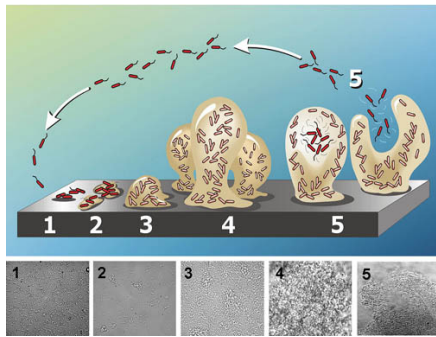
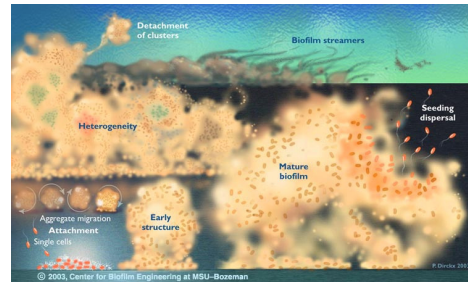


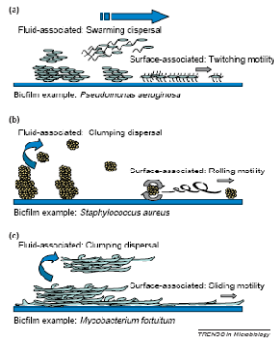
Formação de Biofilme de *Pseudomonas aeruginosa*



Biofilmes: estrutura

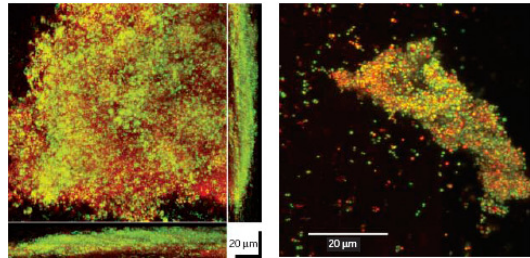


Mecanismos de dispersão de Biofilmes



Hall-Stoodley et al., Trends Microbiol. 13, 7 (2005)

Biofilmes: Dispersão de biofilme de *Staphylococcus aureus*

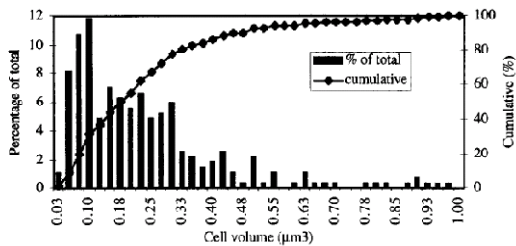


Biofilme

Formas de dispersão do biofilme

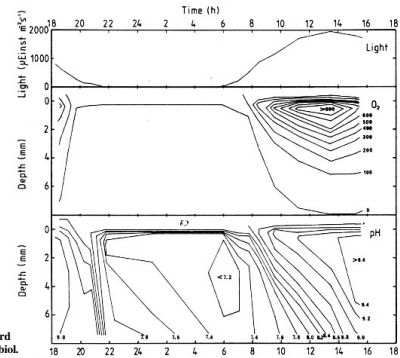
Hall-Stoodley et al., Nature Rev. 2, 95 (2004)

Biofilmes: distribuição do tamanho de células no interior de biofilmes



Jahn, A. and Nielsen, P. H., 1998.

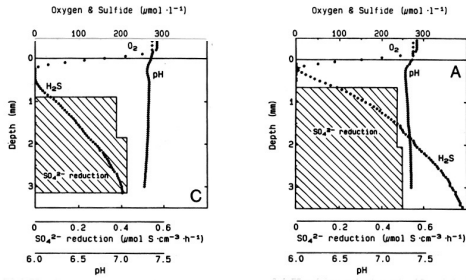
Gradientes criados pela atividade metabólica de células podem alterar radicalmente as condições ambientais no interior de biofilmes



Revsbech & Ward
Appl. Env. Microbiol.
48: 270-275 (1984)

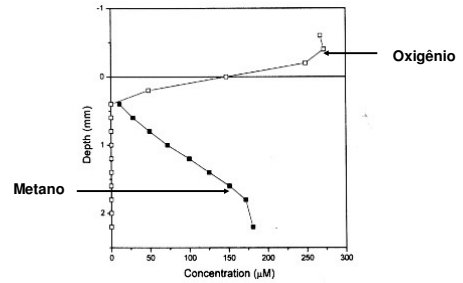
FIG. 2. Isopleths of oxygen (μM) and pH in the 55°C mat measured during a diurnal cycle.

Biofilmes: gradientes gerados pela atividade metabólica permitem coexistência de organismos aeróbios e anaeróbios



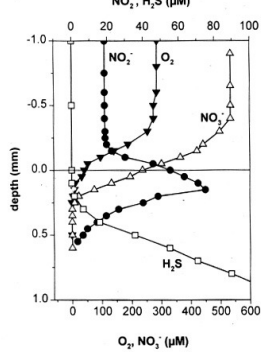
Kühl & Jørgensen Appl. Env. Microbiol. 58: 1164-1174 (1997)

Biofilmes: gradientes gerados pela atividade metabólica permitem coexistência de organismos aeróbios e anaeróbios



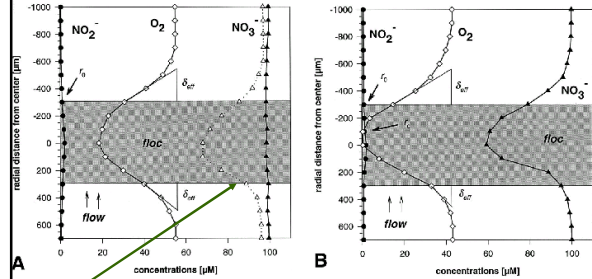
Damgaard et al. Wat. Res. 35: 1379-1386 (2001)

Biofilmes: gradientes gerados pela atividade metabólica permitem coexistência de organismos aeróbios e anaeróbios



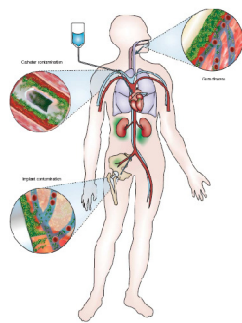
De Beer et al. Appl. Env. Microbiol. 63: 973-977 (1997)

Biofilmes: gradientes gerados pela atividade metabólica permitem coexistência de organismos aeróbios e anaeróbios: flocos de lodo ativado



Schramm Appl. Env. Microbiol. 65: 4189-4196 (1999)

