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Unveiling the multifaceted microbial strategies: Insights into ecological adaptations and interactions

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ABSTRACT

Microorganisms, such as spanning bacteria, archaea and fungi, were ubiquitous and played pivotal roles in shaping ecosystems. This review offered a comprehensive investigation into the multifaceted strategies employed by microorganisms to thrive and adapt within complex ecological niches. Key themes explored in this review encompassed microbial defence mechanisms, biofilm formation, quorum sensing and altruistic behaviours. Microbial defence mechanisms were scrutinized, with a focus on bacteriocin production. Despite the costs associated with production, bacteriocins served as potent weapons that selectively targeted closely related strains, reducing competition and conferring indirect benefits to the producer's genetic kin. Biofilm formation, a critical facet of microbial survival, was discussed in detail. These structured microbial communities encased in self-secreted extracellular matrices provided structural support and protection, demonstrating their significance in diverse ecological contexts. The review further delved into the evolutionary implications of quorum sensing and altruism within microbial communities. Quorum sensing, a mechanism that allowed population density-dependent communication and cooperation, was revealed as essential for microbial survival. In conclusion, this review enhanced our understanding of the intricate strategies microorganisms employed for survival, adaptation and competition in intricate ecosystems. By shedding light on these mechanisms, it advanced our comprehension of microbial community dynamics and their indispensable roles in diverse environments.

Keywords: Communication mechanism, community dynamics, environmental adaptation, microbial interaction

INTRODUCTION

Microorganisms engage in a constant struggle for survival within complex and naturally occurring ecosystems. Within these environments, they vie for access to limited resources, which can lead to either coexistence or domination over other organisms. Microbes also display social tendencies, forming alliances or rivalries as they establish various biological interactions within their respective habitats. Apart from these biological interactions, external factors like temperature, humidity, salinity and the availability of nutrients play a significant role in shaping the composition of microbial communities. As the population of microbes increases and resources become scarcer, they employ diverse strategies to secure the essential nutrients necessary for their sustenance.

Ecosystems host intricate relationships among species, including microorganisms. Multispecies microbial communities are common in nature, fostering essential interactions through signalling molecules and physical contact. Bacteria may employ shared signal molecules to communicate, distinguishing neighbouring cells for cooperation or competition, often forming biofilms cooperatively.

Competition between bacteria includes the production of soluble diffusible factors like bacteriocins and antibiotics. These substances, even at sub-inhibitory levels, facilitate cooperative interactions and signalling within and between species (Destoumieux-Garzón *et al.*, 2002; Davies *et al.*, 2006). Quorum sensing, a cell-to-cell communication mechanism, was discovered through luminescence induction in *Vibrio fischeri* when grown in high-density culture medium. Acylated homoserine lactones serve as autoinducers in this process, but quorum sensing can be disrupted by quorum quenching and inhibitors (Fuqua *et al.*, 1994; Dong *et al.*, 2001; Uroz *et al.*, 2005). Certain microbial growth-inhibitory mechanisms involve cell-to-cell contact. *Escherichia coli* employs a contact-dependent inhibition system utilizing proteins like CdiA and CdiB. The type VI secretion system (T6SS), similar in structure to bacteriophage puncturing devices, breaches bacterial cell walls to deliver effectors (Aoki *et al.*, 2005; Nudleman *et al.*, 2005; Benz *et al.*, 2012; Russell *et al.*, 2014). This review discussed the

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complex strategies utilized by microorganisms to survive, adapt and compete within intricate ecosystems. By illuminating these mechanisms, it has furthered our understanding of microbial community dynamics and their crucial contributions to various environments.

Social behaviour of bacteria

Aristotle once stated, "Human beings are inherently social creatures; an individual who lacks social inclinations by nature, rather than by chance, is either beneath our attention or transcends normal humanity." The study of social behaviour has long captivated biologists. However, it's important to recognize that social behaviours extend beyond humans; they are also observed in animals and plants, both within their own species and in interactions with others. Within the animal kingdom, common social behaviours encompass primate social hierarchies, communication through pheromones and competition among males for mating partners. Recent research has unveiled that plants possess the ability to distinguish between self and non-self, responding accordingly to different stimuli (Dudley and File, 2007). Clearly, social interactions permeate the realm of living organisms, even extending to bacteria.

Prokaryotes, encompassing bacteria and archaea, are traditionally perceived as single-celled organisms. Nevertheless, they exhibit intriguing social behaviours, with variations across species due to distinct evolutionary pathways. These behaviours often involve cell-cell adhesion, division of labour and intercellular cooperation (Claessen *et al.*, 2014; Lyons and Kolter, 2015). Remarkably, these behaviours are observed in organisms lacking neurons or nephrons, typically associated with complex social interactions.

Prokaryotes, despite their structural simplicity and absence of cell differentiation, can display social behaviours akin to multicellular organisms with genuine multicellularity. This behaviour emerges from the differential expression of a common set of genes in response to diverse microenvironments, resulting in varied phenotypes within genetically identical cell populations. Stochastic fluctuations during gene regulation also contribute to cellular variability (Veening *et al.*, 2008; van Vliet and Ackermann, 2015).

Multicellularity offers clear benefits to bacterial populations. The division of labour enables specialized cell types to collaborate, facilitated by intercellular communication. This coordination leads to complex group behaviours that are synchronized, enhancing efficiency and overall functionality, ultimately ensuring better survival for the bacterial population (Aguilar *et al.*, 2015).

