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TOPICAL REVIEW

A very brief note on why bacterial evolution has physiology

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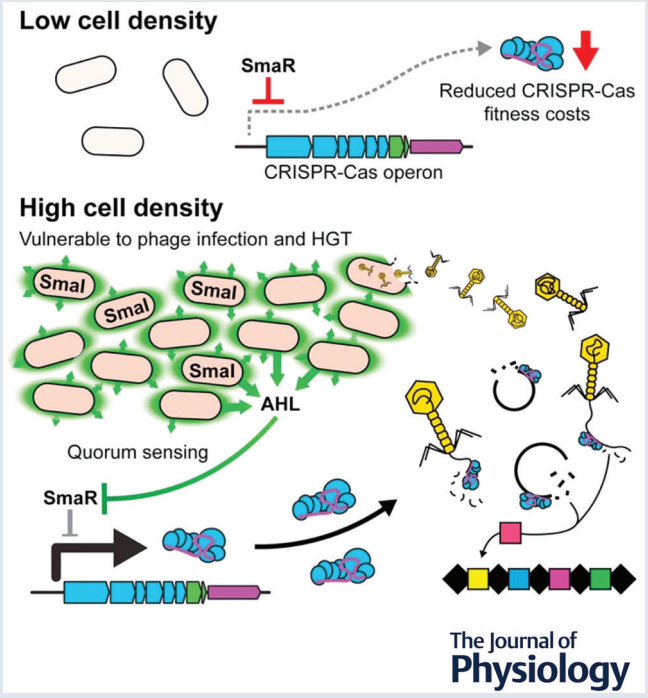

Abstract The majority of bacteria live and evolve in surface biofilms. Both growth in biofilms and horizontal transfer of DNA are regulated by quorum-sensing pheromone signals. The common regulation of bacterial surface growth and DNA transfers illustrates how physiology contributes to bacterial evolution.

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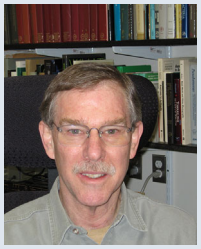

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Abstract figure legend from Patterson et al. (2016) with permission from Creative Commons Attribution (CC BY 4.0). The bacterium *Serratia marcescens* regulates the expression of its CRISPR-Cas adaptive anti-viral defence systems by SmaR repressors, which can be overcome by a density-dependent autoactivator system based on emission of an acyl homoserine lactone (AHL) quorum sensing pheromone (green arrows). Thus, transcription of CRISPR-Cas is repressed at low cell density by SmaR, and the bacteria are quite sensitive to lethal bacteriophage infections. At higher cell concentrations, the AHL pheromone is strongly expressed, and it overcomes SmaR repression to facilitate the expression of the CRISPR-Cas RNA-directed defence molecules (blue blobs attached to the viral DNA). These defence molecules cleave the viral DNA and rescue the infected cells so that they survive the phage attack and can accept any other DNA that may be packaged in phage particles to complete a horizontal gene/DNA transfer (HGT). It has been known since the earliest days of bacterial genetics that a subset of virus particles carry host DNA and are capable of the genetic exchange process known as 'transduction' (Zinder 1958). In this example, the quorum sensing system controls not only bacterial growth but also the potential for evolutionary change by regulating CRISPR-based adaptive bacterial immunity in a way that favours phage-mediated transduction.

When we consider the subject of bacterial evolution, it is commonplace to think about planktonic suspension cultures accumulating mutations that alter the cells' metabolic capabilities. The ongoing Long-Term Evolution project led by Richard Lenski is the paradigm experimental set-up for this way of envisioning how bacteria evolve (Barrick & Lenski 2013; Consuegra et al., 2021). There seems to be little role for physiology in such an evolutionary scenario. However, there are two features of bacterial (and indeed all prokaryotic) life and evolution that indicate the key roles that physiology can play in altering the course of forming novel genomic configurations:

(1) Some of the most important bacterial proliferation occurs in multicellular biofilm populations that form on solid surfaces. Biofilms have been estimated to hold 80% of the planet's bacteria (Penesyan et al., 2021). Therefore, most bacteria have the capacity to alter their physiology to adapt to surface growth, often in mixed populations (Claessen et al., 2014; Mitri et al. 2011; Wang et al., 2020). A significant portion of human microbiome exists as biofilm populations (Macfarlane et al., 2011). Notable among the physiological adaptations to surface growth is the bacterial use of so-called quorum sensing (QS). In QS, bacteria release pheromones that regulate transcription into the environment, and this allows them to assess their population size and density to adjust multiple features of their physiology accordingly (Striednig & Hilbi 2021). Many QS systems have positive feedback 'autoinducer' characteristics that increase their sensitivity to population changes, but this property is not universal (Dogsa et al., 2021). QS pheromones have a wide diversity of chemical structures, so that some are species specific, whereas others like AHLs are shared by many different kinds of bacteria (Aguilar et al., 2021; Mukherjee & Bassler 2019; Spangler et al., 2019). Thus, these physiological tools can stimulate the formation of either single species or multispecies surface populations (Aframian & Eldar 2020; Yi et al., 2021). Multispecies biofilms provide opportunities for horizontal interspecific DNA transfer (Antonova & Hammer 2011).
(2) Contemporary bacteria do not have fixed genetic complements. Instead, they share components of a distributed bacterial 'pan-genome', assembling various DNA components opportunistically to adapt themselves to a given ecological niche (Mira et al., 2010; Rosconi et al., 2022; Sherman & Salzberg 2020; Sonea & Paniset 1976; Sonea & Panisset 1983). In bacteria, therefore, evolution to adapt to a novel environment is largely the process of acquiring the necessary DNA from the pan-genome by horizontal DNA transfer (Banuelos-Vazquez et al., 2017). For example, one recent analysis attributed 3323 'metabolic innovations' of *Escherichia coli* bacteria to acquisition by distinct horizontal transfer events (Pang & Lercher 2019). The same processes of horizontal DNA transfer account for the rapid evolution of antibiotic resistance in pathogenic bacteria (Koraimann 2018; Michaelis & Grohmann 2023; von Wintersdorff et al., 2016).

In addition to transduction, horizontal DNA transfer frequently involves the elaboration of intricate bacterial cell surface structures, most frequently belonging to the type IV secretion system (T4SS) of complex transmembrane molecular export structures (Costa et al., 2021; Le et al., 2014; Pena et al., 2019; Waksman 2019; Christie et al., 2017). T4SS bacterial cell surface appendages are required for most cell–cell direct

Table 1. Examples of bacterial DNA transfer systems regulated by quorum sensing

Organism	DNA transfer system	Reference
Rhizobium	Plasmid transfer initiation, *tra* (transfer) gene expression	(He et al., 2003; McAnulla et al., 2007; Wetzel et al., 2015)
Entereococcus faecalis, Staphylococcus	Plasmid transfer in biofilms	(Cook et al., 2011) (Cook & Federle 2013) (Clewell 2011; Hirt et al., 2018)
Agrobacterium tumefaciens	QS regulates transfer of pAt megaplasmid	(Mhedbi-Hajri et al., 2016)
Vibrio cholera	Competence for DNA uptake, transformation	(Blokesch 2012)
Bacillus subtilis	Short-range horizontal DNA transfer in biofilms	(van Gestel et al., 2021)
B. subtilis	Mosaic life-cycle evolution in biofilm populations	(Staps et al., 2019; van Gestel & Nowak 2016; van Gestel et al., 2019)
Acinetobacter baumannii	Rapid microevolution of antibiotic resistance in biofilms	(Penesyan et al., 2019)
Activated carbon biofilm	QS-dependent plasmid-mediated antibiotic resistance transfer	(Zhu et al., 2020)
Multiple species (review)	Horizontal transfer of antibiotic resistance in mixed biofilms	(Michaelis & Grohmann 2023)
Multiple species (review)	Experimental evolution in biofilms	(Steenackers et al., 2016)
Multiple species (review)	eDNA release	(Ibáñez de Aldecoa et al., 2017)
Multiple species (review)	Relationship between quorum sensing and secretion systems (including type IV)	(Pena et al., 2019)

Abbreviations: eDNA, environmental DNA; QS, quorum sensing.

transfer of chromosomal DNA and autonomously replicating plasmids (Hu, Khara, & Christie 2019). A type 4 pilus structure is also required for the 'competence' of bacteria to incorporate DNA directly from the environment (Blokesch 2012; Costa et al., 2021; Waksman 2019). This competence for DNA acquisition is particularly useful because many biofilm populations are stabilized by release of extracellular environmental DNA (eDNA) from some of the component bacteria (Montanaro et al., 2011; Peterson et al., 2013; Vorkapic 2016), thereby providing readily accessible substrate for horizontal DNA transfers in multispecies biofilms. In addition to their roles in DNA transfers, T4SS surface appendages involved in DNA transfer provide additional stability to the biofilm structure (Patkowski et al., 2023).

Table 1 provides references for the effects of QS pheromones to stimulate horizontal DNA transfer and related processes in bacteria. The role of QS signals in eDNA release and DNA transfers illustrates how deeply intertwined bacterial surface growth and cell physiology are with bacterial acquisition of outside genomic DNA (van Gestel et al., 2021). Without physiology, bacteria's ability to alter cellular DNA would be limited to changes in existing cellular DNA. Actual evolutionary change in bacteria by horizontal DNA transfers engages the full range of growth processes as well as access to phylogenetically distant species. That is, DNA modification in bacteria occurs by fully organic processes subject to physiological regulation. That is the basic message of this brief note.

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Additional information

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Supporting information

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