Biofilmes: patogênese



Infecções associadas a biofilmes

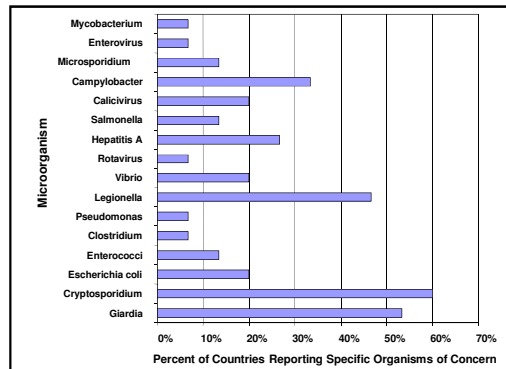
Infection or disease	Common biofilm bacterial species
Dental caries	Acidogenic Gram-positive cocci (e.g., <i>Streptococcus</i>)
Periodontitis	Gram-negative anaerobic oral bacteria
Otitis media	Nontypable strains of <i>Haemophilus influenzae</i>
Musculoskeletal infections	Gram-positive cocci (e.g., staphylococci)
Necrotizing fasciitis	Group A streptococci
Biliary tract infection	Enteric bacteria (e.g., <i>Escherichia coli</i>)
Osteomyelitis	Various bacterial and fungal species—often mixed
Bacterial prostatitis	<i>E. coli</i> and other Gram-negative bacteria
Native valve endocarditis	Viridans group streptococci
Cystic fibrosis pneumonia	<i>P. aeruginosa</i> and <i>Burkholderia cepacia</i>
Melioidosis	<i>Pseudomonas pseudomallei</i>
Nosocomial infections	
ICU pneumonia	Gram-negative rods
Sutures	<i>Staphylococcus epidermidis</i> and <i>S. aureus</i>
Exit sites	<i>S. epidermidis</i> and <i>S. aureus</i>
Arteriovenous shunts	<i>S. epidermidis</i> and <i>S. aureus</i>
Schleral buckles	Gram-positive cocci
Contact lens	<i>P. aeruginosa</i> and Gram-positive cocci
Urinary catheter cystitis	<i>E. coli</i> and other Gram-negative rods
Peritoneal dialysis (CAPD) peritonitis	A variety of bacteria and fungi
IUDs	<i>Actinomyces israelii</i> and many others
Endotracheal tubes	A variety of bacteria and fungi
Hickman catheters	<i>S. epidermidis</i> and <i>C. albicans</i>
Central venous catheters	<i>S. epidermidis</i> and others
Mechanical heart valves	<i>S. aureus</i> and <i>S. epidermidis</i>
Vascular grafts	Gram-positive cocci
Biliary stent blockage	A variety of enteric bacteria and fungi
Orthopedic devices	<i>S. aureus</i> and <i>S. epidermidis</i>
Penile prostheses	<i>S. aureus</i> and <i>S. epidermidis</i>

Costerton et al. Science 284: 1318 (1999)

Biofilmes: Patogênese

- Reservatórios de patógenos
- Interferência com funcionamento de equipamentos (bloqueio de cateteres)
- Deterioração de materiais (cáries, implantes)
- Produção de substâncias imunogênicas (células e componentes celulares, LPS)
 - ⇒ Inflamação crônica, fibrose cística, CLARE, etc.
- Produção de substâncias que inibem reação imunológica (HSL)

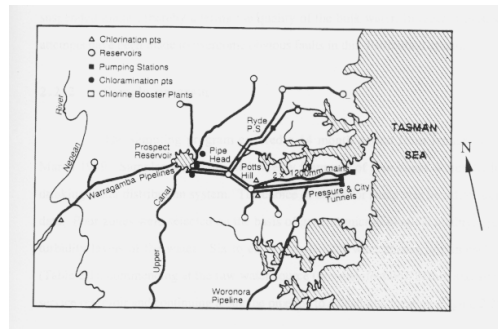
Microorganismos –problema em sistemas de distribuição de água em países que mantêm registros



Patógenos de veiculação hídrica:

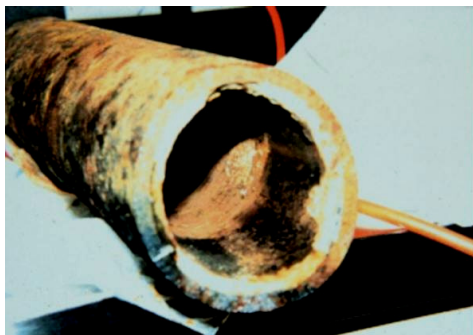
Bactérias	Vírus	Protozoários
Campylobacter	Hepatitis A	Giardia
Escherichia coli	Reovirus	Cryptosporidium
Salmonella	Calicivirus	Entamoeba
Yersinia	Enterovirus	Microsporidium
Vibrio	Coxsackievirus	
Legionella	Adenovirus	
Aeromonas	Echovirus	
Mycobacterium	Poliovirus	
Shigella		
Pseudomonas		

Biofilmes como reservatórios de patógenos

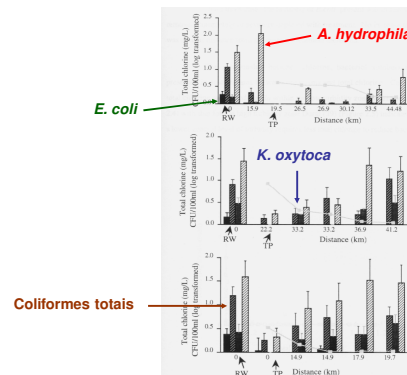


Power, K.N., 1995

Biofilmes como reservatórios de patógenos



Biofilmes como reservatórios de patógenos



Power, K.N., 1995

Biofilmes como reservatórios de patógenos

TABLE 2.16 HPC bacteria isolated from water, biofilms and dispersed biofilms

SITE	HPC BACTERIA (35°C/48 hr)		
	Water /100ml	Biofilms /g	Dispersed biofilms /g
PETERSHAM			
204.6 (a)	9.2	2.2 x 10 ⁴	1.2 x 10 ⁵
204.6 (b)	9.2	1.3 x 10 ⁴	2.2 x 10 ⁵
Average	9.2	1.8 x 10 ⁴	1.2 x 10 ⁵
DURAL			
39.5 (a)	315	< 99	3.2 x 10 ⁵
39.5 (b)	315	6.2 x 10 ⁴	3.2 x 10 ⁵
Average	315	2.5 x 10 ³	3.2 x 10 ⁵

