Abstract

Bacteria, one of the smallest organisms to inhabit the Earth, have some of the largest ecological impacts on their communities. They interact with plants, animals, and other bacteria. Bacteria can be printed using 3D printing methods that recombine different traits of different bacteria. Connell and his research team printed *Staphylococcus aureus* with a *Psuedomonas aeruginosa* shell, increasing its resistance to beta-lactam antibiotics. *Psuedomonas aeruginosa* is found in the lungs of cystic fibrosis patients and loses its social abilities over time (Jiricny et al. 2014). They lose ability of receptor *lasR* in quorum sensing pathways, but biofilm formation is not affected. Recombination could cause such a mutation and increase strain resistance to antibiotics. Some marine bacteria, associated with sponges, have been found to inhibit quorum sensing in *Vibrio* strain bacteria, which down-regulates their virulence (Durai et al. 2013). Biofilm formation in bacterial colonies can be mediated using small molecules that break the cell envelope or matrix, promote cell death, or alter signaling pathways between bacteria (Oppenheimer-Shaanan et al. 2013). The quorum sensing ability of *Psuedomonas aeruginosa* is inhibited by the sub-MICs (antibacterial component) in clove oil by down-regulating virulence factors, such as concentration protease, swimming
motility, *LasB* production, and quorum sensing molecules (Husain et al. 2013). Treating preinfected *Caenorhabditis elegans* with clove oil increased their survival, confirming the antibacterial effect of clove oil. Other essential oils can be synthesized into essential oil sophorolipids (glycolipids). The hydrophobic polar nature of these sophorolipids inhibits quorum sensing molecules and biofilm formation (Mukherji et al. 2013). As a simple essential oil it does not have these traits, but when transformed into sophorolipids they show antibacterial properties. By understanding bacterial interactions and how they are influenced, we can manipulate their communication to benefit human health and maintain a balance in the ecosystem they thrive in.
A Review on Bacterial Communities and Communication

Biofilms:

Structure and Function

Bacteria use a system called “Quorum sensing” to communicate and keep track of population size. Acyl Homoserine Lactones (AHLs) and autoinducing peptides (AIPs) are two common molecules involved in extracellular communication (Blackledge et al. 2013). Quorum sensing (QS) uses small molecules called “autoinducers” when they reach a certain population threshold to change gene expression (Garg et al. 2014). These changes can cause a change from non-virulent to virulent or from a planktonic, free-floating population to a biofilm-protected population (Garg et al. 2014). Biofilm formation in bacterial colonies can seem inactive as a unit with so much life hidden within it, however biofilms contain a hybrid of life, nutrients, and enzymes that all interact. Antibiotic use can be ineffective, if not counter-productive, when the dynamics of the biofilm matrix are not fully understood. If antibiotics are able to penetrate the biofilm, they could react with the free-floating compounds in the matrix, lowering the concentration of the antibiotics making contact and reacting with the target bacterial component (Lazar 2011). Nutrient accumulation in the matrix can support bacteria still in lag phase of the Bacterial Growth Curve (see Fig.1); adhesive, deeper bacterial cells might stay dormant while more surfaced bacteria metabolize the available nutrients.
If the whole matrix is not degraded, bacterial colonies may not be detected since some are dormant and have limited nutrient availability deeper in the biofilm matrix. “Alaromones” have been suggested as a warning molecule secreted when a cell dies that increases resistance in recipients (Lazar 2011). Superficial cells are thought to signal to deeper cells that are harder to detect with current technology and practice (Lazar 2011). A “supragenome” exists in biofilm communities – a collective of DNA among all the organisms in the biofilm – that provides genes for all and lowers the individual energy investment in DNA maintenance (Wolcott et al. 2012). Within the supragenome, DNA
materials and resistance genes can be transferred for competitive advantage between a variety of species and organisms (Wolcott et al. 2012). Interactions can be antagonistic, or synergistic via metabolic cooperation and complementation, resistance, QS systems, byproduct influence, and efficient DNA sharing (Wolcott et al. 2012). The Allee effect is defined as a positive relationship between individual fitness and population density – as the population increases, so does fitness (Smith et al. 2014). Spread is inhibited at high and low dispersal rates, but is maximized when there are an intermediate number of target dispersal sites (Smith et al. 2014). Fluctuating growth rates of populations may be explained by this tradeoff between spread and dispersal (Smith et al. 2014). Only a couple antagonist interactions are evident – competition for nutrients and growth inhibition (Elias and Banin 2012).

