Determinants of synergistic cell-cell interactions in bacteria

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Running title

Determinants of synergistic bacterial interactions

Abstract

Bacteria are ubiquitous and colonize virtually every conceivable habitat on earth. To achieve this, bacteria require different metabolites and biochemical capabilities. Rather than trying to produce all of the needed materials by themselves, bacteria have evolved a range of synergistic interactions, in which they exchange different commodities with other members of their local community. While it is widely acknowledged that synergistic interactions are key to the ecology of both individual bacteria and entire microbial communities, the factors determining their establishment remain poorly understood. Here we provide a comprehensive overview over our current knowledge on the determinants of positive cell-cell interactions among bacteria. Taking a holistic approach, we review the literature on the molecular mechanisms bacteria use to transfer commodities between bacterial cells and discuss to which extent these mechanisms favour or constrain the successful establishment of synergistic cell-cell interactions. In addition, we analyse how these different processes affect the specificity among interaction partners. By drawing together evidence from different disciplines that study the focal question on different levels of organisation, this work not only summarizes the state of the art in this exciting field of research, but also identifies new avenues for future research.

Keywords

Co-aggregation; cooperation; cross-feeding; partner specificity; synergistic interaction

Introduction

Microbial communities are ubiquitous on our planet and play significant ecological roles – for examples as drivers of global biogeochemical cycles or as symbionts of animals and plants (Strickland et al. 2009; Delgado-Baquerizo et al. 2016; Valdes et al. 2018; Vijay and Valdes 2022). These vital functions typically emerge from ecological interactions among different species that exist within taxonomically diverse microbiota (Wagg et al. 2021). Thus, understanding the mechanisms that shape the establishment, stability, and functioning of microbial communities requires knowledge of the underlying ecological interactions.

The tremendous diversity of different ecological interactions that can be observed within microbial communities is generally classified into *antagonistic* or *synergistic* interactions. Antagonistic interactions include all of those cases, in which bacteria harm or kill other bacteria in their vicinity in order to gain a competitive advantage. In contrast, in synergistic interactions, bacteria benefit from the intentional or unwitting behaviour of another individual in their local environment. Antagonistic interactions are generally well understood both in terms of the causal molecular mechanisms (Alteri and Mobley 2016; Peterson et al. 2020) and their eco-evolutionary causes and consequences (Ghoul and Mitri 2016; Granato and Foster 2020; Niehus et al. 2021). However, synergistic interactions have only recently started to move into the focus of attention of a broader research community. The pattern that has started to emerge from applying different methodological approaches is that synergistic interactions are not only highly diverse in form and function, but also that they rely on intimate interactions among bacterial cells. However, what determines the establishment of synergistic interaction among bacterial cells? Do the interactions we see in microbial communities result from rather random encounters between cells or

are there certain rules that structure the emergence and functioning of synergistic interactions within microbial communities? Here we address these issues by drawing together the recent literature on this topic. Taking a holistic approach, we begin by reviewing the different kinds of benefits bacteria exchange in synergistic interactions. After that we analyse which mechanisms bacteria use to exchange benefits between cells, and how the mechanistic nature of the interaction impacts the ecological and evolutionary dynamics within these interactions. By comprehensively summarizing our current knowledge on the factors that determine synergistic cell-cell interactions, this work shall not only provide an overview over this exciting field of research, but also highlight the gaps in our knowledge that can guide the design of future studies.

Benefits of synergistic cell-cell interaction

Synergistic interactions between bacteria of the same or different species are widespread in nature and include a broad range of goods that are exchanged between interacting partners. To provide an overview over the enormous diversity of materials or services that bacteria exchange as part of a synergistic interaction, we classify them into four main categories that are based on the type of exchanged commodity as well as the way this good benefits the receiving cell. The four different types of exchanged goods include: (I) metabolites, (II) energy, (III) services, and (IV) information (Figure 1). In the following, we discuss each of these categories.

The first and probably most important kind of good that is exchanged among bacteria are metabolites (Figure 1). In a process generally referred to as cross-feeding, bacteria transfer substances between cells that derive from the primary or secondary metabolism (Lilja and Johnson 2016; Pacheco et al. 2019; Fritts et al. 2021) and which benefit the receiving cell. These metabolites can either be useful to the donor cell itself

(e.g. vitamins) or be metabolic byproducts that are released by the donor cell and which can be metabolized by the recipient (e.g. sequential degradation of complex substrates (Odom and Wall 1983)). The exchanged compounds can serve as metabolic building blocks (e.g. amino acids, nucleotides) or be used as a source of energy by the recipient (Supplementary Table 1). This is in contrast to metabolites that are produced to induce a certain response in the receiver (i.e. signalling molecules; see 'information exchange' below).

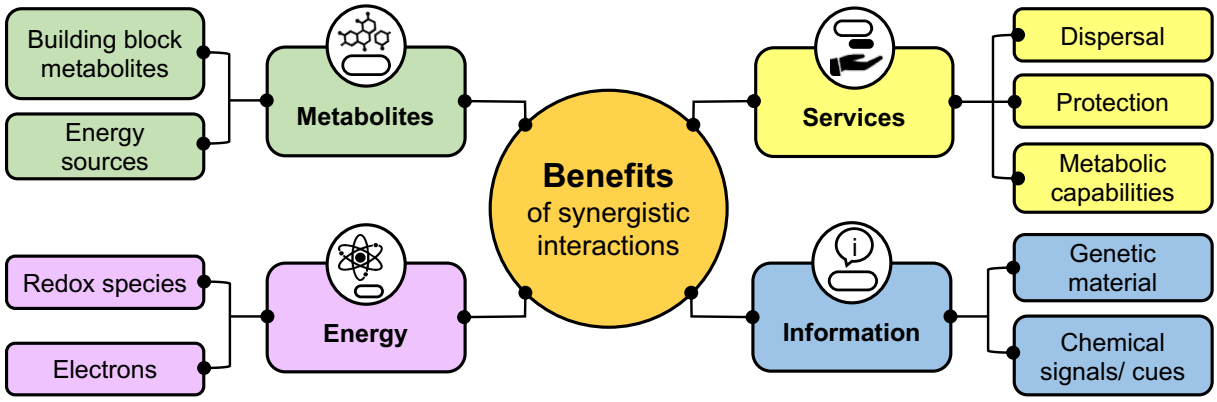

Figure 1: Types of benefits bacteria exchange in synergistic interactions.