Power, K.N., 1995

Biofilmes patogênicos: impacto

- 30% dos pacientes de hospitais com 1 ou mais cateteres intravasculares
- 10% dos pacientes de hospitais com cateteres urinários
- EUA: 2.000.000 infecções hospitalares/ano
- 45% associadas a implantes
- Custo: 11 bilhões US\$/ano

Schierholz & Beuth, J. Hospital Infect. 49: 87 (2001)

Incidência de infecções associadas a implantes

TABLE 1 Incidences of infection of different biomedical implants and devices adapted from Dankert *et al.* [2] and arranged according to body site

Body site	Implant or device	Incidence (%)
Urinary tract	UT catheters	10-20
	CV catheters	4-12
Percutaneous	Temporary pacemaker	4
	Short indwelling catheters	0.5-3
	Peritoneal dialysis catheters	3-5
	Cardiac pacemaker	1
Subcutaneous	Mammary prosthesis	1-7
	Intraocular lenses	0.13
Soft tissue	Prosthetic heart valve	1.88
	Multiple heart valve	3.6
	Vascular graft	1.5
	Artificial heart*	40
Circulatory system	Prosthetic hip	2.6-4.0
	Total knee	3.5-4

*From experiments in calves and sheep.

Gottenbos *et al.* J. Mat. Sci. Mat. Med., 13, 717 (2002)

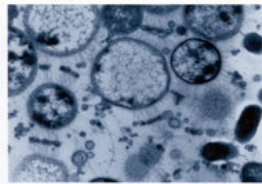
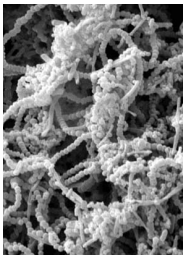
Origem de bactérias que infectaram implantes

TABLE 11 Distant infectious foci of hematogenously infected hip [17] and knee arthroplasties [18]

Distant foci	Hip (n = 27) (%)	Knee (n = 72) (%)
Cutaneous region	19	39
Urinary tract	15	19
Respiratory tract	15	14
Oral cavity	30	7
Gastrointestinal tract	4	6
Septic arthritis	0	3
Abdominal abscess	0	1
Unknown	19	11

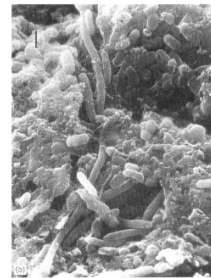
Gottenbos *et al.* J. Mat. Sci. Mat. Med., 13, 717 (2002)

Biofilmes na cavidade bucal



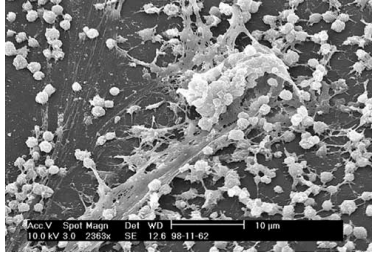
Eastman Dental Research Institute, University College, London, ASM

Entupimento de sonda biliar por biofilme



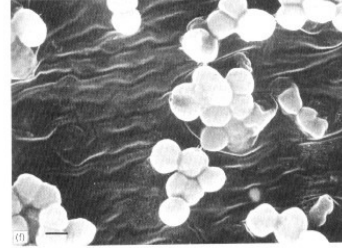
Mittelman, M. W. *In* Fletcher, M. Bacterial Adhesion - Molecular and Ecological Diversity, Wiley-Liss, New York, 1996, p. 97

Biofilme de Staphylococcus sp. na superfície interior de um conector de catéter



Donlan, CDC

Biofilme de Streptococcus epidermidis em catéter de poliuretano



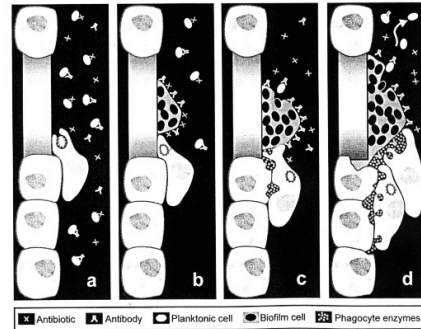
Mittelman, M. W. In Fletcher, M. Bacterial Adhesion - Molecular and Ecological Diversity, Wiley-Liss, New York, 1996, p. 97

Caráter polimicrobiano de infecções por biofilmes!

Cateter	UFC	Ponta de Cateter
01	≥15	<i>Acinetobacter baumannii</i> <i>Enterobacter aerogenes</i> <i>Citrobacter freundii</i>
05	≥15	<i>Enterobacter aerogenes</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus intermedius</i>
09	≥15	<i>Enterobacter aerogenes</i> <i>Pseudomonas aeruginosa</i> <i>Staphylococcus intermedius</i>

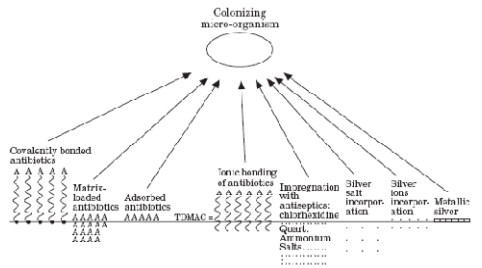
Pizzolitto *et al.*, UNESP Araraquara(2003)

Mecanismo de inflamação por biofilmes



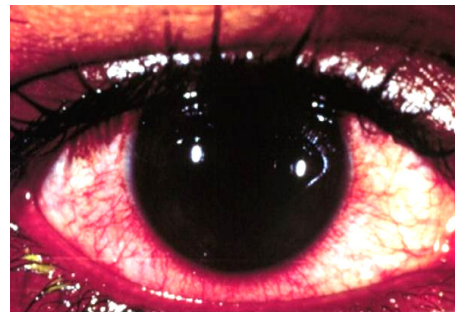
Costerton *et al.*, Science 284: 1318 (1999)

Possíveis alternativas para aumentar a resistência à colonização antimicrobiana de biomateriais

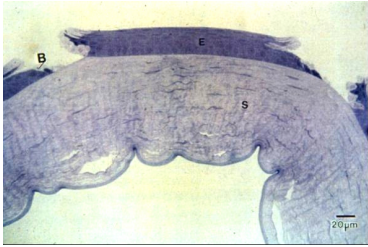


Schierholz & Beuth, J. Hospital Infect. 49: 87 (2001)