Social behaviours are categorized into four classes based on their effects: benefit, altruism, selfishness and spite (Hamilton, 1964; Hamilton, 1970). Mutualism benefits both the performer and recipient, while selfishness benefits the performer but harms the recipient. Altruism, on the other hand, benefits the recipient but not the performer, possibly resulting in harm to the performer. Spiteful interactions, though rare, harm both performer and recipient (Bashey *et al.*, 2012). These classifications should consider long-term reproductive success; altruistic behaviours may not yield immediate benefits but could be advantageous over time. However, complexities arise as behaviours may have multiple outcomes and long-term consequences are challenging to measure. Thus, short-term effects often define behaviours due to uncertain long-term outcomes.

Competition and cooperation in bacteria

Microbes, owing to their diverse ecological niches, often find themselves in competition with other strains and species for limited resources and space. This competition has driven the evolution of various phenotypes aimed at outcompeting and displacing rival microbes. Interestingly, over time, competition has sometimes given way to cooperation, leading to the stable coexistence of microbes, even when they are genetically distinct. This shift in behaviour reflects the selection forces acting on different species or strains based on their specific ecological conditions.

Bacterial interactions frequently involve the exchange of finite, shared public goods. These goods, typically compounds that demand energy and time to produce, become the focal point of bacterial cooperation and competition. Bacterial cells face a choice between competing for these resources or cooperating to maximize their availability. However, this cooperative strategy carries the risk of cheater cells, which exploit the common pool of public goods without contributing to the population's benefit. As cheaters invest nothing in this competition, they can grow over time, potentially dominating the population (Hamilton, 1964; Hibbing *et al.*, 2010; Ghoul and Mitri, 2016).

Reason for competition

Competition among microbial populations arises when they vie for limited resources within ecosystems, a phenomenon widely observed. Genomic investigations have unveiled the prevalence of competition-related elements, such as the type VI secretion system (T6SS) found in 25% of Gram-negative bacteria (Boyer *et al.*, 2009). Actinomycetes allocate a significant portion of their genetic repertoire (5-10%) to the production of secondary metabolites, including antibiotics, which are used in competitive interactions (Nett *et al.*, 2009).

Analyzing the extent of competition often involves constructing and simulating metabolic models based on sequence data. Freilich *et al*. (2011) pioneered this approach, revealing that competition is a dominant feature in mixed bacterial cultures, with relatively few instances of positive interactions. Experiments using bacterial isolates from tree-holes have validated these findings (Fiegna *et al.*, 2015). Several conditions favor the prevalence of competition: (i) overlapping metabolic niches and resource requirements, (ii) spatial mixing of

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different bacterial strains with intermingled nutrients and secretions, and (iii) the limitation of resources relative to the microbial population (Ghoul and Mitri, 2016).

Environmental factors significantly influence these conditions. Complex nutrient structures with multiple resources or niches can reduce competition within populations, but resource ratio theory posits that an abundance of one resource may not preclude others from acting as limiting factors (Miller *et al.*, 2005). Phylogenetic relationships among bacterial species in a community also contribute to resource niche differentiation, with distantly related species often coexisting due to differences in their resource needs (Hardin, 1960), although lateral gene transfer can eventually lead to niche overlap (Shapiro *et al.*, 2012; Niehus *et al.*, 2015).

Spatial mixing depends on various factors, including nutrient availability and mechanical characteristics of the environment. Investigations with *Pseudomonas aeruginosa* have shown that nutrient levels influence spatial structuring of bacterial colonies (Mitri *et al.*, 2016). However, spatial mixing often leads to a reduction in diversity over time, suggesting that competition intensifies as resources become depleted. Mechanical aspects, such as fluid dynamics and surface properties, also influence spatial organization (Persat *et al.*, 2015). For instance, Cardinale (2011) demonstrated that a mixture of algae can cooperate to remove nitrate from stream water only under heterogeneous flow conditions; uniform flow results in competitive exclusion (Cardinale, 2011).

Cell density can serve as a trigger for competitive behaviours. As bacterial cell density increases, physiological stress mounts due to nutrient depletion or cellular damage from competitive actions like bacteriocin secretion (Cornforth and Foster, 2013; LeRoux *et al.*, 2015a). In response to this stress, bacteria regulate competitive phenotypes to ensure survival. For example, *P. aeruginosa* forms protective biofilms upon detecting antibiotics (Oliveira *et al.*, 2015) and deploys its T6SS when neighbouring cells are eliminated (LeRoux *et al.*, 2015b). Similar responses are observed in *B. subtilis*, which secretes lethal compounds upon detecting a *Bacillus simplex* biofilm in close proximity (Rosenberg *et al.*, 2016). Soil bacteria can also modify competitive behaviours in response to neighbouring colonies by regulating antibiotic production (Abrudan *et al.*, 2015; Kelsic *et al.*, 2015).

Consequences of competition over time

Competition among microbial populations can lead to a reduction in local diversity and an increase in ecological stability (Allesina and Levine, 2011; Coyte *et al.*, 2015). This competition can manifest in various ways, resulting in three possible outcomes: less competitive strains may be driven out, different strains may coexist by specializing in distinct metabolic niches and resource types, or they may split into different spatial niches within the environment.

Niche differentiation is exemplified in the tree-hole microbial community evolution experiment, where initially competing bacterial species evolved to utilize each other's waste products, increasing overall productivity and reducing competition strength (Fiegna *et al.*, 2015). Spatial separation, common on surfaces like mucus, soil, leaf surfaces or agar, allows different spatial niches to coexist as microbial populations slowly differentiate from a homogeneous competition to distinct spatial patterns (Hallatschek *et al.*, 2007; Mitri *et al.*, 2016).