Besides metabolites, bacteria can also exchange electrons as a form of non-material energy between cells (Figure 1, Table 1, Supplementary Table 1). This can be achieved by shuttling redox compounds such as H_2 or formate via diffusion through the extracellular environment from electron-donating to electron-accepting cells (Stams et al. 2006; Stams and Plugge 2009; Shi et al. 2016). Alternatively, a transfer of electrons can be mediated by a direct physical contact of cell surfaces between interacting cells (Jiang et al. 2018; Meysman et al. 2019) or via long (µm-cm range) electrical connections between cells (Summers et al. 2010; Shi et al. 2016; Walker et al. 2020). The latter includes (i) electrically conductive nanowires that are formed by

Table 1: Mechanisms bacteria use to transfer synergistic benefits between bacterial cells. Both the commodity (i.e. M = metabolites, E = electrons, S = services, and I = information) that has been shown to be exchanged using the different mechanisms and whether the exchange has been observed within members of the same or different species is indicated (- = no example found, + = few cases found (< 50% of examples), ++ = many cases found (>50%)).

Transfer mechanism	Commodity	Within species	Between species	References
Passive diffusion	M, E, S, I	+	++	Abisado et al. 2018; Bernier et al. 2011; Pacheco et al. 2019; van Tatenhove-Pel et al. 2021; Sher et al. 2011; Grandclement et al. 2016; Harcombe et al. 2014; Bridges and Bassler 2019
Active transport	M, E, S, I	+	++	Abisado et al. 2018; Sokolovskaya et al. 2020; Butaite et al. 2021; Grandclement et al. 2016
Vesicle exchange	M, I	+	+	Woith et al. 2019; Toyofuku et al. 2017; Berleman et al. 2014; Schwechheimer and Kuehn 2015; Brown et al. 2015; Biller et al. 2014; Kim et al. 2015
Vesicle chains	M, I	++	-	Remis et al. 2014; Pirbadian et al. 2014; Berleman et al. 2014
Nanowires	E	+	+	Shi et al. 2016; Summers et al. 2010; Wang et al. 2019; Wegener et al. 2015; Pirbadian et al. 2014
Nanotubes	M, I	+	++	Shi et al. 2016; Dubey and Ben-Yehuda 2011; Dubey et al. 2016; Pande et al. 2015; John et al. 2017
Pili	M, E, I	+	++	Shi et al. 2016; Walker et al. 2020; Virolle et al. 2020; Wang et al. 2005
Flagella	S	-	++	Muok et al. 2021; Marx 2009; Ishii et al. 2005; Shimoyama et al. 2009; Friedlander et al. 2013
Cell-cell attachment	M, E, S, I	++	+	Shi et al. 2016; Drescher et al. 2014; van Tatenhove-Pel et al. 2021; Benomar et al. 2015; Sah and Wall 2020; Pande et al. 2016; Marchal et al. 2017; Cao et al. 2015; Kim et al. 2008; Nadell et al. 2016; Hobley et al. 2015

unicellular bacteria (e.g. *Geobacter* species) (Summers et al. 2010; Wang et al. 2019), (ii) conductive conduits that consist of multicellular, filamentous bacteria (Pfeffer et al. 2012; Shi et al. 2016; Lovley 2017; Meysman 2018), or (iii) extracellular mineral particles that connect redox reactions that are catalysed by different microbial species (Reguera et al. 2005; Kato et al. 2012; Shi et al. 2016). In all of these cases, the

transferred electrons are used to establish ion-gradients across the cellular membrane, which in turn is used for ATP synthesis. Although in most cases, the exact mechanisms of electron transfer between cells are not completely understood, the available evidence suggests that this is a very widespread and ecologically important way of how two different species interact synergistically (Morris et al. 2013).

The third type of benefit bacteria exchange with each other are services (Figure 1, Table 1, Supplementary Table 1). A service results from the behaviour one bacterium performs, typically to enhance its own Darwinian fitness, and which benefits another bacterium in its immediate vicinity. Such services can include a range of different traits such as transport (e.g. hitchhiking of a non-motile bacteria by attaching to the flagellum of another motile bacterium (Muok et al. 2021)), detoxification (e.g. degradation of an antibiotic (Sorg et al. 2016; Cubillos-Ruiz et al. 2022), or niche construction (e.g. changing pH in a favourable direction (Aranda-Díaz et al. 2020)). Other processes falling into this category are the production of biofilms (Madsen et al. 2016; Dragoš et al. 2018), the release of digestive enzymes (e.g. proteases, chitinases (Drescher et al. 2014; Smith and Schuster 2019)) to degrade complex molecules or of siderophores to sequester iron (Amin et al. 2009).

The final commodity that is exchanged between bacteria is information (Figure 1, Table 1, Supplementary Table 1). Information can be transferred between cells in the form of genetic material, which encodes a certain biochemical capability that is beneficial to the receiver (e.g. a gene conferring antibiotic resistance (Kent et al. 2020)). Genetic information such as chromosomal or plasmid DNA can be passed from one bacterium to another one using a variety of mechanisms including conjugation (Lederberg and Tatum 1946), transformation (Griffith 1928), or transduction (Lederberg 1952; Zinder and Lederberg 1952). Besides these canonical pathways of