Viewing biofilms as a community is beneficial to understanding and monitoring their growth in population and resistance. With these insights, chronic illnesses can be better understood in their evolution and those specific strain populations can be more effectively targeted with antibiotics. Individual bacteria contribute to the biofilm formation, thus increasing numbers increases biofilm strength and size (Elias and Banin 2012). Biofilms provide more means for fitness than planktonic bacteria or mono-species colonies can achieve (Hoiby et al. 2010). Biofilms can take on many structures: separate microcolonies that communicate in open systems, co-aggregate species that communicate in biofilms, or layered species that separate in biofilms to maximize their overall community fitness (Elias and Banin 2012). Biofilms allow species to communicate quickly and privately (Elias and Banin 2012), as compared to open systems, where anti-QS drugs and antibiotics can interfere. Looking at the diversity of life in biofilms in
comparison to the structure and origin of their biofilm could prove useful in preventing future biofilm formations harboring chronically infectious bacteria.

**QS and Biofilm Control**

There are a few methods being researched that could reveal biofilms as a source to a problem and inhibit formation to fix it. Biofilm formation in bacterial colonies can be mediated using small molecules that break the cell envelope or matrix, promote cell death, or alter signaling pathways between bacteria (Oppenheimer-Shaanan et al. 2013). Dairy spoilage may be encouraged by an enzyme produced in a biofilm in milk; no biofilm has been identified, but if the enzyme causing spoilage is, it could be linked back to a bacteria found in milk and/or cows known to produce said enzyme (Teh et al. 2014). The enzyme is cell-associated in the biofilm, before colony maturation, but may become extracellular as the mature biofilm disperses (Teh et al. 2014). We can develop tests to recognize the presence and identify the spoilage-causing strain of bacteria in milk and potentially remove the biofilms from milk or chemically break down QS enzymes before spoilage to lengthen their shelf life in stores.

Indian spice *Cuminum cyminum* has natural anti-biofilm and QS inhibiting (QSI) properties, effective on gram-negative strains, that inhibits acyl homoserine lactone (AHL) QS signal functions related to biofilm exopolysaccharide (EPS) formation as well as flagellar motility (Packiavathy et al. 2012). Specifically, it is the methyl eugenol (ME) component of *C. cyminum* that interferes with AHL functioning, preventing bioluminescence, violacein production, and biofilm formation (Packiavathy et al. 2012). ME is a naturally derived compound and a good target for AHL-influenced EPS-biofilm formation in various Gram-negative strains. The EPS synthesis is promoted by low
concentrations of nitrogen, potassium and phosphate, and high concentrations of carbon (Sutherland 2001). Red seaweed, *Chondrus crispus*, has water-soluble antibacterial properties sourced from Kappa-carrageenan that enhance host immunity to *Pseudomonas aeruginosa* QS and virulence factors (Liu et al. 2013). It in itself cannot deplete or inhibit bacterial growth, but it can increase the host’s chance for survival (Liu et al. 2013). Nitrogen has recently been utilized as an antibacterial component toward gram-negative strains as well. Fimbrolides are halogenated furanones found in marine algae that exhibit QSI properties, particularly in conjunction with nitric oxide (NO) (Kutty et al. 2013). Biofilm dispersal and susceptibility to antibiotics are induced by NO sublethal concentrations (Kutty et al. 2013). Fimbrolide-NO hybrids are the first synthesized antimicrobial agent to target both QS signaling and changing the environment from its optimum for bacterial growth (Kutty et al. 2013). Using NO in combination with other various QSI compounds could be a practical way to increase targeted bacterial susceptibility to QSI.

Ajoene, a sulfur-containing chemical found in garlic extract, works very well with tobramycin to destroy biofilms and stop lytic necrosis of polymorphonuclear leukocytes in *Psuedomonas aeruginosa* pulmonary infections (Jakobsen et al. 2012). Whereas AHL QS molecules are attributed to gram-negative bacteria, AIPs are the QS molecule most used by gram-positive strains (Blackledge et al. 2013). AIPs are hydrophobic molecules and bind proteins to ultimately direct gene expression; AIPs can upregulate RNA translation that causes biofilm adhesion and development (Blackledge et al. 2013). An inhibitor of RNA-III activating protein (RAPi) in *Staphylococcus aureus*, that prevents interaction between RAP and its target protein, reduces adhesion and biofilm formation.
and has been considered for its ability to prevent infections, even by resistant strains (Blackledge et al. 2013). In another study, a couple modified and unnaturally occurring AHL compounds were introduced to gram-negative strains and effectively reduced biofilm formation (Blackledge et al. 2013). AHLs can be targeted enzymatically for decomposition, a process known as “quorum quenching”, observed in many eukaryotic and bacterial life forms (Blackledge et al. 2013).