In microbial competition, three established outcomes exist: the dominance of more competitive strains, niche differentiation to reduce competition and spatial separation of strains. Recent scenarios propose additional dynamics. The Black Queen Hypothesis suggests stable coexistence within a niche, where one species produces essential public goods to avoid extinction, benefiting competitors (Morris *et al.*, 2012; Morris, 2015). Similar dynamics occur in intraspecies cooperation and cheating, as observed in siderophores production and cyclic rock-paper-scissor interactions (Czárán *et al.*, 2002; Narisawa *et al.*, 2008).

Strains in competition may engage in an arms race, favouring spatial differentiation (Czárán *et al.*, 2002; Bucci *et al.*, 2011; Biernaskie *et al.*, 2013). Environmental conditions and competitive phenotypes influence stability and diversity (Schlatter and Kinkel, 2015).

Warfare between two strains may be neutralized by other community members, as seen in antibiotic antagonism among producers (Abrudan *et al.*, 2015). The equilibrium, where different antibiotic producers cancel each other's effects, may be short-lived as strains evolve for competitive advantage (Kelsic *et al.*, 2015). Ultimately, competition tends to reduce diversity and increase ecological stability, influenced by environmental factors, but multiple outcomes can coexist within the same environment (Ghoul and Mitri, 2016).

Cooperation

Cooperation is a fundamental aspect of bacterial social behaviour, encompassing various activities that benefit individuals and their communities. This includes actions like dispersal, foraging, biofilm construction, reproduction, chemical warfare and signalling (Crespi, 2001). *P. aeruginosa*, for example, regulates 6 to 10% of its genes through cell-cell signalling, highlighting the importance of communication and cooperation (Schuster *et al.*, 2003).

Cooperative behaviours often involve the production of public goods, which can be exploited by cheaters-individuals who benefit without contributing to production (West *et al.*, 2006). This apparent paradox, where cooperation appears to defy the survival of the fittest, poses a significant challenge. The Tragedy of the Commons theory underscores the potential instability of cooperation, as individual selfishness can undermine collective benefits. Siderophores production in *P. aeru*ginosa exemplifies this conflict, where cheaters exploit the costly siderophores produced by cooperators, gradually increasing in frequency and potentially outcompeting cooperators (Griffin *et al.*, 2004).

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Cooperation in bacterial populations can be categorized into two types: "whole group traits" and "others only traits" (Pepper, 2000). Whole group traits benefit the entire population, including producers, whereas others only traits involve co-operators sacrificing themselves for the benefit of others. Examples of whole group traits include the production of public goods that enhance resource utilization efficiency (Pfeiffer *et al.*, 2001; Kreft, 2004). Others only traits are exemplified by cellular slime molds and bacteria like *Myxococcus xanthus* forming fruiting bodies or undergoing autolysis to aid in nutrient sharing, sporulation and dispersal (Strassmann *et al.*, 2000; Webb *et al.*, 2003).

The rationale for social cooperation in bacteria can be explained through direct and indirect fitness benefits. Direct benefits occur when cooperation directly enhances the fitness of the co-operator, often through mutual benefits or mechanisms that reward cooperation and punish cheating (Sachs *et al.*, 2004). Indirect benefits, on the other hand, occur when cooperation benefits other individuals carrying the cooperative gene, often related through kin selection (Hamilton, 1964). Genetically related individuals may cooperate to pass down shared genes, facilitated by mechanisms like kin discrimination and limited dispersal (Hamilton, 1964). However, distinguishing between direct and indirect benefits can be complex, particularly in the case of whole group traits like siderophore production (Jansen and van Baalen, 2006). The key question is how such cooperative behaviour can remain stable in the presence of cheaters due to migration or mutation (West and Buckling, 2003).

Kin selection

Kin selection, initially introduced by Smith in 1964, elucidates how relatives collaborate in reproductive efforts to gain indirect fitness advantages. This concept encompasses two categorizations: a more stringent interpretation, where interactions are confined to individuals sharing a common genetic lineage and a broader interpretation encompassing interactions among individuals sharing a particular gene of interest, whether through ancestral connections or alternative mechanisms (Hamilton and Fox, 1975). Hamilton argued in favour of distinguishing general inclusive fitness from kinship effects, hence advocating for the narrower definition of kin selection (Hamilton and Fox, 1975). However, modern researchers predominantly prefer the broader term, as kinship usually underpins the rationale for achieving indirect fitness benefits. In contemporary scientific discourse, the broader interpretation of kin selection is the more commonly employed terminology due to its applicability in various scenarios involving shared genes or genetic relatedness (Jansen and van Baalen, 2006).

Mutual benefit

Mutualism is traditionally defined as a social behaviour that has fitness benefits on both the actor and the recipient (Hamilton, 1964; Lehmann and Keller, 2006). The term cooperation and mutualism are sometimes used interchangeably but this may cause confusions as mutualism is generally used to refer to specific interspecies cooperation (Brown, 1983; Herre *et al.*, 1999; Foster and Wenseleers, 2006). The two terms describe two different ideas. Cooperation describes a simple mutually beneficial social behaviour between an actor and recipient which generally explains direct benefits. This does not explain the possibility of indirect benefits where such interaction may bring harm in the short term but benefits in long term (West *et al.*, 2006). On the other hand, interspecific mutualism describes a bigger picture of the impact of each party on each other. While it is easy to explain how mutually beneficial interactions evolve, interspecific mutualism is a complex issue to address. Hence, the term mutual benefit is a more suitable description of a behaviour that is generally beneficial to both actor and recipient.