156 horizontal gene transfer, several alternative mechanisms such as nanotubes (Dubey
157 and Ben-Yehuda 2011) and membrane vesicles (Woith et al. 2019; Dell'Annunziata et
158 al. 2021) have been described (Table 1, Supplementary Table 1). The diversity of
159 mechanisms bacteria use to exchange information in the form of genetic material
160 underscores its importance for the ecology and evolution of microbial communities.
161 Besides a transfer of genetic material that potentially leads to a long lasting
162 modification of a cell's phenotype, bacteria also exchange information as a form of
163 communication. For this, metabolites are released into the surrounding gaseous or
164 aqueous phase, which induces an immediate yet rather momentary response in the
165 receiving cell. The physico-chemical properties (e.g. size, polarity) of the molecules
166 that are used for this purpose determine their diffusion rates and thus the radius around
167 the emitter, within which the molecule takes its full effect (Netzker et al. 2020).
168 Chemical communication of this kind is used to coordinate behaviours within species
169 (e.g. quorum sensing (Cook and Federle 2014; Mould et al. 2020)) or between different
170 species (Abisado et al. 2018; Laganenka and Sourjik 2018; Ranava et al. 2021). In
171 both cases, chemical communication systems regulate the expression of genes that
172 are involved in, for example, the production of biofilms (Laganenka and Sourjik 2018)
173 or virulence factors (Mould et al. 2020). Because members of the same species
174 strongly benefit from a coordinated expression of these genes, these communication
175 systems are frequently highly species-specific (Hawver et al. 2016). Nevertheless, it is
176 well established that chemical signals can also induce responses in unrelated species
177 (Federle and Bassler 2003; Abisado et al. 2018). However, in these cases, the potential
178 benefits resulting for both sender and receiver of the message remain frequently
179 unclear (Bernier et al. 2011).

180

Mechanisms of metabolite transfer between bacterial cells

Bacteria use a wide array of different mechanisms to exchange metabolites or services between cells. These mechanisms are generally subdivided into two main categories, depending on whether the transfer of materials depends on a direct physical contact between bacterial cells (i.e. *contact-dependent* exchange) or not (i.e. *contact-independent* exchange) (D'Souza et al. 2018; Pacheco et al. 2019; van Tatenhove-Pel et al. 2021; Figure 2). In addition, these mechanisms differ with regard to the spatial distance that is covered during the transfer process. While interactions that require a close contact of cell surfaces for the interaction to occur result in a direct transfer of metabolites between cells (distance: < 1 µm) (Benomar et al. 2015; Sah and Wall 2020; López-García and Moreira 2021), other mechanisms that depend on the formation of, for example, cell membrane protrusions (e.g. nanowires, pili, nanotubes) can cover intermediate distances between cells (i.e. ~ 1-60 µm) (Remis et al. 2014; Dubey et al. 2016; Fischer et al. 2019). In contrast, contact independent interactions that for example rely on an exchange of materials via diffusion through the extracellular environment, can cover gaps between cells that range from direct cell proximity up to very large distances (i.e. > 100 µm) (Be'Er et al. 2009; Cordero and Datta 2016; van Tatenhove-Pel et al. 2021). In the following, we will provide an overview over the different kinds of mechanisms bacteria use to transfer materials between bacterial cells (Figure 2, Table 1, Supplementary Table 1).

The first group of mechanisms bacteria use to exchange materials are summarized under the umbrella term contact-independent mechanisms. Processes that fall into this category typically cover longer distances between bacterial cells and rely on a diffusion of exchanged goods either as a free molecule or as compounds that are encapsulated within membrane vesicles.