The quorum sensing ability of *Psuedomonas aeruginosa* is inhibited by the sub-MICs (minimum inhibitory concentration of antibacterial component) in clove oil by down-regulating virulence factors, such as concentration protease, swimming motility, *LasB* production, and quorum sensing molecules (Husain et al. 2013). Essential oils and methanol in plants are effective in lowering the integrity of bacteria based on their relative concentrations (sub-MICs) (Morteza-Semnani et al. 2006). Gram-positive bacteria are more susceptible to methanol than gram-negative and essential oils vary in effectiveness based on their chemical makeup and relative concentrations (Morteza-Semnani et al. 2006). Rosemary and tea tree oils are effective against QS signaling and violacein production in bacteria when applied at higher concentrations (Alvarez et al. 2012). They are effective against food-borne pathogens, such as *E. coli* and *L. monocytogenes*, and have good potential to be used as a food preservative (Alvarez et al. 2012). Other essential oils can be synthesized into essential oil sophorolipids (glycolipids) that inhibit QS molecules and biofilm formation due to the hydrophobic polar nature of oils (Mukherji et al. 2013). Simple essential oils do not always function as an antibiotic, but when transformed into sophorolipids they can show antibacterial properties.
Ecological Perspectives:

Plant Interactions

Essential oils and secondary metabolites in plants have been heavily studied in recent years due to the discovery of their natural antibacterial properties. Different oils work in different ways to lower the integrity of bacterial communities. Flavonoids interact with extracellular and soluble proteins and bacterial membranes (Savoia 2012). Alkaloids work in a variety of ways and are characterized by their interactions with bacterial components (Savoia 2012). The chemical composition can be highly variable and interact differently with microbial membranes based on their permeability (Savoia 2012). Terpenes are not readily understood, but it is thought that the lipophilic compounds in terpenes disrupt membranes of bacteria (Savoia 2012). Phenolics and polyphenols work by interacting with bacterial enzymes and disrupting bacterial envelopes (Savoia 2012). Coumarins are highly variable in their function, often found in spices and plants, and are effective based on the sub-MICs of the specific volatiles (Savoia 2012). Once more organic compounds are identified as anti-bacterial, we can further understand how the classes of secondary metabolites function to decrease bacterial integrity, whether by inhibiting efflux pumps, biofilm formation, or QS activity (Savoia 2012). This different compounds are found in a variety of forms in nature and at specific concentrations to maximize the producer’s fitness.

Bacteria-plant relationships show various composition dynamics relative to location of bacterial colonization on the plant and the volatile organic compounds (VOCs) emitted from that location (Junker and Tholl 2013). VOCs decrease the integrity
of microbial membranes and alter the metabolic and regulatory cellular processes, which are fine-tuned based on type of volatile emitted and concentration (Junker and Tholl 2013). There are three areas of a plant that emit secondary metabolites and VOCs: the rhizosphere (roots), phyllosphere (leafs and stem), and Anthosphere (flowering parts) (Junker and Tholl 2013). VOCs can be effective over small or long ranges and help establish niches for bacterial colonies to grow in plant tissues and are usually synergistic, but can be antagonistic (Junker and Tholl 2013).

Rhizospheric bacteria fix nutrients in the soil for the plant and use the carbon from terpene hydrocarbon volatiles for their own metabolism; the VOCs emitted by the roots are selective for certain bacteria and inhibit colonization of unwanted microbes (Junker and Tholl 2013). Since bacteria in the rhizosphere live in biofilms, viewing their matrix as part of the population mass, a perspective called Quorum regulated Biofilm Biomass (QRBB) that views it as a transient “supraorganism”, gives important information on QS products and functioning (Hoagland 2013). Environmental triggers are often biofilm triggers in soil, such as how much water is available (Hoagland 2013). Fungi and archaea also contribute to soil biofilms, significantly increasing their size as well as carbon capacitance (Hoagland 2013).

