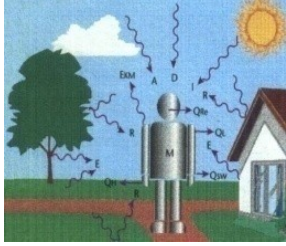
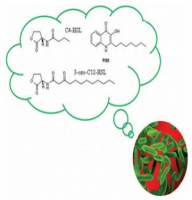
5TH APIQUALITY & 6TH APIMEDICA

INTERNATIONAL SYMPOSIUM

ROME, 22-25 NOVEMBER 2016



Propolis: Is there a potential for the development of new and efficient antimicrobial agents ?



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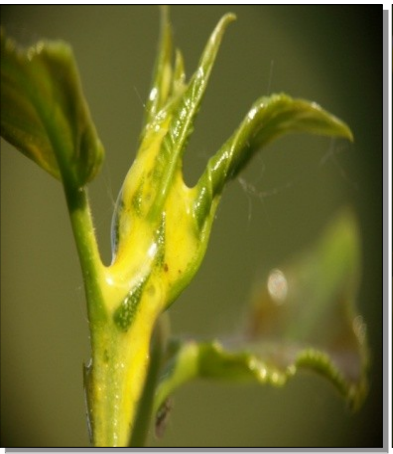
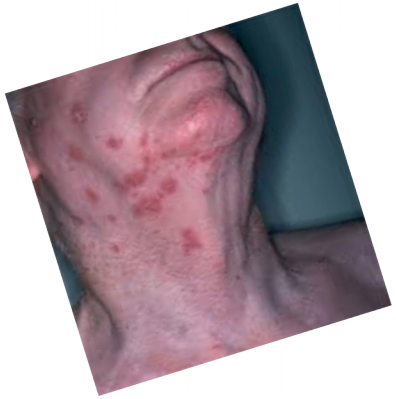
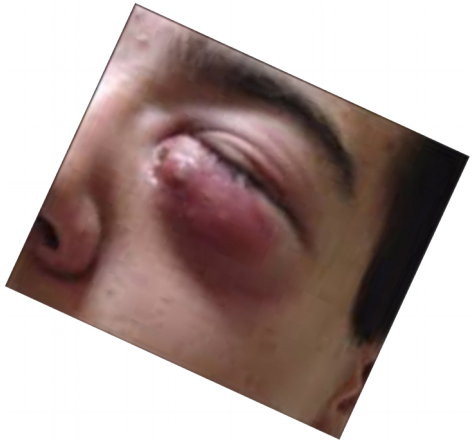


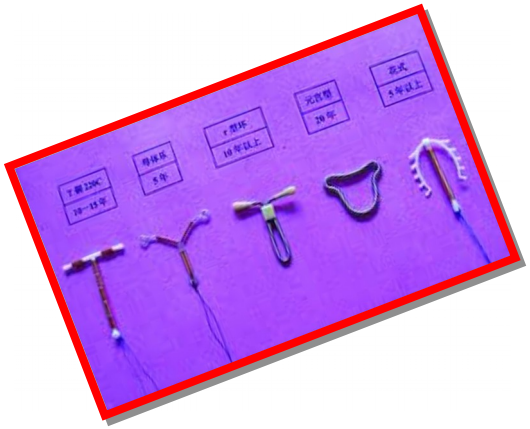


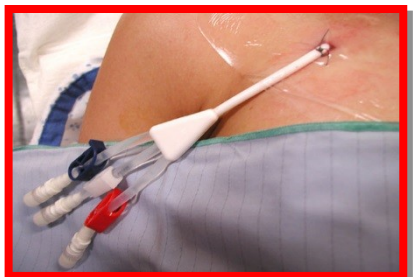
Recent public announcements stated that 60% to 85% of all microbial infections involve biofilms developed on natural intact or damaged tissues (skin, mucosa, endothelial epithelia and teeth)

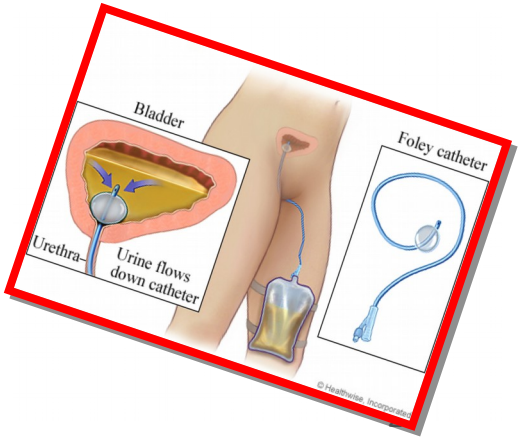




or artificial devices: (central venous catheters, peritoneal, urinary catheters, dental materials, cardiac valves, intrauterine contraceptive devices, contact lenses and other implants)







The insertion of the prosthetic medical devices for different exploratory or therapeutically purposes especially in severe pathological conditions represents a risk factor for the occurrence of chronic infections in developed countries.

being characterized by slow onset, middle intensity symptoms, chronic evolution and **resistance to antibiotic treatment**

molecules

MDPI

Article

Impact of Biohybrid Magnetite Nanoparticles and Moroccan Propolis on Adherence of Methicillin Resistant Strains of *Staphylococcus aureus*

Abstract: Biofilm bacteria are more resistant to antibiotics than planktonic cells. Propolis possesses antimicrobial activity. Generally, nanoparticles containing heavy metals possess antimicrobial and antibiofilm properties. In this study, the ability of adherence of Methicillin Resistant Strains of *Staphylococcus aureus* (MRSA) to catheters treated with magnetite nanoparticles (MNPs), produced by three methods and functionalized with oleic acid and a hydro-alcoholic extract of propolis from Morocco, was evaluated. The chemical composition of propolis was established by gas chromatography mass spectrometry (GC-MS), and the fabricated nanostructures characterized by X-ray diffraction (XRD), transmission electron microscopy (TEM), Mossbauer spectroscopy and Fourrier transform infrared spectroscopy (FTIR). The capacity for impairing biofilm formation was dependent on the strain, as well as on the mode of production of MNPs. The co-precipitation method of MNPs fabrication using Fe^{3+} and Na_2SO_3 solution and functionalized with oleic acid and propolis was the most effective in the impairment of adherence of all MRSA strains to catheters ($p < 0.001$). The adherence of the strain MRSA16 was also significantly lower ($p < 0.001$) when the catheters were treated with the hybrid MNPs with oleic acid produced by a hydrothermal method. The anti-MRSA observed can be attributed to the presence of benzyl caffeate, pinocembrin, galangin, and isocupressic acid in propolis extract, along with MNPs. However, for MRSA16, the impairment of its adherence on catheters may only be attributed to the hybrid MNPs with oleic acid, since very small amount, if any at all of propolis compounds were added to the MNPs.

Keywords: co-precipitation; hydrothermal; functionalization; propolis; flavonoids; diterpenes

Badiaa Lyoussi , Soukaina El-Guendouz , Smail Aazza ,, Vassya Bankova , Maria G. Miguel, Molecules, 2016

Table 1. Chemical composition of hydro-alcoholic extract of Moroccan propolis.