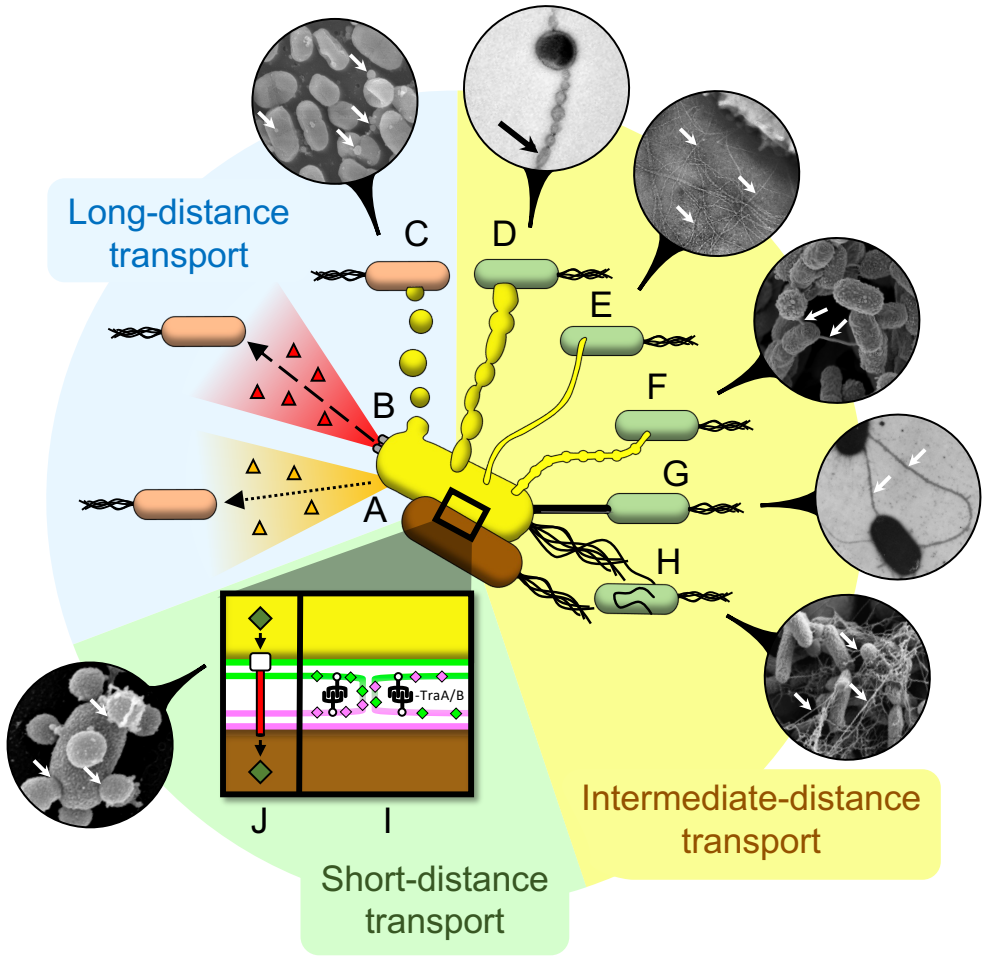

Figure 2: Mechanisms bacteria use to exchange synergistic benefits between bacterial cells. The different transfer mechanisms are subdivided into three categories based on the distance they can bridge. (A-C) Contact-independent transport mechanism that can cover longer distances (blue background) include (A) passive diffusion, (B) active transport, and (C) vesicle-mediated exchange. (D-H) Contact-dependent transport mechanisms covering short to intermediate distances between bacteria (yellow background) comprise (D) vesicle chains, (E) nanowires, (F) nanotubes, (G) pili, and (H) flagella. (I-J) The last category of contact-dependent processes involves mechanisms that facilitate transport over relatively short-distances (green background) such as the (I) TraA/B-mediated fusion of outer membranes and (J) type-III, -IV or -VI secretion systems. Electron micrographs show the corresponding structures (arrows). Photos reproduced with permission from (C) Prof. Dr. Steven Biller, (D) (Fischer et al. 2019), (E) (Dahl et al. 2022), (G) (Curtiss III, Roy, et al. 1969), (H) (Friedlander et al. 2013), (I,J) (López-García and Moreira 2021).

Emission of materials from cells can either be passive through an unintended leakage of certain compounds through the cellular membrane (Be'Er et al. 2009; Pacheco et al. 2019; Figure 2A) or be due to an active, transporter-mediated export of compounds out of cells (Sokolovskaya et al. 2020; Butaite et al. 2021; Figure 2B). Functional reasons for an active transport can be the disposal of accumulating waste products to prevent autointoxication (Lilja and Johnson 2016), the externalization of

specific metabolites to alter the environment (e.g. β-lactamase to degrade ampicillin (Yurtsev et al. 2016)), or the triggering of specific biological responses in other cells (e.g. quorum sensing (Abisado et al. 2018)). Another example of actively released compounds are siderophores that chelate iron. By taking up the iron-siderophore complex, bacteria scavenge this essential nutrient from iron-deplete environments (Sandy and Butler 2009; Butaite et al. 2021). In addition, bacteria can secrete enzymes into the extracellular environment to harvest nutrients by degrading complex polymers (e.g. chitin (Beier and Bertilsson 2013) or lignin (Vicuña 1988)) or to modify environmental conditions (e.g. adjusting the pH (Aranda-Díaz et al. 2020)).

Active secretion mechanisms require energy-dependent transporters (e.g. ABC transporters (Rees et al. 2009)) and can be modulated to regulate social behaviours within a group of interacting microorganisms. One example for this is intraspecific (Kalamara et al. 2018) and interspecific (Ayrapetyan et al. 2014) quorum sensing via the secretion of signalling molecules called autoinducers that coordinate physiological processes of a community in a cell density-dependent manner. In *Bacillus subtilis* for example, it is known that this system regulates the production of metabolites (e.g. surfactin, lipopeptides) that are released into the environment and are available to all community members within a population, thereby controlling cooperative behaviours such as swarming or biofilm formation, which are crucial for the cells' survival (Kalamara et al. 2018).

The third diffusion-based transfer mechanism is an exchange of materials between cells via membrane-encapsulated vesicles (Kim et al. 2015; Figure 2C). These vesicles are bilayered structures that can either be derived from a cell's outer membrane or be outer-inner membrane vesicles that result from membrane blebbing or the explosive lysis of cells (Toyofuku et al. 2019). Both types of vesicles encapsulate

the transferred commodity, thereby not only protecting it from environmental influences, but also preventing its dilution into the surrounding medium (Woith et al. 2019).

The second main group of mechanisms bacteria use to transfer materials between cells depends on a direct physical contact between interacting individuals. The group of processes that are summarized in this category can cover short to intermediate distances between bacterial cells. This can be achieved for example by cell protrusions (Kaplan et al. 2021) that stretch out to the next interaction partner to either connect cells or even bridge the cytoplasm between them.

The first mechanism in this category are so-called *vesicle chains*. By interlinking outer membrane vesicles, bacteria (e.g. *Myxococcus*) generate a tubular structure with a continuous lumen (Remis et al. 2014; Wei et al. 2014; Figure 2D). In this way, vesicle chains not only connect cells, but also facilitate a targeted exchange of metabolites between cells (Ducret et al. 2013; Remis et al. 2014). More detailed studies have identified two different types of vesicle chains. The first one consists of outer membrane protrusion that then transform into a vesicle chain by a process called *pearling* (Fischer et al. 2019). The second type of vesicle chains seems to originate from inner membrane vesicles that remain enclosed by the outer membrane and are then transported away from the cell due to the elongation of the outer membrane tube (Fischer et al. 2019). Vesicle chains not only mediate interactions between bacteria, but also extend the cell's surface. In this way, additional surface space is generated for catalytic/ enzymatic reactions that can also increase the likelihood to detect and import nutrients (Fischer et al. 2019).

The second type of structure bacteria use to interact with the environment (Cheng and Call 2016; Shi et al. 2016) or other cells of the same or different species (Summers

et al. 2010; Wegener et al. 2015; Lovley 2017) are so-called *nanowires* (Figure 2E). These membrane protrusions facilitate the exchange of electrons by directly shuttling electrons or substitute metabolites like H_2 or formate, thus enabling further redox reactions. The formation of nanowires seems to be species-specific and currently two main models exist. In *Geobacter sulfurreducens,* nanowires have been suggested to be a micrometer-long polymerization of the hexaheme cytochrome OmcS (Wang et al. 2019), while in *Shewanella oneidensis* MR-1, nanowires are considered to be outer membrane extensions containing soluble periplasmic components together with multiheme cytochromes (Pirbadian et al. 2014). Generally, a broad variety of bacterial and archaeal species are known to use different cytochromes to facilitate the extracellular transport of electrons (Cheng and Call 2016; Shi et al. 2016).