Altruism

Altruism, traditionally defined as selfless behaviour entailing costs to the actor while benefiting others, requires a more nuanced consideration. It should be evaluated based on long-term consequences and absolute fitness outcomes. For instance, if a cooperative behaviour incurs short-term costs but yields future benefits, it should be viewed as mutually beneficial rather than purely altruistic. Figure 1 summarised the mechanism of altruism.

Reciprocal altruism, involving nonrelatives who take turns aiding each other, is not genuinely altruistic because it yields direct fitness advantages over time (Trivers, 1971). It entails individuals investing in cooperation now to gain future benefits, making it mutually beneficial rather than purely altruistic.

Altruism has been redefined based on the actor's fitness relative to other group members (Wilson, 1975; Colwell, 1981). Weak altruism describes behaviours that reduce the actor's fitness compared to other group members. Examples include public goods production, where actors bear costs but all group members, including the actors, benefit. This is often termed whole-group or group-beneficial traits (Pepper, 2000; Dugatkin *et al.*, 2003; Dugatkin *et al.*, 2005). The altruistic or mutually beneficial nature of whole-group traits depends on cost-benefit ratios and population structure.

Defining altruism relative to the local group rather than the whole population poses challenges since natural selection acts on entire populations, not arbitrarily defined subsets. Assessing altruism within a group context ignores benefits that spread equally throughout the population. Traits benefiting the entire population are termed altruistic, although these benefits should not be overlooked.

Microbial social behaviour – The biofilm

Biofilms, common in microbial communities, have garnered extensive research attention due to their

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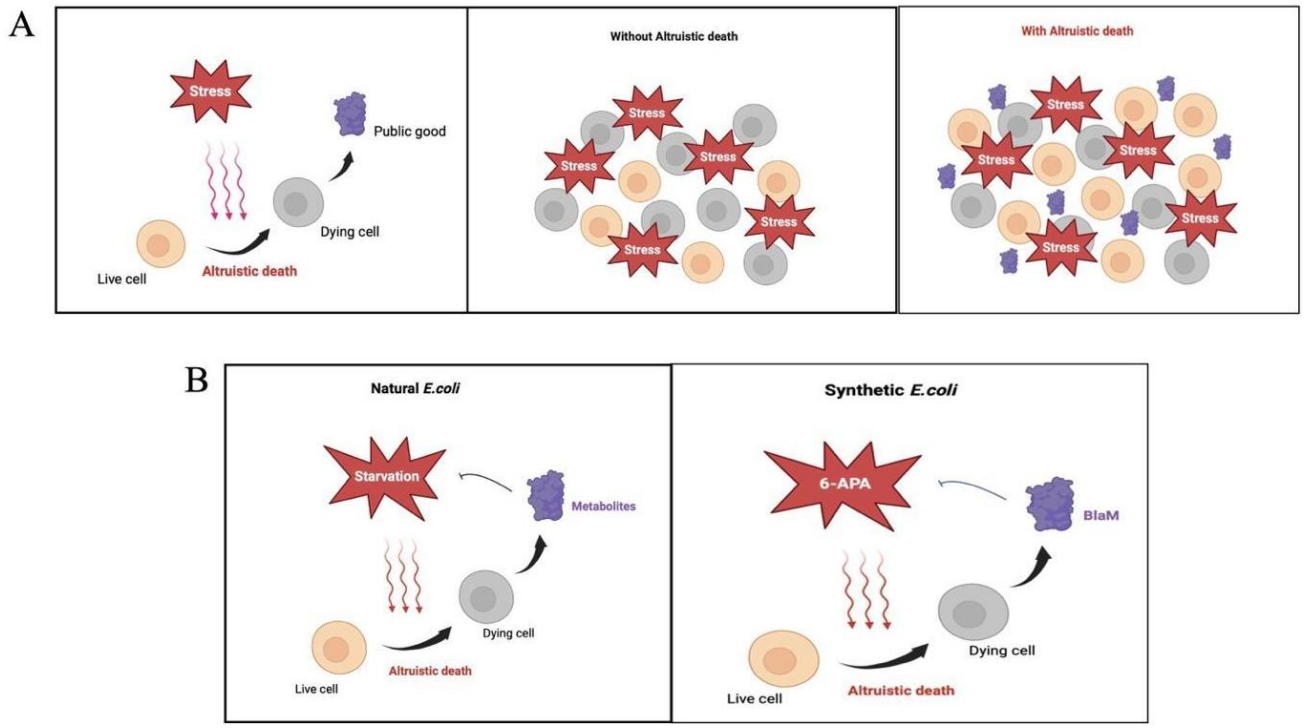

Figure 1: Altruistic cell death serves as a social interaction mechanism that amplifies collective stress resistance. (A) This diagram illustrates an abstract model of altruistic death, where cells succumb to environmental stress but, in the process, release a stress-relieving public good that benefits the survivors. (B) Real-life instances of this phenomenon include *E. coli* cells dying due to starvation, releasing metabolites that nourish the surviving cells. Adapted and modified from Carmona-Fontaine and Xavier (2012). Figure was generated using BioRender software (https://biorender.com).

ubiquity, impact on various processes and potential applications (Stewart, 2002; Davies, 2003). While biofilms can be problematic, they also offer benefits, including applications in wastewater treatment and biological fuel cells (Singh *et al.*, 2006; Logan, 2009; Erable *et al.*, 2010).