Biofilmes: produção de substâncias imunogênicas

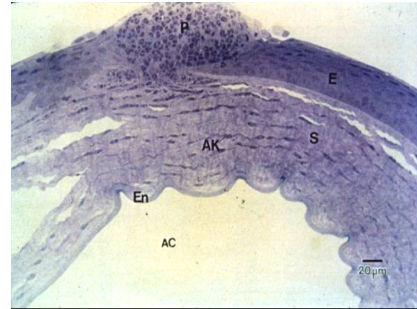


Biofilmes: produção de substâncias imunogênicas



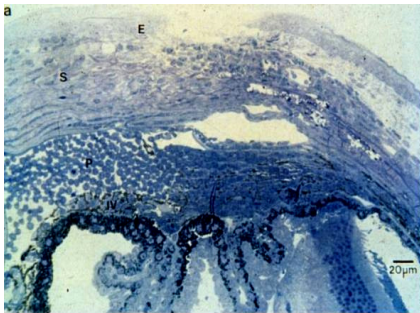
Córnea infectada com Paer 1 – tempo 0

Biofilmes: produção de substâncias imunogênicas



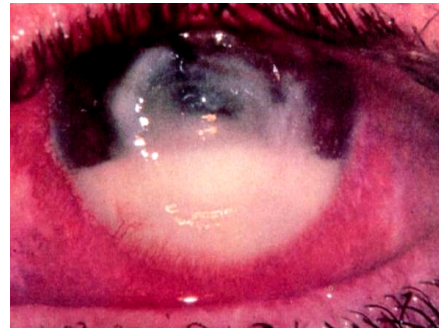
Córnea infectada com Paer 1 – tempo 24h

Biofilmes: produção de substâncias imunogênicas

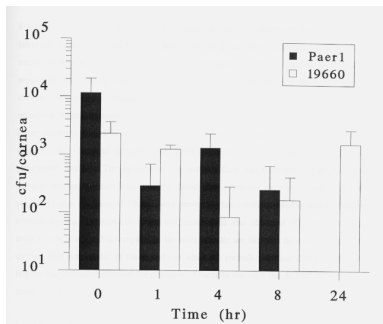


Córnea infectada com Paer 19660 – tempo 24h

Biofilmes: produção de substâncias imunogênicas

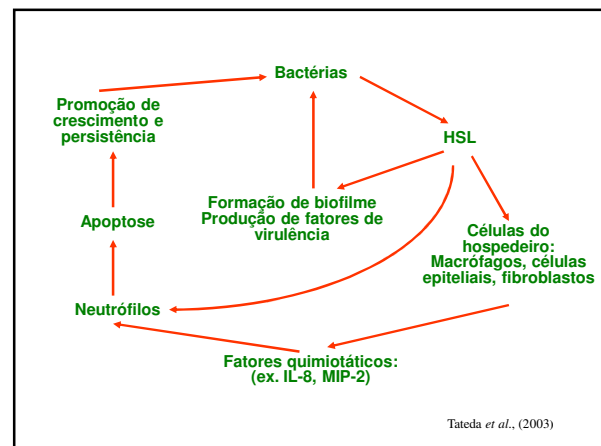
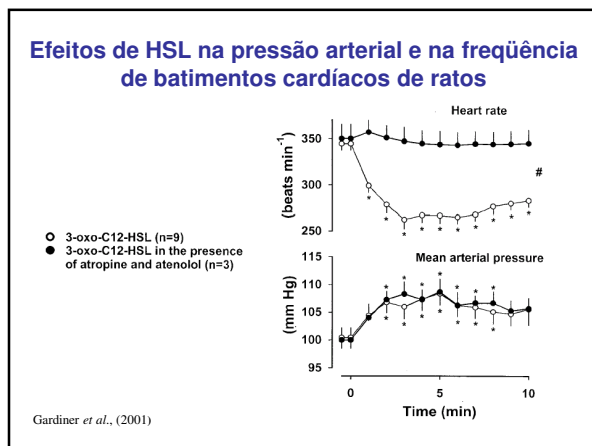
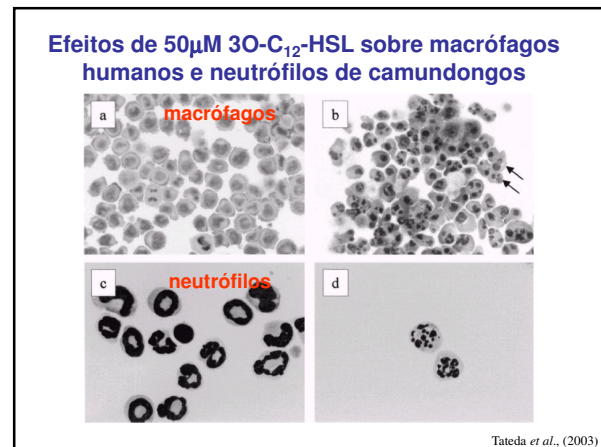
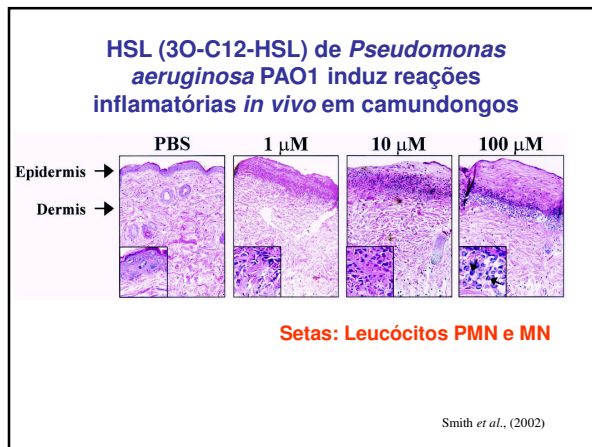
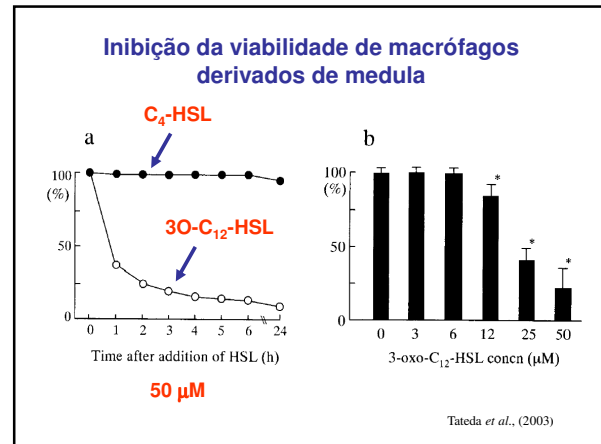
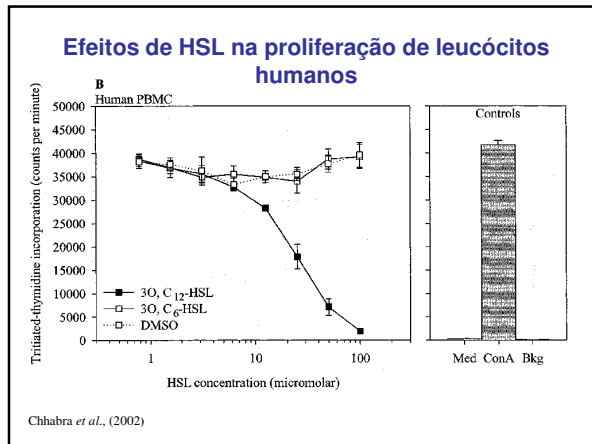