The third mechanism bacteria employ to establish cell-cell connections are *nanotubes*. These more recently discovered structures are composed of chains of continuous membranous segments traversed by an uninterrupted lumen (Dubey et al. 2016; Figure 2F). As such, their structure shows a striking similarity to the previously discussed vesicle chains. Two types of nanotubes have been described. First, *intercellular nanotubes* that connect neighbouring cells and, second, *extending nanotubes* that reach into the surrounding of the cell and seem to function like plant roots by searching for nutrients and interaction partners (Dubey et al. 2016). In general, nanotubes are known to facilitate cell-cell communication and mediate the exchange of cytoplasmic metabolites and plasmid DNA between cells of the same or different bacterial species (Dubey and Ben-Yehuda 2011; Pande et al. 2015; Dubey et al. 2016; Stempler et al. 2017).

The fourth structure used by bacteria to interconnect cells are *pili* (Figure 2G), which are non-flagellar, proteinaceous, multi-subunit surface appendages (Kline et al. 2010).

Pili facilitate the attachment of the pili-forming individual to other bacteria, host cells, or environmental surfaces. In addition, these structures can be involved in biofilm formation, cell motility, signalling, as well as protein and DNA transport across membranes. Pili can be categorized into five main classes, namely (1) chaperone-usher pili, (2) curli, (3) type III secretion pili, (4) type IV pili, and (5) type IV secretion pili (Fronzes et al. 2008; Kline et al. 2010). Pili cover a wide range of functions and can be involved in both synergistic and antagonistic interactions (Fronzes et al. 2008).

The fifth mechanism bacteria use to establish connections over intermediate distances between cells is mediated by *flagella* (Figure 2H). These filaments, which mainly consist of flagellin, serve the main purpose to propel bacteria forward (Silverman and Simon 1977). However, more recent studies have revealed that flagella can also be used by bacteria to bind interaction partners in order to reduce the spatial distance for an otherwise diffusion-based exchange (Marx 2009; Ishii et al. 2005). One study even suggested that the flagellar cap protein FliD was involved in synchronizing the metabolism of interacting cells of *Pelotomaculum thermopropionicum* and *Methanothermobacter thermautotrophicus*, thus pointing to an important role of flagella for triggering the syntrophic interaction between interacting strains (Shimoyama et al. 2009).

The final way of how bacteria can transfer synergistic benefits between cells is by interacting with each other via immediate cell surface connections (Benomar et al. 2015; Charubin et al. 2020; Figures 2I,J). For this, cells frequently produce an extracellular matrix that mainly consists of polysaccharides, proteins, and DNA (Flemming and Wingender 2010), thus leading to the formation of a free-floating (Gilbertie et al. 2019) or surface-attached bacterial aggregate (Madsen et al. 2016). Direct cell surface connections are the closest interaction two cells can form (Benomar

et al. 2015; López-García and Moreira 2021) and have been shown to be involved in the exchange of membrane material and proteins (Sah and Wall 2020; Figure 2I) as well as cytoplasmic constituents (Benomar et al. 2015; Figure 2J).

Although mechanism to exchange synergistic benefits between bacterial cells abound in bacterial communities, the factors determining which mechanism is used under what circumstances still remain unclear. Besides the type of the traded good or physiological constrains operating on cells, it is likely that also eco-evolutionary factors play an important role in determining which mechanism is used given certain environmental condition. In the following, it will be discussed how the interplay between these factors shapes synergistic interactions.

Transfer mechanisms determine the specificity of synergistic interactions

Given the functional and structural diversity of synergistic interactions in bacteria, a key question is which factors determine whether a certain interaction can successfully establish and be maintained in the long-run. The answer to this question is strongly determined by the mechanism that is used to transfer the traded commodity between cells.

In the case of contact-independent interactions, goods are mostly unspecifically released into the extracellular environment (e.g. biofilm production, enzymes to degrade toxins, metabolites). These so-called *public goods* are equally accessible to both the cells producing them and other cells that are present in the local environment. Specificity with regards to the type of cells taking advantage of the traded good can emerge based on the molecular nature of the secreted substance. For example, the majority of quorum sensing signals can only be understood by members of the same species (McCaig et al. 2013). Moreover, a nutrient that has been released into the

361 extracellular environment can benefit some cells more than others – for example
362 because of strain-specific preferences for this metabolite (Pacheco et al. 2019). Also
363 vesicle-encapsulated cargos limit the availability of the exchanged materials to certain
364 strains, because the recipient requires a specific machinery to capture and fuse with
365 the membrane vesicle (Toyofuku et al. 2017). Despite these exceptions, contact-
366 independent interactions are, in general, less specific than interactions that rely on a
367 physical contact between cells. A consequence of this is that unintended recipients
368 can take advantage of the public good, potentially resulting in significant costs to the
369 producing cells (van Tatenhove-Pel et al. 2021).

370 In contrast, contact-dependent interactions are intrinsically more specific with
371 regards to the choice of suitable interaction partners. This link stems from the fact that
372 in contact-dependent interactions, the producer of a synergistic benefit can, in
373 principle, actively decide with which other cell it initiates a certain interaction and
374 potentially also the time at which it terminates the relationship. This possibility puts the
375 producer of the beneficial good in control over the establishment and the duration of
376 the interaction. A second factor that contributes to the increased specificity of contact-
377 dependent transfer mechanisms is the fact that the molecular mechanisms that are
378 used to adhere to other cells and establish intercellular interactions are frequently
379 highly species-specific. While a nanotube-mediated exchange of metabolites and
380 protein has been demonstrated to function in a wide range of interspecific interactions
381 (Dubey and Ben-Yehuda 2011; Pande et al. 2015), other transfer mechanisms such
382 as vesicles (Tashiro et al. 2017) or pili (Low et al. 2022) are known to operate within a
383 taxonomically restricted set of species. Also the exchange of genetic information is
384 more likely to be successful within bacterial taxa that are more closely related to each
385 other (Gogarten et al. 2002; Lawrence and Hendrickson 2003; Polz et al. 2013),
386 suggesting that either the mechanisms used to transfer genetic material (e.g.

conjugation pili, viruses) or the transferred genetic material itself (e.g. ability of a plasmid to replicate in a new host cell (Caspi et al. 2000), fitness costs for expressing foreign genes (San Millan and Maclean 2017)) limits the flow of genes to evolutionarily more distant taxa. Taken together, the presented evidence suggests that the kind of mechanism that is used to transfer synergistic benefits between bacterial cells potentially operates as a filter that biases the taxonomic distribution of synergistic interactions within microbial communities. However, systematic studies that analyse the species-specificity of different transfer mechanisms remain scarce.

