Title

Bacterial call-to-arms for warfare at the infection site.

Authors

Vitor Cabral[1] and Karina B. Xavier[1]

Authors affiliation

[1]Instituto Gulbenkian de Ciência, Oeiras, Portugal

Corresponding author: Karina B. Xavier (kxavier@igc.gulbenkian.pt)

Summary

Bacterial sensing is important to perceive environmental cues and activate responses. In this issue of Cell Host & Microbe, Hertzog et al. (2018) show that group A Streptococcus can couple the ability to respond to host cues with autoinduction of a quorum sensing system, leading to killing of bacterial competitors.

Main Text

Cell-cell communication is of utmost importance for any given organism. Communication between cells is a hallmark for the success of multicellularity, as it allows individual cells to coordinate their activities and support the function of the entire organism. The importance of such communication in single-cell organisms, such as bacteria, however, has been elusive. The discovery of bacterial communication systems, termed quorum sensing (Miller and Bassler 2001), has changed the way we think about bacteria, unveiling the social capacity of these microorganisms. Not only can bacteria communicate with their kin, but some can also communicate with unrelated bacteria, expanding the social circle of these organisms. Cell-cell signaling mediated by quorum sensing enables bacterial populations to synchronize gene expression, allowing a community to act as more than a collection of individuals.

Most bacteria can communicate within their species (intra-), or with other species of bacteria (interspecific communication), and this communication can play an important role in complex bacterial communities (Pereira et al 2013; Thompson, Oliveira et al 2015). Since its discovery, quorum sensing has been associated with bacterial behaviors that are important for bacteria-host relationships (Whiteley et al 2017). These interactions can be beneficial to the host, as in the classic example of the luminescent symbiont *Vibrio fischeri* and the bobtail squid (Ruby and McFall-Ngai 1992), where the bacteria have a safe and nutrient-rich niche inside the host, and the

host uses the light produced by the bacteria to better escape from predators. It can also be hostile, as it happens with many human and plant pathogens where quorum sensing regulates virulence gene expression (Papenfort and Bassler 2016; Jimenez and Federle 2014). Additionally, it is becoming increasingly apparent that bacteria can also respond to host cues, and that these responses are particularly important for bacteria during infection. It is, therefore interesting to find that bacteria can also integrate their capacity to detect quorum sensing autoinducers with signals or cues from their host organisms (Hertzog, Kaufman et al 2018).

In previous work, the Hanski lab showed that the pathogen group A Streptococcus (GAS) induces an endoplasmic reticulum stress response through the production and release of streptolysins into host cells. As the host alleviates the stress imposed by this attack, target cells increase the production and release of asparagine (Baruch et al 2014). GAS cells that are in close proximity to these host cells sense the released asparagine and respond to this cue, resulting in toxin production and proliferation (Baruch et al 2014). Hanski and colleagues have also shown that a subset of GAS strains have a quorum sensing system (Sil) that is associated with this host-sensing mechanism (Baruch et al 2014; Fig. 1A). They were intrigued by the fact that *in vitro* bacteria are unable to induce the quorum sensing system without exogenous addition of an autoinducer peptide (SilCR). Building on their previous work, Hertzog, Kaufman *et. al.* now show that asparagine, produced by host cells in response to GAS-induced stress could promote the production of the SilCR autoinducer signal (Hertzog, Kaufman et al 2018). They further showed that the bacteria that are close to the host cells use the asparagine to kickstart their quorum sensing system, leading to increased production of SilCR and subsequent autoinduction of the quorum sensing system. This autoinduction can then propagate to all the bacteria with this quorum sensing system and thus synchronize the *sil*-positive population. Additionally, induction of *sil* by asparagine and SilCR leads to the associated production of bacteriocins (bacterially-derived antimicrobial proteins) and corresponding immunity proteins that protect these producers from the killing effect of bacteriocins. Interestingly, in infections with a mixture of *sil*-positive and *sil*-negative strains, the bacteriocins kill the non-producers, conferring an advantage to the producers. As a result, the bacteria capable of inducing the Sil quorum sensing system out-compete the strains that do not have the *sil* operon (Fig. 1B).

These results highlight the role of quorum sensing during infections with heterogeneous populations, as seen in other cases (Whiteley et al 2017; Valente et al 2017). We tend to think of infections as homogeneous, not taking into account that different strains and sub-populations of the same species can co-inhabit an infectious site. Moreover, these results demonstrate how microorganisms can integrate host responses with population sensing, in this case by both triggering and sensing a host response and then propagating the resultant signals to the whole population to mount a successful infection.

Hertzog, Kaufman *et. al.* have evidence that this *sil* operon might have been incorporated through horizontal gene transfer, namely because it is in a low GC genomic island and is present in only 18% of clinical isolates. In contrast, the sensing system for host-released asparagine, and consequent increase in bacterial growth, occurs independently of *sil*. The coupling of the asparagine-sensing system with the quorum sensing-induced bacteriocin production seems to be a fortunate connection. The first seems to enable the bacteria to increase their numbers without leading to dramatic and fatal damage to the host cells, until they are ready to successfully invade. The second allows the *sil*-positive sub-population to get rid of competitors in order to dominate the tissue invasion (Fig. 1B).