Biofilmes: sobrevivência da cepa queratogênica (19660) e colonizadora de lentes de contato Paer1 no olho do rato

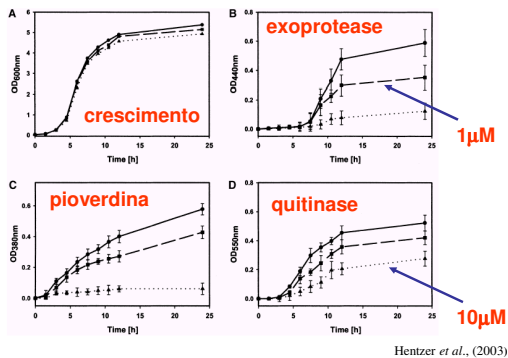


Homoserinalactonas na infecção hospitalar

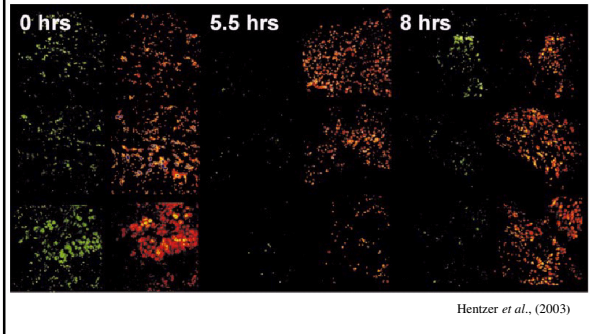
- Catéteres com biofilmes de *Pseudomonas aeruginosa* estão impregnados com homoserinalactonas (Stickler *et al.*, 1998).
- Homoserinalactonas produzidas por processos de QS em biofilmes: ação imunomodulatória.



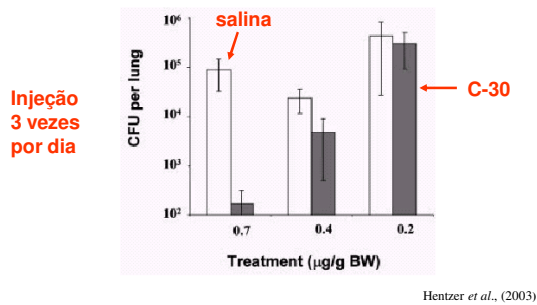
Efeitos de furanona C-30 na expressão de fatores de virulência de *Pseudomonas aeruginosa* PAO1.



Inibição de QS de *Pseudomonas aeruginosa* PAO1 pela furanona C-30 em pulmões de camundongo (1,7 μg/g, injeção intravenosa)



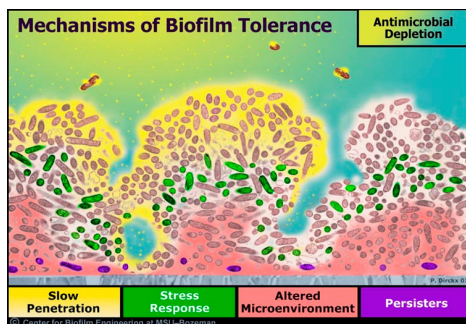
Eliminação de *Pseudomonas aeruginosa* PAO1 de pulmões de camundongos pela dosagem de furanona C-30



Biofilmes: Resistência a biocidas e antibióticos

- Glicocálix limita a penetração do antibiótico no interior do biofilme
- Limitação de nutrientes dos microrganismos no interior do biofilme aumenta a sua resistência a antibióticos
- Fenótipo mais resistente no interior do biofilme

