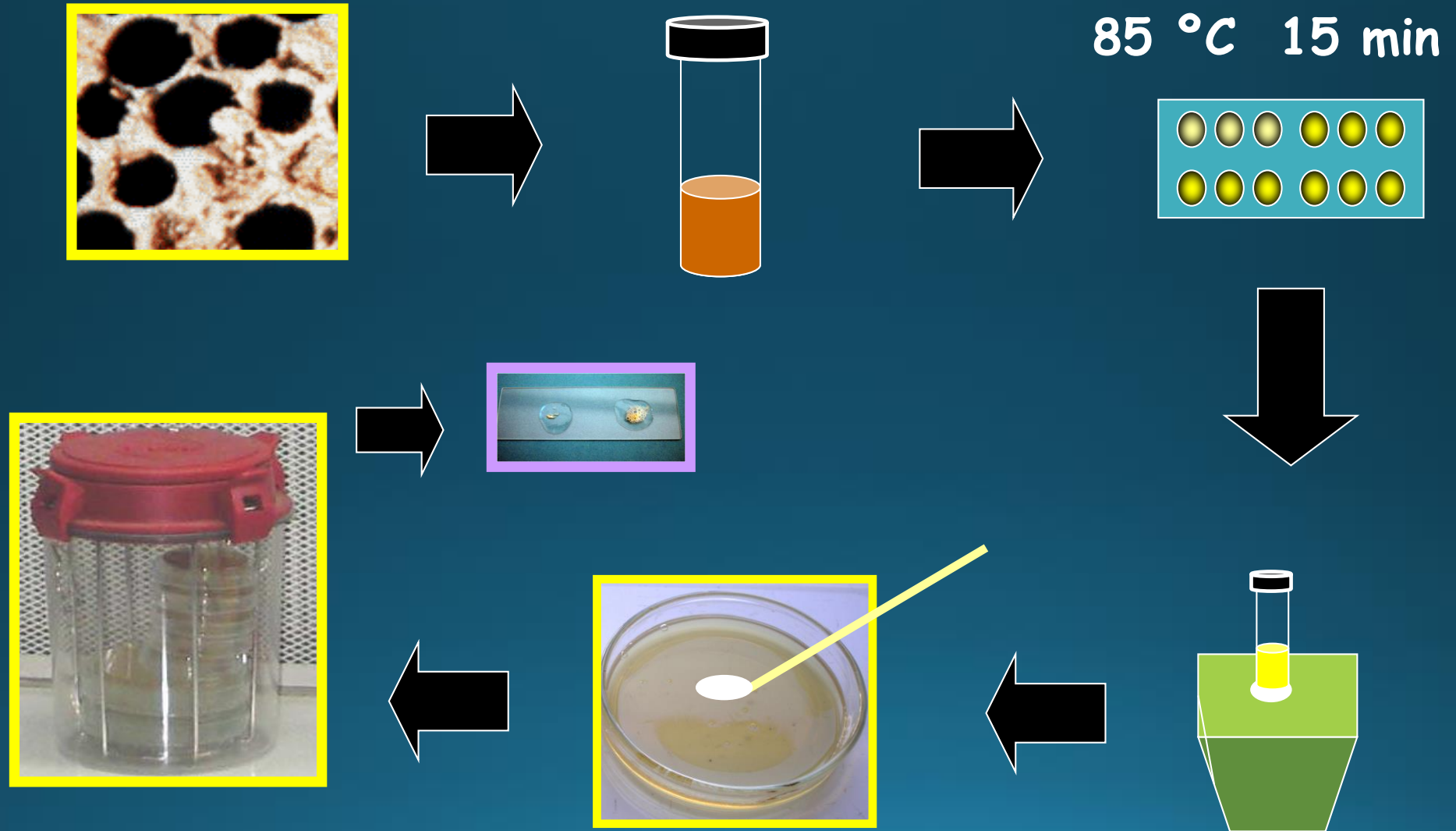


Loque americana: Aislamientos a partir de panales con síntomas

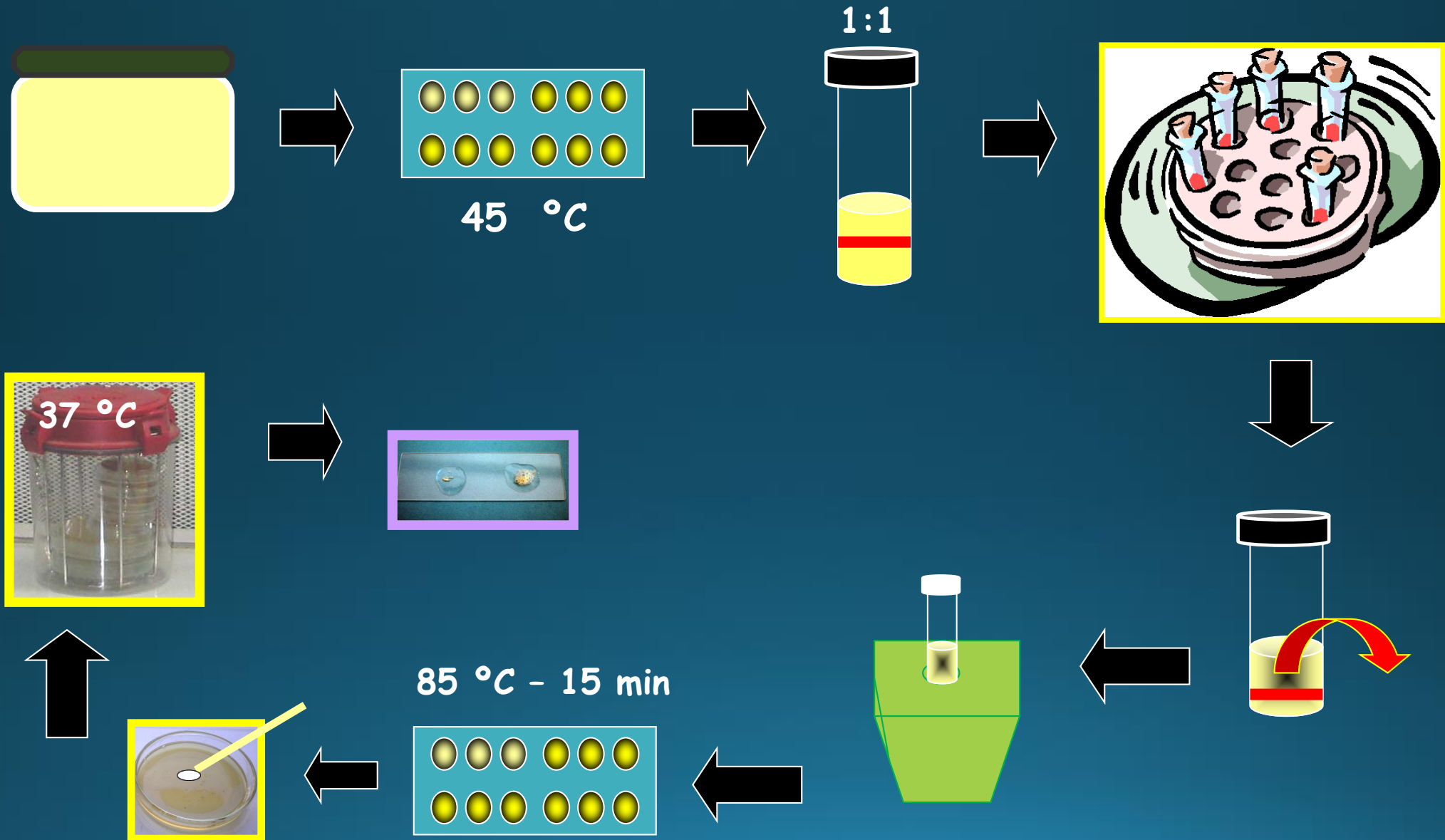




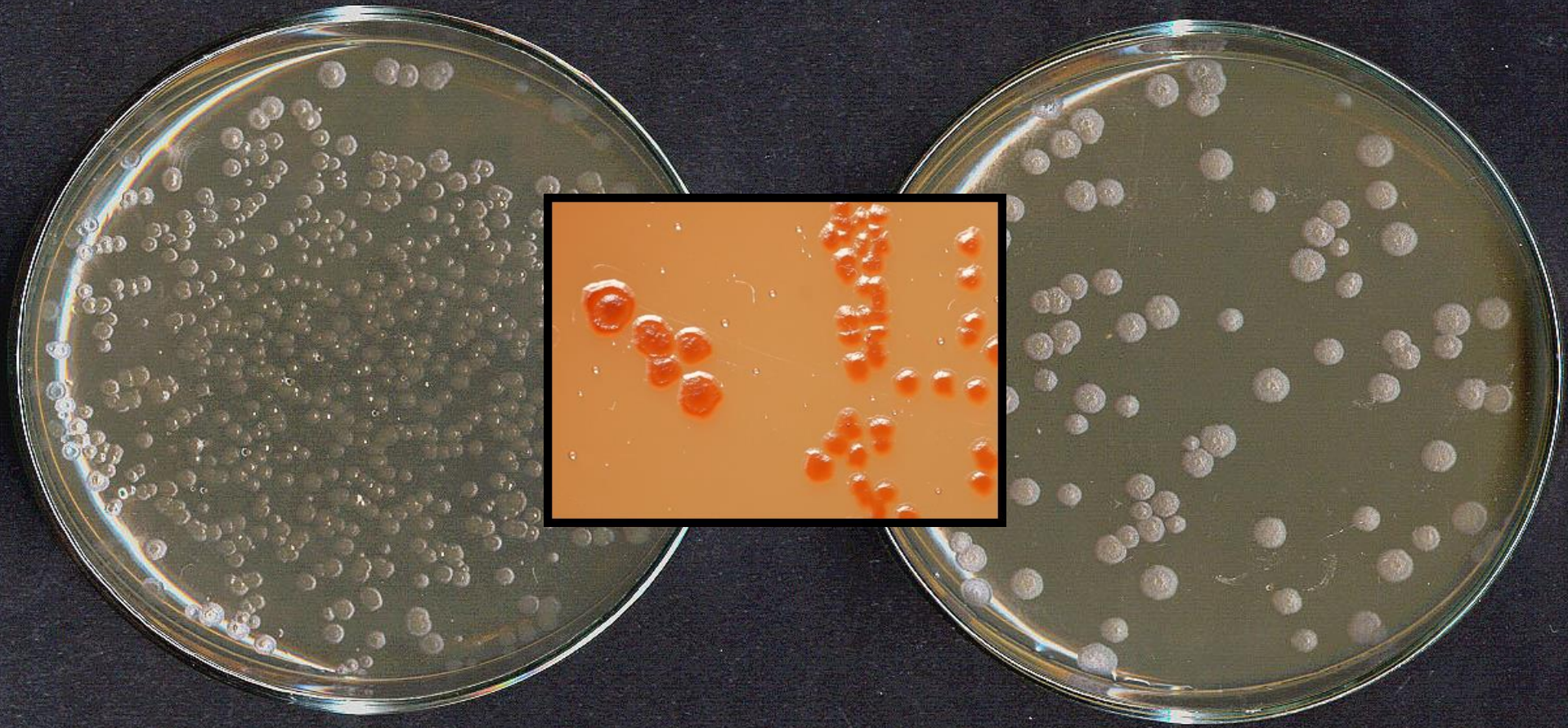
Aislamiento de esporas viables de restos larvales



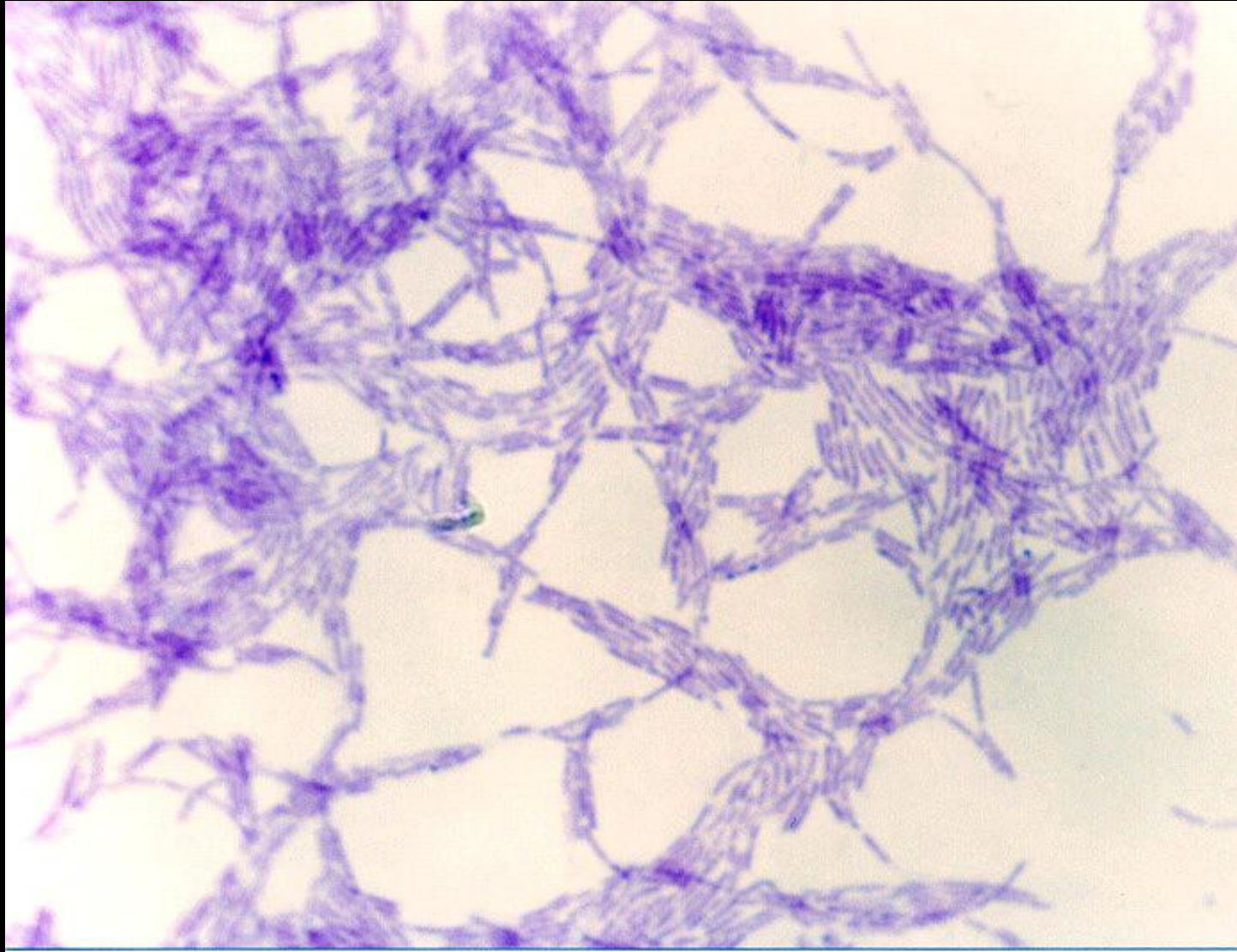
Aislamiento de esporas viables a partir de miel



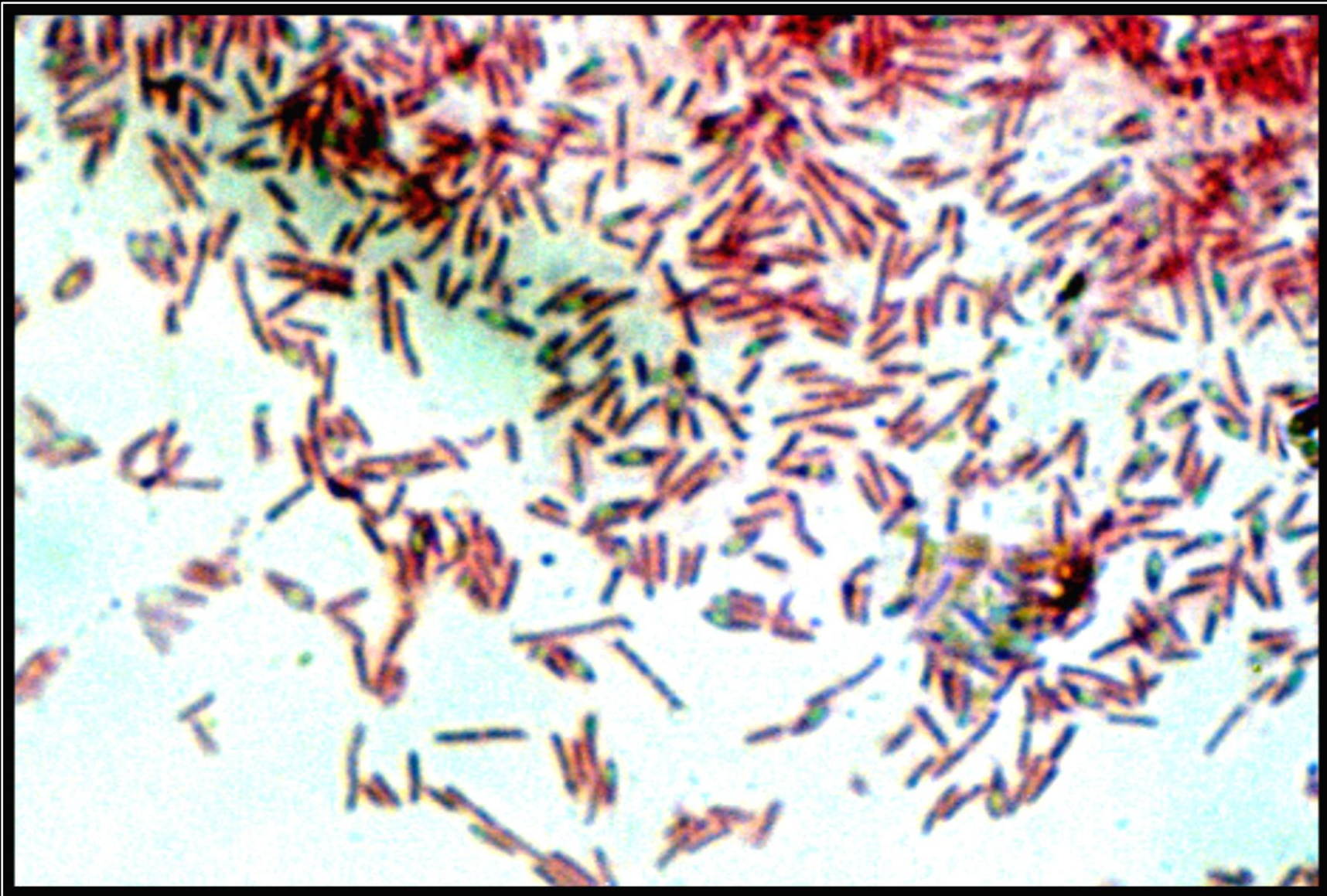
Aislamiento de *Paenibacillus larvae* en medios semi-selectivos