The authors think that this operon has been transferred to a group A Streptococcus ancestor, and that the reduced number of clinical isolates that keep this system indicates that it is being lost due to adaptation to the host. The results, however, seem to show an advantage of this operon during infection, which makes the former assumption harder to understand. It would be interesting to investigate if there are conditions where the strains that have maintained this system have a disadvantage compared to strains that lost it, what that disadvantage and cost is to these strains (e.g. higher sensitivity to antimicrobial treatment, lower tolerance and/or lower resistance to host immune response, etc), and how much cost is associated with losing the competitive benefit shown in this work.

Another interesting aspect of the system dissected and described by Hertzog, Kaufman *et. al.* is the importance of the spatial localization of some members of the sub-population. Indeed, some bacteria within the sub-population need to be adherent or close to the mammalian cells that are attacked by streptolysins, and consequently release asparagine. These bacteria need to take up asparagine, which has a limited radius of action, to kickstart their coupled quorum sensing/bacteriocin system. Upon uptake of asparagine, the bacteria start producing the quorum sensing autoinducer that will diffuse, and due to its bigger radius of action, induce the same system in neighboring cells. Whether bacteria are activated by asparagine (closer to the mammalian cells) or by the autoinducer (farther away), they will produce and release bacteriocins that will kill competitors. Thus, the bacterial spatial localization in this system is important. As some microbial communities are even more complex, notably the gut microbiota where different species occupy different stratified niches, it will be important to examine the coupling of bacterial quorum sensing systems with host-cue sensing mechanisms across different microbial populations.

Overall, the work by the Hanski lab describes a system where in reaction to the host response to a bacterial attack, the induction of the quorum sensing system results in the production of bacteriocins and killing of non-producers. This strongly supports the interpretation that this quorum sensing system plays an important role in the competition among the different bacterial populations within the infected host. Therefore, this work highlights bacterial competition as an additional level of

complexity, beyond the already intricate challenges imposed by the host, that bacteria face during the course of an infection.

Acknowledgments

VC is supported by Fundação para a Ciência e Tecnologia (FCT) by a Postdoctoral Fellowship (SFRH/BPD/116806/2016). We thank Rita A. Oliveira for the helpful comments to the manuscript.

References:

Miller, M.B. and Bassler, B.L. (2001) Quorum Sensing in Bacteria. Annual Review of Microbiology 55:1, 165-199.

Pereira, C.S., Thompson, J.A., Xavier, K.X. (2013) AI-2-mediated signalling in bacteria, FEMS Microbiology Reviews, Volume 37, Issue 2, Pages 156–181

Thompson, J.A. and Oliveira, R.A., Djukovic, A., Ubeda, C., Xavier, K.B. (2015) Manipulation of the Quorum Sensing Signal AI-2 Affects the Antibiotic-Treated Gut Microbiota, Cell Reports, Volume 10, Issue 11, Pages 1861-1871

Whiteley, M., Diggle, S.P. & Greenberg, E.P. (2017). Progress in and promise of bacterial quorum sensing research. Nature volume 551, pages 313–320

Valente R.S., Nadal-Jimenez P., Carvalho A.F.P., Vieira F.J.D., Xavier K.B. (2017) Signal Integration in Quorum Sensing Enables Cross-Species Induction of Virulence in Pectobacterium wasabiae. MBio ;8(3). pii: e00398-17

Ruby, E.G. and McFall-Ngai, M.J. (1992). A squid that glows in the night: development of an animal-bacterial mutualism. Journal of Bacteriology, p. 4865-4870

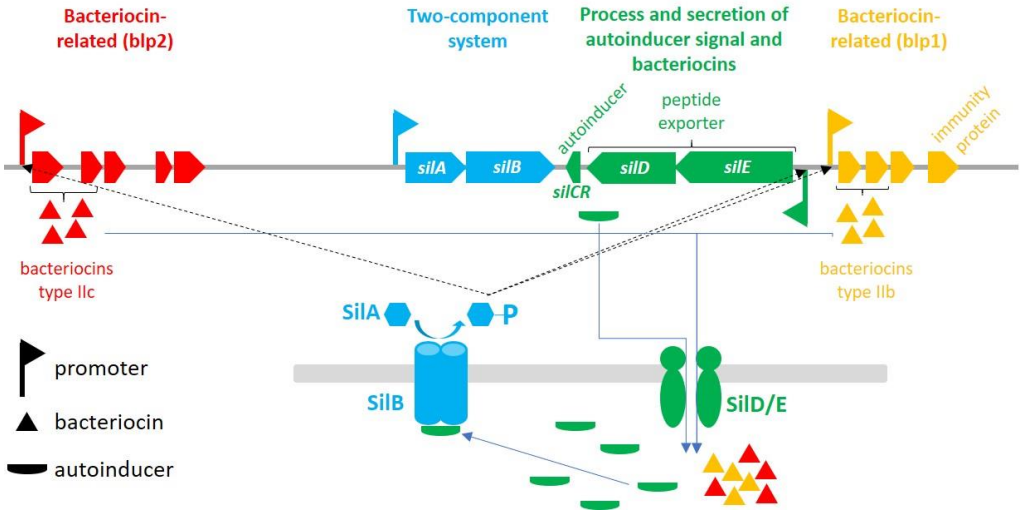
Papenfort, K. and Bassler, B.L. (2016). Quorum sensing signal–response systems in Gram-negative bacteria. Nature Reviews Microbiology volume 14, pages 576–588

