

IMPACT OF SALICYLIC ACID ON BIOFILM FORMATION BY PHYTOPATHOGENIC BACTERIA

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Most of bacteria commonly survive in nature by forming biofilms, surface-attached microbial agglomerations. Bacteria in such multicellular assemblages are protected from fluctuations in environmental conditions, antibiotics and host defenses by complex matrix. So it's not surprising that biofilm formation is a major virulence factor among human and plant pathogenic bacteria.

Biofilm formation is controlled by population density-dependent regulatory mechanism of cell-to-cell communication called quorum-sensing (QS). Such communication among Gram-negative bacteria involves acyl homoserine lactones (AHLs), small secreted molecules that can be self-recognized in dose-dependent manner, and a complex set of transcription factors of QS-controlled genes. QS plays a significant role in attachment of bacteria, biofilm development and dispersal.

A plant-produced phenolic compound salicylic acid (SA) is widely known as a primary plant immune response signal but yet little is known about its effect on production of virulence factors by phytopathogenic bacteria. In this study we tested the effect of SA on biofilm formation, swimming motility and AHL production by different plant pathogens.

The obtained data showed that SA showed strong inhibitory effect on the AHL production, swimming motility and biofilm formation by *Pectobacterium carotovorum* 29 and *Pseudomonas syringae* pv *syringae* 13. Taking into account the fact that there're structural similarities between SA and LasR regulator ligand (LasR is a transcriptional regulator of LasI/LasR QS system in *Pseudomonas aeruginosa*) we may suggest that SA inhibits swimming motility and biofilm production by inhibiting the activity of LuxR-like regulator in these bacteria. The reduced ability to produce AHLs may be explained by the fact that LuxR-like regulator up regulates *luxI* (AHL-synthase) gene expression, otherwise it remains basal and amplification of QS signal doesn't occur. However, it was surprising that SA inhibited swimming motility by *Pseudomonas corrugata* 3'M, while increasing its ability to form a biofilm. SA showed also no effect on AHL production by this bacterium, maybe due to its ability to utilize SA as a carbon source.

Neither biofilm formation nor swimming motility was affected by SA in *Erwinia amylovora* 1/79. Unfortunately, we couldn't study the effect of SA on its AHL production as no violacein production by the biosensor strain was

observed. Apparently, AHLs of *Erwinia amylovora* 1/79 strains have N-acyl side chains from C₁₀ to C₁₄ in length and thus they are unable to induce violacein production by *C. violaceum* CV026, which can only be used to detect AHLs with N-acyl side chains from C₄ to C₈.

The AHL production by *Xanthomonas campestris* pv *campestris* 2.5 was not detected either, which was not remarkable as it has been shown earlier that *Xanthomonas campestris* lacks AHL signaling system and uses diffusible signal factors as autoinducers. In our study, SA induced the swimming motility of this strain and had no particular effect on biofilm formation.

ИДЕНТИФИКАЦИЯ ФИТОПАТОГЕННЫХ МИКРООРГАНИЗМОВ С ПОМОЩЬЮ СЕКВЕНИРОВАНИЯ СЛУЧАЙНЫХ ПОСЛЕДОВАТЕЛЬНОСТЕЙ ДНК

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Ежегодно Республика Беларусь несет серьезные потери урожая в связи с развитием инфекционных заболеваний сельскохозяйственных растений. Ситуацию усугубляет так же тот факт, что традиционные для нашей страны фитопатогены постоянно дополняются занесенными из других регионов (*Clavibacter michiganensis*, *Bacillus pumilus*, *Erwinia amylovora*). Четкая идентификация фитопатогенных микроорганизмов поможет определить в дальнейшем правильную стратегию борьбы с ними, а также разработать рациональные решения по сохранению сельскохозяйственных культур.

Стандартным методом идентификации микроорганизмов является секвенирование 16S рРНК, однако данная методика не способна дать достоверный результат для многих групп микроорганизмов, особенно грамположительных бактерий. Помимо этого, многие непатогенные виды могут стать патогенными за счет горизонтального переноса генов; подобные изменения невозможно определить исключительно с помощью секвенирования 16S рРНК.

Для проведения данной работы были использованы штаммы фитопатогенов, выделенные сотрудниками Института микробиологии НАН РБ и Биологического факультета БГУ на территории РБ в течение 2008-2012 гг. Всего было отобрано 30 штаммов бактерий, предположительно относящихся к 7 родам (*Bacillus*, *Clavibacter*, *Dickeya*, *Erwinia*, *Pectobacterium*, *Pseudomonas*, *Xanthomonas*). Целью данного исследования является определение точного систематического положения данных штаммов с помощью секвенирования случайных последовательностей