Tinción de Gram



Coloración de esporas



Nitrato-reductasa

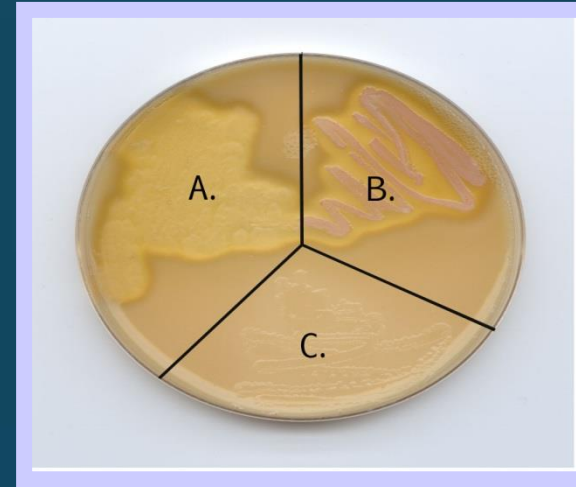


Positivo



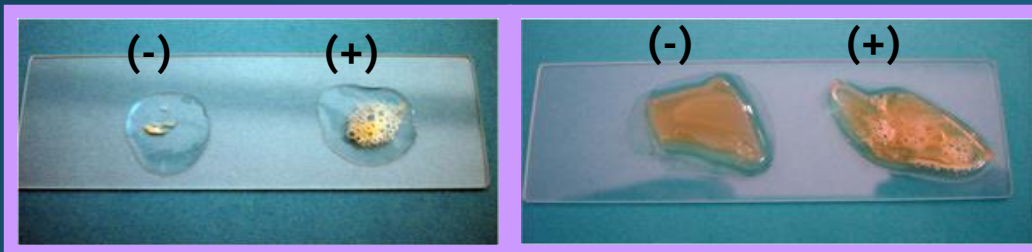
Negativo

Hidrólisis de caseína



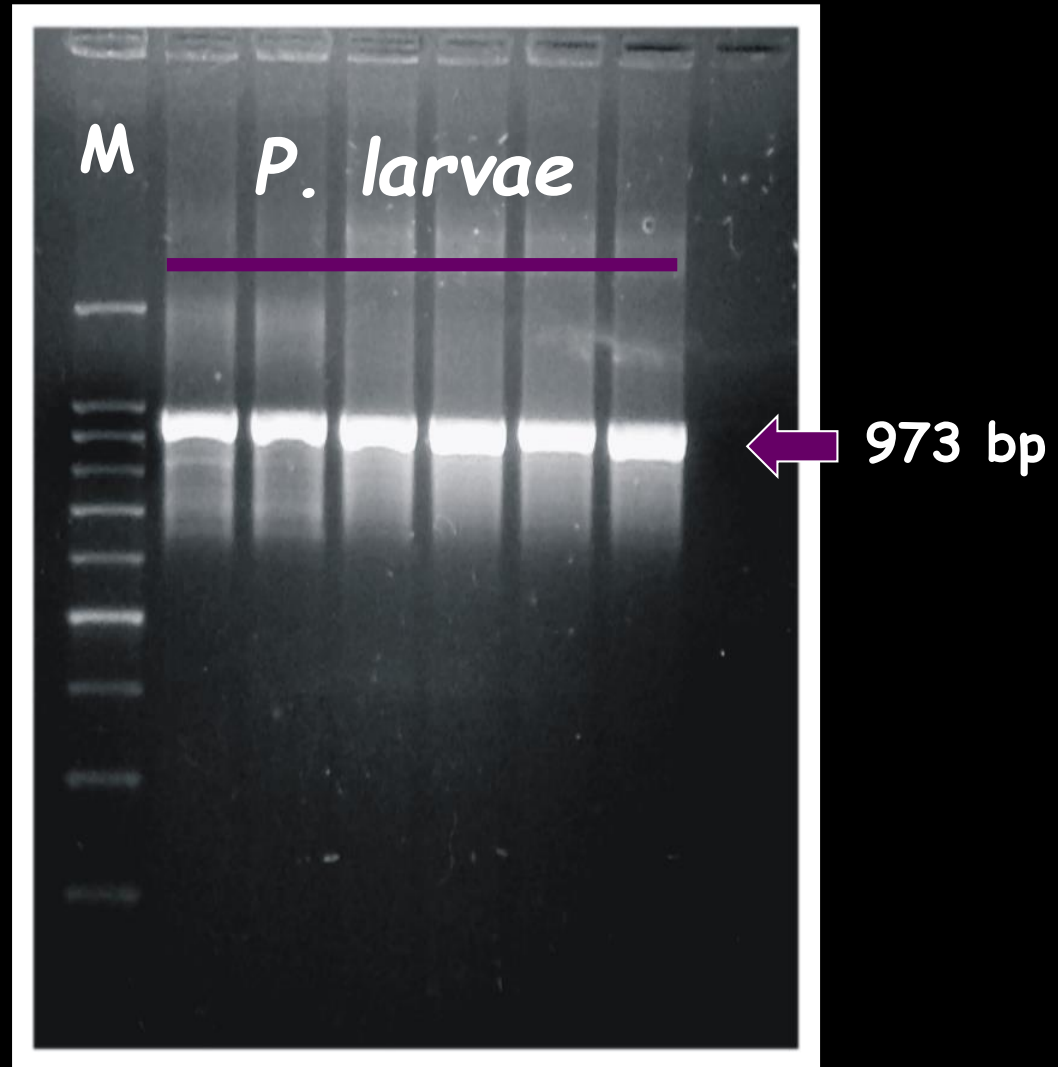
A y B: Caseinasa (+)
C: Caseinasa (-)

Catalasa



Citocromo-oxidasa

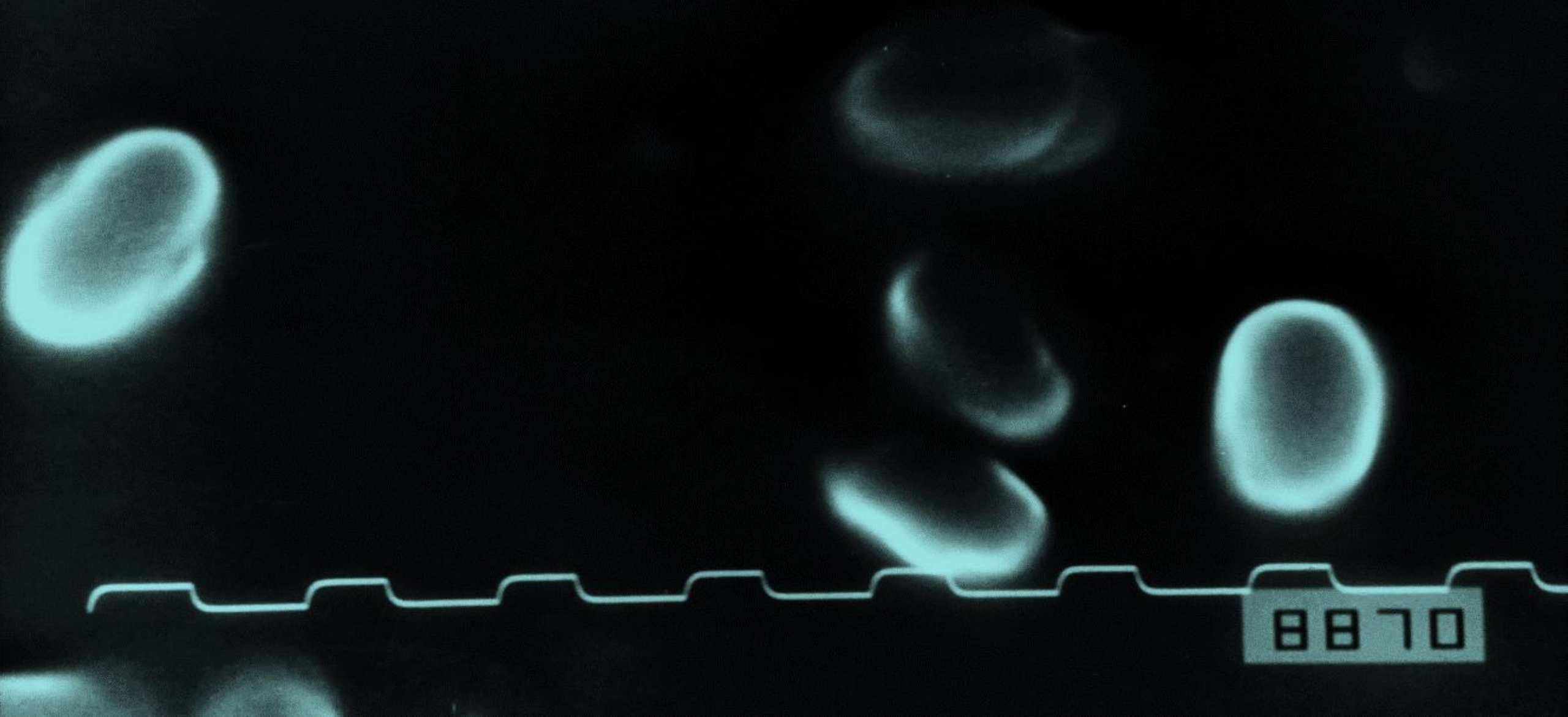
Amplificación específica del gen 16S rDNA primers PI1/PI2