Jimenez, J.C. and Federle, M.J. (2014) *Quorum sensing in group A Streptococcus.* Front Cell Infect Microbiol. 2014 Sep 12;4:127.

Baruch, M., Hertzog, B.B., Ravins, M., Anand, A., Cheng, C.Y., Biswas, D., Tirosh, B., Hanski, E. (2014) Induction of endoplasmic reticulum stress and unfolded protein response constitutes a pathogenic strategy of group A streptococcus. Front Cell Infect Microbiol.;4:105.

Hertzog, B.B. and Kaufman Y., Biswas, D., Ravins, M., Ambalavanan, P., Wiener, R., Angeli, V., Chen, S.L., Hanski, E. (2018) A sub-population of group A streptococcus elicits a population-wide production of bacteriocins to establish dominance in the host. Cell Host and Microbe.

A



B

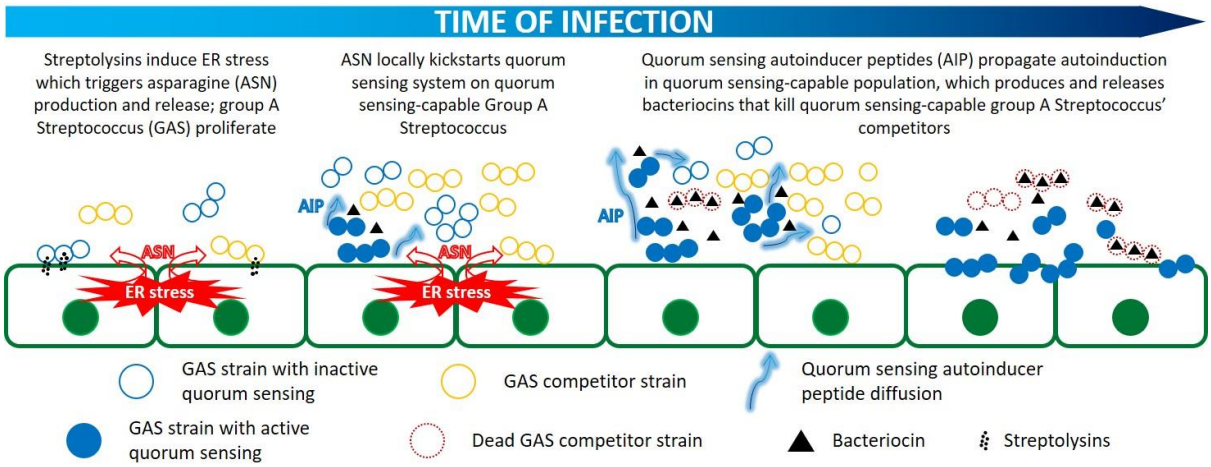


Figure 1. The GAS *sil* quorum sensing system controls the production and release of bacteriocins that kill bacterial competitors. (A) The *sil* operon, similar to the quorum sensing operons found in other gram-positive bacteria, encodes a two-component system, a peptide transporter, and an autoinducer peptide signal molecule. *silCR* encodes an autoinducer whose expression is under the same promoter that controls the expression of SilD/E, a peptide exporter. The autoinducer leaves the cell through this exporter and accumulates extracellularly, where it interacts with the two-component system, being recognized by the SilB receptor, and inducing the phosphorylation of SilA. Phosphorylated SilA triggers autoinduction, by promoting more expression of SilD/E and SilCR. Full induction of the system induces the expression of both blp1 and blp2 loci, which encode for bacteriocins type IIb and IIc, respectively. These bacteriocins are exported, like the autoinducer, through SilD/E. **(B)** During infection, group A Streptococcus (GAS) cells that are close to soft tissue epithelial layers produce streptolysins that induce ER stress in host cells, resulting in the production and release of asparagine (ASN). GAS cells can sense ASN and proliferate. A sub-population of GAS that has the *sil* operon and is in proximity to host

cells will activate the quorum sensing autoinduction by sensing ASN. This autoinduction, mediated by SilCR, will propagate to other quorum sensing-capable GAS. This will result in the production and release of bacteriocins that eliminate GAS competitor strains that lack the immunity proteins encoded in the *sil* operon. As a result, quorum sensing-capable GAS dominate the population and invade the tissue.