Volatile in the phyllosphere inhibit airborne colonization of bacteria on leaves in some plants and are in higher concentrations around damaged tissue, likely to prevent infection of microbes since exposed tissue has less of a barrier for microbes to pass to reach nutrients (Junker and Tholl 2013). Tolerance to volatiles is necessary for bacteria to occupy plant tissues, creating niches that only certain bacteria can occupy (Junker and Tholl 2013). In some cases, the volatiles are used as an energy and carbon source for the
bacteria; these bacteria have been shown to support growth and germination in many plant species (Junker and Tholl 2013). Samioside is a phenyethanoid glycoside produced in aboveground plant tissues of Phlomus samia that shows antibacterial properties to gram-negative and gram-positive bacteria alike (Morteza-Semnani et al. 2006). Two essential oils were analyzed using GC-MS and revealed sesquiterpenes as the active component in the oils, with weak to moderate sub-MICs (Demirci et al. 2008). Most VOCs are selective toward strains and types of bacteria; identifying a single compound, samioside, which is effective against both, may allow regulation of more bacterial colonies at once and increase economic efficiency of antibiotics.

In the anthosphere, nectarins and pollen containing hydrogen peroxide and VOCs are produced to inhibit colonization of bacteria in the reproductive organs, even in small concentrations (Junker and Tholl 2013). The bacterial colonization on leaves is much more diverse than on petals and longer-lived (Junker and Tholl 2013); seeing as petals are modified leaves, the volatile production change from leaf to flower may kill off most bacteria that thrive on the leaf so that only a few can survive on the petal. Bacteria on petals are less susceptible to floral plant volatiles than those on leaves (Junker and Tholl 2013). Flower production is seasonally temporary, so the bacteria must have a faster colonization rate as well – otherwise the petal is shed before substantial colonization can occur (Junker and Tholl 2013). The colonization populations are more selective on petals than any other part of the plant.

**Bacterial Integrity Control**

Flagellar morphogenesis in Burkholderia glumae is coded for in the bacterial genome, however, if the temperature is not optimized, the flagellar placement is non-
polarized (Jang et al. 2014). At optimum temperature, flagellar placement is polarized to one side of the bacteria, revealing a strong sensitivity of QS-mediated morphogenesis to temperature (Jang et al. 2014). Bacterial strain *Lactobacillus plantarum* establishes itself within *Drosophila melanogaster* via ingestion and influences mating preferences by altering cuticular hydrocarbon sex pheromones (Sharon et al. 2010). It is unclear whether it is the array of life in their diet causes this or if it is a rapid switch in diet that allows *L. plantarum* to amplify its QS signals and population (Sharon et al. 2010). This could be due to less competition, which allows easier nutrient and spatial acquisition or more stress to establish populations because of competition between varieties of life occupying the same niche. The direct benefit to the bacteria is unclear, but perhaps the bacteria give an evolutionary or fitness advantage to Drosophila that encourages feeding on the food source *L. plantarum* inhabit (Sharon et al. 2010). Inhibiting *Drosophila* feeding on the food source bacteria live in could artificially select for certain strains of bacteria and select against others. Environmental manipulations are sufficient to control bacterial growth and spread.

A chloroform isolate found in some marine sponges, has been found to inhibit quorum sensing in *Vibrio* strain bacteria, which down-regulates their virulence (Durai et al. 2013). The isolate is produced from mutualistic bacteria that live on the sponges (Durai et al. 2013). A pathogen of aquatic crustaceans, *Vibrio campbellii*, uses three different QS systems that utilize the same signal cascade, but vary virulence based on their host type (Pande et al. 2013). The way QS signals are targeted needs to be evaluated for each host type, not as a single mechanism, to obtain the desired effects of inhibitors (Pande et al. 2013). *Burkholderia cenocepacia* has two main QS systems; one uses AHL
compounds, the other uses cis-2-dodecenoic acid (BDSF) (Udine et al. 2013). Single, double, and triple mutants reveal that increasing the number of mutations in the strain decreases its virulence, biofilm and protease production, and antibiotic susceptibility (Udine et al. 2013). By adding BDSF to triple mutant populations, some of the knocked-down functions can be restored, even in the AHL QS-pathway (Udine et al. 2013). Seemingly, AHL is at least partially controlled by BDSF and they utilize most of the same genes (Udine et al. 2013). Monoclonal antibodies (MAbs) can be synthesized to target gram-negative QS signals in the bloodstream, allowing earlier detection of Pseudomonas aeruginosa infection (Palliyyil et al. 2014). These antibodies are sensitive enough to detect the presences of homoserine lactonase QS molecule in urine as well as blood (Palliyyil et al. 2014). This method could also be used to test for other strain’s QS signals or modified QS molecules and the antibodies could be switched out. This would not influence genetic mutations and resistance in strains, but could detect early colonization before the biofilm is established. Future research may find concentration-sensitive MAb testing to determine the stage of infection of bacterial strains and can then be treated with BDSF inhibiting molecules.