Biofilms typically represent surface-associated microbial communities enclosed within a self-produced extracellular matrix. Although their structures vary among species and even strains of the same species, certain fundamental characteristics are shared (Monds and O'Toole, 2009). All biofilms consist of an extracellular matrix comprising polysaccharide biopolymers, proteins and nucleic acids that bind cells together (Branda *et al.*, 2005). Biofilm development can also be influenced by growth conditions, substrates and culture medium. While single-species biofilms are theoretically clonal, they may exhibit genotypic similarity but phenotypic diversity due to differences in gene expression arising from shared gene compositions (Stewart and Franklin, 2008). Such cell differentiation is driven by various factors affecting gene expression. Figure 2 summarised the general biofilm process in bacteria.

Biofilm in Gram-positive bacteria

Bacillus subtilis serves as an excellent model for studying biofilm formation among Gram-positive organisms. Unlike Gram-negative bacteria, *B. subtilis* can undergo developmental processes leading to biofilm production. This process begins with the activation of matrix-secreting genes in response to external signals (Branda *et al.*, 2006). The extracellular matrix plays a crucial role in maintaining the structure and integrity of the biofilm (Marvasi *et al.*, 2010). Initially, short, motile rod-shaped cells form extended chains of stationary cells, which adhere to each other and the surface through the secreted extracellular matrix as biofilms develop (Kobayashi, 2007). As differentiation occurs, the biofilm becomes heterogeneous, with various cell types dynamically localized within it (Vlamakis *et al.*, 2008). This includes spores and motile cells, alongside matrix-producing cells. However, cells can adapt their gene expression in response to different conditions, and lab-generated biofilms have a limited lifespan, disintegrating in response to self-generated signals, allowing spore dispersion (Kolodkin-Gal *et al.*, 2010). It's important to note that biofilm formation is not a prerequisite for sporulation (Branda *et al.*, 2001; Hamon and Lazazzera, 2001).

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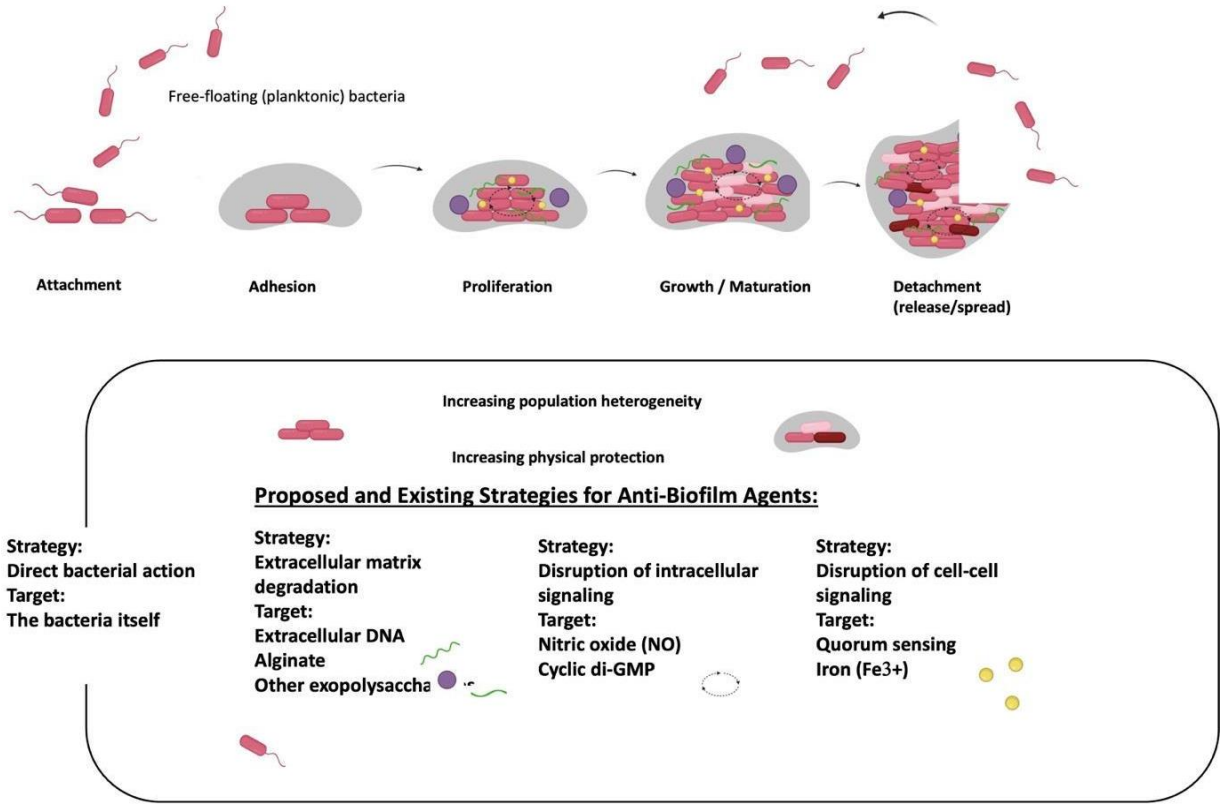

Figure 2: General biofilm process in bacteria. Figure was generated using BioRender software (https://biorender.com).

Biofilm formation in *B. subtilis* is regulated by multiple pathways due to its encounters with shifting soil microenvironments. The Spo0A pathway is the main regulator, influencing matrix production through the SinR-SinI epigenetic switch and repressing the *tapA* and *eps* operons through AbrB (Chu *et al.*, 2006). AbrB also represses matrix protein BslA and regulatory proteins SlrR and Abh (Chu *et al.*, 2008; Verhamme *et al.*, 2009). Dual control by SinR and AbrB allows Spo0A to fine-tune gene expression in response to changing conditions. Additionally, the DegU-DegS two-component system also plays a role in biofilm formation (Verhamme *et al.*, 2007; Verhamme *et al.*, 2009).