Detection and recognition mechanisms are often highly specific

A key issue for the establishment of synergistic interactions between bacterial cells is the detection and recognition of potential interaction partners. The two main steps that are important in this process are (i) the detection of suitable cells from a larger distance and (ii) the recognition of target cells upon closer contact.

Bacteria feature highly sensitive olfactory systems that they use to detect and localize suitable food sources (Yawata et al. 2020) or symbiotic partners (Taylor and Stocker 2012). In this context, the successful localisation of the source of a chemical gradient depends on properties of the individual cell (e.g. the sensitivity of a strain's chemotaxis pathway or its swimming speed) as well as on environmental factors such as the viscosity of the surrounding medium or the amount of chemoattractant that is emitted from the source (Keegstra et al. 2022). To localize a certain target, bacteria require sufficiently strong emission levels of the corresponding chemoattractant. Thus, over longer distances, bacteria are more likely to be attracted to chemicals that are produced by larger assemblages of bacteria (e.g. biofilms (Moore-Ott et al. 2022)) or food particles than to individual cells. Nevertheless, chemotaxis-based mechanisms

412 are likely sufficient to attract a specific set of bacterial species from the surrounding
413 environment to a certain location (Lambert et al. 2019).

414 Once bacteria get into closer contact, a suit of mechanisms starts to operate that
415 further enhances the specificity of cell-cell interactions. Three main principles have
416 been described in this context (Rendueles and Ghigo 2012; Troselj et al. 2018).

417 First, bacteria recognize suitable interaction partners by using adhesive structures
418 (e.g. adhesins (Rickard et al. 2003)) that specifically bind to receptors on the surface
419 of other cells (i.e. co-aggregation (Rickard et al. 2003)). This kind of mechanism is
420 typically highly species-specific. The resulting consortia of aggregating cells then
421 benefit from an enhanced ability to colonize a certain environment (e.g. tooth biofilms
422 (Kolenbrander et al. 2006)) or from metabolic interactions between interconnected
423 partners (Bradshaw et al. 1994; Palmer Jr et al. 2001)).

424 Second, bacteria produce structures which allow them to rather unspecifically
425 adhere to other bacteria (e.g. exopolysaccharides) (Burdman et al. 1998). Once other
426 species start to attach, specificity can be introduced by favouring the growth of some
427 strains (Culotti and Packman 2014; Ren et al. 2015), while inhibiting the attachment or
428 growth of others (Rendueles and Ghigo 2012).

429 The third kind of specificity-enhancing mechanisms increases the relatedness within
430 a local assemblage of bacteria (i.e. kin groups) by killing other unrelated individuals.
431 Antagonizing behaviours that aim at obliterating competitors of the same or different
432 species typically rely on the production of toxins. Due to the fact that the survival of
433 cells hinges upon their resistance to the killing mechanism used, cognate pairs of toxin
434 and immunity-conferring proteins contribute to the establishment of genetically well-
435 defined bacterial groups. This kind of mechanism can be beneficial on the consortium-
436 level, because the division of labour among for example toxin-producing cells and the

faster growing toxin-resistant individuals enhances the competitive ability of the whole group (Zhang et al. 2020). Moreover, the local elimination of susceptible genotypes can efficiently prevent third parties from exploiting other synergistic behaviours among group members such as swarming (Kraigher et al. 2022) or public goods cooperation (McNally et al. 2017).

This kind of toxin-based cell-cell recognition mechanisms can be contact-dependent or contact-independent. Contact-dependent recognition of other cells can be mediated by receptors that specifically bind other cells (e.g. interactions between TraA and TraB in myxobacteria (Cao and Wall 2017) or CdiA of the contact-dependent inhibition system in *Escherichia coli* (Aoki et al. 2005)). In a second step, the toxin is then delivered to target cells where it takes full effect. This can be achieved by a transfer of outer membrane material that contains the toxic protein (Vassallo et al. 2018; Figure 2I) or via the delivery of the toxic protein into the cytoplasm of the target cell using type III (Zhu et al. 2006), IV (Souza et al. 2015), VI (Crisan and Hammer 2020), or VII (Cao et al. 2016) secretion systems (Figure 2J). Alternatively, other strains that try to invade a local group of bacteria can also be eradicated by releasing toxins (e.g. bacteriocins) into the extracellular environment (Tait and Sutherland 2002). Again, only bacteria that are resistant to the toxin will be able to survive, thus representing a strong filter that favours closely related kin. In the absence of other, potentially exploitative genotypes, synergistic interactions among the members of the local communities can thrive unrestrictedly.

Taken together, all of the abovementioned mechanisms confine the taxonomic diversity within groups of interacting bacteria to only include those that manage to attach to other resident strains and/ or are resistant to the antagonistic behaviours shown by members of the local group. While certain types of cell-cell recognition

systems only allow the establishment of interactions among conspecific genotypes (Aoki et al. 2005; Cao and Wall 2017), others also include interspecific interactions (e.g. co-aggregation).

Relatedness among partners determines the establishment of synergistic interactions

The above analysis suggests that the mechanism bacteria use to exchange synergistic benefits and recognize suitable partners likely impacts the structure of synergistic interactions within microbial communities. If this is the case, a clear correlation between the probability for a successful establishment of a synergistic interaction and the phylogenetic relationship among interacting partners should be detectable. In theory, three potential patterns can be expected (Figure 3).