Aromatic Acids	**%**	**Phenolic Acid Esters**	**%**	**Flavonoids**	**%**	**Diterpenes**	**%**	**Sugars and Sugar Derivatives**	**%**	**Fatty Acids**	**%**	
Benzoic acid	0.4	Pentenyl *p*-coumarate	0.7	Pinostrobin chalcone	2.7	Ferruginol	1.2	Monosaccharides	0.4	Hexadecanoic acid	-	
Hidroxybenzoic acid	0.1	Isopentenyl caffeate	1.8	Pinocembrin chalcone	5.9	Communic acid	2.7	Disaccharides	-	Octadecanoic acid	1.0	
Cinnamic acid	0.3	Pentenyl caffeate	0.9	Pinocembrin	7.4	Totarol	1.1	Glycerol	0.1	Octadecenoic acid	0.5	
p-Coumaric acid	0.3	Dimethylallyl caffeate	1.2	Pinobanksin	3.6	Imbricataloic acid	3.2	Inositol	Tr	Tetracosanoic acid	-	
Dimethoxycinnamic acid	0.6	Pentenyl ferulate	0.9	Pinobanksin 3-*O*-acetate	3.4	13-*epi*-Cupressic acid	2.2	Total	0.5	Total	1.5	
Ferulic acid	0.4	Benzyl ferulate	1.7	Galangin	5.3	Ferruginolon	1.2					
Isoferulic acid	0.4	Benzyl *p*--coumarate	1.3	Chrysin	3.6	Dehydroabietic acid	Tr					
Caffeic acid	0.8	Benzyl caffeate	4.7	Total	31.9	Isocupressic acid	8.1					
Total	3.3	Caffeic acid phenetyl ester	1.7			Junicedric acid	1.8					
		Cinnamyl ferulate	0.4			Total	21.5					
		Cinnamyl caffeate	1.2									
		Total	16.5									

Standard deviation does not succeed 6% for any of the constituents

Chemical composition of the propolis

Phenolic acid esters	**%**	**Flavonoids**	**%**	**Diterpenes**	**%**
Pentenyl *p*-coumarate	0.7	Pinostrobin chalcone	2.7	Ferruginol	1.2
Isopentenyl caffeate	1.8	Pinocembrin chalcone	5.9	Communic acid	2.7
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Benzyl caffeate	4.7	**Total**	**31.9**	Isocupressic acid	8.1
Caffeic acid phenetyl ester	1.7			Junicedric acid	1.8
Cinnamyl ferulate	0.4			**Total**	**21.5**
Cinnamyl caffeate	1.2				
Total	**16.5**				

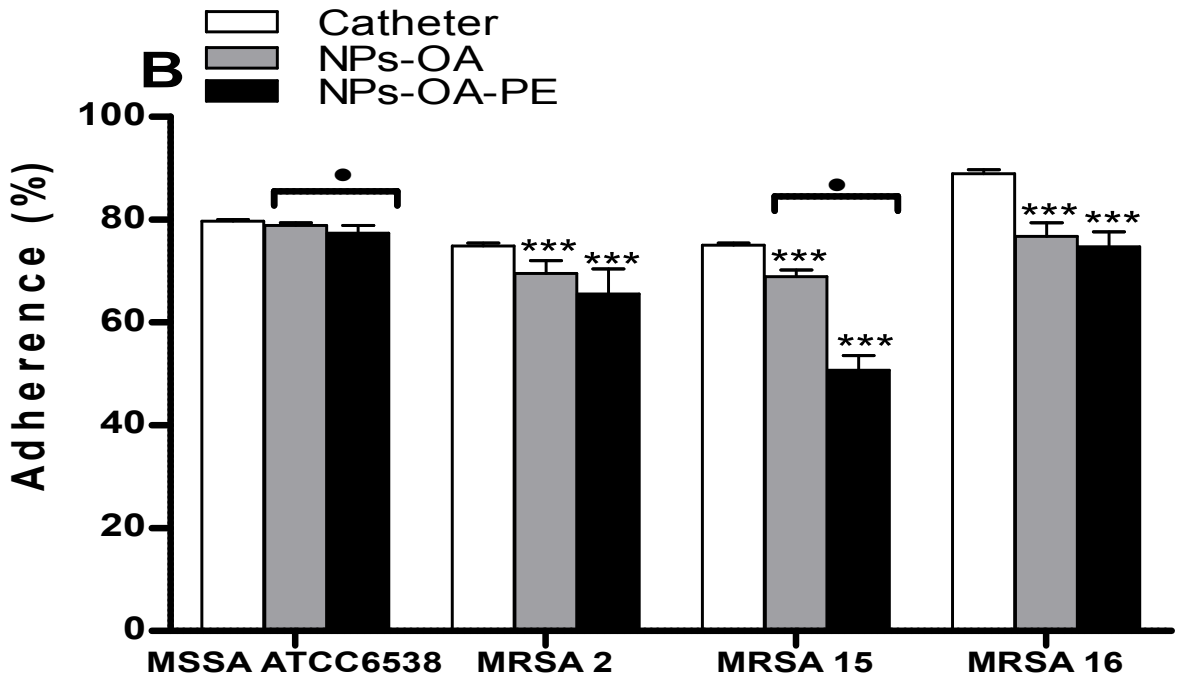


Figure . Percentage of adherence of microorganisms on catheter after contact with functionalized MNPs obtained by different methods: (**A**) Method #1; (**B**) Method #2; and (**C**) Method $3. OA—oleic acid, PE—propolis extract, Data represent the mean ± S.D from two separated experiments, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ ($n = 6$), statistically significant when compared with catheter not submitted to any treatment. Square brackets indicate the use of One Way ANOVA for each group. • $p < 0.05$ ($n = 6$).

The antibacterial properties of propolis have been correlated with the presence of: flavonoids, phenolic compounds and aromatic acids in general ...

Table 3 Solvents used for active component extraction. Compounds in bold are commonly obtained only in one solvent [adapted from 34].

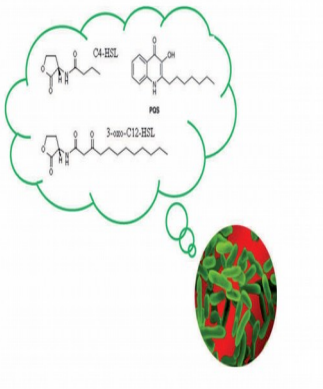
Water	Methanol	Ethanol	Chloroform	Dichloromethane	Ether	Acetone
Anthocyanins	Anthocyanins, Terpenoids,	Tannins	Terpenoids	Terpenoids,	Alkaloids	Flavonols
Starches	Saponins, Tannins,	Polyphenols	Flavonoids	Tannins,	Terpenoids	
Tannins	**Xanthoxylline, Totarol,**	**Polyacetyle**		Polyphenols,	**Coumarins**	
Saponins	**Quassinoids, Lactones,**	**nes**		**Polyacetylenes**,	**Fatty acids**	
Terpenoids	Flavones, **Phenones,**	Flavonols		Flavonols,		
Polypeptides	Polyphenols, **Polypeptides,**	Terpenoids		**Sterols**,		
Lectins	**Lectins**	**Sterols**		Alkaloids		
		Alkaloids				

However, mechanisms of action are not yet elucidated

The antibacterial properties of propolis: Two strategies

1. By affecting bacterial growth :

Bactericidal and / or bacteriostatic



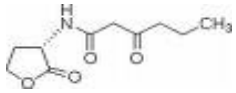
2. Not affecting bacterial growth :

Compounds that affect bacterial virulence: ***anti-quorum sensing***

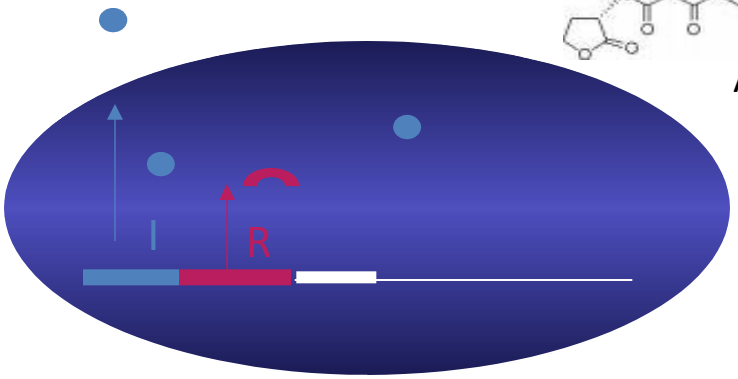
Compounds that affect **the bacterial lifestyle: *anti-biofilm***

Bacteria can communicate with members of their own species and others to **coordinate their behavior** in response to cell density.