Biofilm in Gram-negative bacteria

E. coli and *Salmonella*, both Gram-negative bacteria, are also capable of forming biofilms and this process is linked to their pathogenicity. The initiation of biofilm formation in *E. coli* involves adhesion to a surface. While flagellar movement aids in motility and dispersal along the surface, it's noteworthy that non-motile *E. coli* strains can also form biofilms (Pratt and Kolter, 1999; Sheikh *et al.*, 2001). Strong adhesion factors can replace motility during the initial attachment phase between the bacteria and the surface (Donlan, 2002). Initial attachment depends on physicochemical and electrostatic interactions (Dunne, 2002), while permanent attachment in *E. coli* relies on structures such as type 1 fimbriae, curli and conjugative pili. Type 1 fimbriae adhere in a mannose-dependent manner to various surfaces (Duncan *et al.*, 2005) and are essential for pathogenicity in *E. coli* (Kaper *et al.*, 2004). Curli fimbriae also play a role by aiding attachment to extracellular matrix proteins during biofilm formation (Olsén *et al.*, 1989). Finally, conjugative pili facilitate horizontal gene transfer, promoting biofilm formation and allowing *E. coli* to acquire essential genes from the environment or other *E. coli* strains (Reisner *et al.*, 2006).

Following secure attachment, *E. coli* undergoes maturation to construct a three-dimensional structured architecture, secreting surface proteins and extracellular matrix components. The type V secretion pathway facilitates the translocation of proteins into the extracellular medium, contributing to protein maturation (Henderson *et al.*, 2004). *E. coli* employs numerous adhesins to promote colonization and biofilm maturation. While not directly involved in cell-to-surface adhesion, Antigen 43 (Ag43), a self-recognizing surface autotransporter protein, plays a key role in cell-to-cell adhesion, significantly impacting biofilm maturation (Kjaergaard *et al.*, 2000a). *Ag43* promotes cell-to-cell adhesion in liquid culture, resulting in auto aggregation, clump formation, sedimentation and ultimately biofilm formation (Schembri *et al.*, 2003). Moreover, *Ag43* facilitates heterogeneous biofilm formation between different bacterial species, such as *E. coli* and *P.*

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aeruginosa (Kjaergaard *et al.*, 2000a; 2000b). *Ag43* is complemented by two adhesins, AidA and TibA, commonly found in pathogenic *E. coli*, further promoting aggregation and enhancing biofilm formation (Sherlock *et al.*, 2005). Together, these three proteins, referred to as self-associating autotransporters (SAAT), collectively contribute to biofilm development (Klemm *et al.*, 2006). Additionally, cell surface glycoconjugates, including lipopolysaccharide O antigen and capsular polysaccharide K antigen, are crucial in determining bacterial interactions with their environment and influencing biofilm formation (Beloin *et al.*, 2008). Lipopolysaccharides (LPS), located on the outer membrane of Gram-negative bacteria, affect adhesion processes between bacteria and surfaces, while *E. coli* capsules also play a significant role in biofilm formation by influencing adhesion processes (Beloin *et al.*, 2006).

E. coli biofilms, like those of *B. subtilis*, consist of matrix polysaccharides, proteins, nucleic acids, lipids/phospholipids, nutrients and metabolites (O'Toole and Ghannoum, 2004). Matrix polysaccharides offer structural support and protection to the biofilm. Three crucial exopolysaccharides in *E. coli* biofilm formation are β-1,6-N-acetyl-D-glucosamine polymer (PGA), colanic acid and cellulose (Danese *et al.*, 2000; Agladze *et al.*, 2005; Uhlich *et al.*, 2006). Biofilm formation in *E. coli* is highly regulated. The *cpxRA* system detects environmental changes and responds to envelope stress, promoting early adaptation to stresses and modulating flagellar gene expression (De Wulf *et al.*, 2002). This system also senses abiotic surfaces and neighboring bacteria, contributing to biofilm maturation by modulating cell-to-cell adhesion (Beloin *et al.*, 2004). The EnvZ/OmpR two-component pathway, in collaboration with the *CpxRA* system, senses surface osmolarity, a significant driver of biofilm formation on abiotic surfaces, leading to increased surface adhesion and curli expression (Jubelin *et al.*, 2005). Additionally, the *Rcs* two-component system, including membrane proteins RcsC and RcsD and response regulator RcsB, plays a crucial role in bacterial surface remodelling and biofilm maturation in response to various signals (Majdalani and Gottesman, 2005).

Biofilm as a social interaction

Biofilm formation can be regarded as a form of social interaction, necessitating communication and cooperation among closely situated individuals for development and survival. Within a biofilm, cellular specialization can occur, Boles *et al.* (2004) successfully identified phenotypically distinct cell variants in a wrinkly *P. aeruginosa* biofilm, demonstrating different behaviours such as faster biofilm formation and greater stress resistance. The secretion of the extracellular matrix is a collaborative effort to provide protection against environmental factors or predation, either by expanding the biofilm or through chemical defences (Matz and Kjelleberg, 2005).