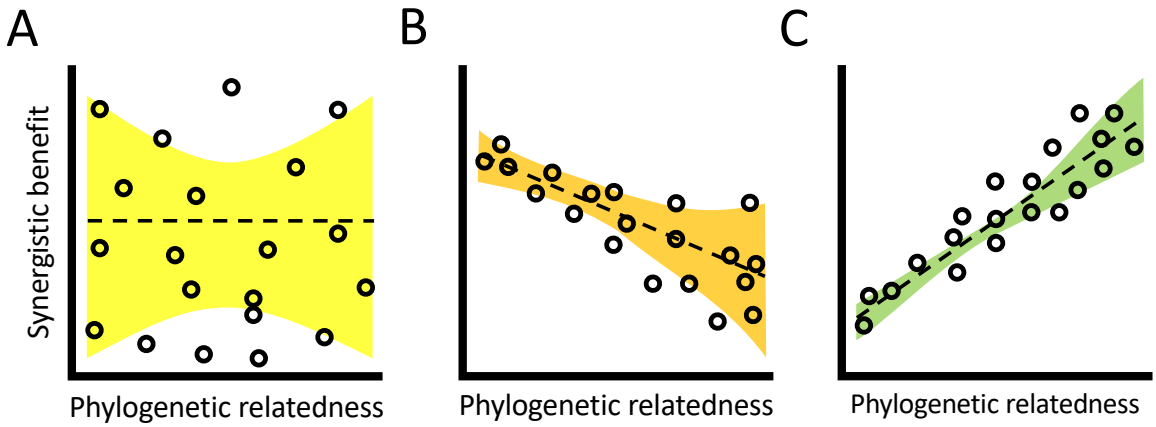

Figure 3: Potential statistical relationships between the phylogenetic relatedness of two interaction partners and their propensity to establish a synergistic interaction (here shown as synergistic benefit). (A) No statistical relationship. (B) Negative correlation between both parameters (i.e. closely related species are more likely to establish a synergistic interaction). (C) Positive correlation between both parameters (i.e. more distantly related species are more likely to establish a synergistic interaction). In these hypothetical graphs, circles represent the results of independent replicates and differently coloured ribbons the respective 95% confidence interval.

First, the phylogenetic relatedness between interacting partners might not predictably influence the formation of synergistic interactions (Figure 3A). Not finding a significant relationship between both parameters could be due to problems of the experimental setup. For example, an imbalanced or biased phylogenetic distribution of strains used to experimentally test this hypothesis could explain the inability to detect a significant statistical relationship (Horner-Devine and Bohannan 2006; Cadotte et al. 2017; Mahon et al. 2021). Alternatively, high rates of horizontal gene transfer within communities could erode the taxonomic specificity of synergistic interactions (Butaite et al. 2021). Finally, also other factors such as the simultaneous operation of multiple effects (e.g. different interactions) with opposing consequences or the confounding effect of a previous coevolutionary history could distort the ability to detect a clear relationship between both parameters (Fritschie et al. 2014; Venail et al. 2014).

The second possible outcome is a negative relationship between the phylogenetic relatedness between partners and the propensity for the establishment of a synergistic interaction (Figure 3B). In other words, synergistic interactions are more likely to occur between close relatives. A causal explanation for this could be that phylogenetically more closely related bacteria are also more similar in their metabolic capabilities and therefore to occupy a similar ecological niche (Goberna et al. 2019). This could enhance the ecological opportunity to establish synergistic interactions among more closely related individuals (Horner-Devine and Bohannan 2006). In addition, closely related species are also more likely to share the same mechanisms to exchange materials, such as certain contact-dependent interactions, which could introduce a taxonomic bias (Horner-Devine and Bohannan 2006; Sher et al. 2011). Finally, so-called *kin selection mechanism* that promote interactions among closer relatives (e.g. the production of toxins that kill non-resistant strains) could explain the observed pattern (Sah and Wall 2020).

517 The third possible outcome is that synergistic interactions between species are more
518 likely to establish than interactions within species (Figure 3C). This pattern could be
519 due to the fact that bacteria, which occupy different ecological niches, also show a
520 reduced competition for nutrients and other resources. Consequently, growth of
521 interspecific cocultures should, on average, be higher than the one of more closely
522 related taxa that experience enhanced competition (Venail and Vives 2013; Russel et
523 al. 2017). Another important point is that two phylogenetically more dissimilar species
524 are also more likely to differ in the architecture of their metabolic network (Salles et al.
525 2012) A consequence of this can be that the cost to produce certain metabolites and
526 the rates at which they are produced can differ between bacterial species. Hence, two
527 strains can significantly benefit by reciprocally trading cheaper/ easier to produce
528 compounds against more valuable ones. The probability for this kind of synergistic
529 metabolic complementarity is increased in interspecific interactions. Interestingly,
530 entering into an obligate metabolic relationship with another species can also extend
531 the biochemical capabilities of a strain such as the ability to use a new carbon source
532 (Ona et al. 2021).

533 Unfortunately, explicit experimental verifications of the role of phylogenetic
534 relatedness on the establishment and functioning of synergistic interactions are rare
535 so far. One study that addressed this issue in unidirectional cross-feeding interactions
536 between an amino acid donor and an auxotrophic recipient found clear evidence that
537 this kind of synergistic interaction was more likely to establish between
538 phylogenetically more dissimilar species (Giri et al. 2021). However, the experiments
539 performed in this study did not distinguish whether the exchange of amino acids
540 between partners was contact-dependent or contact-independent. The same result
541 was corroborated by a recent study, in which thousands of pairwise interactions were
542 analysed in different carbon sources using a droplet-based cocultivation platform

(Kehe et al. 2021). The comprehensive data set of this study also suggested that positive interactions among strains were more likely to emerge among taxonomically dissimilar strains. A third study, in which different strains of *Pseudomonas* bacteria were sampled from natural soil and pond communities found that low or non-producers of the siderophore pyoverdine were more likely to benefit when exposed to the pyoverdine that derived from a more closer related strain (Butaite et al. 2021). This pattern was likely caused by the fact that a strain that is closer related to the siderophore producer is also more likely to share the receptor that is required to take up the iron-siderophore-complex from the environment. Taken together, the above studies suggest that multiple forces - such as a metabolic complementarity between strains or biochemical constrains restricting the access to the exchanged good - are operating simultaneously to determine the establishment and functioning of synergistic interactions among bacteria. However, more studies are needed to unravel how different kinds of transfer mechanisms affect the specificity of the focal synergistic interaction.