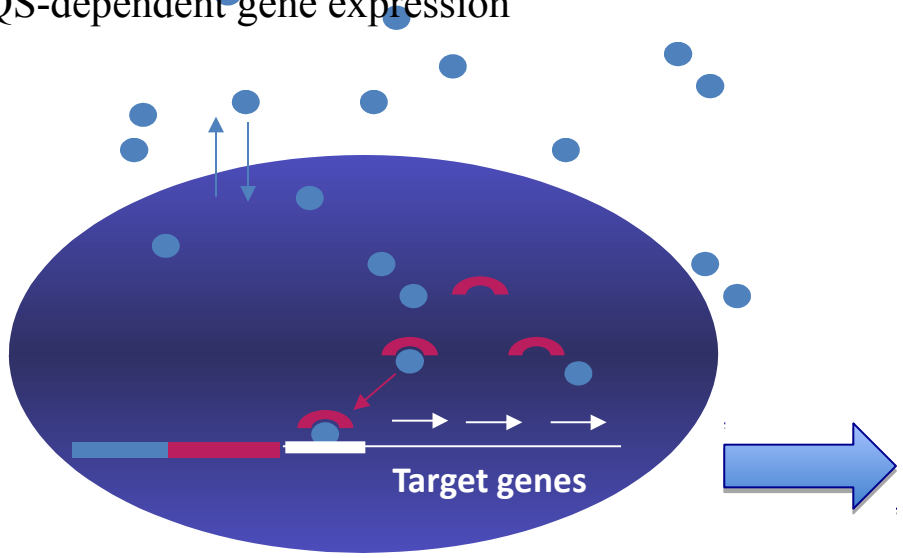
Low cell density
No QS-dependent gene expression

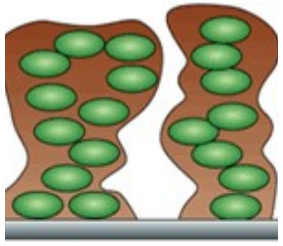


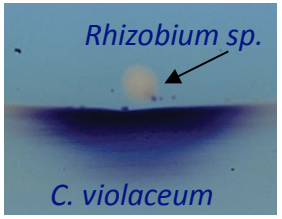
AHL



High cell density
QS-dependent gene expression





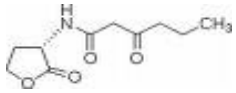


Bacterial responses to QS signals

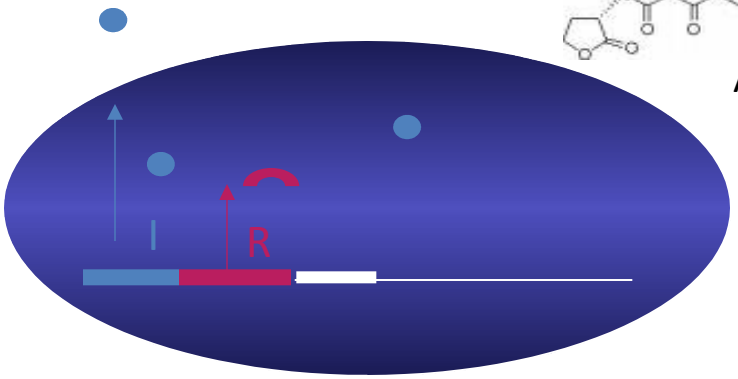
Virulence	Stress response
Biofilm dynamics	Protein folding
Plasmid transfer	EPS synthase
Motility	

Bacteria can communicate with members of their own species and others to **coordinate their behavior** in response to cell density.

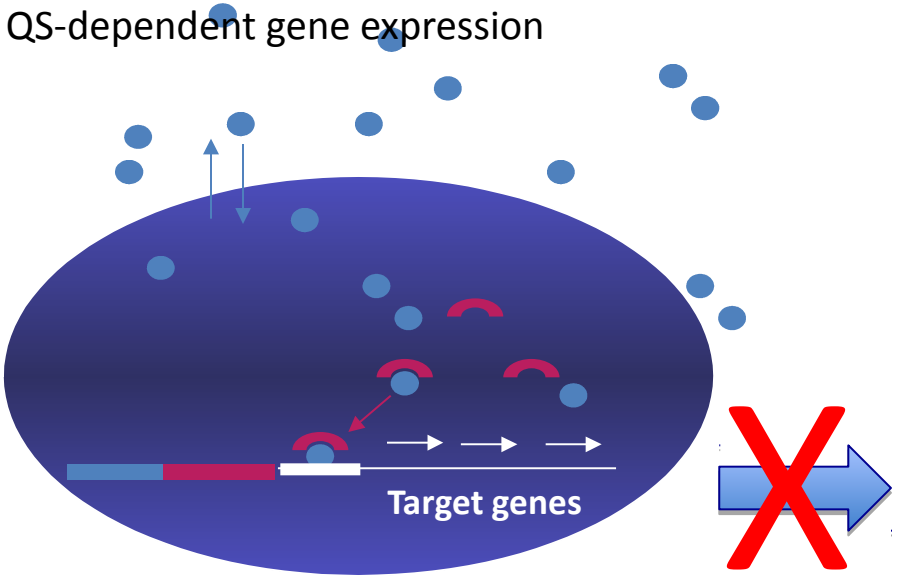
Low cell density
No QS-dependent gene expression

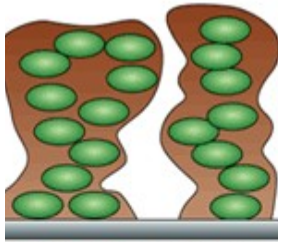


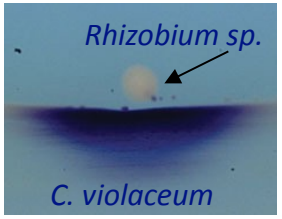
AHL



High cell density
QS-dependent gene expression





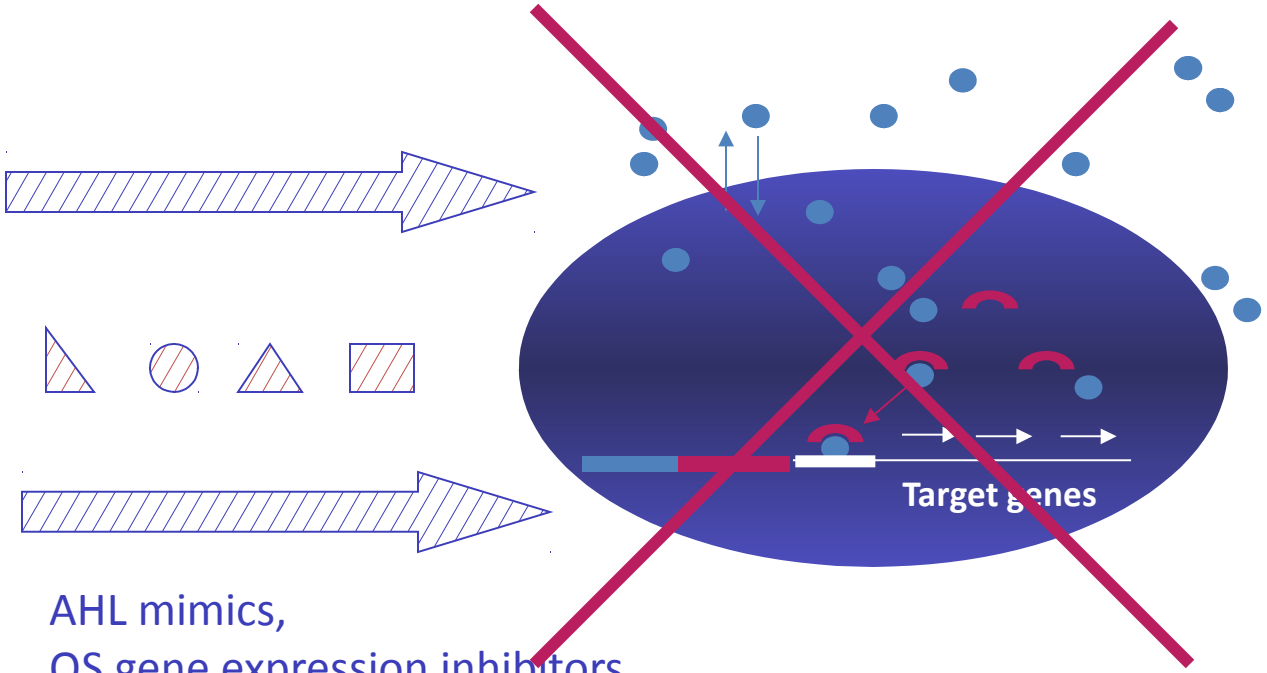


Bacterial responses to QS signals

Virulence	Stress response
Biofilm dynamics	Protein folding
Plasmid transfer	EPS synthase
Motility	