VITA Diagnostic Kit



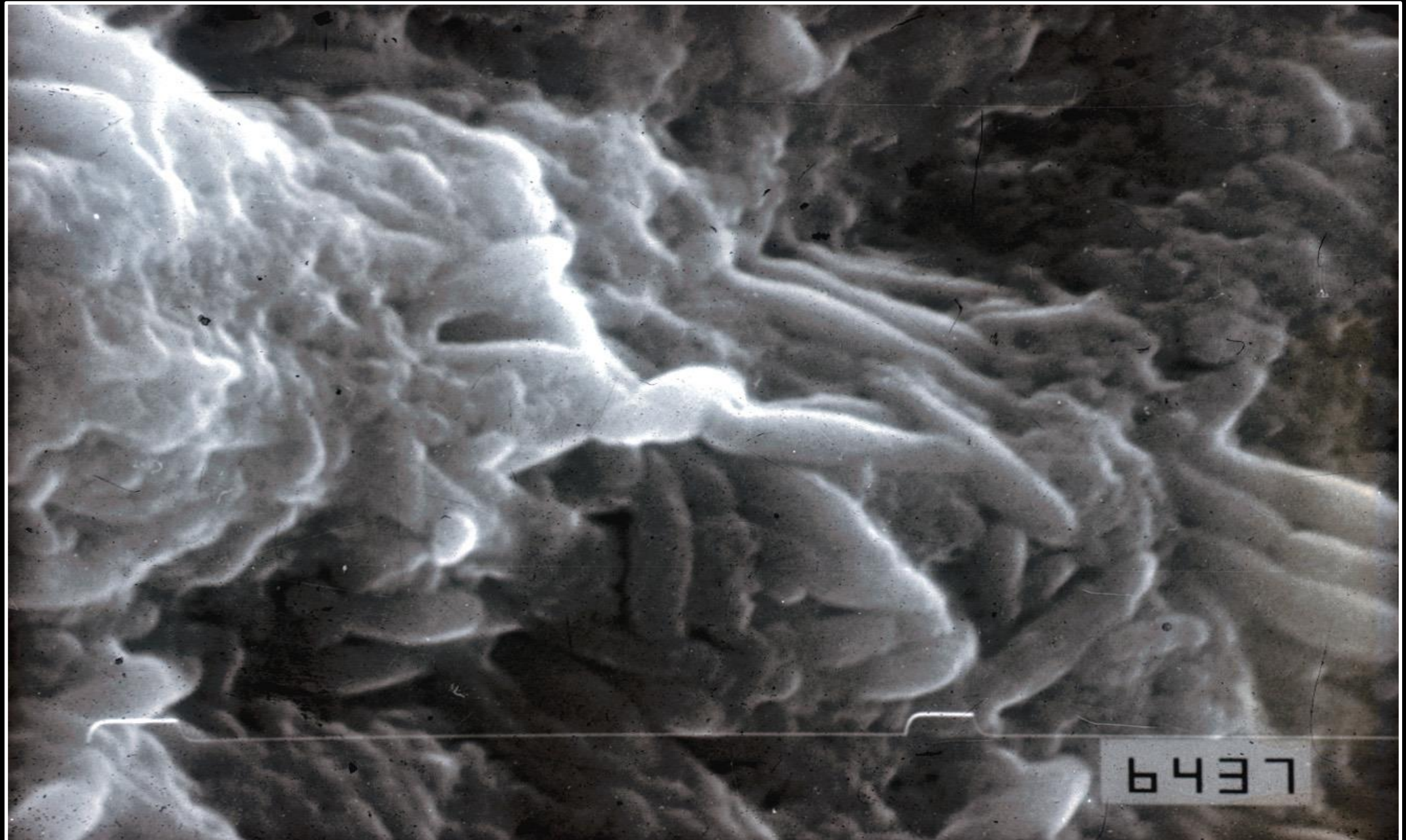
Loque Americana: Patogénesis

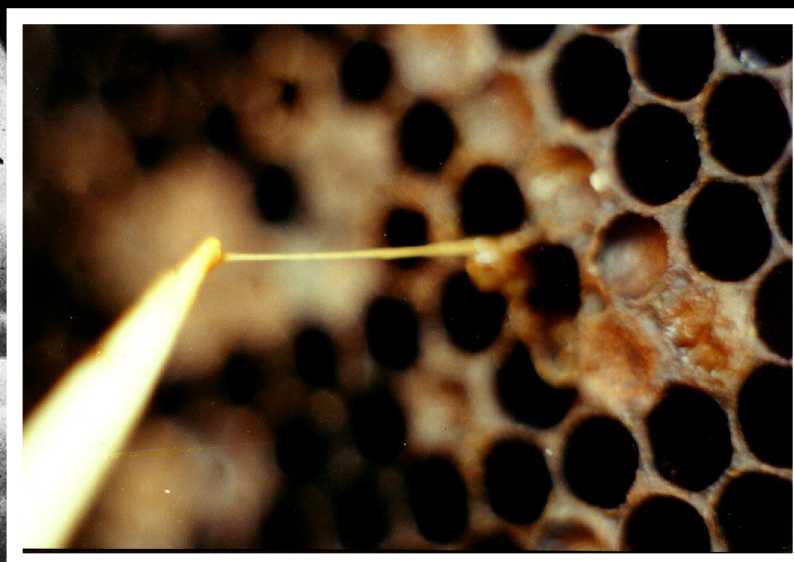
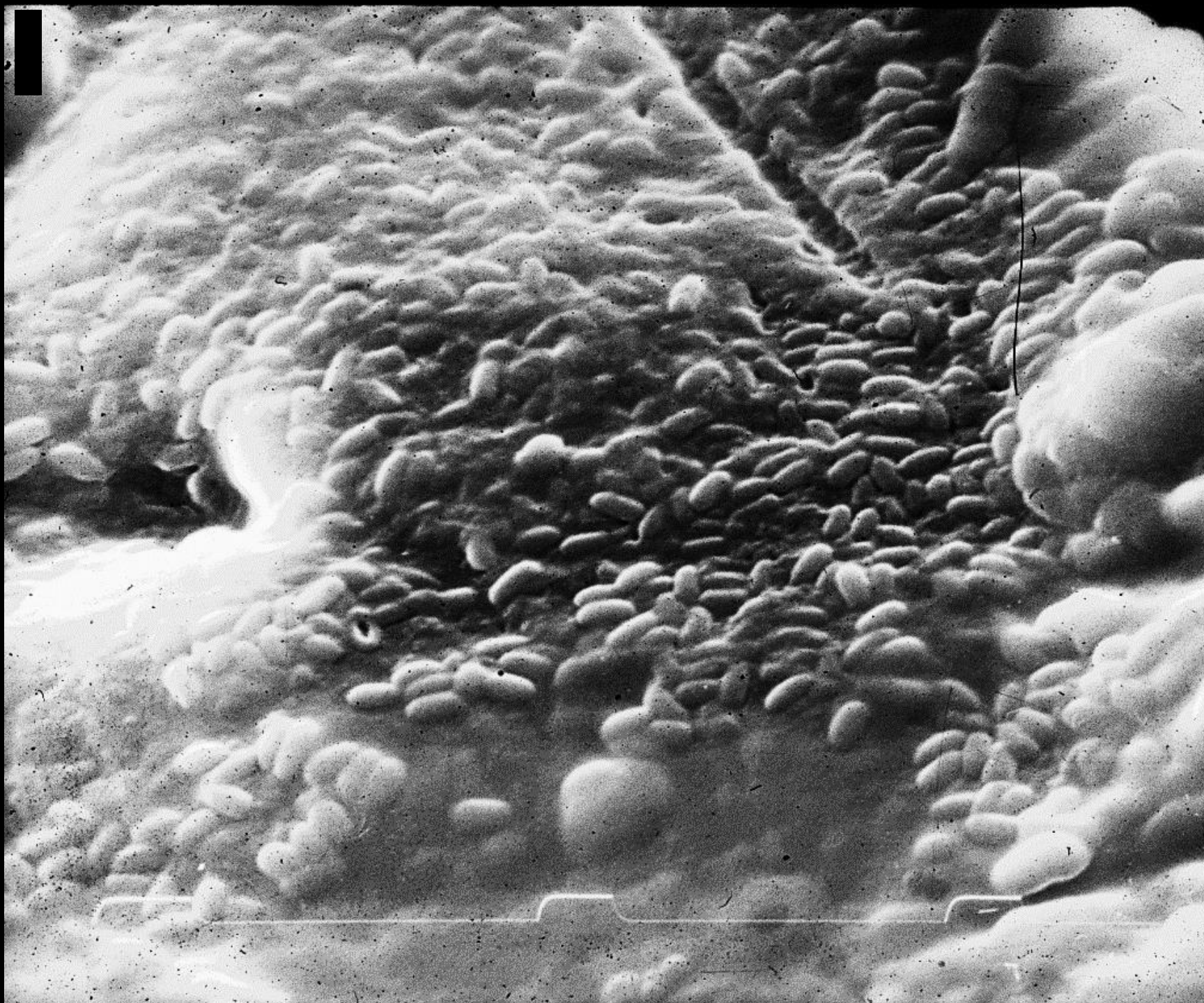


Células vegetativas flageladas



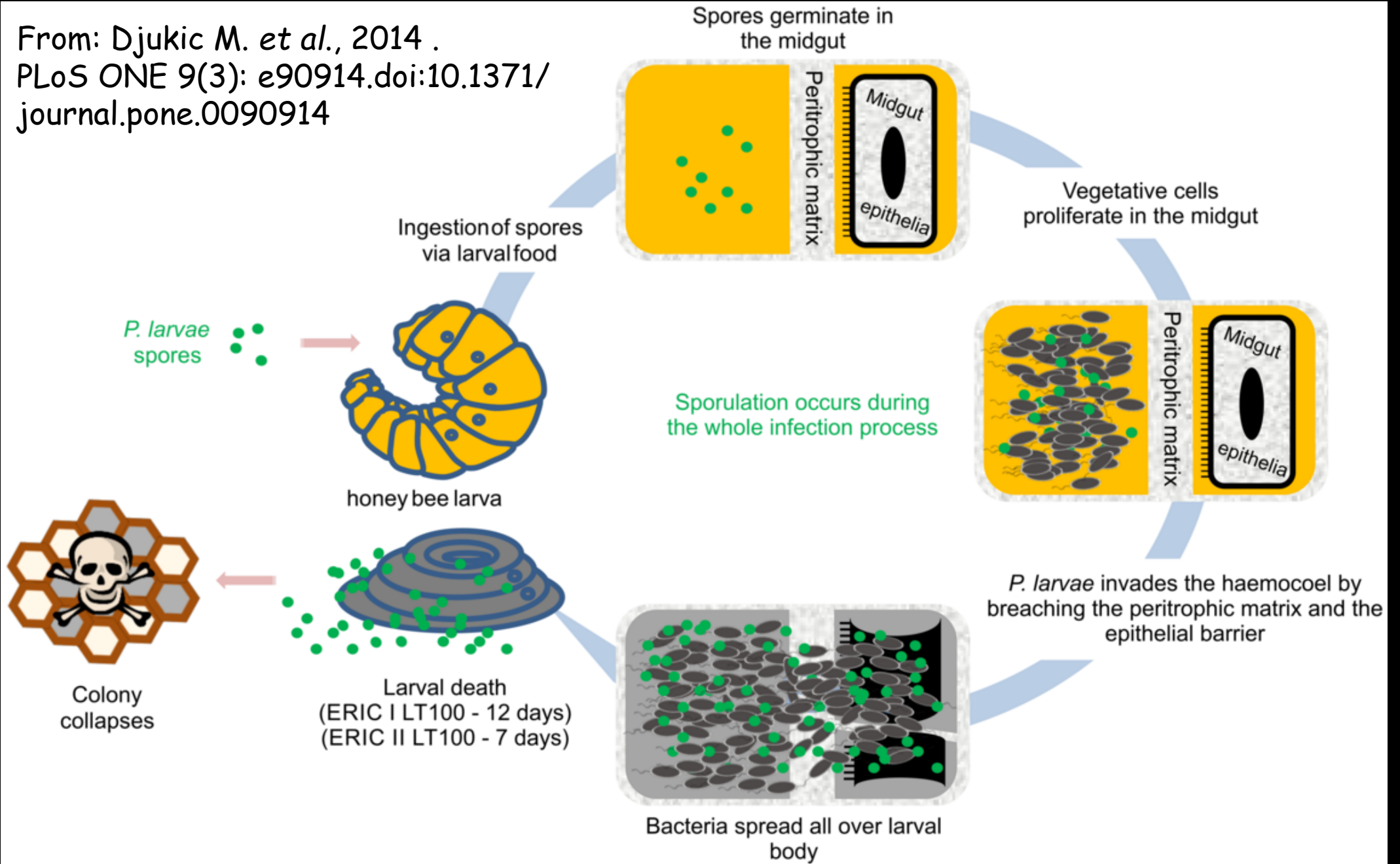
Las formas vegetativas proliferan en el lumen del intestino larval





1665

From: Djukic M. *et al.*, 2014 .
PLoS ONE 9(3): e90914.doi:10.1371/
journal.pone.0090914



Diferencias en la patogénesis entre genotipos ERIC I y II

Según Genersch, 2017

	<i>P. larvae</i> , genotype ERIC I	<i>P. larvae</i> , genotype ERIC II																																																												
<i>P. larvae</i> virulence: time course of larval mortality in laboratory infection assays	<p>cumulative larval mortality in % (100% = all AFB-dead larvae)</p> <table border="1"><caption>Estimated data for ERIC I mortality</caption><thead><tr><th>Days post infection</th><th>Cumulative larval mortality in %</th></tr></thead><tbody><tr><td>1</td><td>0</td></tr><tr><td>2</td><td>0</td></tr><tr><td>3</td><td>5</td></tr><tr><td>4</td><td>35</td></tr><tr><td>5</td><td>60</td></tr><tr><td>6</td><td>65</td></tr><tr><td>7</td><td>70</td></tr><tr><td>8</td><td>75</td></tr><tr><td>9</td><td>78</td></tr><tr><td>10</td><td>85</td></tr><tr><td>11</td><td>95</td></tr><tr><td>12</td><td>100</td></tr><tr><td>13</td><td>100</td></tr><tr><td>14</td><td>100</td></tr></tbody></table>	Days post infection	Cumulative larval mortality in %	1	0	2	0	3	5	4	35	5	60	6	65	7	70	8	75	9	78	10	85	11	95	12	100	13	100	14	100	<p>cumulative larval mortality in % (100% = all AFB-dead larvae)</p> <table border="1"><caption>Estimated data for ERIC II mortality</caption><thead><tr><th>Days post infection</th><th>Cumulative larval mortality in %</th></tr></thead><tbody><tr><td>1</td><td>0</td></tr><tr><td>2</td><td>0</td></tr><tr><td>3</td><td>10</td></tr><tr><td>4</td><td>55</td></tr><tr><td>5</td><td>75</td></tr><tr><td>6</td><td>90</td></tr><tr><td>7</td><td>100</td></tr><tr><td>8</td><td>100</td></tr><tr><td>9</td><td>100</td></tr><tr><td>10</td><td>100</td></tr><tr><td>11</td><td>100</td></tr><tr><td>12</td><td>100</td></tr><tr><td>13</td><td>100</td></tr><tr><td>14</td><td>100</td></tr></tbody></table>	Days post infection	Cumulative larval mortality in %	1	0	2	0	3	10	4	55	5	75	6	90	7	100	8	100	9	100	10	100	11	100	12	100	13	100	14	100
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Social immune response: removal of diseased larvae through nurse bees in experimentally infected mini colonies	<p>cumulative removal rate in % (100% = all AFB-infected larvae)</p> <table border="1"><caption>Estimated data for ERIC I removal rate</caption><thead><tr><th>Days post infection</th><th>Cumulative removal rate in %</th></tr></thead><tbody><tr><td>1</td><td>8</td></tr><tr><td>2</td><td>15</td></tr><tr><td>3</td><td>30</td></tr><tr><td>4</td><td>42</td></tr><tr><td>5</td><td>45</td></tr><tr><td>6</td><td>46</td></tr><tr><td>7</td><td>47</td></tr><tr><td>8</td><td>48</td></tr><tr><td>9</td><td>55</td></tr><tr><td>10</td><td>60</td></tr><tr><td>11</td><td>62</td></tr><tr><td>12</td><td>63</td></tr><tr><td>13</td><td>65</td></tr></tbody></table>	Days post infection	Cumulative removal rate in %	1	8	2	15	3	30	4	42	5	45	6	46	7	47	8	48	9	55	10	60	11	62	12	63	13	65	<p>cumulative removal rate in % (100% = all AFB-infected larvae)</p> <table border="1"><caption>Estimated data for ERIC II removal rate</caption><thead><tr><th>Days post infection</th><th>Cumulative removal rate in %</th></tr></thead><tbody><tr><td>1</td><td>5</td></tr><tr><td>2</td><td>18</td></tr><tr><td>3</td><td>58</td></tr><tr><td>4</td><td>78</td></tr><tr><td>5</td><td>85</td></tr><tr><td>6</td><td>85</td></tr><tr><td>7</td><td>85</td></tr><tr><td>8</td><td>85</td></tr><tr><td>9</td><td>85</td></tr><tr><td>10</td><td>85</td></tr><tr><td>11</td><td>86</td></tr><tr><td>12</td><td>87</td></tr><tr><td>13</td><td>90</td></tr></tbody></table>	Days post infection	Cumulative removal rate in %	1	5	2	18	3	58	4	78	5	85	6	85	7	85	8	85	9	85	10	85	11	86	12	87	13	90				
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Clinical diagnosis: differentially patchy brood nest patterns from diseased colonies (field samples)																																																														

1. Lumen intestinal
2. Membrana peritrófica
3. Células del epitelio intestinal
4. Hemocelo

Factores de virulencia:

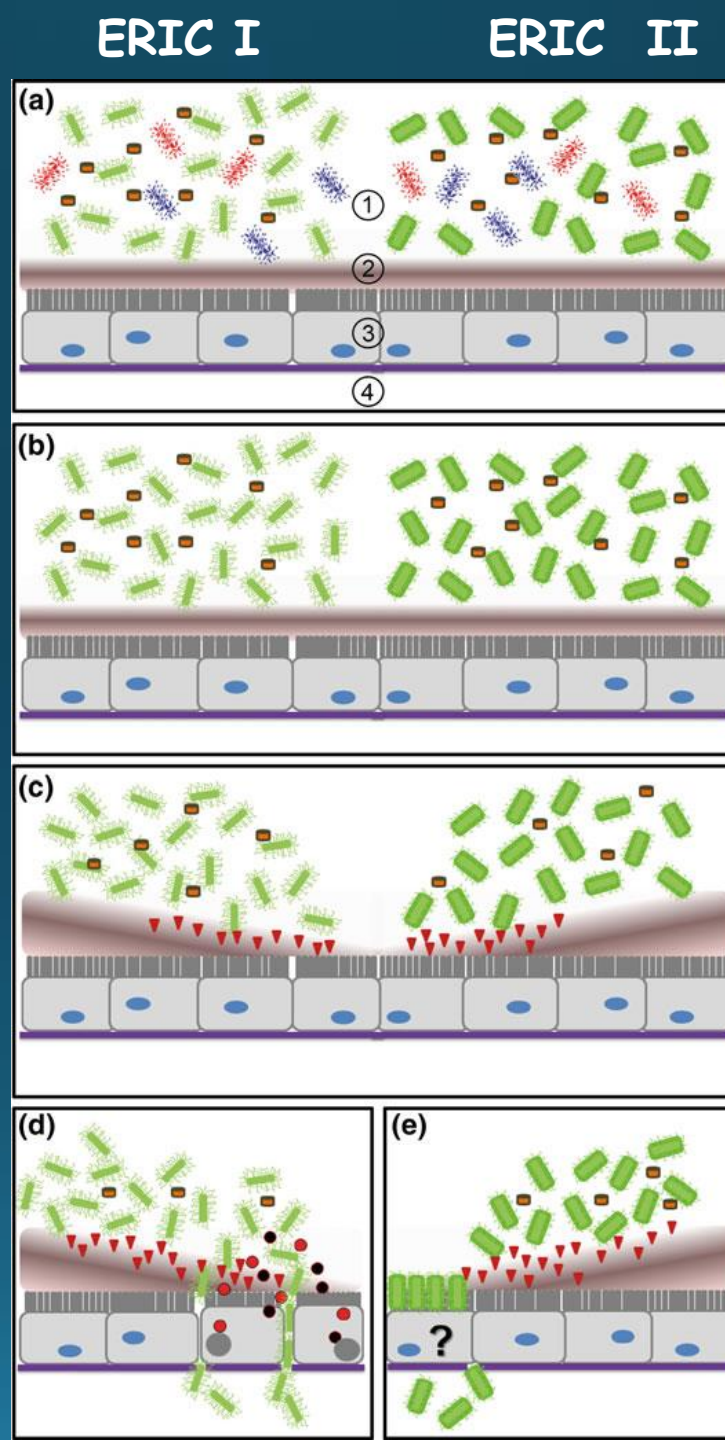
Triángulos rojos: quitinasas
PICPB49 ERIC I y II