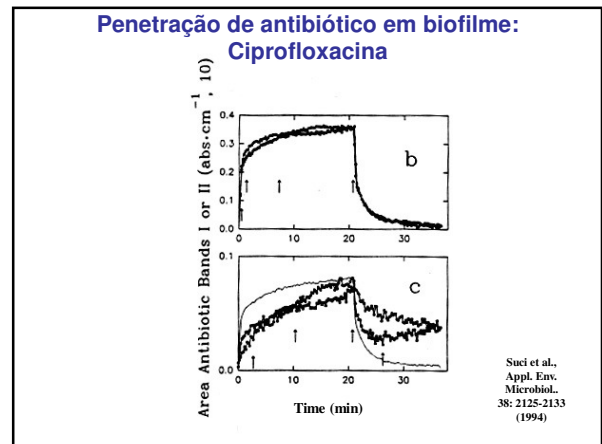
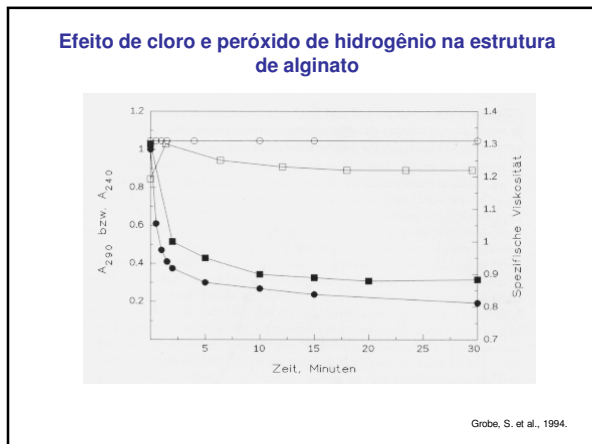
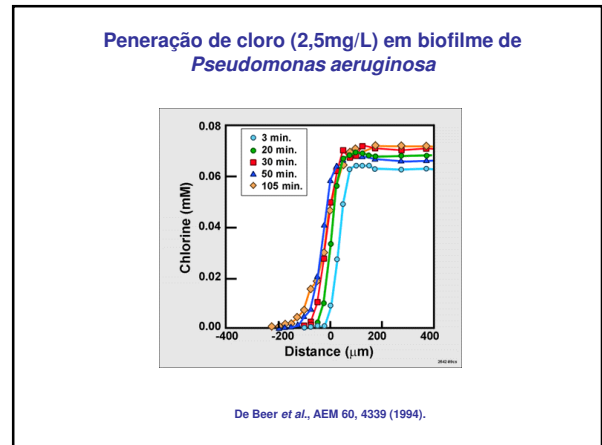
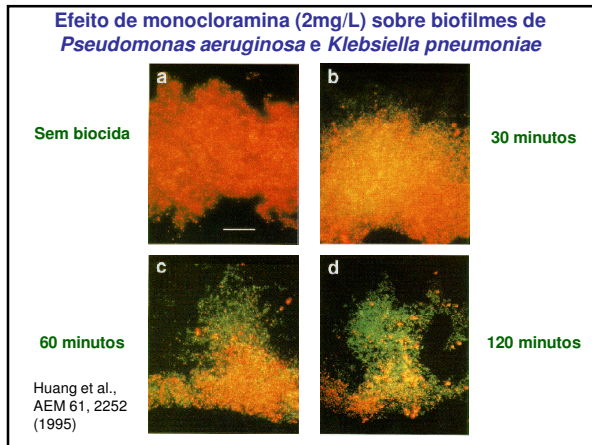
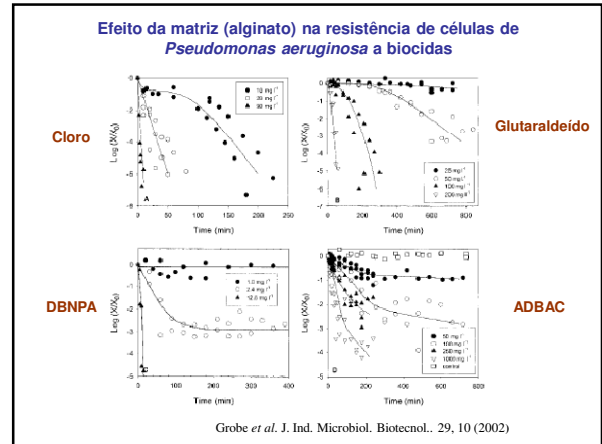
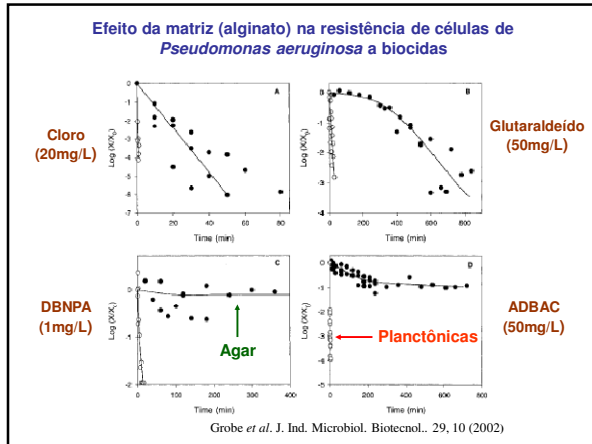
Mecanismos de resistência de Biofilmes

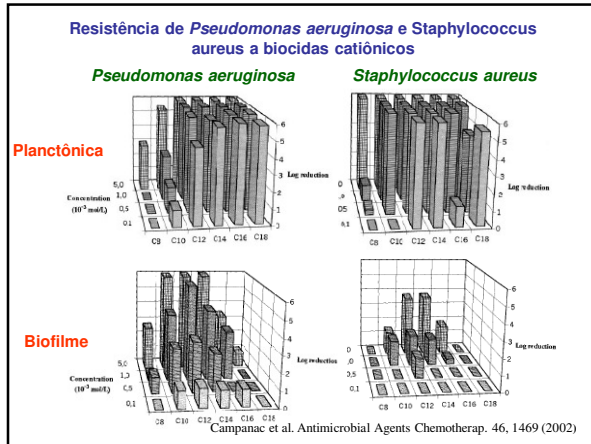


Biofilmes: resistência a antibióticos

Antibiótico (mg/ml)	UFC inicial	UFC final	Fase
0	4,7x10 ⁸	2,7x10 ⁹	Biofilme
100	5,8x10 ⁸	4,2x10 ⁷	Biofilme
1000	2,0x10 ⁸	6,1x10 ⁶	Biofilme
0	1,1x10 ⁹	1,4x10 ⁹	Planctônica
20	1,2x10 ⁹	3,6x10 ⁶	Planctônica
20	8,2x10 ⁸	3,0x10 ⁶	Planctônica
50	1,2x10 ⁹	0	Planctônica

Nickel et al., Antimicrob. Agents Chemother., 27, 619-624, (1985).