Bacterial response to host mimics

Propolis



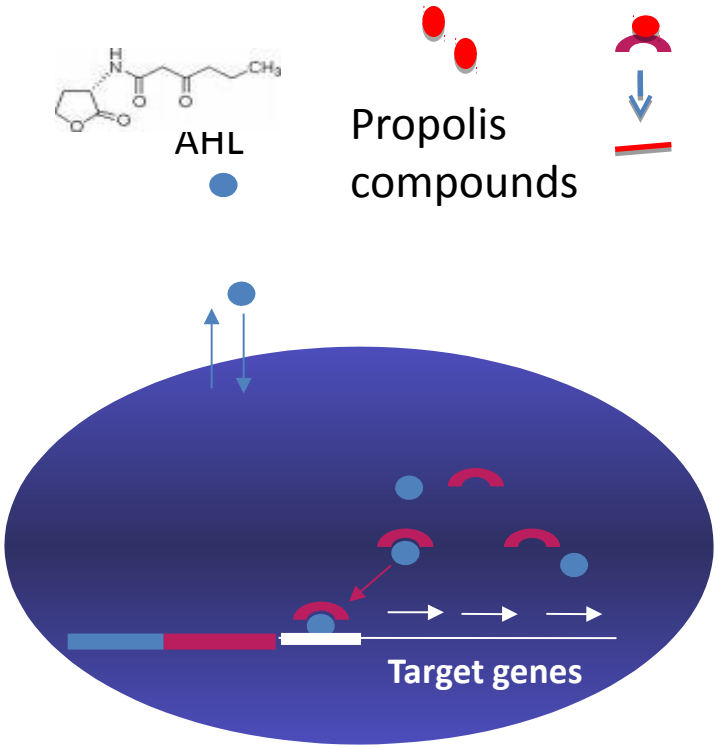
AHL mimics,
QS gene expression inhibitors
…

There is some evidence that **propolis** may be able to make equivalent (but chemically different) signals that can be detected by the bacterial communication systems and in some cases can **interfere with the bacterial conversations** and **even inhibit virulence gene expression.**

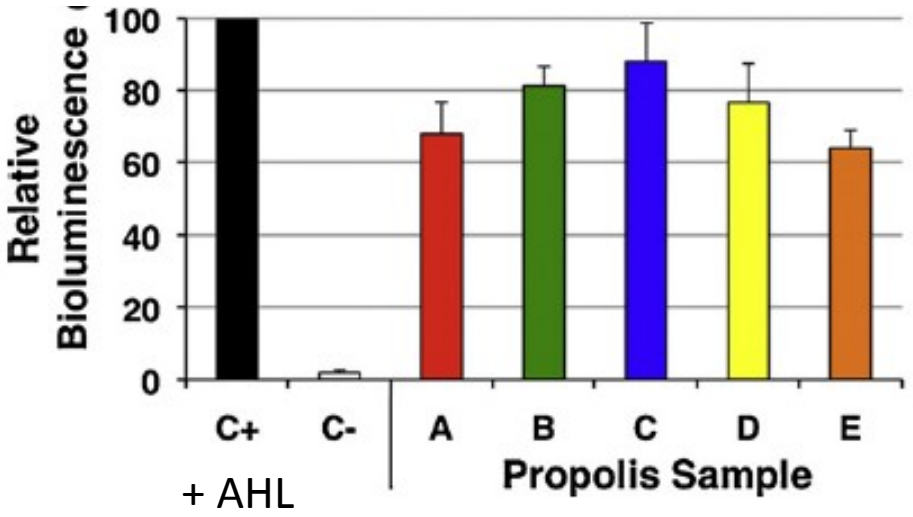
A novel property of propolis (bee glue): Anti-pathogenic activity by inhibition of *N*-acyl-homoserine lactone mediated signaling in bacteria

Zackery Bulman, Phuong Le, André O. Hudson, Michael A. Savka*

Molecular Bioscience and Biotechnology Program, School of Life Sciences, Rochester Institute of Technology, Rochester, NY 14623, USA