Rectángulos naranjas: metabolitos secundarios bacterianos ERIC I y II

Círculos rojos y azules: Toxinas
Plx1 y Plx2 ERIC I

Proteína S-layer ERIC II

Antibiótico paenibalicina ERIC II



Rep-PCR

M

1

2

3

4

5

6

7

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10

11

12

13

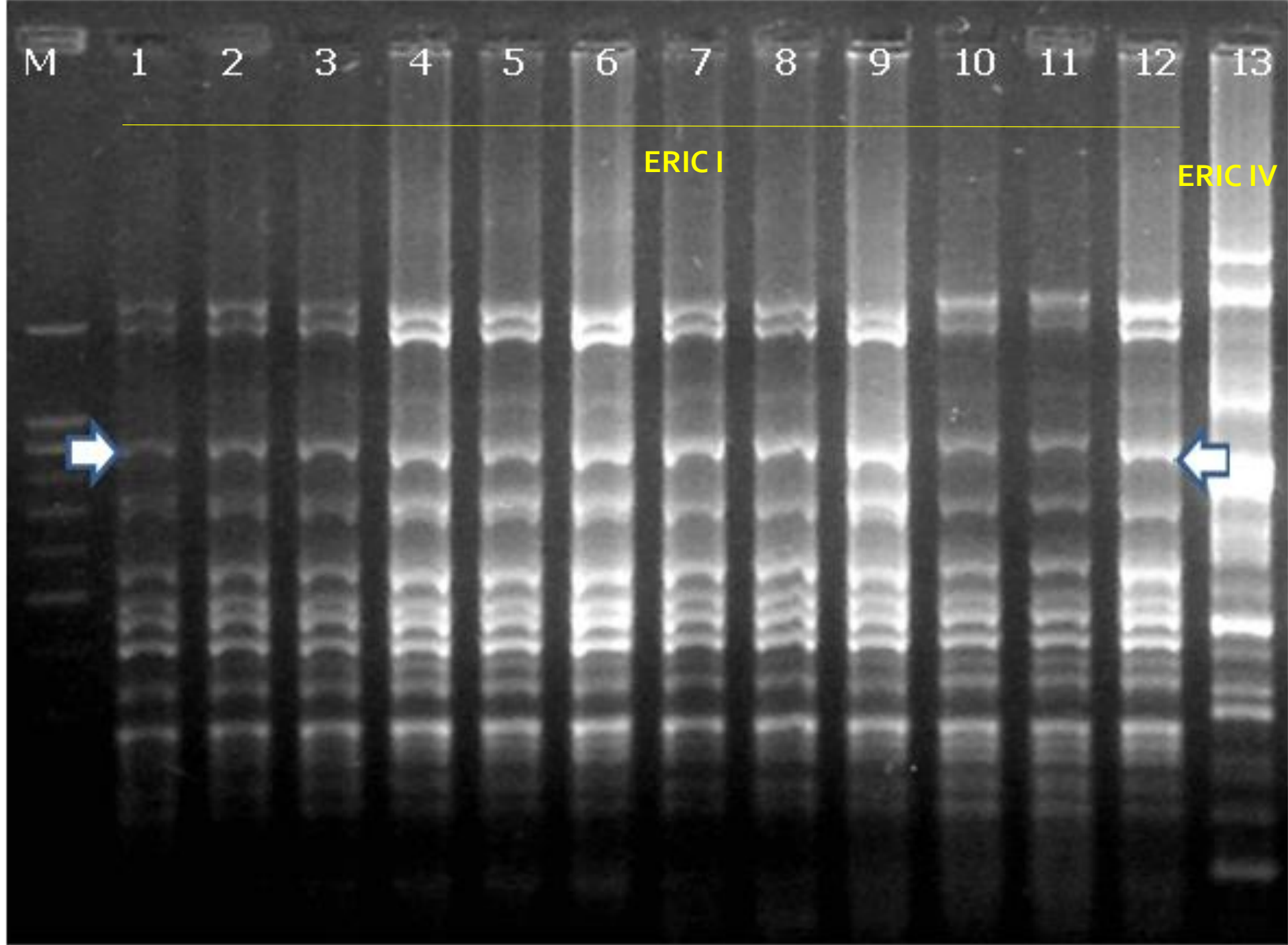
ERIC

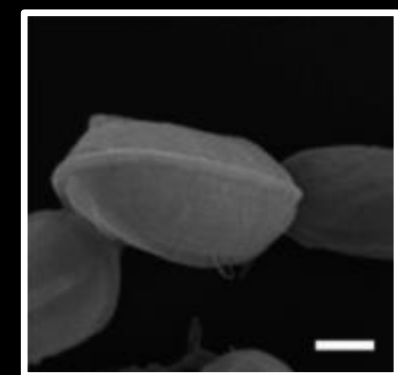
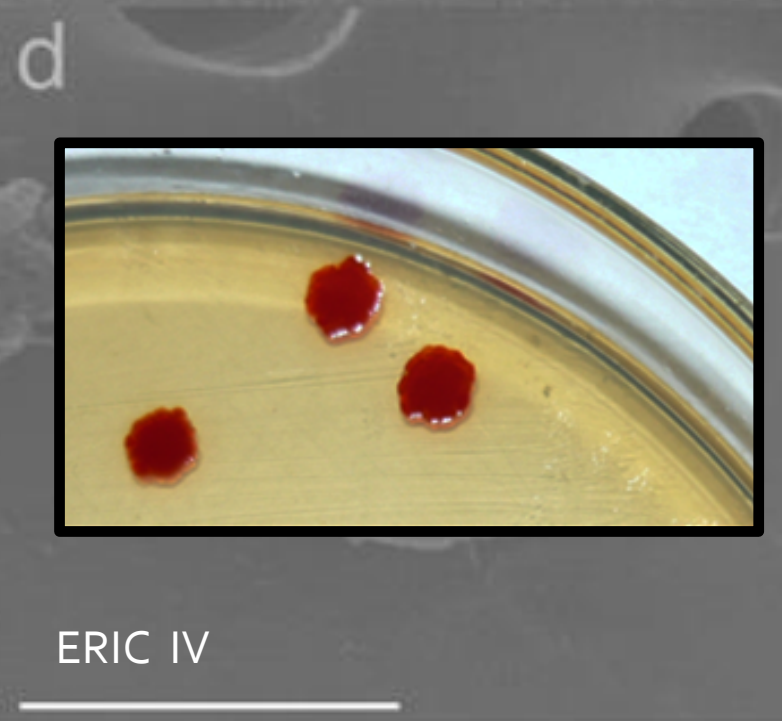
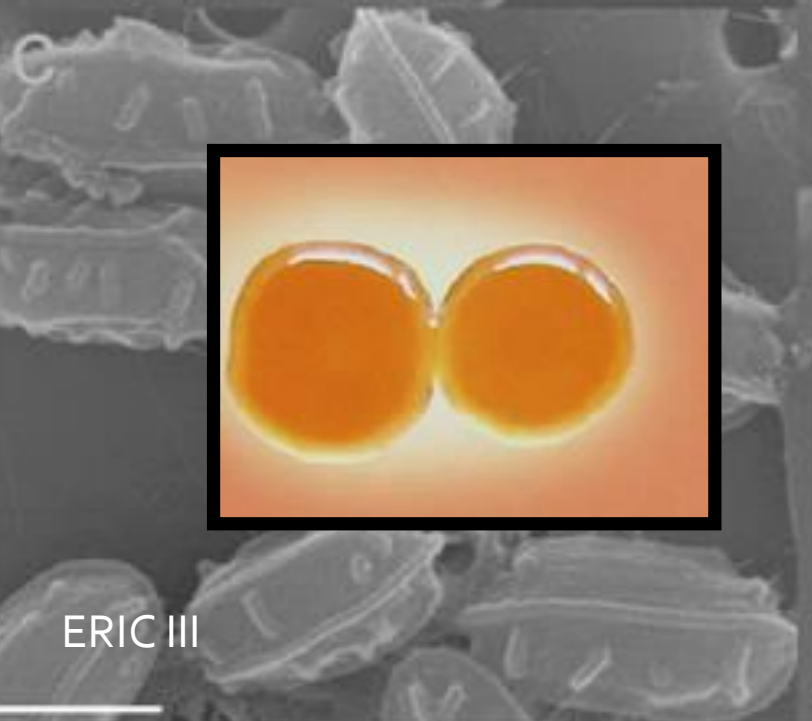
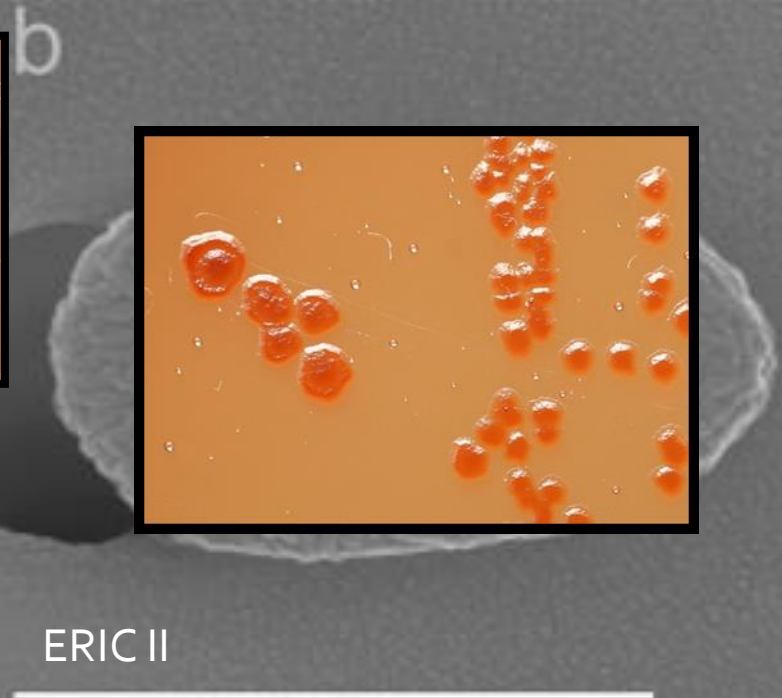
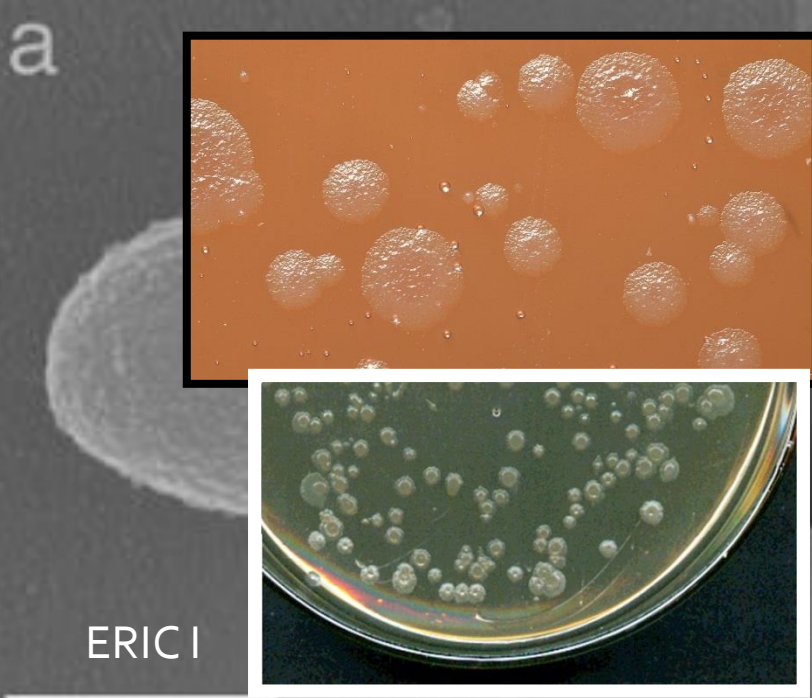
ERIC I