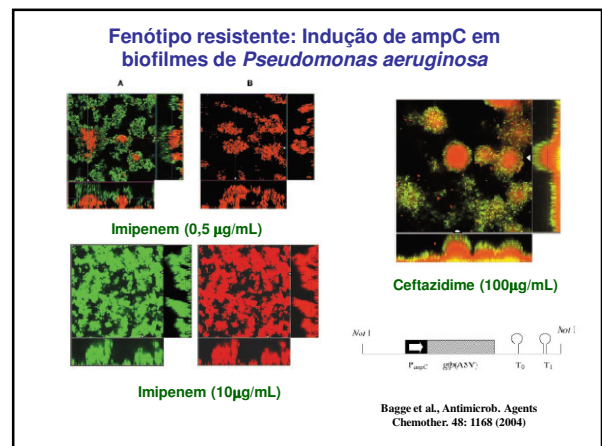
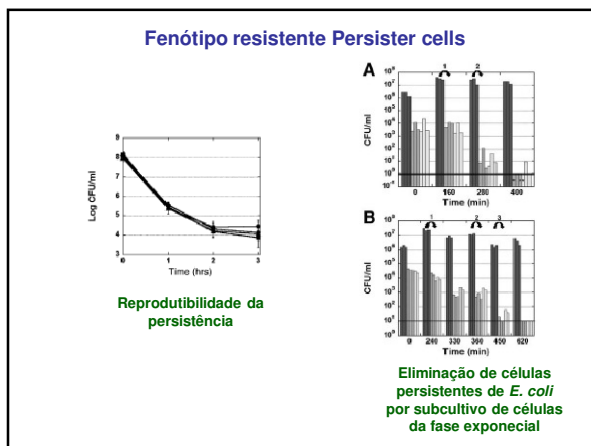
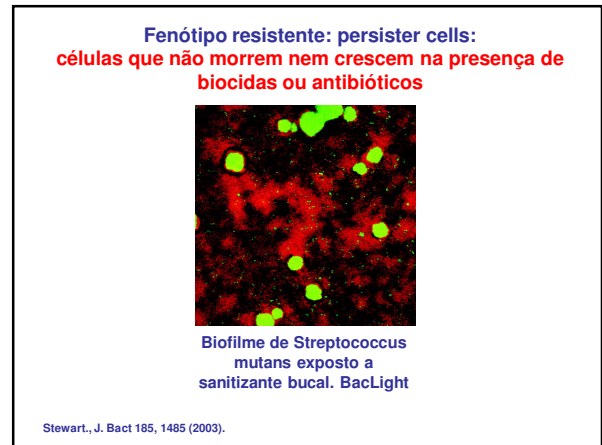
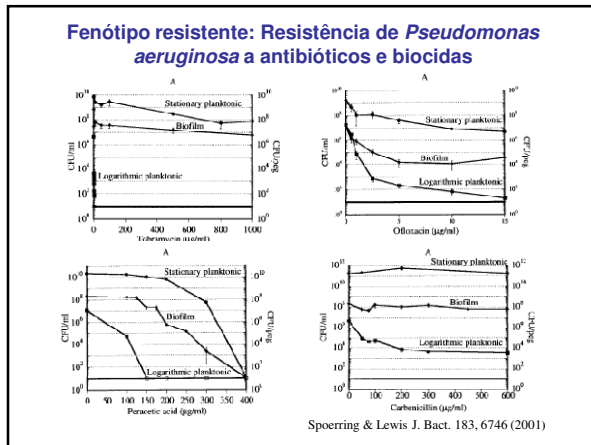




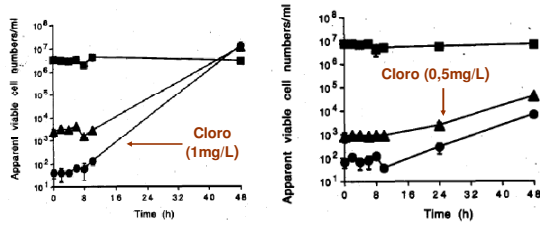
Biofilmes: resistência a antibióticos: Efeito de campo elétrico na eficiência do antibiótico

Antibiótico (mg/ml)	Corrente ($\times 10^3$ A)	UFC final
1	0	$1,11 \times 10^8$
1	1	$8,63 \times 10^7$
5	0	$2,67 \times 10^7$
5	1	0

Wellman et al., Antimicrob. Agents Chemother. 40:2012-2114 (1996).



Recuperação de *Klebsiella oxytoca* após exposição ao cloro

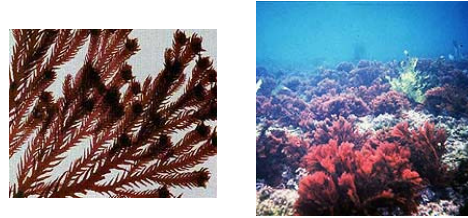


Células cultivadas sob limitação de carbono

Células cultivadas em TSB

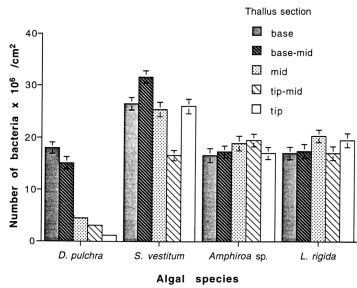
Power et al. Water Res. 31, 135 (1997)

Controle de Biofilmes pela inibição de processos de QS: as furanonas



Delisea pulchra

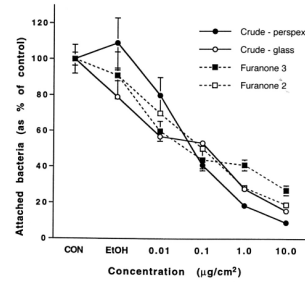
Controle de Biofilmes pela inibição de processos de QS: as furanonas



Número de bactérias nas superfícies de algas: *Delisea pulchra*, *Sargassum vestitum*, *Amphiroa sp.* and *Laurencia rigida*.

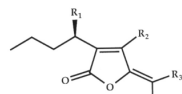
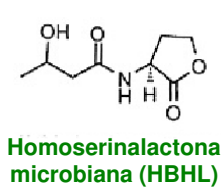
Maximilien et al., Aquat. Microb. Ecol. 15: 233 (1998)

Redução da adesão de bactérias por extratos crus e furanonas purificadas de *Delisea pulchra*.



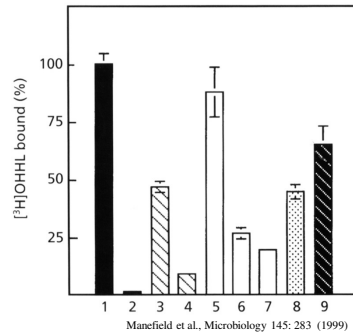
Maximilien et al., Aquat. Microb. Ecol. 15: 233 (1998)

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Compound	R1	R2	R3	R4
1	H	Br	Br	Br
2	H	Br	H	Br
3	Oac	Br	H	Br
4	OH	Br	H	Br
5	Oac	Br	H	I
6	H	H	Br	Br

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In vivo displacement of $[^3\text{H}]\text{OHHL}$ from *E. coli* cells overproducing LuxR. (1) 0.5µM $[^3\text{H}]\text{OHHL}$ alone; 3-9: 0.5µM $[^3\text{H}]\text{OHHL}$ + (3) 0.5µM OHHL, (4) 5µM OHHL, (5) 10µM compound 4, (6) 50µM compound 4, (7) 100µM compound 4, (8) 100µM compound 2 and (9) 100µM compound 8. (2): *E. coli* treated with 0.5µM $[^3\text{H}]\text{OHHL}$ alone.