Furthermore, the secretion of various public goods essential for biofilm formation, including rhamnolipids, biosurfactants, macro vesicles containing signalling molecules and proteases, is an outcome of social behaviour (West *et al.*, 2006). Cell death can contribute to the entire community by providing nutrients and beneficial genes, either through cooperative self-sacrifice or competitive elimination (Webb *et al.*, 2003). Biofilm dispersal may also result from social behaviour to reduce competition with non-dispersing relatives. Quorum sensing plays a vital role in the coordinated effort of biofilm formation, as evidenced by the inability of quorum sensing-deficient cells to effectively develop a mature biofilm (Davies *et al.*, 1998).

Quorum sensing as a social interaction

Quorum sensing was initially characterized in luminescent marine bacteria, specifically *V. fischeri* and *V. harveyi* (Nealson and Hastings, 1979). In these bacteria, bioluminescence, mediated by the luciferase enzyme luxCDABE, is triggered when cell population density reaches a threshold due to the accumulation of autoinducer signalling molecules (Miyamoto *et al.*, 1988). Quorum sensing is a widespread phenomenon in the bacterial world, with examples including *Streptomyces* spp. coordinating antibiotic production, *Enterococcus faecalis* using it for conjugation, and *Myxococcus xanthus* employing it in fruiting body development (Dworkin and Kaiser, 1985).

Bacteria engage in cell-to-cell communication through the secretion of chemical molecules to coordinate communal behaviours. A diverse range of chemicals and signalling molecules has been identified, and many bacteria can employ multiple signal types for communication. Bacteria have evolved intricate hierarchical regulatory networks to integrate and process sensory information, allowing them to differentiate between species within heterogeneous populations. Such intra- and inter-species communication is vital for bacterial survival in their natural habitats.

The evolutionary significance of quorum sensing is a compelling but often overlooked subject. Microbiologists typically assume that quorum sensing is readily favoured by natural selection because of its positive effects on the entire population (Henke and Bassler, 2004). However, evolutionary theory offers an alternative viewpoint, considering quorum sensing as a mode of communication and cooperation.

Bacteriocin secretion

Microbes utilize a diverse range of defence mechanisms, which include traditional broad-spectrum antibiotics, bacteriocins, metabolic by-products, lytic substances and various protein exotoxins (James *et al.*, 2013). Unlike classical antibiotics, bacteriocins have a relatively narrow spectrum of activity, targeting only bacteria closely related to the producing strain. Bacteriocins are produced by the majority of bacteria and, more recently, have been found in Archaea as well (Torreblanca *et al.*, 1994).

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Bacteriocin in Gram-negative bacteria

The bacteriocin family includes a diverse group of proteins that vary in size, target microorganisms, mode of action and immunity mechanisms. Among them, colicins produced by *E. coli* have been extensively studied. Colicin gene clusters are typically found on plasmids and typically consist of a colicin-encoding gene, a specific immunity-conferring gene and a lysis gene responsible for colicin release through cell lysis (James *et al.*, 1996). The production of colicins is mediated by the SOS regulon under stressful conditions and these toxins are lethal to both the producing cell and neighbouring cells recognized by colicins. Colicins recognize their targets through the interaction between specific colicin protein domains and cell surface receptors, limiting their killing range to phylogenetically related strains. Colicins employ various mechanisms, including pore formation in the cell membrane and nuclease activity against DNA, rRNA and tRNA targets.

It's worth noting that while colicins are classical Gram-negative bacteriocins, they can vary within subgroups of this family. In *E. coli*, bacteriocin genes are exclusively found on plasmids, while nuclease pyocins in *P. aeruginosa* are exclusively encoded on the chromosome. Nuclease pyocins share sequence similarity with *E. coli* colicins but remain uncharacterized. Additionally, genes encoding bacteriocins in *Serratia marcesens*, closely related to the colicin family, are located on both plasmids and chromosomes (Enfedaque *et al.*, 1996).

In general, bacteriocins isolated from Gram-negative bacteria often result from recombination between existing bacteriocins, facilitated by the domain structure of bacteriocin proteins (Lau *et al.*, 1992). The central domain, comprising approximately 50% of the colicin protein, is responsible for recognizing specific cell-surface receptors. The N-terminal domain, making up roughly 25% of the protein, is typically involved in translocating the protein into the target cell. The remaining portion of the protein contains the killing domain and a short immunity region for binding to an immunity protein. Notably, pyocins from *P. aeruginosa* have a reversed order of the translocation and receptor recognition domains but share a similar overall domain structure (Sano *et al.*, 1993).

Bacteriocin in Gram-positive bacteria

Gram-positive bacteria produce a wider variety and higher abundance of bacteriocins compared to Gram-negative bacteria. Unlike Gram-negative bacteriocins, Gram-positive bacteriocins may not be lethal to producer cells. They have a dedicated bacteriocin-specific regulation network and a transport mechanism that includes sec-dependent pathways.

The majority of bacteriocins are produced by lactic acid bacteria (LAB) and can be categorized into three classes (Klaenhammer, 1988). Class I bacteriocins are known as lantibiotics, characterized by post-translational modifications involving amino acids such as lanthionine and B-methyllanthionine (Guder *et al.*, 2000). Lantibiotics can be further divided into subgroups A and B based on their structural features and mode of killing (Jung and Sahl, 1991). Type A lantibiotics, like Nisin, are larger and depolarize the target cell's cytoplasmic membrane (Schüller *et al.*, 1989). Type B lantibiotics, such as mersacidin, disrupt cell wall biosynthesis and are generally smaller with a globular secondary structure, functioning through enzyme inhibition (Brötz *et al.*, 1995).