ERIC IV

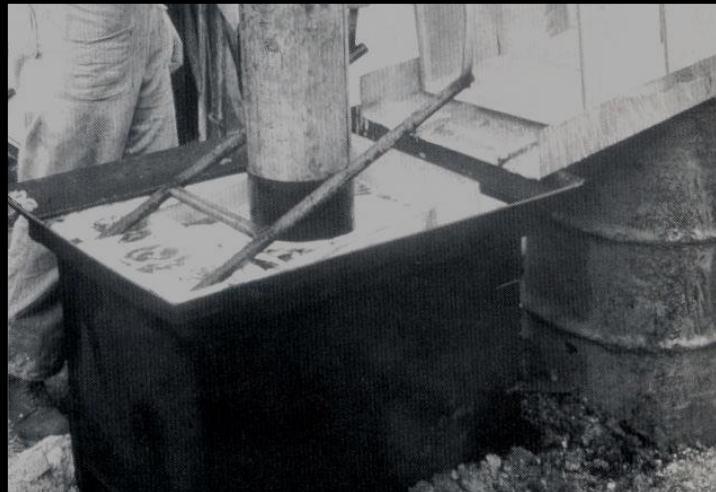
1.500 pb

900 pb

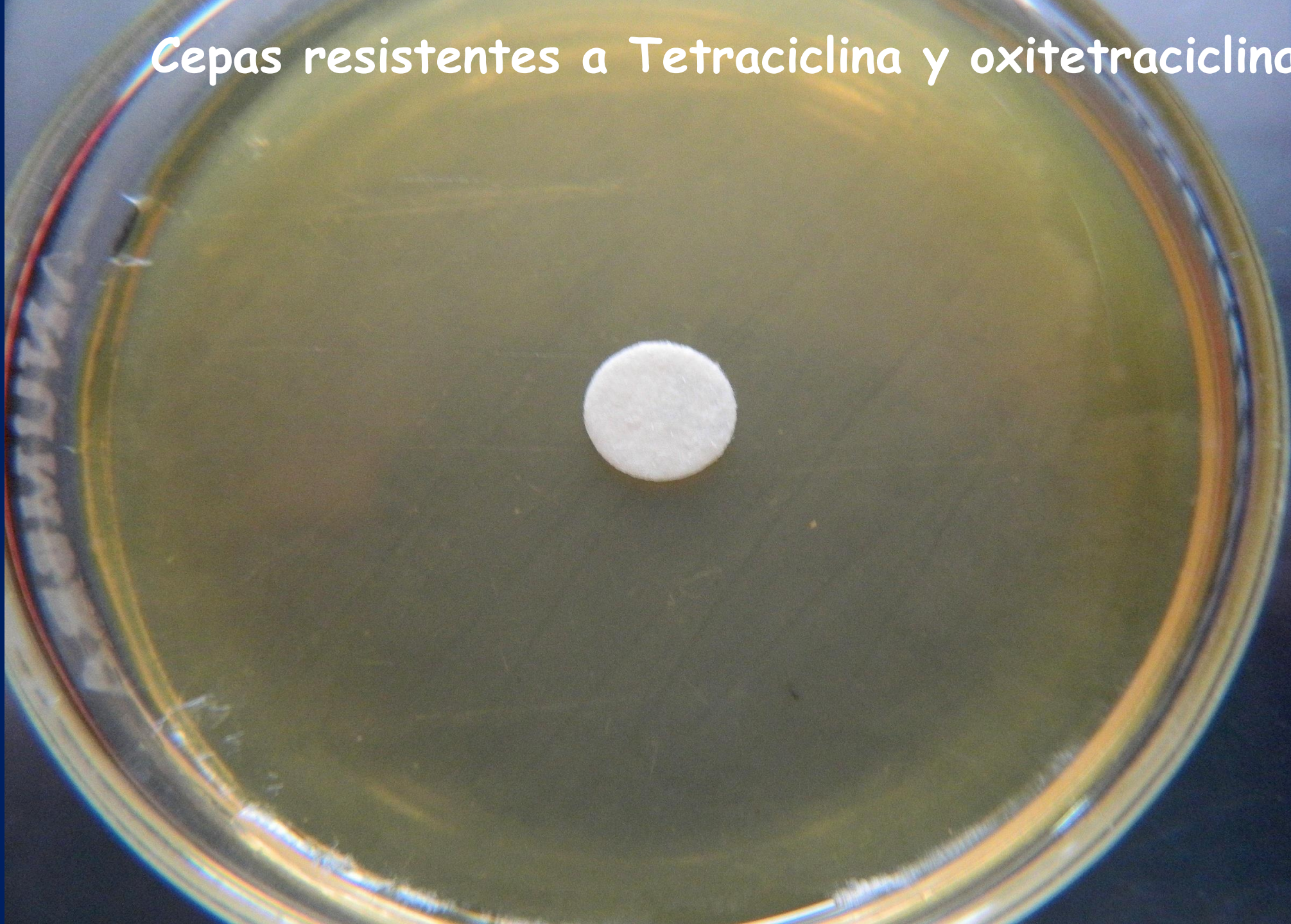




Control

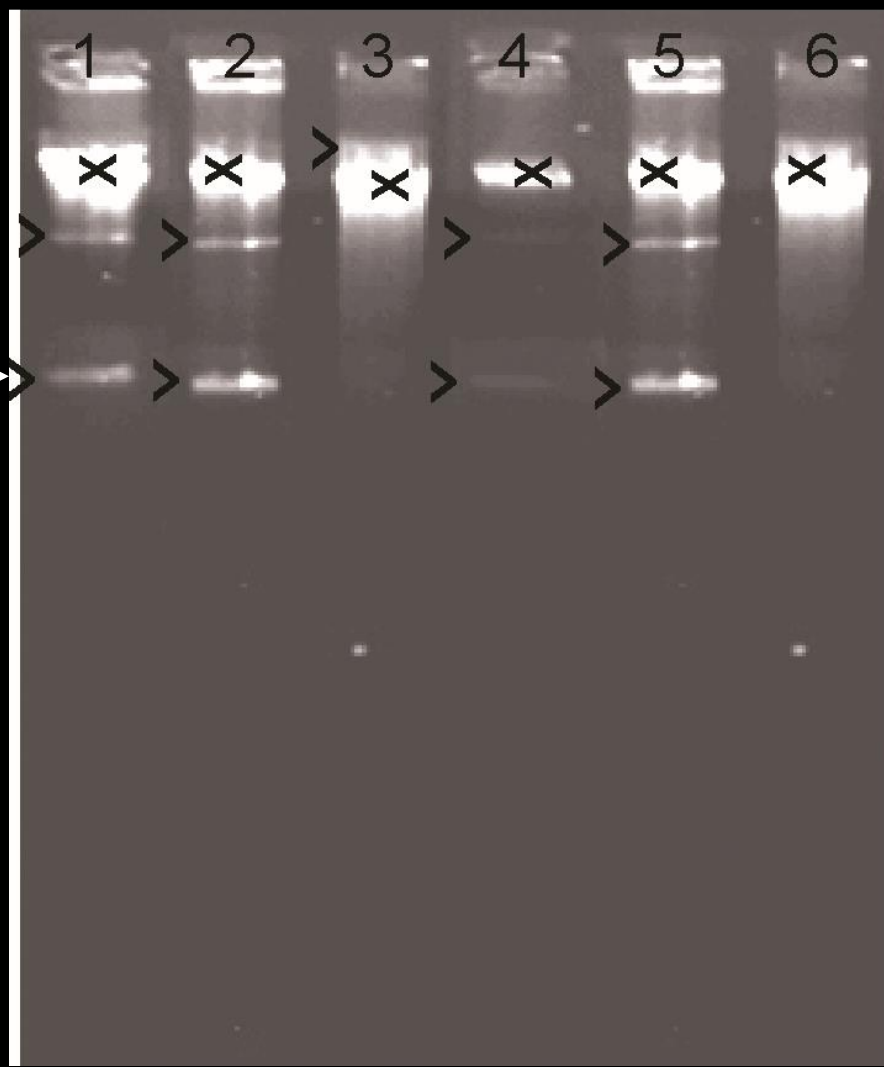


Cepas resistentes a Tetraciclina y oxitetraciclina

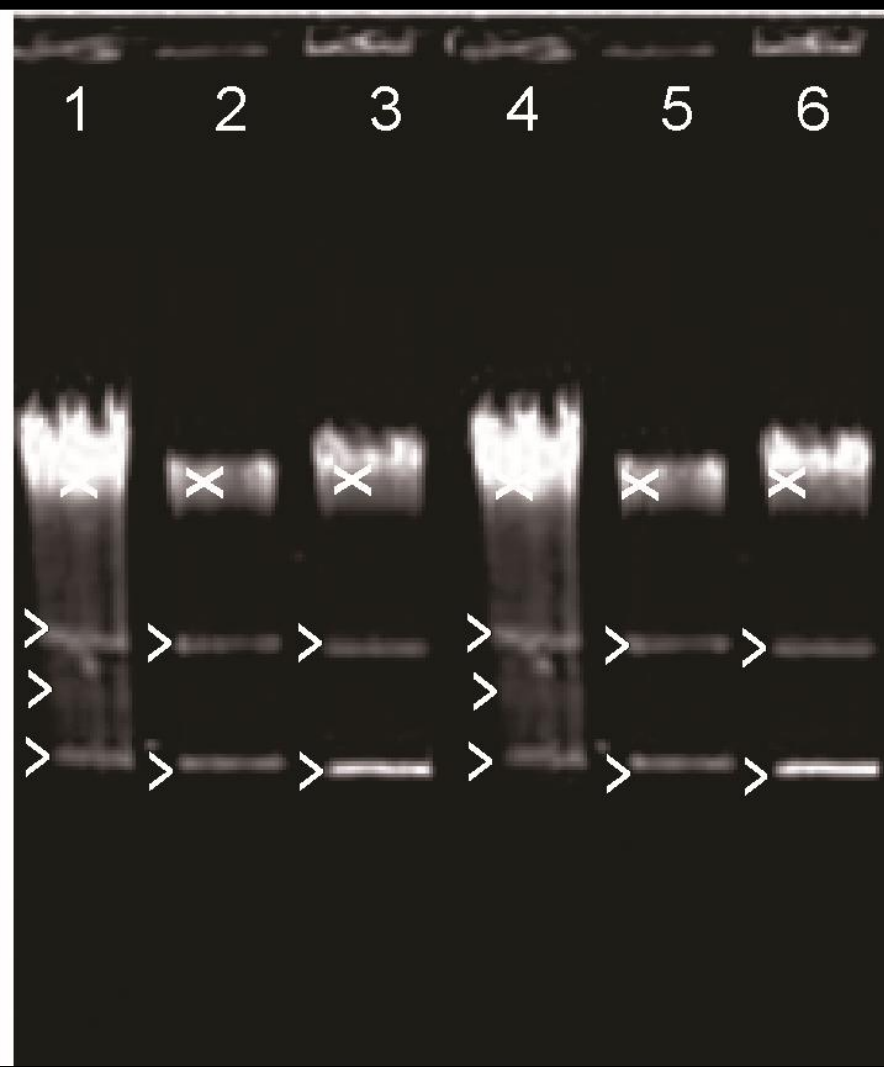


Perfiles plasmídicos en cepas de *Paenibacillus larvae*

A



B



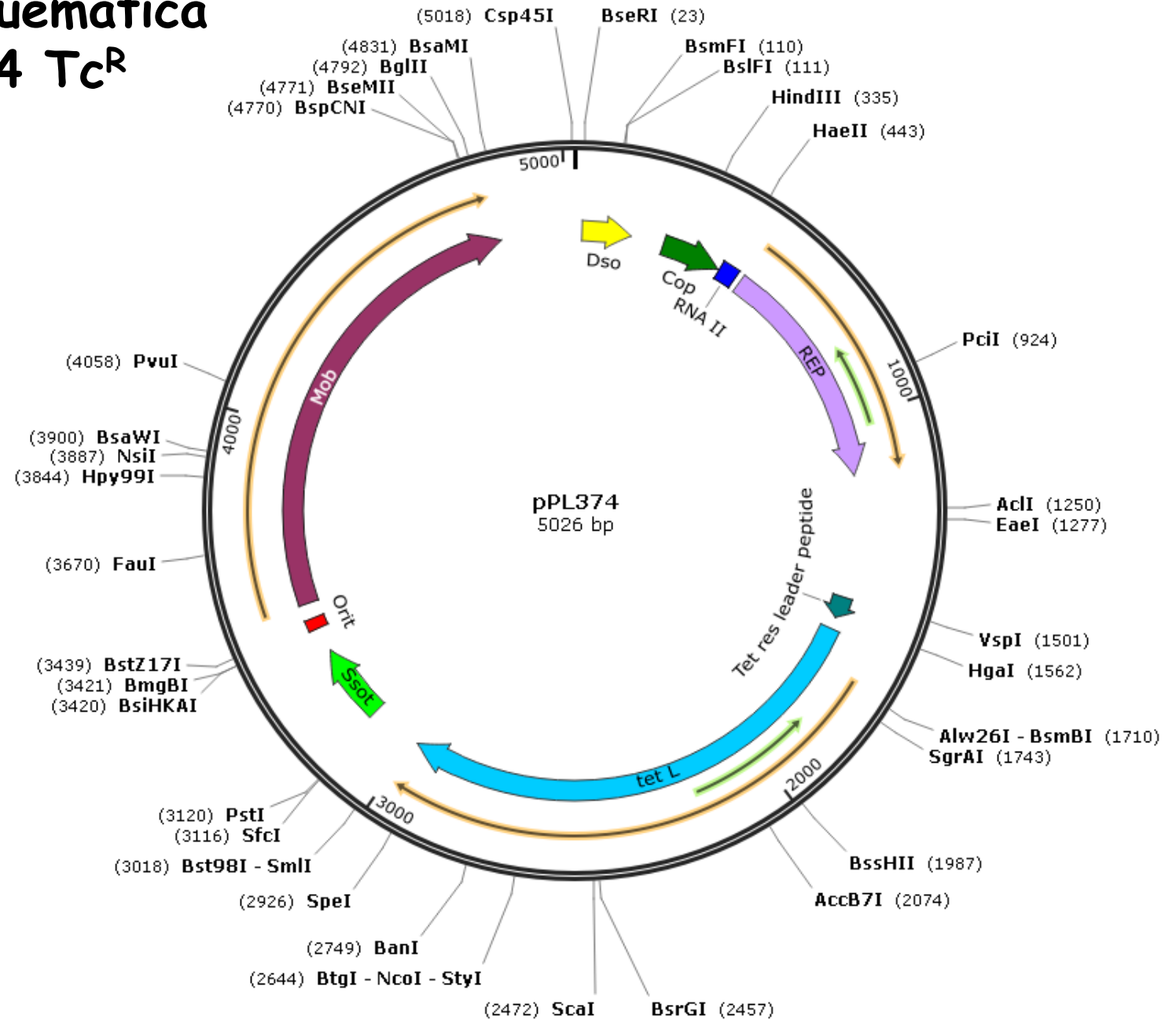
5.000 pb

8.000 pb

5.000 pb

Representación esquemática del plásmido pPL374 Tc^R

- dso:** Origen de replicación de doble hebra
- Cop:** Represor transcripcional (gen Cop)
- RNA II:** ARN contra transcrito
- rep:** Inicio de replicación
- Tet r.l. peptide:** péptido TetL
- tetL:** Gen de resistencia a tetraciclina por eflujo activo
- sso:** Origen de replicación de simple hebra
- oriT:** Origen de transferencia
- mob:** Función de movilización (gen mob)



One Health

PRODUCTIVITY

HUMAN
HEALTH
(HH)

E.g. Food Safety
(HACCP)

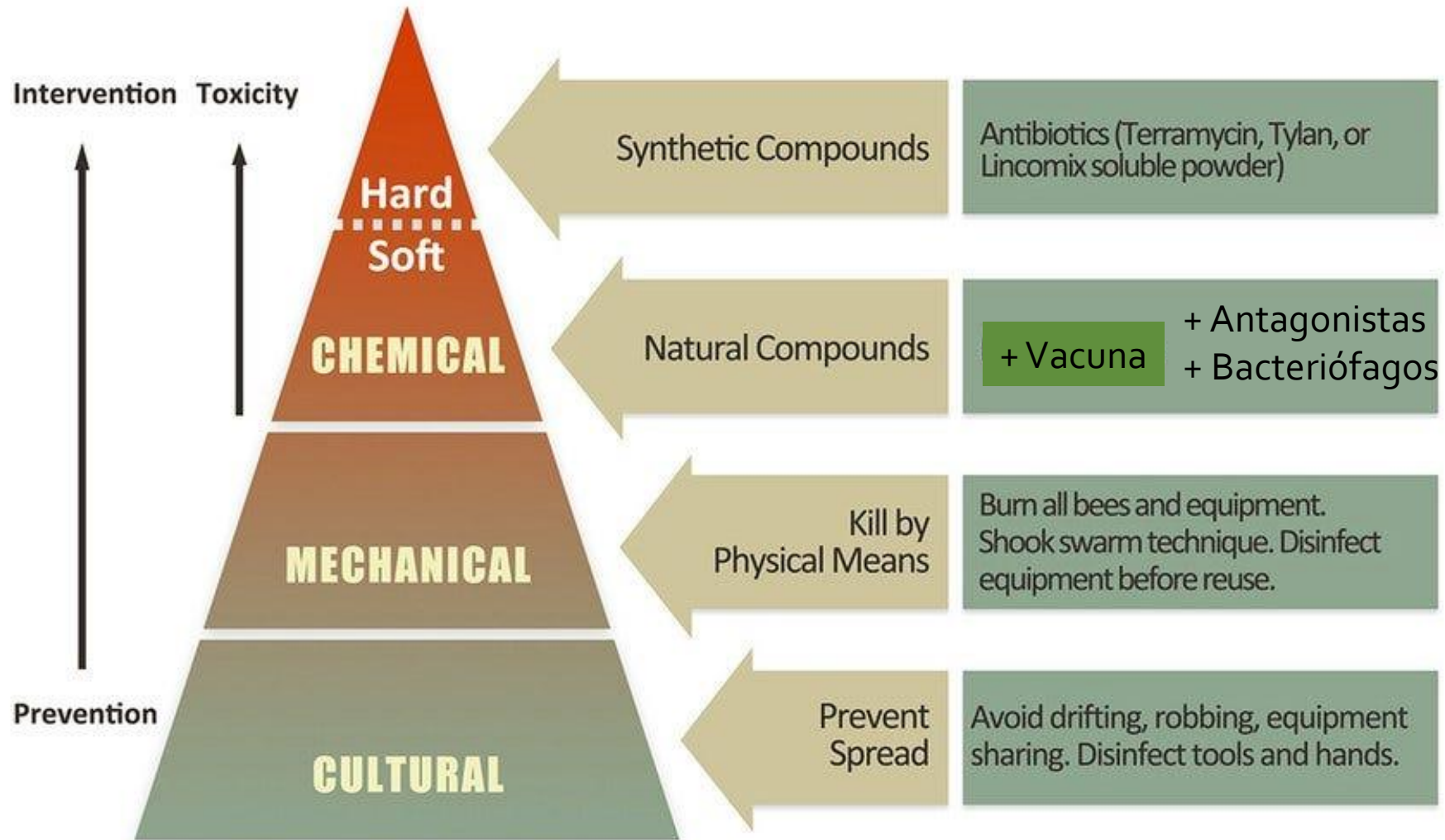
HONEY BEE
HEALTH
(HBH)

E.g. Biosecurity
Measures in
Beekeeping (BMBs)

ENVIRONMENTAL
PROTECTION
(EP)

GOOD BEEKEEPING PRACTICES (GBPs)





IPM for American Foulbrood

Daland Animal Health Inc. Vacuna para abejas contra loque americana Basada en el principio de inmunidad adquirida por la reina.



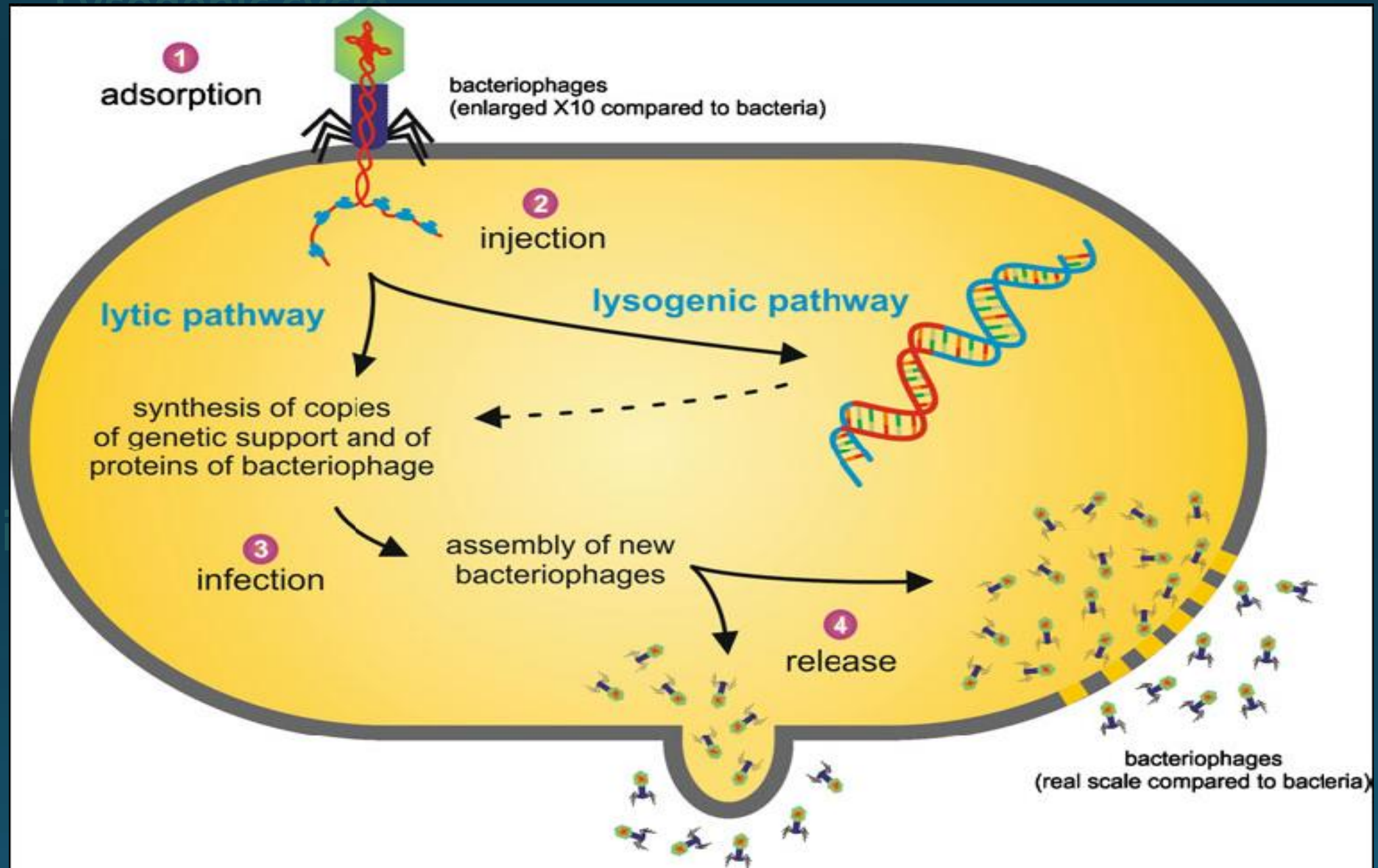
Otras alternativas de control de la enfermedad ecológicamente limpias

- Empleo de bacteriófagos
- Empleo de bacterias antagónicas o sus metabolitos bioactivos

Que son los bacteriofagos ?

- Los bacteriófagos son virus que solo infectan y se replican en bacterias.
- Los fagos aprovechan la maquinaria de las bacterias para dirigirla a la síntesis de nuevos fagos capaces de inducir la lisis bacteriana y liberar nuevos fagos al ambiente.
- Poseen una extrema especificidad infectando sólo a la bacteria "blanco" y sin afectar otras bacterias ni organismos eucarióticos.
- No son tóxicos para los insectos ni para las plantas ni para los animales ni para los humanos.