Propolis contains compounds that suppress QS responses



Propolis constituent(s) antagonize signaling in *lasR*-dependent biosensors

Journal of Ethnopharmacology 138 (2011) 788–797

P. aeruginosa biofilm lifestyle

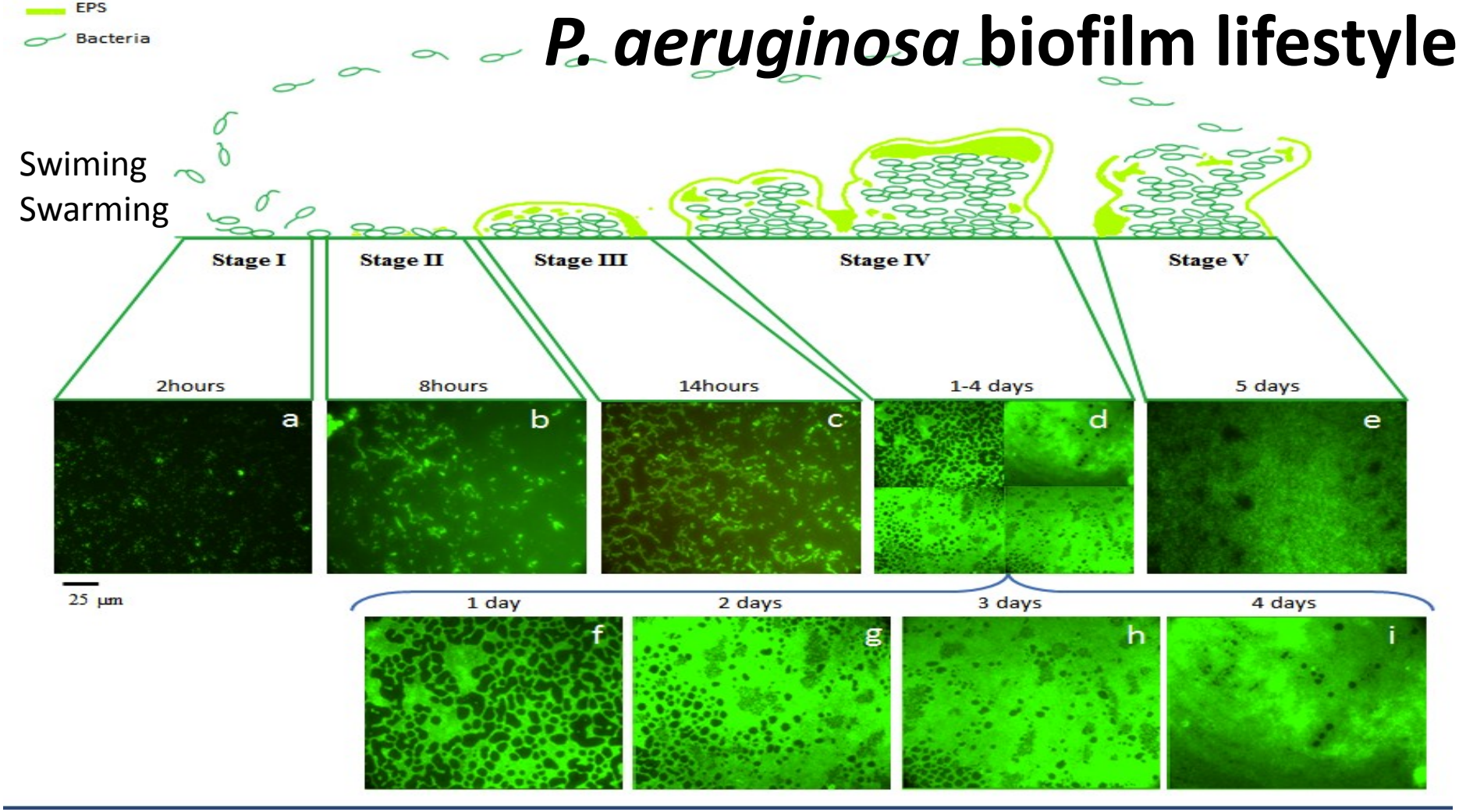
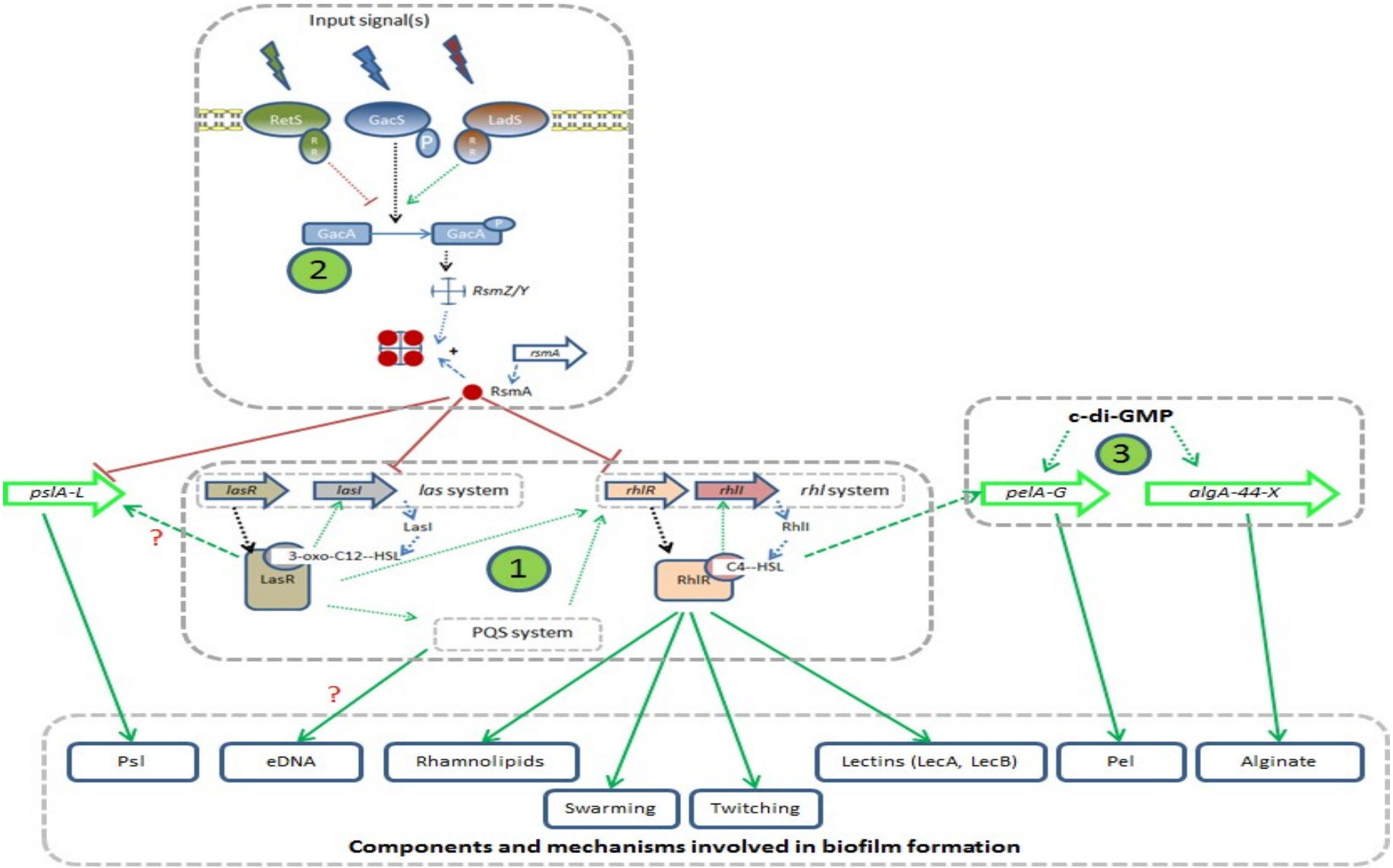


Figure 1: Biofilm lifestyle cycle of P. aeruginosa grown in glucose minimal media. In **stage I**, planktonic bacteria initiate attachment to an abiotic surface, which becomes irreversible in **stage II**. **Stage III** corresponds to microcolony formation. **Stage IV** corresponds to biofilm maturation and growth of the three-dimensional community. Dispersion occurs in **stage V** and planktonic bacteria that are released from the biofilm to colonize other sites. The biofilm formation by P. aeruginosa PAO1 was revealed with Syto9 and visualized in Leica DM IRE2 inverted fluorescence microscope with 400x magnification at 2 h (Stage I), 8 h (Stage II), 14 h (Stage III), 1 to 4 days (Stage IV), and 5 days(Stage V). Images represent a 250×250-□□field.

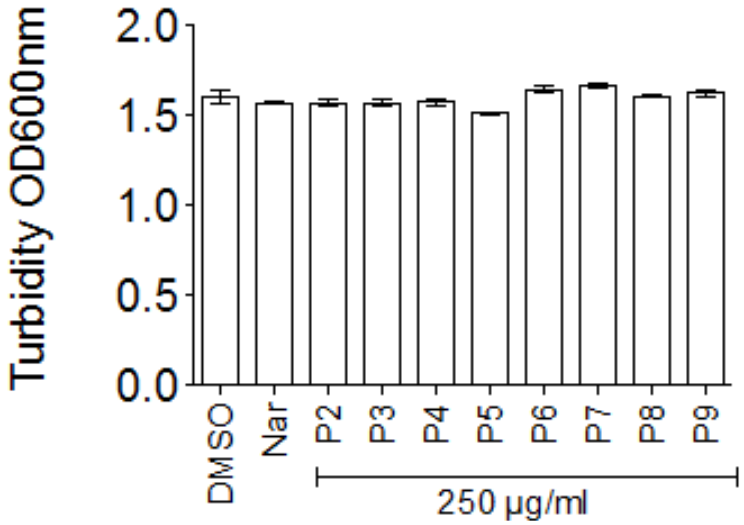
Razamiravaka & El Jaziri 2014

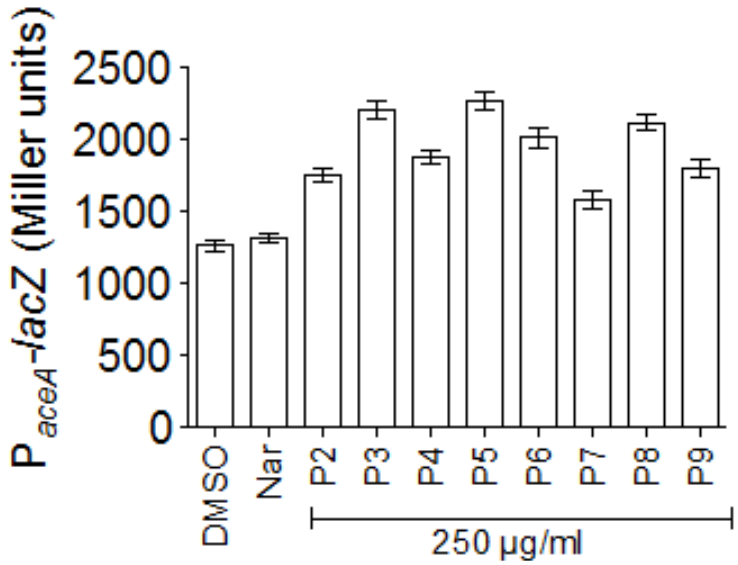
Quorum sensing and biofilm regulation in *Pseudomonas aeruginosa*