Class II LAB bacteriocins are small, heat-resistant peptides that lack lanthionine modifications (Jung and Sahl, 1991). They are further categorized into Class IIa and Class IIb. Class IIa bacteriocins share a conserved amino-terminal sequence (YGNGVXaaC) and are known for their activity against *Listeria*, functioning by forming pores in the cytoplasmic membrane of target cells (Hastings *et al.*, 1991). Class IIb bacteriocins also form pores in target cell membranes but are composed of two different proteins (Nissen-Meyer *et al.*, 1992). Recently, a third subgroup of Class II bacteriocins has been proposed to include sec-dependent bacteriocins like acidocin B (Leer *et al.*, 1995).

Class III LAB bacteriocins are large, heat-sensitive proteins, such as helveticins J and V, and lactacin B (Vaughan *et al.*, 1992). A more recent Class IV LAB bacteriocin classification includes bacteriocins that require lipid or carbohydrate moieties, like leuconocin S and lactocin 27 (Bruno and Montville, 1993).

Gram-positive bacteriocins typically require a greater number of genes for their production compared to Gram-negative bacteriocins. For instance, the nisin gene cluster includes genes for a precursor peptide, modification enzymes, leader peptide cleavage protein, secretion, immunity and expression regulation (Engelke *et al.*, 1994). These gene clusters are predominantly found on plasmids but can also be located on chromosomes or transposons (Dodd *et al.*, 1990).

It has traditionally been believed that Gram-positive bacteriocins primarily target other Gram-positive bacteria. For example, lactococcins A, B and M specifically kill *Lactococcus* (Mota-Meira *et al.*, 2000). In contrast, type A lantibiotics like nisin A and mutacin B-Ny266 have demonstrated activity against a wide range of organisms, including Gram-positive species like *Actinomyces*, *Bacillus*, *Clostridium*, *Corynebacterium*, *Enterococcus*, *Gardnerella*, *Lactococcus*, *Listeria*, *Micrococcus*, *Mycobacterium*, *Propionibacterium*, *Streptococcus* and *Staphylococcus*, as well as medically important Gram-negative bacteria like *Campylobacter*, *Haemophilus*, *Helicobacter* and *Neisseria* (Ross *et al.*, 1999).

The production of Gram-positive bacteriocins usually occurs during the transition from the logarithmic growth phase to the early stationary phase. For instance, nisin production typically takes place between the mid-log phase and early stationary phase (Buchman *et al.*, 1988). However, this production pattern is not solely dependent on the cell cycle but is rather influenced by cell population density. Nisin A, for example, can regulate its own expression by acting as a quorum sensing signalling molecule, impacting its two-component systems *nisR* and

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nisK, which consist of a response regulator and a sensor kinase, respectively (Chung *et al.*, 1989). Remarkably, nisin transcription can be controlled by adding nisin to the culture medium, with transcription levels directly correlating with the amount of nisin added (Kuipers *et al.*, 1995).

Bacteriocin and social interaction

The production of bacteriocins can be seen as a potentially antagonistic interaction, involving costs for both producers and recipients (Gardner *et al.*, 2004). Producers may face the expense of diverting resources from other cellular functions to support bacteriocin production. In the case of Gram-negative bacteria, cell death is a necessary step for the release of bacteriocins (Mader *et al.*, 2015). However, it's important to note that bacteriocin production can indirectly benefit the relatives of the producer cell. Since relatives are shielded from the harmful effects of bacteriocins, only unrelated competitors will be eliminated, thereby reducing the intensity of competition experienced by relatives. Consequently, bacteriocin production can also be seen as a form of indirect altruism. The extent of bacteriocin production may be influenced by the degree of genetic relatedness among individuals (Gardner *et al.*, 2004). Optimal bacteriocin production is likely to be favoured when genetic relatedness is at an intermediate level, as there are fewer relatives to enjoy the advantages of reduced competition. Conversely, if genetic relatedness is high, bacteriocin production may be reduced because there are fewer competitors to target.

CONCLUSION

In conclusion, this review delves into the fascinating world of microbial interactions, focusing on various aspects of bacteriocins and biofilm formation. Bacteriocins, produced by both Gram-negative and Gram-positive bacteria, represent a diverse array of antimicrobial peptides and proteins that play essential roles in microbial competition and survival. While Gram-negative bacteriocins, such as colicins, tend to have a narrow killing range and are often associated with plasmids, Gram-positive bacteriocins exhibit greater diversity and are typically regulated by dedicated systems. These bacteriocins may not always be lethal to producer cells and can have broader target ranges.

On the other hand, biofilm formation is a complex process involving microbial communities that cooperate and communicate effectively. Biofilms are structured communities encased in an extracellular matrix that provide protection and facilitate survival in various environments. Microbes within biofilms display cooperative behaviours, such as the secretion of public goods, which benefit the entire community. Quorum sensing, a form of bacterial communication, plays a pivotal role in coordinating these social behaviours within biofilms.

Furthermore, the study highlights the intricate balance between competition and cooperation in microbial communities. Bacteriocin production can be seen as a form of spiteful interaction, incurring costs for both producers and recipients, but also providing indirect benefits to relatives by reducing competition with non-relatives. This dynamic interplay between competition, cooperation, and communication is essential for understanding the survival and adaptation of microorganisms in their natural habitats.

Overall, this study sheds light on the multifaceted strategies employed by microorganisms to thrive and adapt in complex ecological niches. It underscores the importance of considering both the individual and collective behaviours of microbes when studying their interactions and ecological roles.

CONFLICTS OF INTEREST

All authors declare no conflict of interest.

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