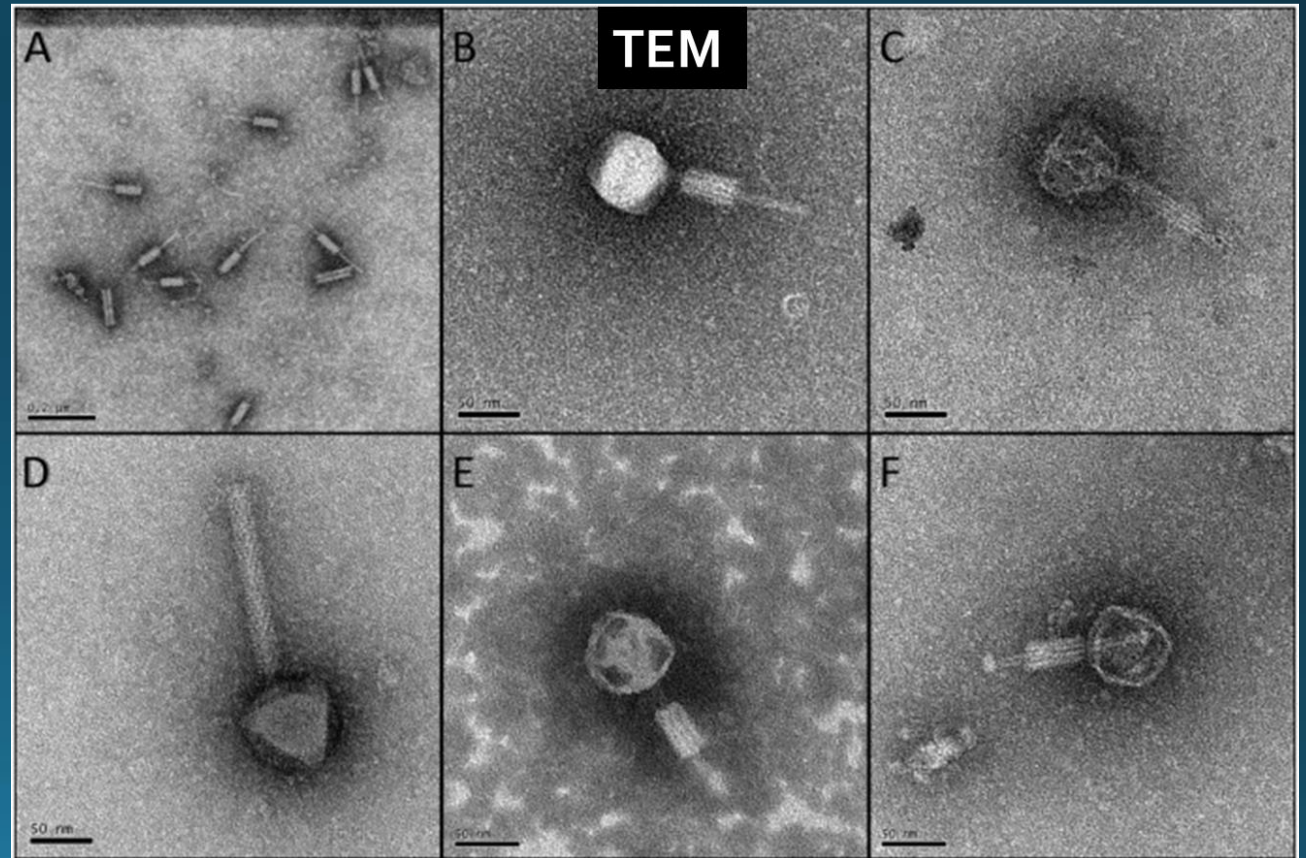
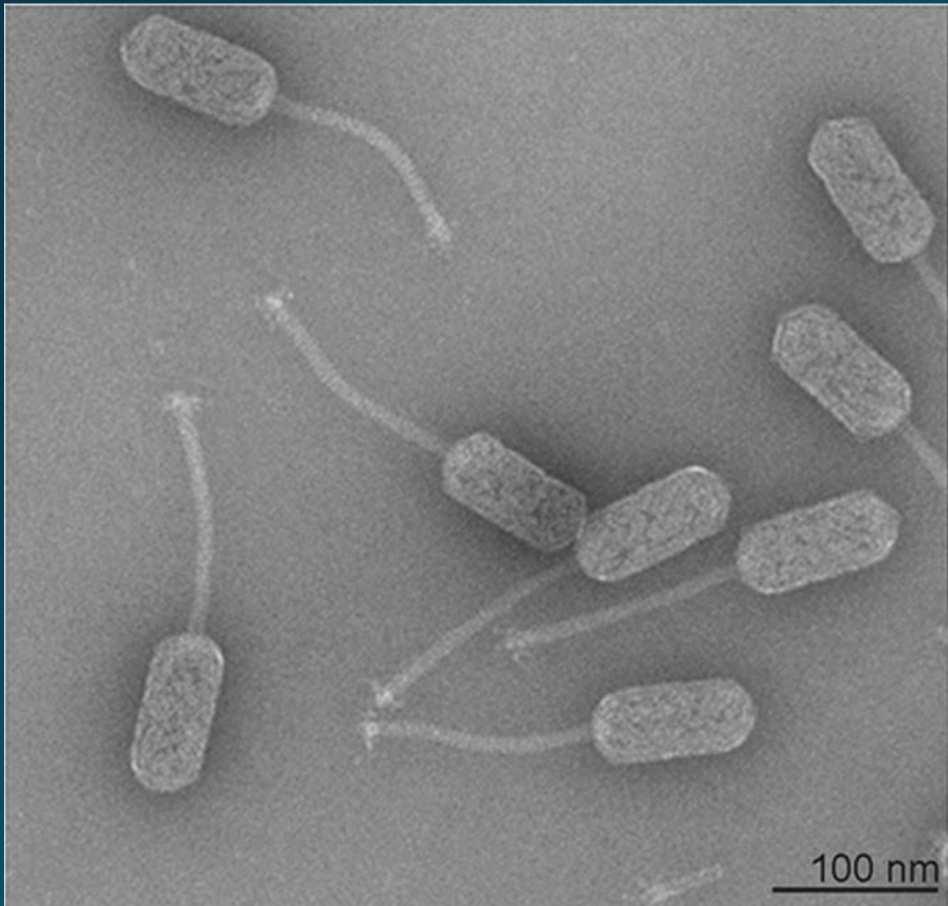
Lysogenic cycle



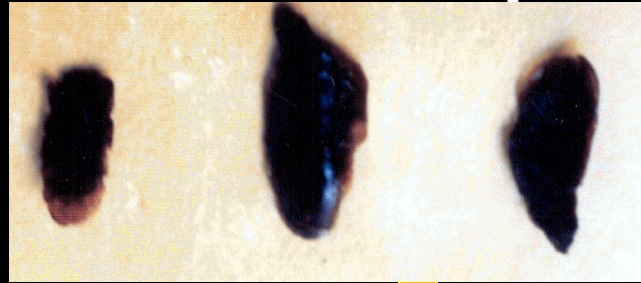
Lytic

Fagoterapia

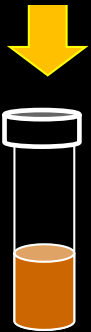
Es el empleo terapéutico de los bacteriófagos para el control de enfermedades bacterianas



Aislamiento de bacteriófagos de larvas con síntomas de loque americana



1-5 g
+ PBS



Incubación 36-
37 °C
24 h / 100 rpm

1-5 g
+ PBS



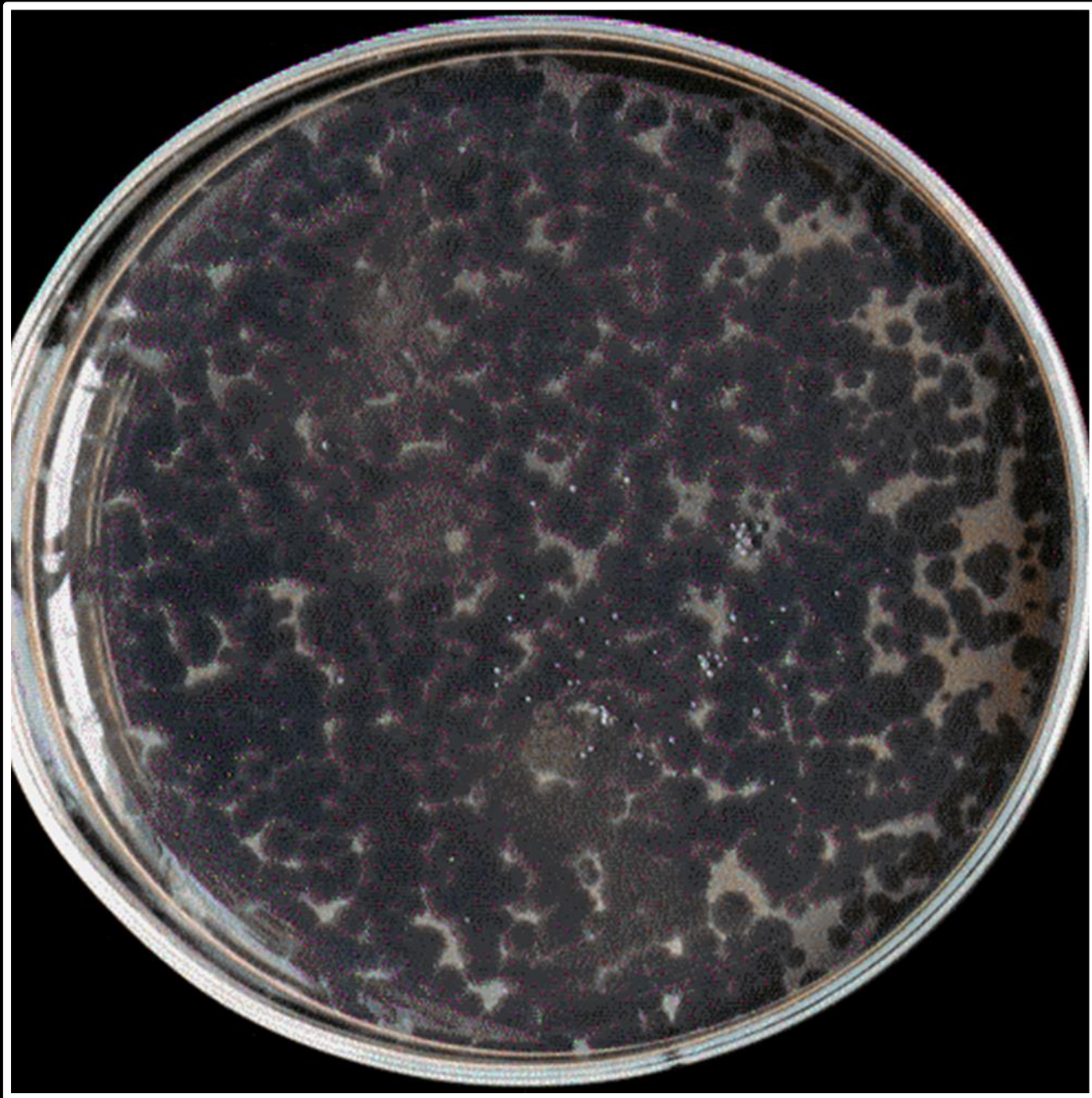
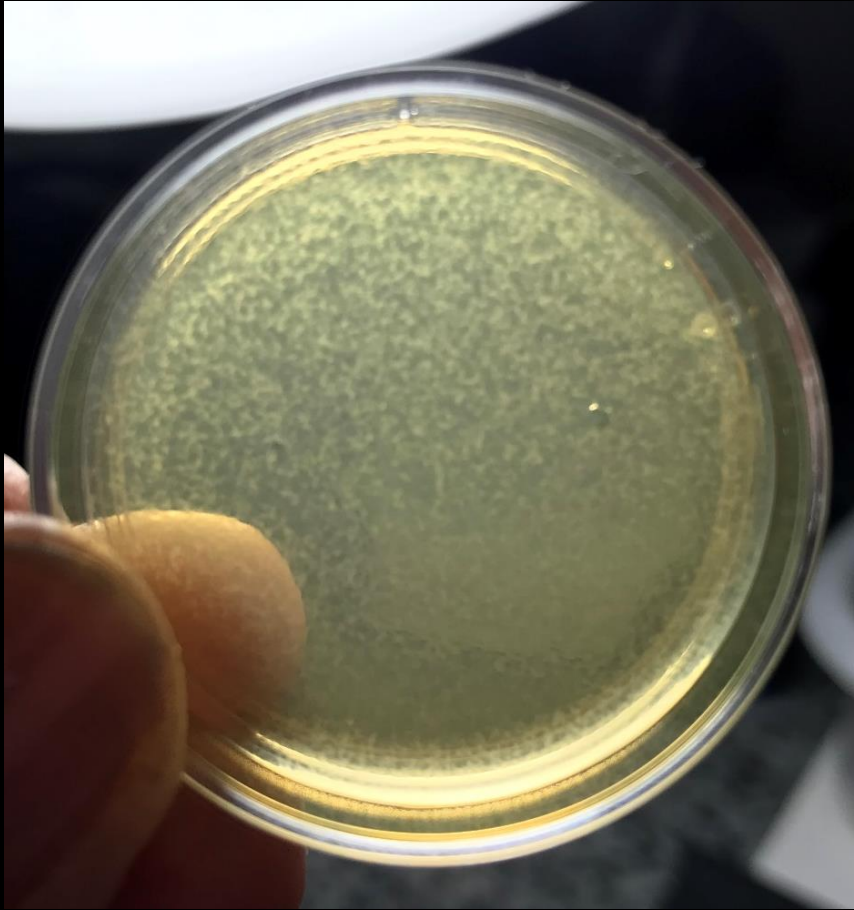
Centrifugación

Filtración
0.45 μm



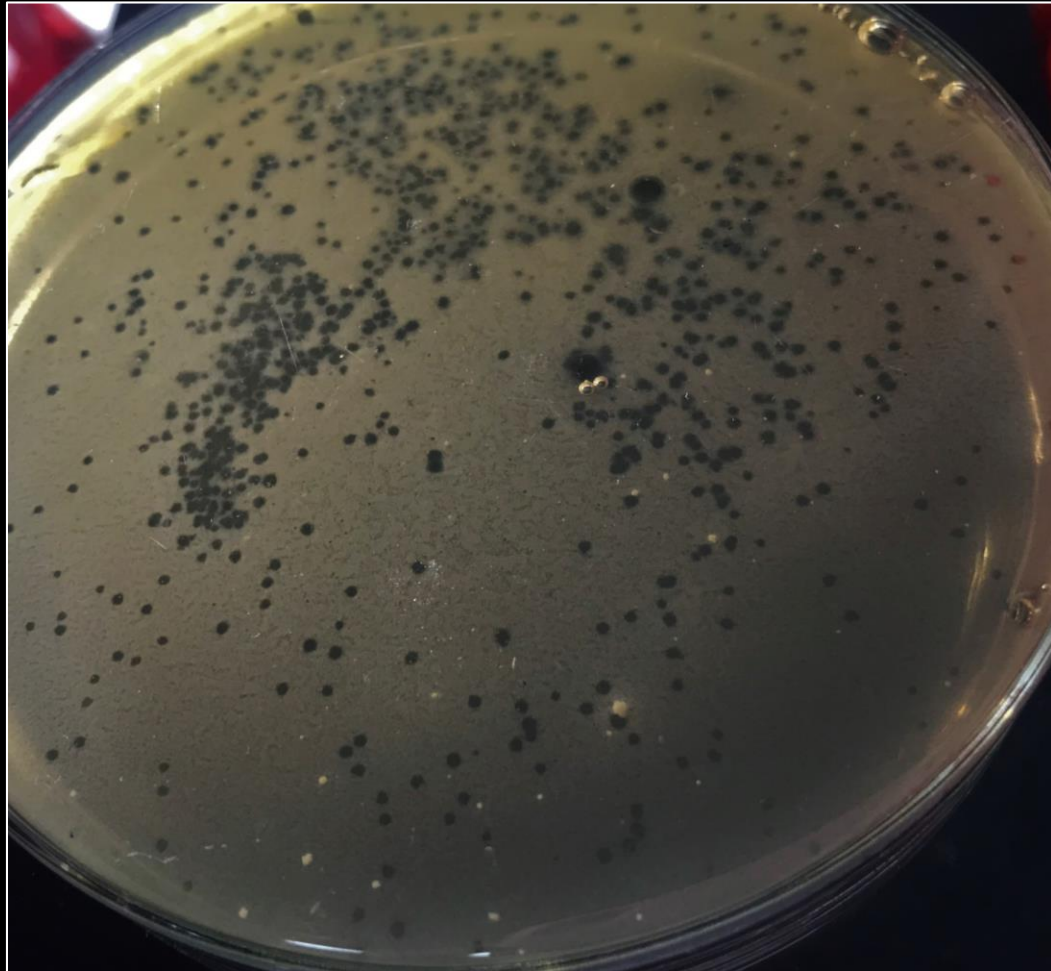
Spot test para verificar presencia de bacteriófagos líticos en la muestra