Manefield et al., Microbiology 145: 283 (1999)

Furanonas aceleram a degradação da proteína LuxR

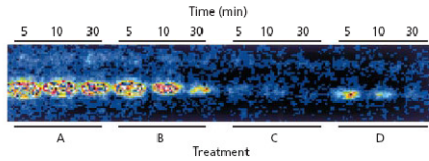
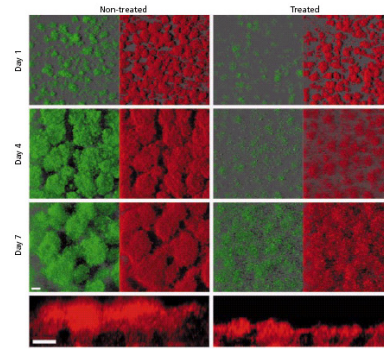


Fig. 6. Western blot following the decay of the LuxR protein over time (5–30 min) in XL-1 Blue (pH7.2) (pGFP::30) cultures in the presence and absence of 3-oxo-C6-HSL and compound 30. Treatments are: A, an untreated negative control; B, 2 μM 3-oxo-C6-HSL; C, 10 μM compound 30 + 2 μM 3-oxo-C6-HSL for that order; and D, 2 μM 3-oxo-C6-HSL + 10 μM compound 30 (in that order). Addition of 3-oxo-C6-HSL prior to treatment with the halogenated furanone protects the protein from degradation. This blot is representative of three separate experiments.

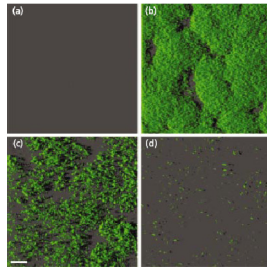
Manefield et al., Microbiology 148: 1119 (2002)



Efeito de furanona 56 na formação de biofilme de *Pseudomonas aeruginosa* PAO1 (reporter *lasB*)

Hentzer et al., (2002)

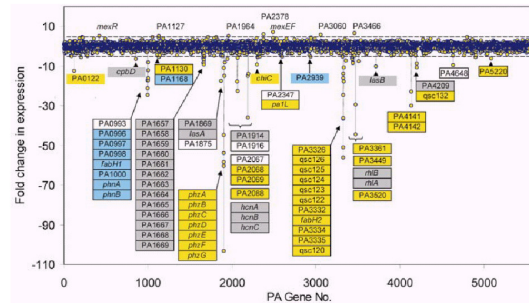
Inibição da expressão de *Vibrio fischerii* luxR (em *Pseudomonas aeruginosa* PAO1) pela furanona 56



- a: sem indução do luxR;
- b: 2h após adição de 250nM OHHL ao meio;
- c: 2h após adição de 15μM furanona,
- d: 2h após adição de 15mM furanona

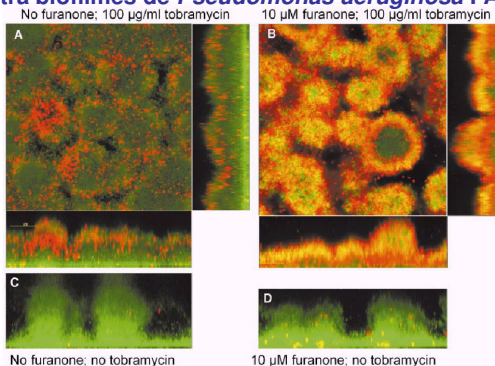
Hentzer et al., (2002)

Efeitos de furanona C-30 na expressão de genes de *Pseudomonas aeruginosa* PAO1.



Hentzer et al., (2003)

Efeitos de furanona C-30 na eficácia de antibióticos contra biofilmes de *Pseudomonas aeruginosa* PAO1.



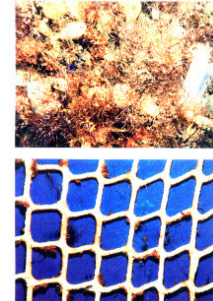
Hentzer et al., (2003)

Controle de Biofilmes pela inibição de processos de QS: as furanonas

Redes expostas durante 105 dias ao meio ambiente em fazendas de criação de salmão na Tasmânia

controle

Rede coberta com polímero contendo furanonas (Wattyl NetClear antifouling)



Lactonases: Enzimas que degradam HSL

Table 1. Occurrence of AHL degradation enzymes in prokaryotes and eukaryotes

Species	Gene*	Enzyme	Reference
Prokaryotes			
<i>Bacillus</i> sp. 240B1	<i>ahA</i>	AHL lactonase	Dong <i>et al.</i> , 2000
<i>B. thuringiensis</i>	<i>ahA</i> homologues	AHL lactonase	Dong <i>et al.</i> , 2002; Lee <i>et al.</i> , 2002
<i>B. cereus</i>	<i>ahA</i> homologues	AHL lactonase	Dong <i>et al.</i> , 2002; Reinmann <i>et al.</i> , 2002
<i>B. mycoides</i>	<i>ahA</i> homologues	AHL lactonase	Dong <i>et al.</i> , 2002
<i>B. anthracis</i>	<i>ahA</i> homologues	AHL lactonase	Ulrich, 2004
<i>Agrobacterium tumefaciens</i>	<i>ahM</i> , <i>ahB</i>	AHL lactonase	Zhang <i>et al.</i> , 2002; Carlier <i>et al.</i> , 2003
<i>Arthrobacter</i> sp. BEN110	<i>ahD</i>	AHL lactonase	Park <i>et al.</i> , 2003
<i>Erwinia pneumoniae</i>	<i>ahK</i>	AHL lactonase	Park <i>et al.</i> , 2003
<i>Hericortia paradonata</i> VAI-C	ND	AHL acylase?	Leadsitter and Greenberg, 2000
<i>Ralstonia</i> strain XJ12B	<i>ahD</i>	AHL acylase	Lin <i>et al.</i> , 2003; Hu <i>et al.</i> , 2003
<i>Pseudomonas</i> strain PA1-A, <i>P. aeruginosa</i> PAO1	<i>pruQ</i>	AHL acylase	Huang <i>et al.</i> , 2003.
Eukaryotes			
Human (airway epithelia)	<i>PQN3</i>	Lactonase	Chan <i>et al.</i> , 2003; Greenberg <i>et al.</i> , 2004
Porcine (kidney)	<i>ACT1</i>	Acylase I	Xu <i>et al.</i> , 2003

*ND, not determined.

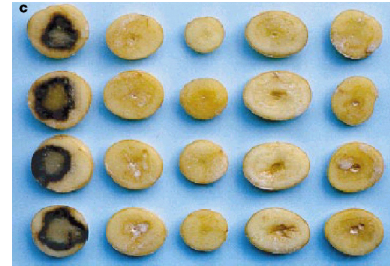
Batatas transgênicas transformadas com HSL-lactonase, infectadas com *Erwinia carotovora* SCG1

25.000 CFU

50.000 CFU

75.000 CFU

100.000 CFU



Dong *et al.*, (2001)