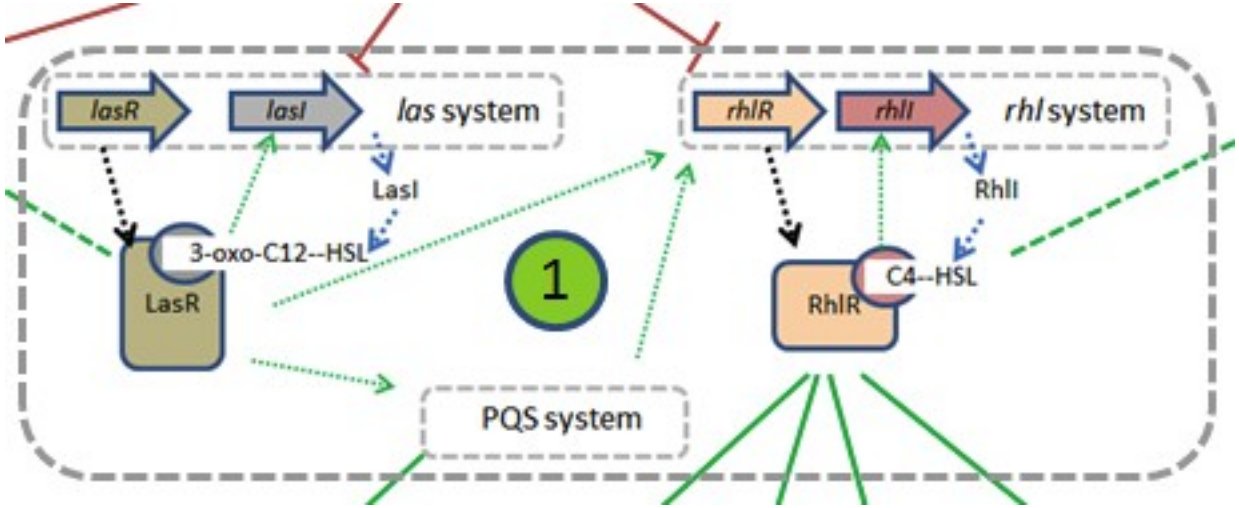
Razamiravaka & El Jaziri 2014

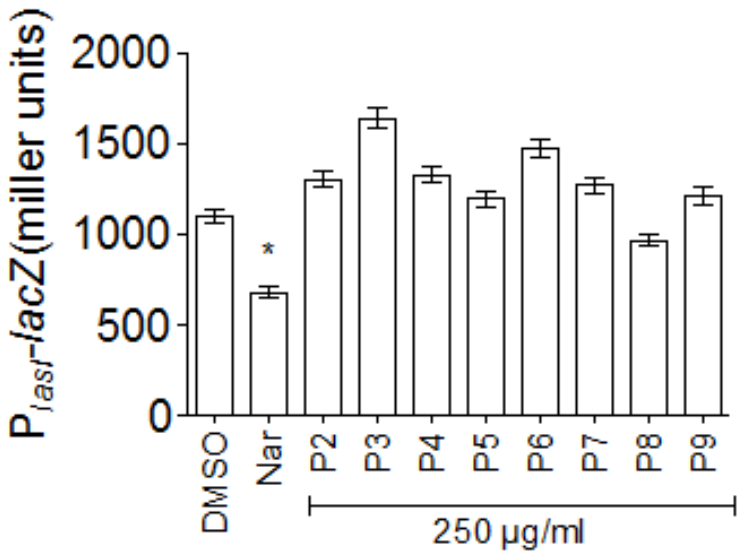
P7 have no effect on growth and on gene transcription in *P. aeruginosa*

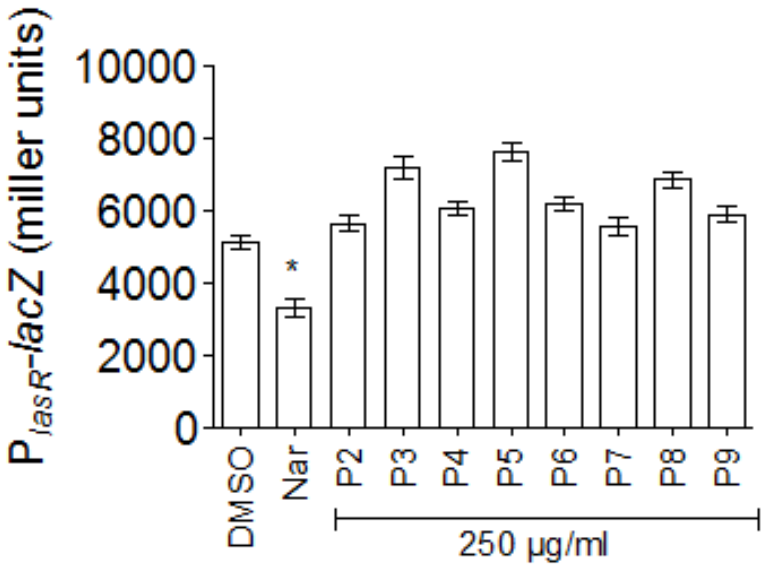




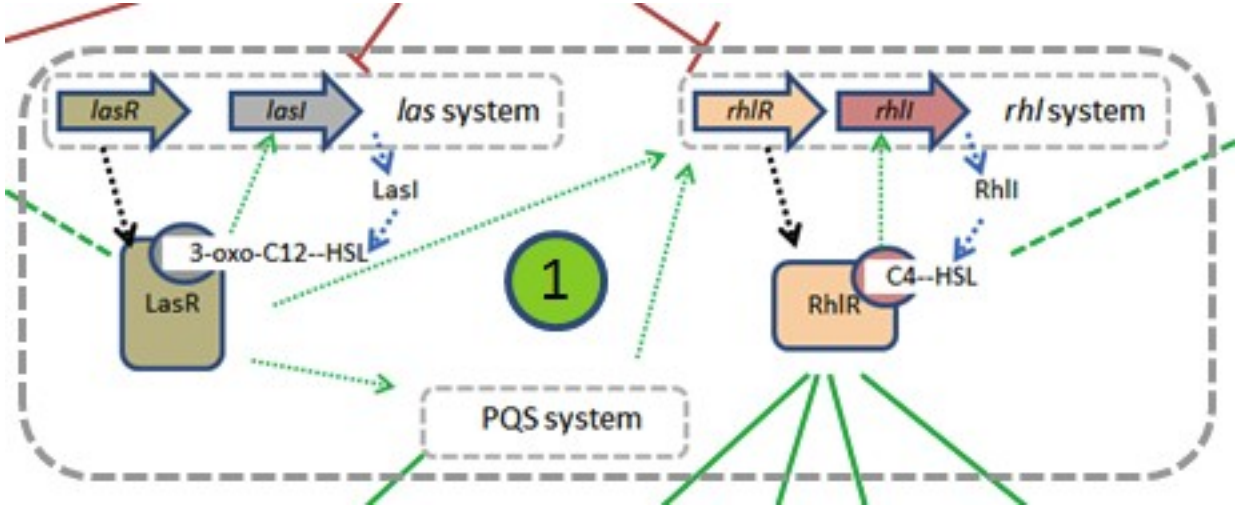
P7 have no effect on *lasI/R* system in *P. aeruginosa*