Preparación de stocks de fagos concentrados

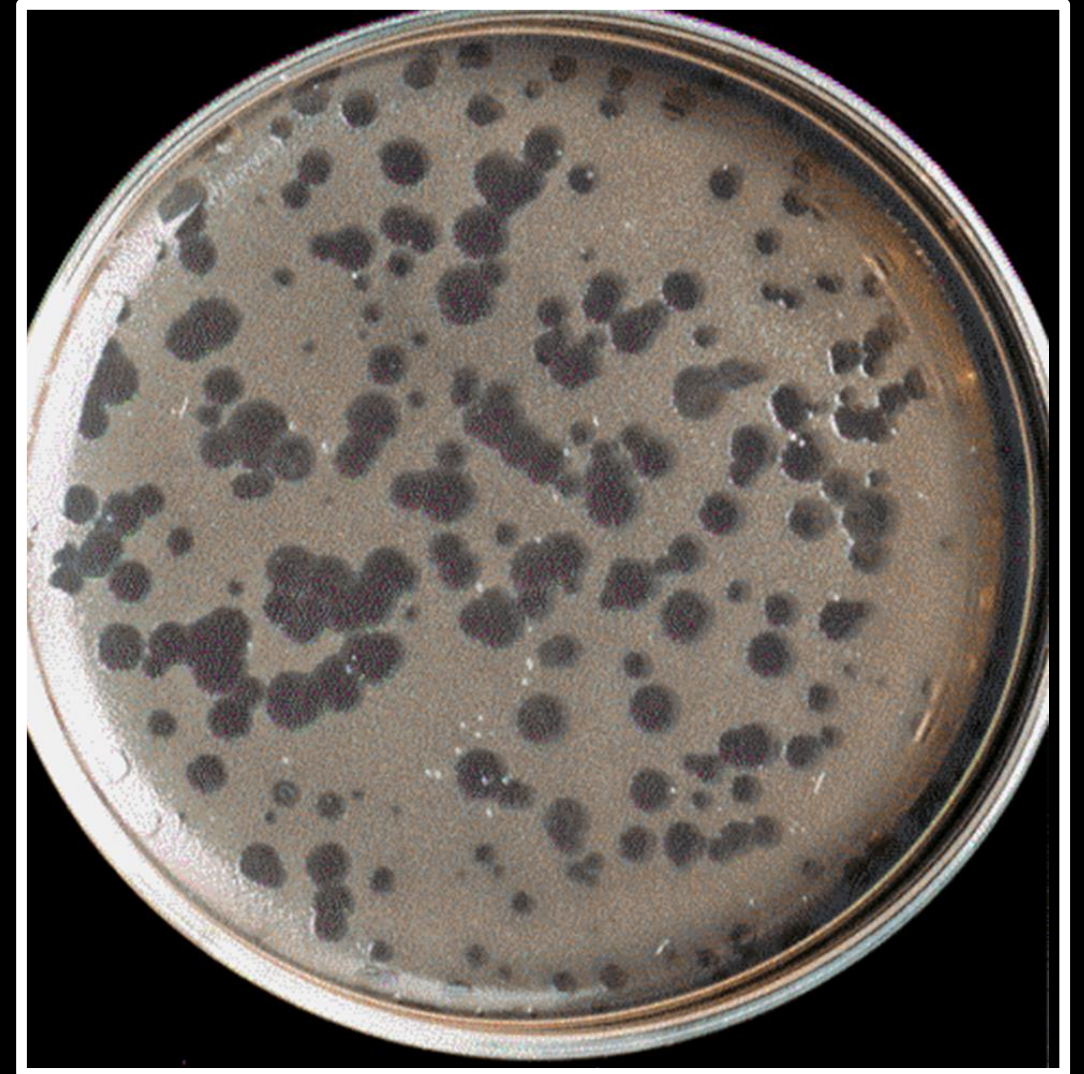


Conteo de unidades formadoras de placas (UFP) por ml

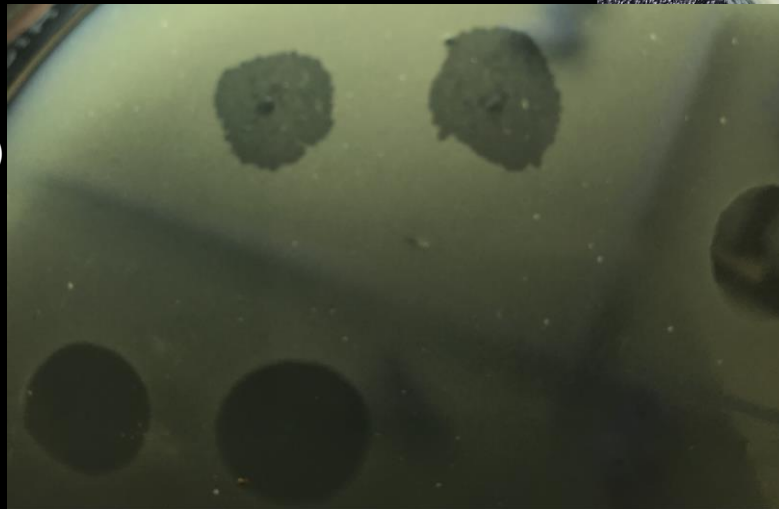
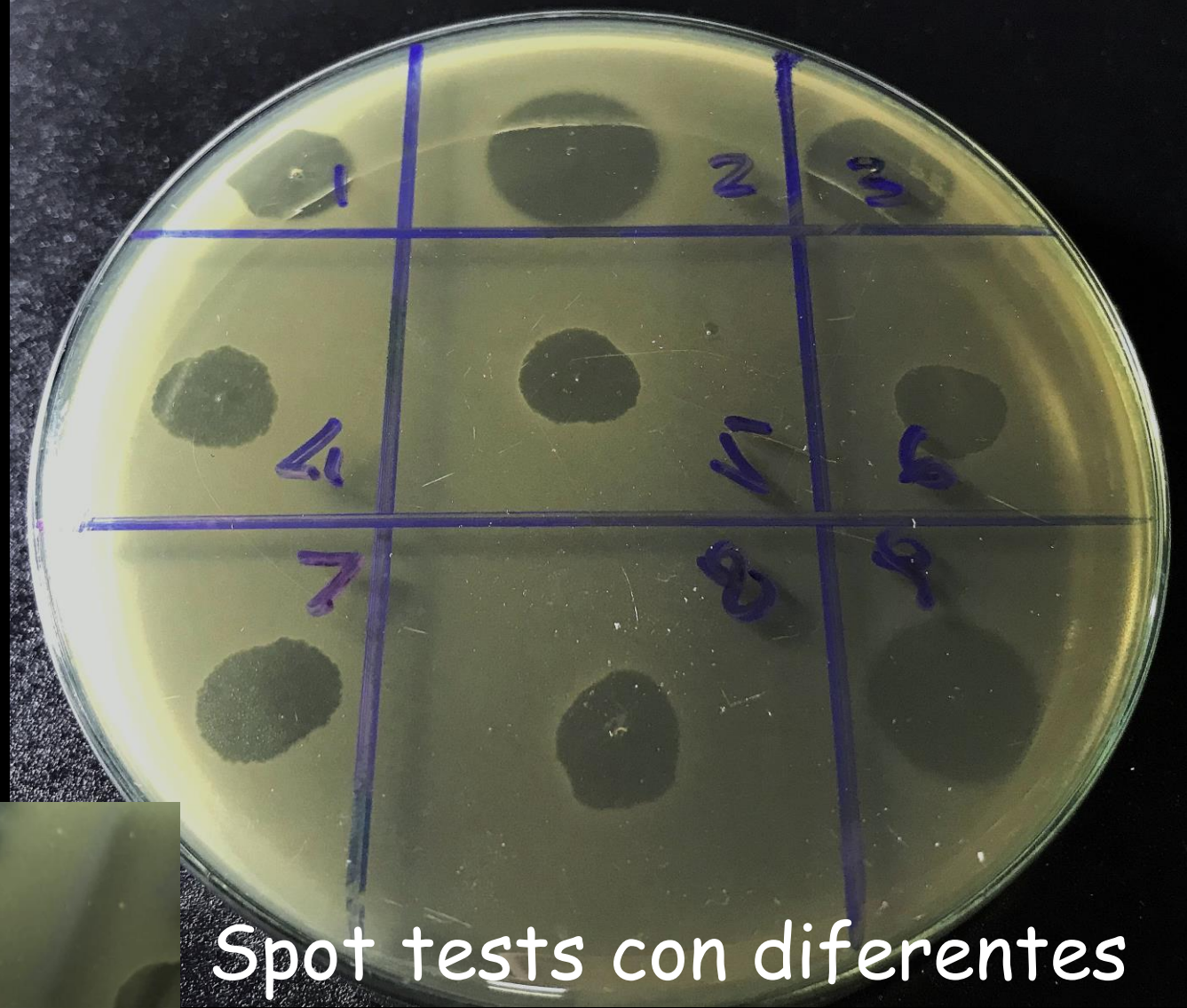
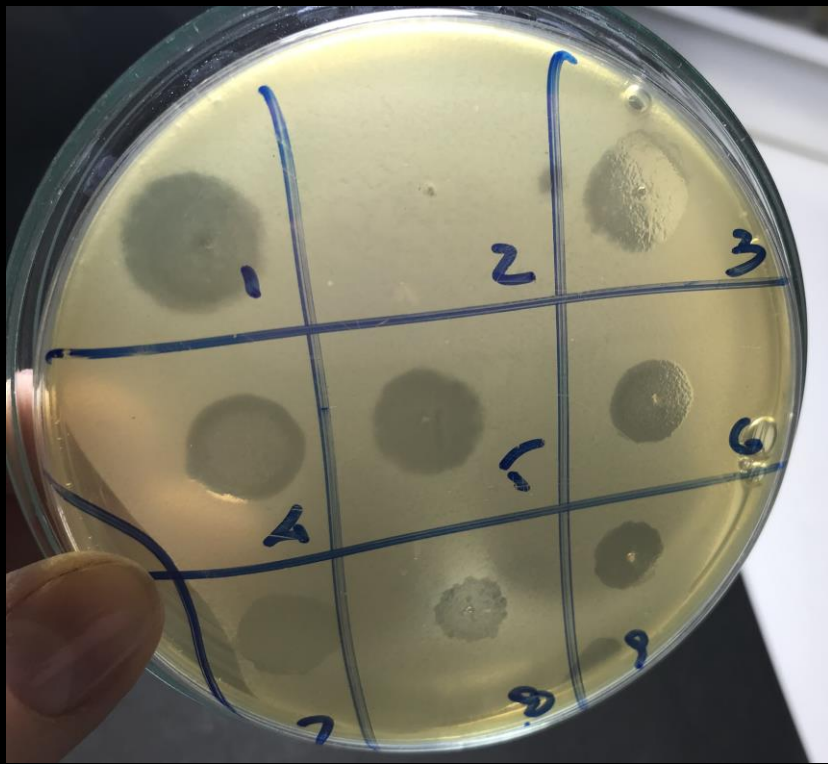
Se preparan diluciones de los stocks de alto título y se calcula el número de fagos por ml UFP/ml



Phage Sweden



Phage PBL1c (USA)



1. PBL₁ phage (USA)
2. Phage 38 (Argentina)
3. Phage Sweden (Sw)
4. Phage Bavio (Argentina)
5. Phage Ohio (USA)
6. Phage Arkansas (USA)
7. Phage Chascomus (A)
8. Phage Lobos (A)
9. Phage Modena (Italy)

Spot tests con diferentes fagos aislados en nuestro laboratorio frente a 55 cepas de *P. larvae*



Bacteriophages as an alternative to conventional antibiotic use for the prevention or treatment of *Paenibacillus larvae* in honeybee hives

T. Scott Brady, Bryan D. Merrill, Jared A. Hilton, Ashley M. Payne, Michael B. Stephenson, Sandra Hope

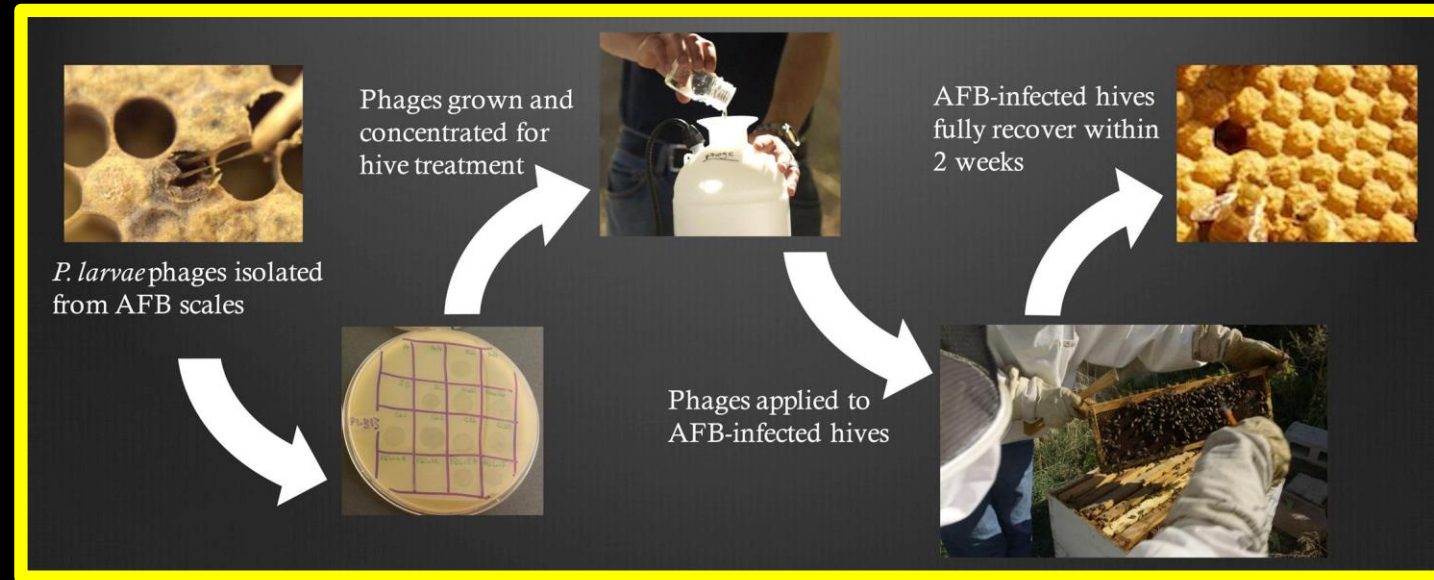
Show more

<https://doi.org/10.1016/j.jip.2017.09.010>

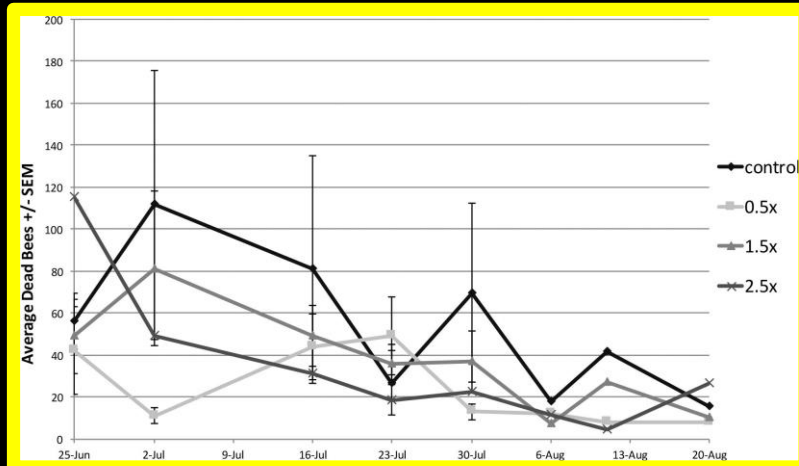
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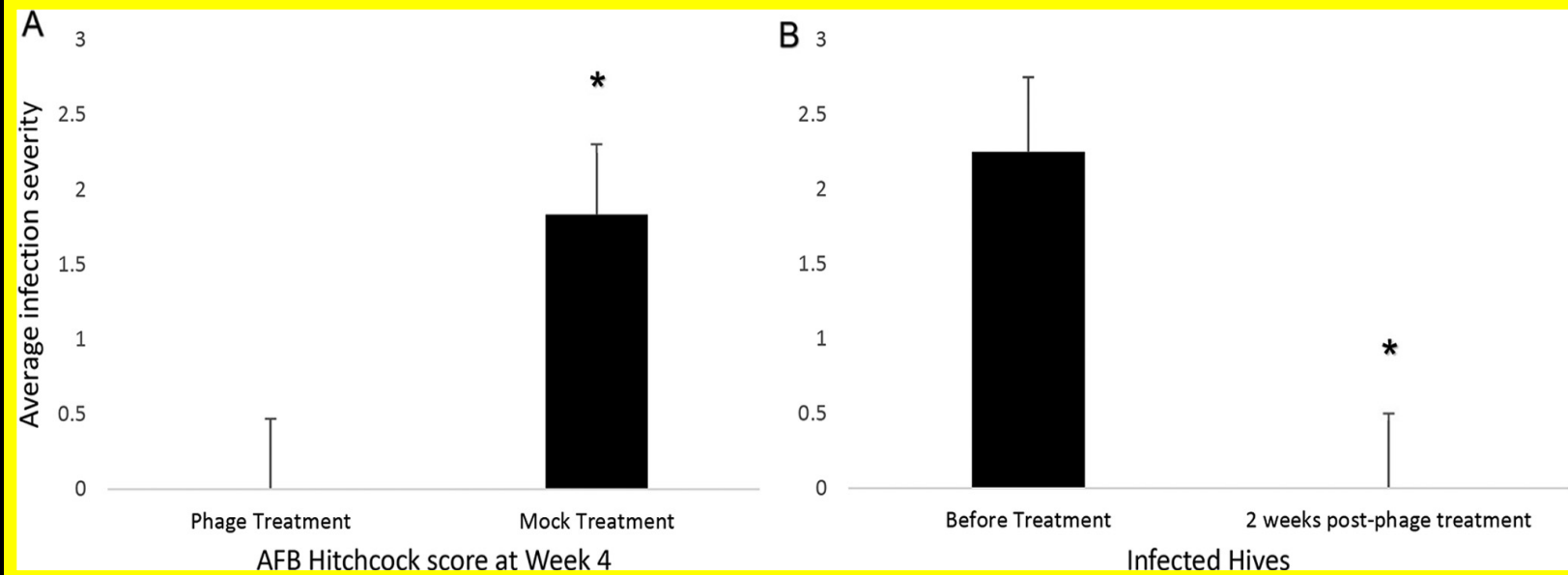
open access



