

Identification of a Solo Acylhomoserine Lactone Synthase from the Myxobacterium *Archangium gephyra* (DSM 2261)

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Background

Myxobacteria are a unique group of Proteobacteria that are best known for their large genomes, well-coordinated social behavior, and ability to predate other microbes. Our recent survey of the unexplored, biosynthetic gene clusters from myxobacteria included in the antiSMASH database determined that the potential for such discovery from cultivable myxobacteria remains high. An oddity reported by this survey was the presence of a solo acylhomoserine lactone (AHL) synthase within the genome of the myxobacterium *Archangium gephyra* (DSM 2261). AHL quorum signaling (QS) systems are abundant throughout Proteobacteria enabling the bacteria to monitor their environment and alter their gene expression in response to fluctuations in cell-population density. AHLs are diffusible, extracellular chemical signals that bind to LuxR-type receptors which in turn induce expression of LuxI-type AHL synthases. No evidence of AHLs production by myxobacteria has been reported, but data suggests that the model myxobacterium *Myxococcus xanthus* responds to exogenous AHLs produced from their potential prey and demonstrates enhanced predatory features despite having no obvious LuxR receptor within its genome. In addition, the presence of putative LuxR receptors within numerous members of *Myxococcus* and *Coralloccoccus* has been reported in a recent survey of myxobacterial signaling proteins.

Hypothesis

Eavesdropping, the interception of signals by unintended receivers, has become a generally accepted cornerstone in hypotheses surrounding interspecies cross talk within polymicrobial communities. Considering the abundance of AHL QS systems throughout Proteobacteria other than myxobacteria, and the generalist diet of predatory myxobacteria that includes large swaths of AHL signaling proteobacteria, supports the assumption that predatory disruption of QS-associated physiological functions in the prey would likely benefit the fitness of *A. gephyra* by improving predation of quorum signaling prey. Without an AHL receptor also apparent in the genome of *A. gephyra*, we hypothesize that AgpI is an orphaned functional AHL synthase. Herein we report bioinformatic analysis, functional assessment, and heterologous expression of the myxobacterial AHL synthase AgpI

Results

- AgpI is highly homologous to functional AHL synthases: (Figure 1)
 - Absence of a cognate AHL receptor in the genome of *A. gephyra*
- Utilizing blastp, the genomes of *A. gephyra* was assessed for features homologous to different AHL receptors: LuxR, AinR, and LuxN. No homologues with significant homology (>30% identity) were observed. We also queried the Hidden Markov Model (HMM) associated with autoinducer binding domains deposited in Pfam against the proteome of *A. gephyra* using HMMSEARCH. No hit with significant sequence homology with LuxR-type, AHL receptors was found.

Results (cont.)

- A. gephyra* does not produce AHLs during axenic cultivation or addition of exogenous deuterated AHLs
- Heterologous expression of AgpI confirms functional production of AHLs

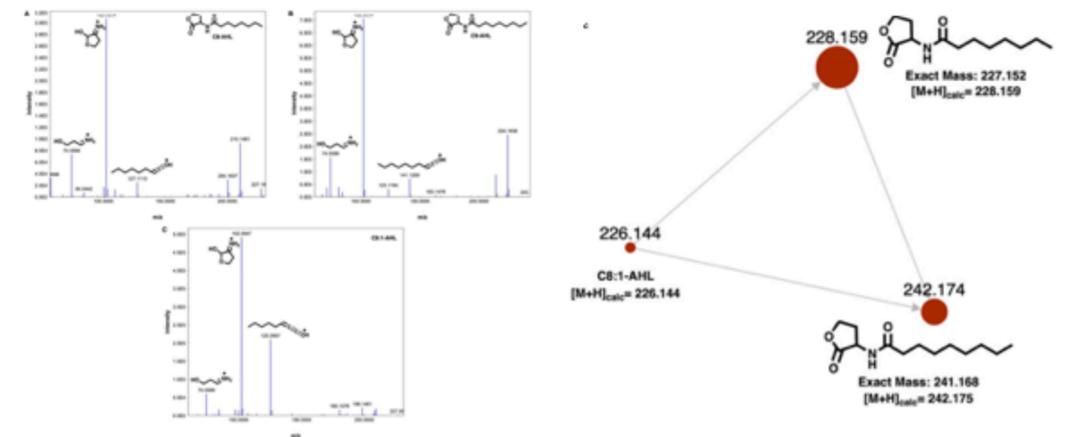


Figure 2: A, B, C) MS/MS fragmentation spectra with diagnostic fragments indicated for each AHL detected in extracts from heterologous *E. coli* expressing AgpI. D) Molecular family from the molecular network of LC-MS/MS datasets from extracts of heterologous *E. coli* expressing AgpI rendered by GNPS. Detected m/z values from raw data positioned over each node with node diameter depicting associated intensities for each AHL.

Conclusions

- A. gephyra* possess functional solo AHL synthases that produce the AHL signals C8-AHL and C9-AHL when heterologously expressed in *E. coli*.
- Production of AHL metabolites from a solo AHL synthase without any cognate AHL receptor with homology to LuxR also present in the genome of *A. gephyra* is the first to be reported.

References

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Strategies

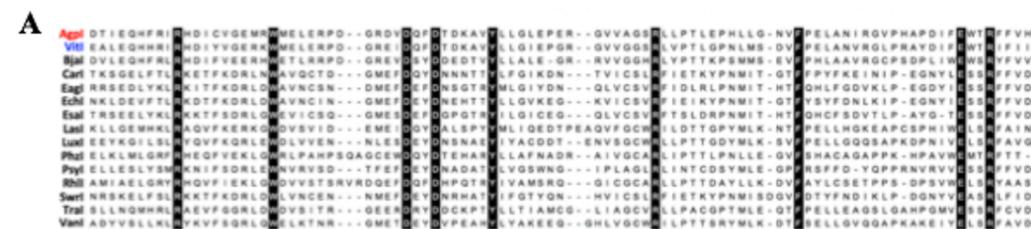
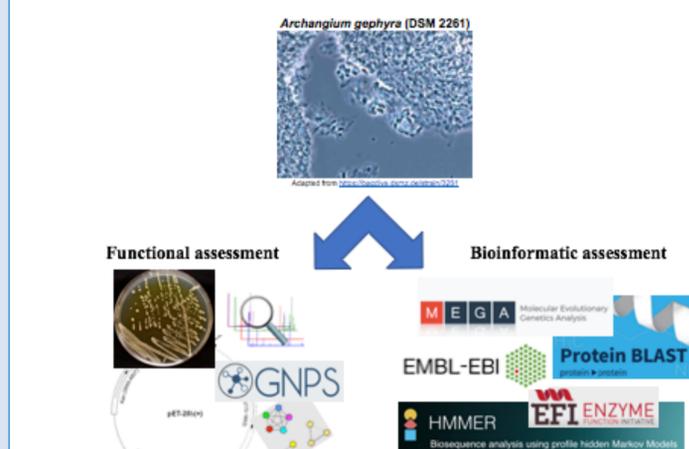


Figure 1: A) Alignment of LuxI synthases including AgpI with conserved residues boxed in black. B) Maximum Likelihood phylogenetic tree including AgpI rendered in MEGA7 using ClustalW aligned with AHL synthases experimentally confirmed to produce AHLs

Acknowledgements

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