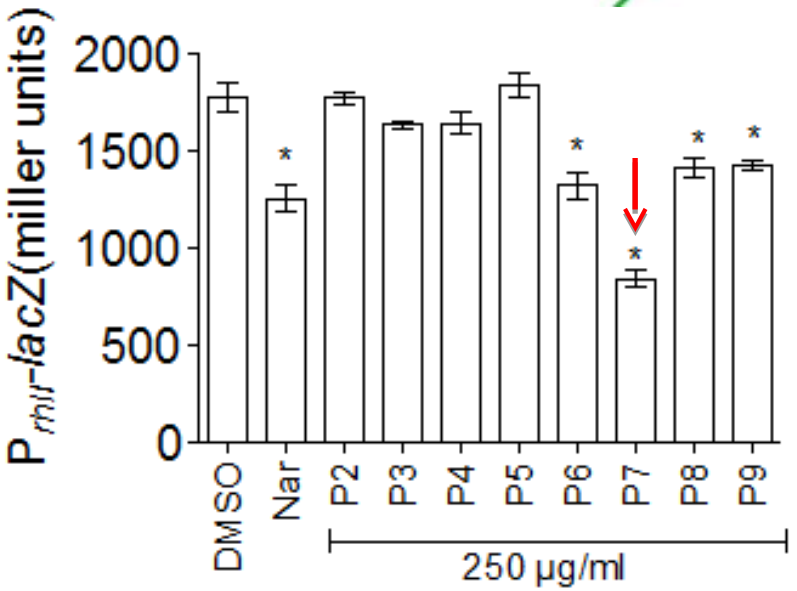


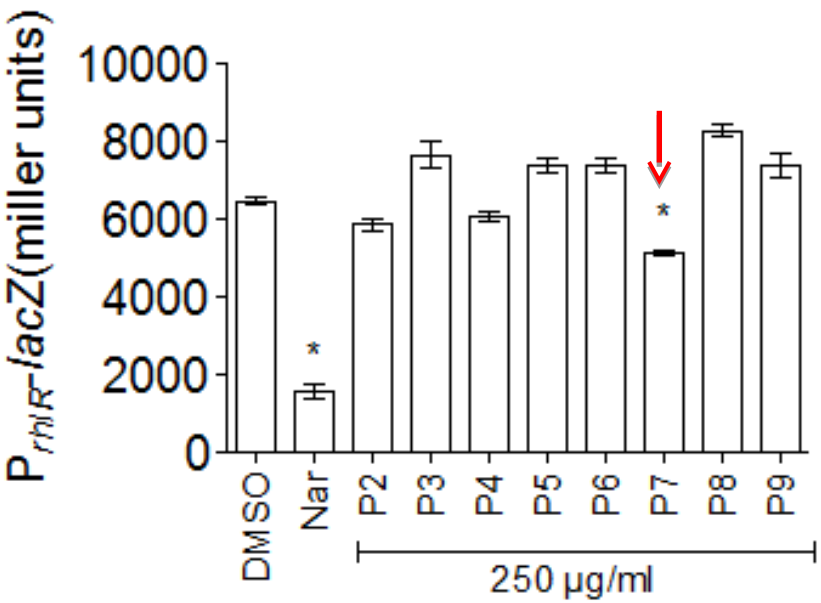




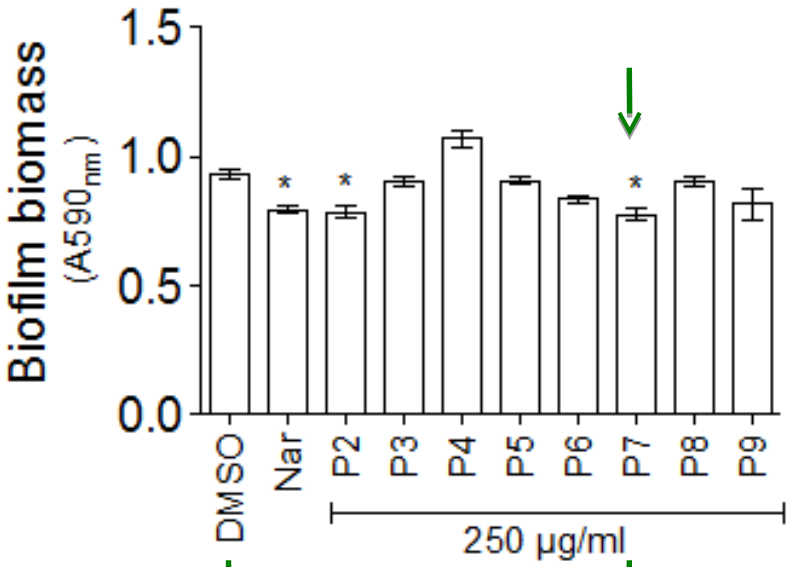
P7 affects *rhlI/R* system in *P. aeruginosa*



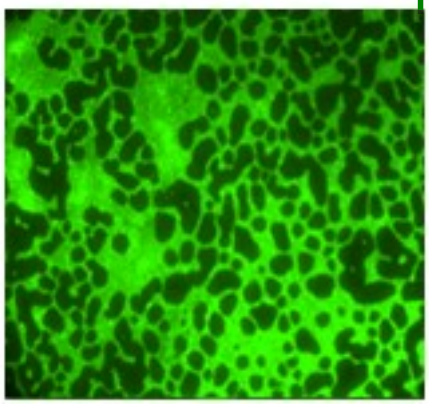




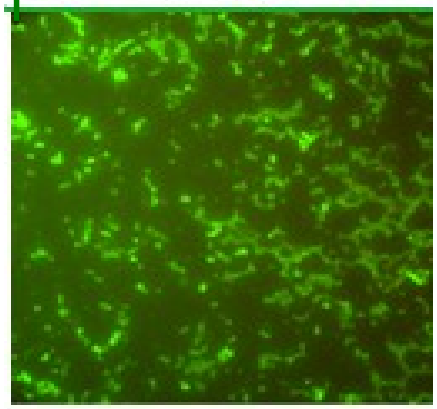
P7 reduces biofilm production in *P. aeruginosa*



Affects biofilm lifecycle/maturation



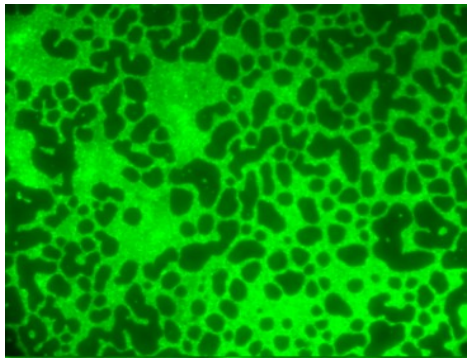
Biofilm (2 x 24 h)



Biofilm (24 h)
treated with P7 for 24 h

P7 improves tobramycine penetration in *Pseudomonas aeruginosa* biofilm

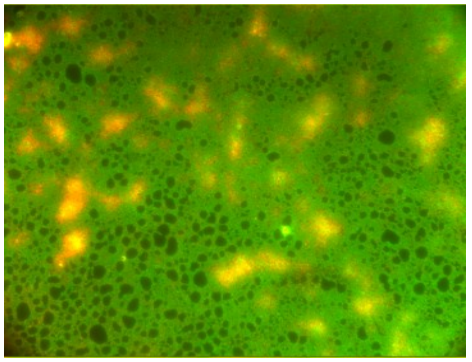
(a)