Phage cocktail effectively prevent and clear AFB



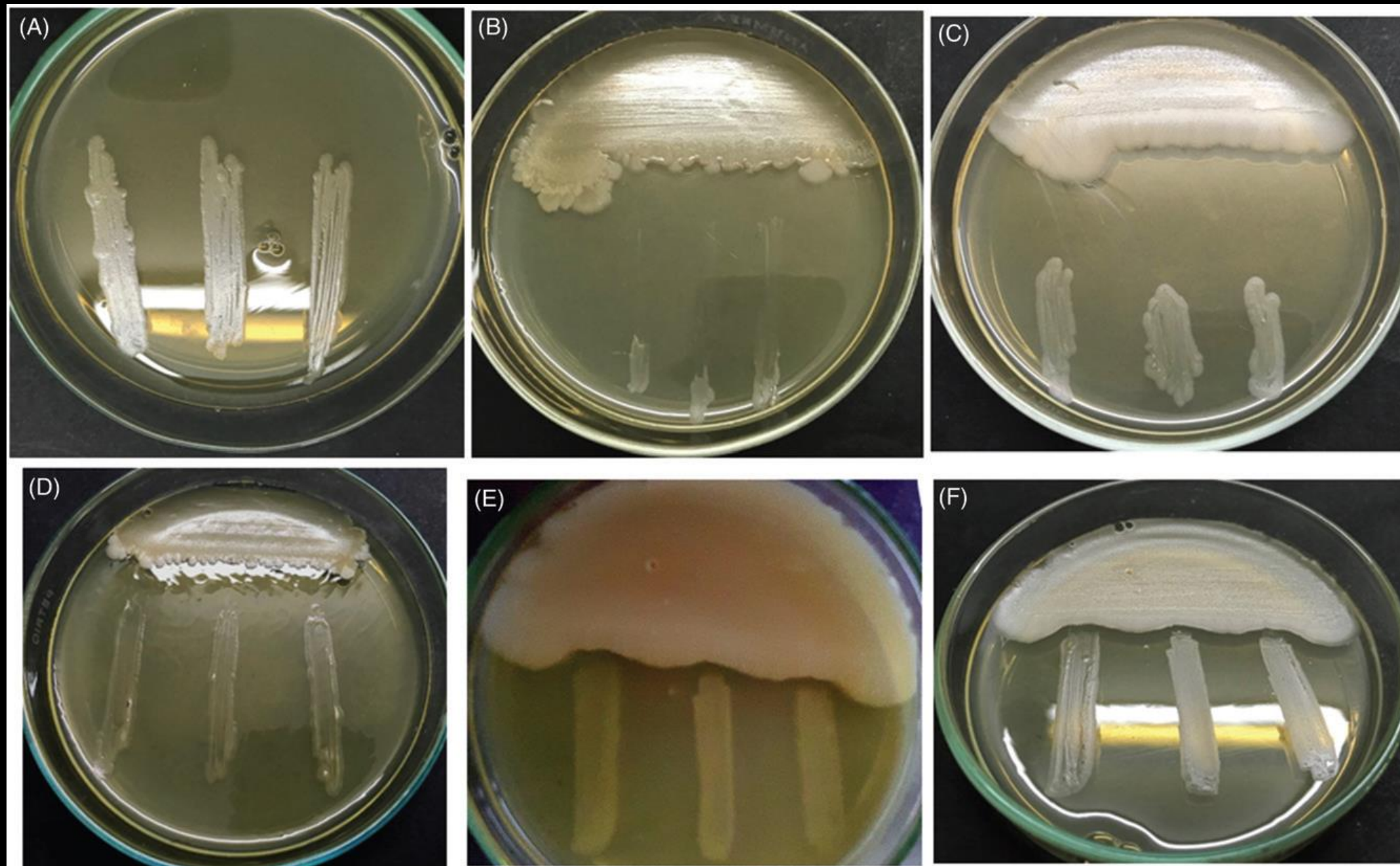
Average bee death over time in control and *P. larvae* phage - overdose beehives . No statistical difference (P=0.639) was observed.



A) AFB Infected hives immediately treated with phages
B) AFB infected hives treated after clinical signs observed

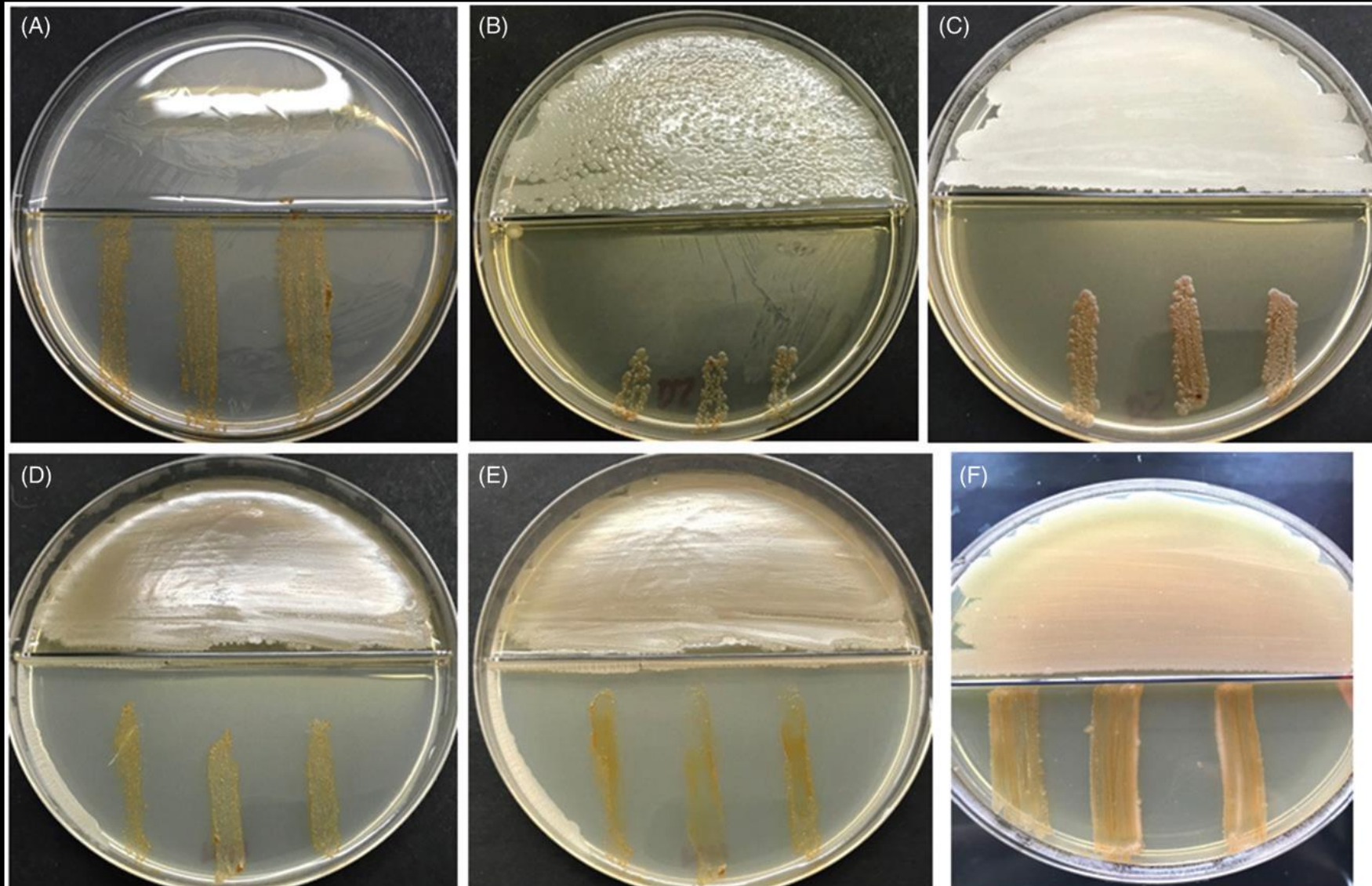
Biocontrol de *Paenibacillus*
larvae mediante bacterias
antagónicas y sus metabolitos
activos

Patrones de inhibición probando compuestos difusibles frente a *P. larvae* ERIC I



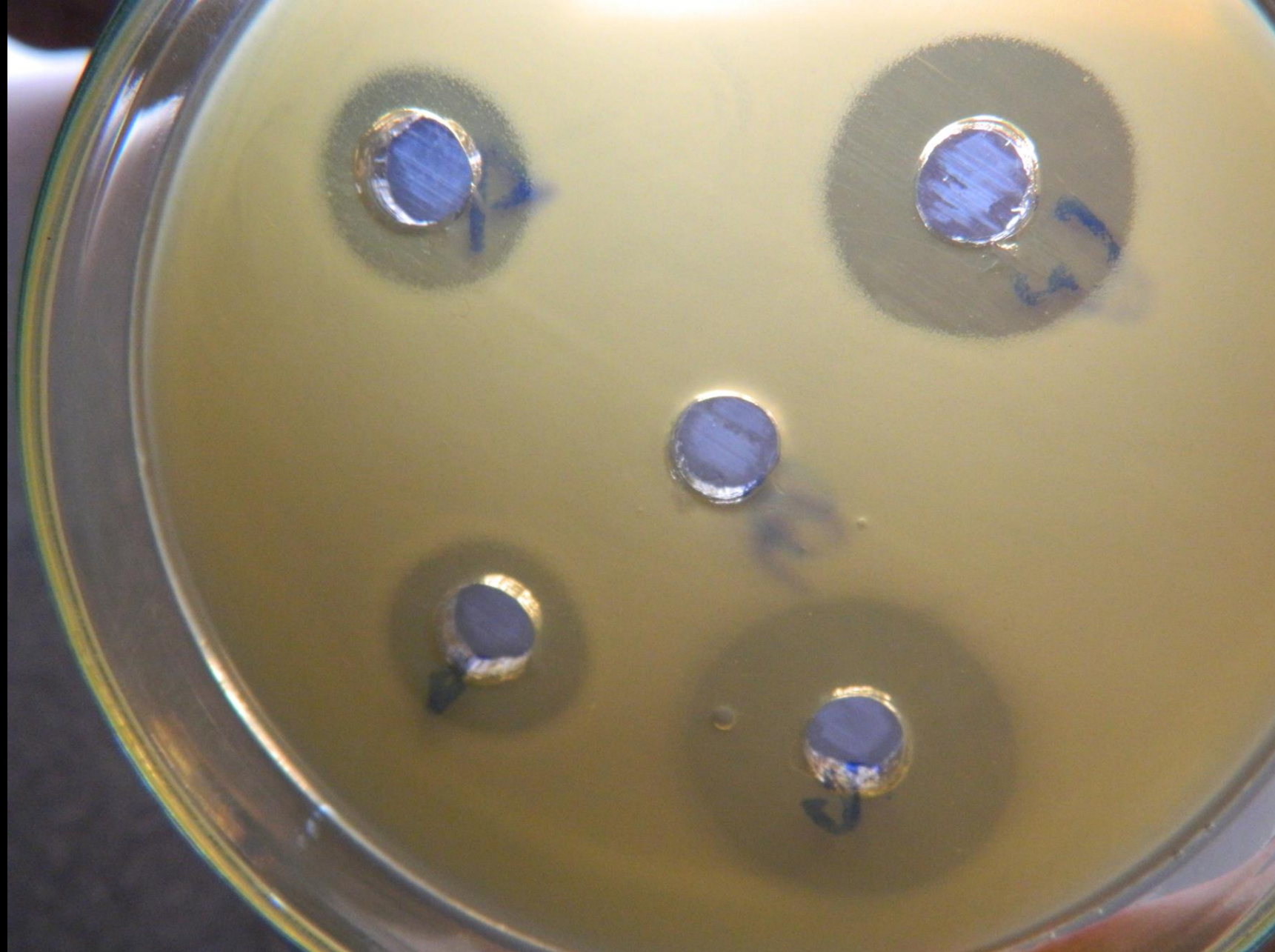
- A) Control
- B) *B. pumilus* mv81
R:4
- C) *B. megaterium*
m435 R:3
- D) *Br. laterosporus*
BLAT16g R:2
- E) *B. cereus* m395
R:1
- F) *B. cereus* mv33
R:0

Patrones de inhibición por co-inoculación probando metabolitos volátiles frente a *P. larvae* ERIC II

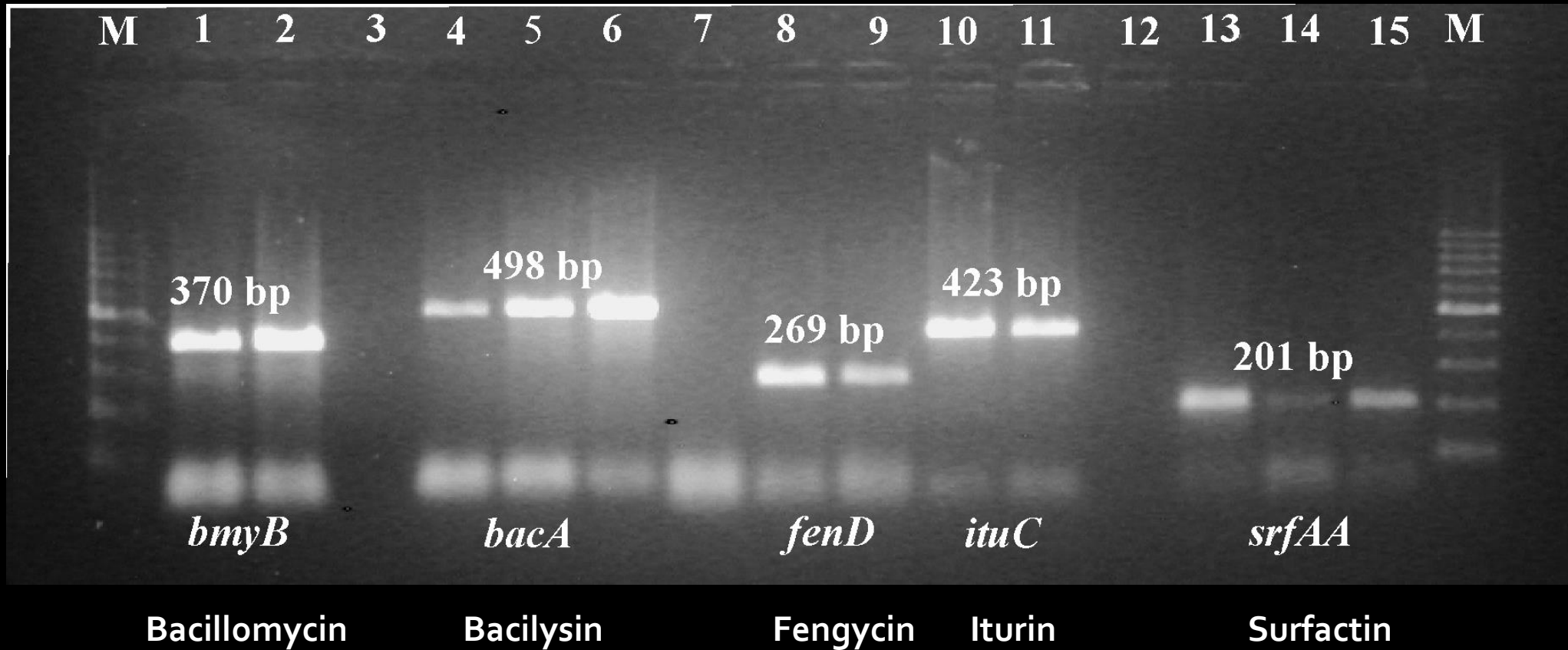


- A) Control: agua
- B) *B. pumilus* m116 R:4
- C) *B. subtilis* m334 R:3
- D) *B. pumilus* m363 R:2
- E) *B. megaterium* m435 R:1
- F) *Br. laterosporus* BLAT 170. R:0

Spot tests empleando lipopéptidos purificados frente a *P. larvae*



Detección por PCR de genes que codifican para la síntesis de péptidos antimicrobianos



Muchas gracias!!!!!!



Preguntas ?