Resistance in bacteria P. aeruginosa is not solely due to QS ability, but also to its ability to form a biofilm to avoid detection and degradation (Friman et al. 2013). This supports other research indicating QS ability is independent of ability to form a biofilm based on infection duration (Jiricny et al. 2014). In cystic fibrosis patients, Pseudomonas aeruginosa has evolved from a cooperative to a non-cooperative strain (Jiricny et al. 2014). The change occurs over time – it depends on how long the infection has persisted. (Jiricny et al. 2014) The social ability of older infections is diminished, so they are less
readily detectable and targeted by antibiotics (Jiricny et al. 2014). They lose ability of receptor lasR in quorum sensing pathways, but biofilm formation is not affected. (Jiricny et al. 2014) Biofilm-mediated recombination could cause such a mutation and increase strain resistance to antibiotics. \textit{P. aeruginosa} produces 2-AA infochemical at all stages of infection that causes host tolerance to the infection as well as pathogen persistence in Cystic Fibrosis patients (Tzika et al. 2013). From a genetic level, it increases insulin resistance and decreases mitochondrial functioning in skeletal muscles (Tzika et al. 2013). ATP synthesis is lowered, so energetic efficiency in muscles is decreased as well, opening up the host to \textit{P. aeruginosa} chronic and persistent infections (Tzika et al. 2013).

A gram-negative marine bacterium, \textit{Photobacterium halotolerans}, produces Solonamide B, which interferes with MRSA \textit{agr} QS signaling pathways and RNAIII virulence gene expression (Nielsen et al. 2014). This is the first organic compound found to be effective in lowering integrity of MRSA strain bacteria (Nielsen et al. 2014). The genetic manipulations induced by resistance can be reversed if the mechanism for resistance is clearly understood.

With the invention of 3D printing, bacterial structure and communities can be replicated and observed for deep interactions among different strains in a biofilm (Connell et al. 2013). Bacterial communities can be printed so strains are adjacent, layered, or free-floating in their environment (Connell et al. 2013). \textit{Staphylococcus aureus} shows increased resistance to beta-lactam antibiotics when printed with a \textit{Pseudomonas aeruginosa} shell using 3D printing (Connell et al. 2013). This method mimics the supragenome concept of biofilm communities, where genes can be shared and mutated to select for resistivity. In \textit{Pseudomonas aeruginosa} infections, the human host
peptide LL-37 is produced as a defense compound, but is actually counter-productive to the host system (Strempel et al. 2013). The peptide promotes secretion of virulence factors, upregulation gene clusters that produce QS signals and efflux pumps, and LPS toxin modification (Strempel et al. 2013). The peptide was also found to be involved in adaptive resistance to fluoroquinolone and aminoglycoside antibiotics (Strempel et al. 2013). *P. aeruginosa* hijacks the host defense system to increase its own resistance and virulence. Structure-based Virtual Screening (SBVS) of *Psuedomonas aeruginosa* revealed five active inhibitors of QS involved in the LasR receptor (Tan et al. 2013). The most active one, 5-imino-4,6-dihydro-3H-1,2,3-triazolo[5,4-d]pyrimidin-7-one (G1), inhibits 46 proteins from being produced in *P. aeruginosa* PAO1 and reduced QS-regulated virulence factor expression (Tan et al. 2013). SBVS may be a useful approach to identifying active QS-related molecules, which can be targeted with antibiotics to knock down particular strains. With advancing technology, bacterial printing and restructuring is a possible way to control bacterial communities.

**Conclusion**

There is still a lot of work to be done in pursuit to understand bacterial community functioning. The systems used are very complex and may not be so obvious initially in their processes. By investing time and funding in research, the methods of communication are better understood and targeted with antibiotics. To understand biofilms, all life harbored must be considered as well as their metabolic pathways, the biofilm matrix must be observed as a unique and essential part of their community’s functioning, the causes of increased resistance need to be understood, and physical
distribution within biofilms of various life need to be mapped. Bacteria can be used to lower the integrity and colonization of other bacteria. Essential oils and spices contain compounds that actively degrade bacterial membranes, toxins, communication molecules, and various other chemical markers. Plant-bacteria interactions can model natural antibacterial methods; if taken up in practice, resistance in strains may be less encouraged by the antibiotics. Monitoring nutrient acquisition and excretion of different bacteria in a biofilm is crucial to understanding what part of their environment they rely on the most. Small changes in the environment can drastically down-regulate communication and virulence of bacteria. By integrating information about bacterial biofilms and communities, we are one step closer to reforming medicinal practice to safer methods and not encouraging resistance in bacteria.

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