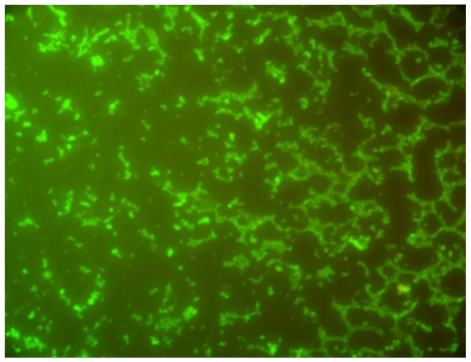
One-day old culture
+DMSO at culture initiation

+Tobramycin (100 µg/ml)

(b)

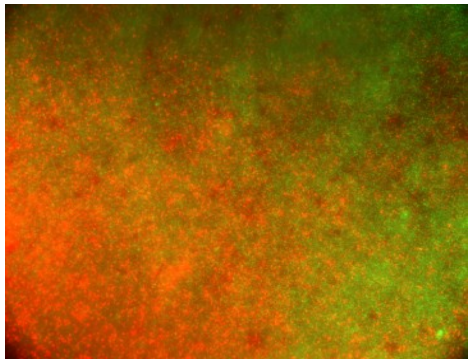


Two-day old culture



One-day old culture
+ P7 at culture initiation

+Tobramycin (100 µg/ml)



Two-day old culture

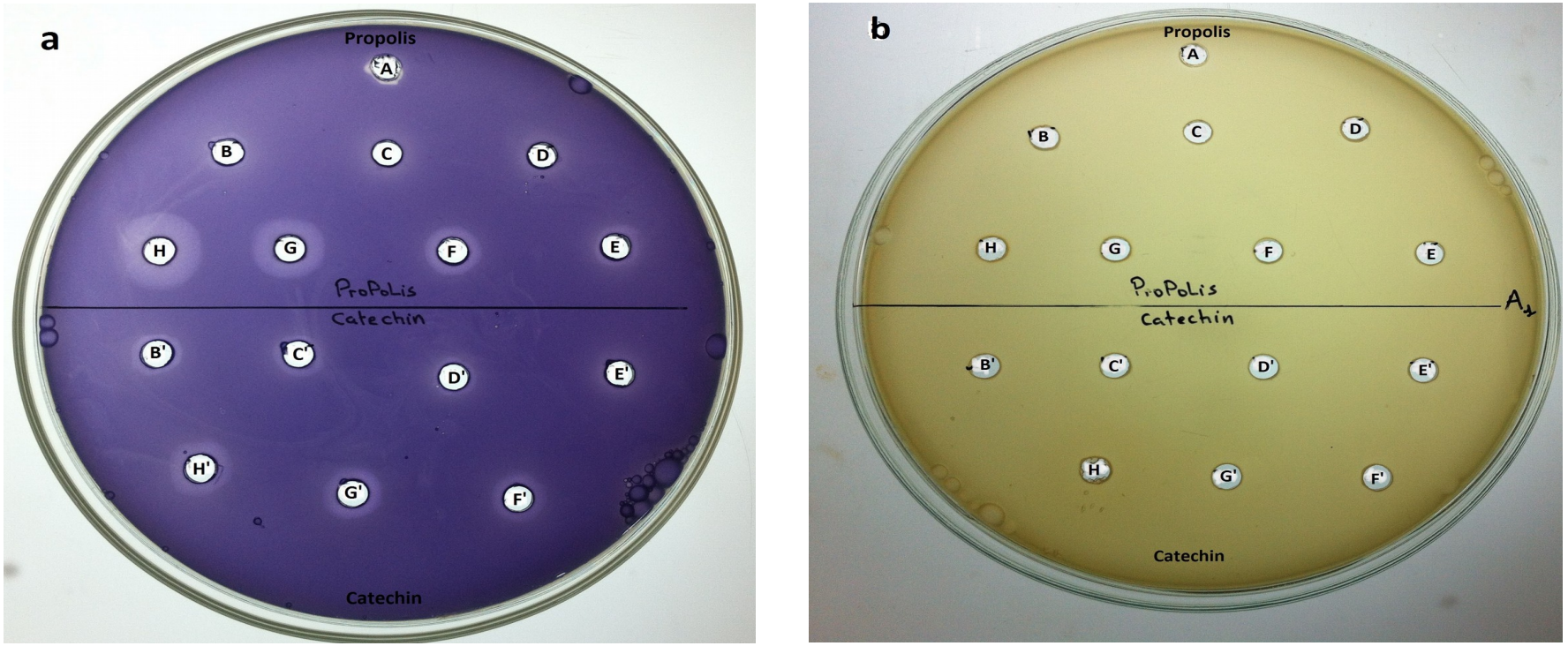


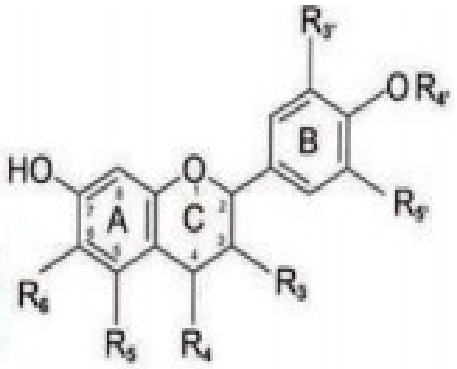
Figure 4. Anti-QS properties of propolis and catechine. (**A**): ethanol 70%; (**B**): propolis at 0.24 mg/ml; (**C**): propolis at 0.36 mg/ml; (**D**): propolis at 0.49 mg/ml; (**E**): propolis at 0.61mg/ml; (**F**): propolis at 0.73 mg/ml; (**G**): propolis at 0.98 mg/ml; (**H**): propolis at 1.22 mg/ml. (**B'**): (+)-catechin at 0.24 mg/ml; (**C'**): (+)-catechin at 0.36 mg/ml; (**D'**): (+)-catechin at 0.49 mg/ml; (**E'**): (+)-catechin at 0.61mg/ml; (**F'**): (+)-catechin at 0.73 mg/ml; (**G'**): (+)-catechin at 0.98 mg/ml; (**H'**): (+)-catechin at 1.22 mg/ml.

The anti-QS activity of propolis extract against bacterial QS was determined using violacein production by *Chromobacterium violaceum*. Loss of purple pigment in *C. violaceum* indicates the inhibition of QS by the propolis extract. Control wells containing catechin and ethanol were included. The seven of propolis showed halo zone on the purple background. Therefore the highest inhibition zone was observed for the concentration (**H** in the picture). No inhibition was apparent with ethanol . (b) showed no growth inhibition zone at all tested concentration of propolis, this is demonstrate that propolis extract has not affect the growth of *C. violaceum* CV026.

❖ In this study, we analyzed several propolis samples from different geographic regions of Morocco for QSI activity that disrupts QS AHL bacterial communication mechanism, correlated the QSI activity of propolis with its chemical composition, and identified the flavonoid **pinocembrin** as a potential propolis active principle that disrupts AHL-dependent QS in bacteria.

❖ There are indications that additional flavonoids are important propolis constituents in this respect. It is obvious that propolis from Morocco deserves further studies as a promising source of compounds and compound mixtures in the search for new approaches for antipathogenic treatments of bacterial pathogens based on natural products.

The Flavanone naringenin reduced the production of virulence factors controlled by the QS mechanism in Pseudomonas aeruginosa PAO1



Propolis: Is there a potential for the development of new and efficient antimicrobial agents ?

- Propolis plays a key role in the prevention and control of bacterial invasions

- Propolis has an inhibitory effect on the expression of virulence genes of some pathogenic bacteria without affecting bacterial growth

- Propolis also affect the production of biofilm

This alteration of the biofilm architecture allowed a better penetration of tobramycin into the biofilm and increases the accessibility of the antibiotic to encapsulated bacteria in the extracellular matrix

شكرا..

Bee Healthy and Happy..