Conclusion and future perspectives

Synergistic interactions among bacteria are ubiquitous in natural microbial communities (Strickland et al. 2009; Delgado-Baquerizo et al. 2016; Valdes et al. 2018; Vijay and Valdes 2022). However, the factors that determine their establishment remain poorly understood. By summarizing the knowledge that is currently available on this topic, we aimed at identifying major gaps in our understanding of the mechanisms that govern the establishment of synergistic interactions between bacterial genotypes. In particular, we analysed (i) the types of benefits that are exchanged in synergistic interactions, (ii) the molecular mechanism bacteria use to

transfer benefits between cells, (iii) the ways of how bacteria choose their interaction partner, and (iv) the consequences these mechanisms have for the phylogenetic relatedness among interaction partners.

The overall pattern that emerged from our holistic treatment is that first synergistic interactions are highly diverse in their form and function. This includes both the commodities that are traded between bacteria and the mechanisms that are used to transfer these goods between cells. Second, several lines of evidence suggest that synergistic interactions among bacteria should be rather species-specific (i.e. some combinations of strains perform better than others). Both the majority of mechanisms bacteria use to exchange synergistic benefits as well as the means to recognize and select certain interaction partners revealed a strong element of specificity, albeit the degree of specificity depended on the details of the particular interaction considered. While some mechanisms favoured exclusively interactions among members of the same species, others also included specific combinations of different species. However, studies that systematically analyse the taxonomic specificity of transfer and recognition mechanisms were generally rare. Finally, the few existing studies that experimentally verified the statistical relationship between the phylogenetic relatedness among two bacterial genotypes and their propensity to engage in synergistic interactions did not explicitly analyse which mechanism bacteria used to select a partner and transfer synergistic benefits between cells. However, knowledge on these details is critically important to fully understand the forces that structure microbial communities.

Future work should not only aim to further our understanding of the molecular mechanisms bacteria use to choose suitable interaction partners and trade synergistic benefits with them, but also link this information to experimental tests, in which the

dependence of these mechanisms on the taxonomic identity of the respective interaction partners is explicitly considered. A better understanding of these mechanistic links will help to explain the taxonomic structure of natural microbial communities and thus aid the design of synthetic communities for biotechnological or medical purposes (Giri et al. 2020). Moreover, more detailed knowledge on the causes and consequences of synergistic interactions within microbial communities will shed new light on the biology of the bacterial lifestyle in general. In this context, it will be particularly interesting to unravel the forces that shape the establishment and functioning of intercellular metabolic networks in bacteria (Pande and Kost 2017).

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Supplementary information

Determinants of synergistic cell-cell interactions in bacteria

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Supplementary Table 1: Examples of synergistic benefits exchanged by bacteria.

Exchanged benefit	Traded commodity	Transfer mechanism	Within species	Between species	Species	Reference number
Metabolites	Acetate, methionine, ammonia	Diffusion		●	*Escherichia coli*, *Salmonella enterica*, *Methylobacterium extorquens*	1
	Acetate, carbon dioxide, hydrogen	Diffusion		●	*Desulfovibrio vulgaris*, *Methanococcus maripaludis*	2
	Organic acids, ammonia	Diffusion		●	*Escherichia coli, Rhodopseudomonas palustris*	3
	Vitamins	Diffusion		●	*Lobomonas rostrate, Mesorhizobium loti*	4
	Nucleotides	Diffusion	●		*Saccharomyces cerevisiae*	5
	Amino acids, proteins	Nanotubes		●	*Acinetobacter baylyi, Escherichia coli*	6
	Amino acids	Cell-cell attachment		●	*Acinetobacter baylyi, Escherichia coli*	7
	Amino acids	Cell-cell attachment	●		*Escherichia coli*	8
	ATP	Cell-cell contact observed		●	*Ignicoccus hospitalis*, *Nanoarchaeum equitans*	9
	Industry intermediates coniferol, caffeate	Diffusion	●		*Escherichia coli*	10
	Cobamides	Active transport		●	Several bacterial and eukaryotic species	11
	Glucose as carbon source	Diffusion, cell-cell attachment	●		*Lactococus lactis*	12
	Vesicles as carbon source	Vesicles	●		*Prochlorococcus*	13
	Biofilm as carbon source	Biofilm	●		*Cyanobacterium* sp.	14
	Carbon and Nitrogen source	Cell-cell attachment		●	*Azotobacter vinelandii, Bacillus licheniformis, Paenibacillus curdlanolyticus*	15
	Carbon and Nitrogen source	Physical contact observed	●		*Cyanobacterium* sp.	16
	Proteins	Vesicles	●	●	*Myxococcus xanthus*	17
	Proteins	Cell-cell attachment		●	*Clostridium acetobutylicum, Desulfovibrio vulgaris Hildenborough*	18
	Membrane proteins	Cell-cell attachment	●		*Myxococcus xanthus*	19
	Membrane proteins	Cell-cell attachment	●		*Myxococcus xanthus*	20
Electrons	Electrons	Physical contact	●		Cable bacteria	21
	Electrons	e-Pili, nanowires, physical contact		●	*Geobacter metallireducens, Geobacter sulfurreducens*	22
	Electrons	e-Pili		●	*Syntrophus aciditrophicus,*	23

					Geobacter sulfurreducens	
	Electrons	Nanowires	●		*Shewanella oneidensis*	24
	Electrons	Nanowires	●		*Lysinibacillus varians*	25
	Electrons	Diffusion, nanowires, cell-cell attachment	●	●	Several species	26
	Electrons	Nanowires		●	ANME-1 archaea, SRB HotSeep-1	27
	Electrons	Nanowires	●		*Geobacter sulfurreducens*	28
	Electrons	Conductive minerals		●	*Geobacter sulfurreducens, Thiobacillus denitrificans*	29
	Electrons	Conductive minerals		●	*Geobacter metallireducens, Methanosarcina barkeri*	30
Services	Antibiotic protection, β-lactam	Diffusion		●	*Lactococcus lactis,* gut microbiota	31
	Antibiotic protection, chloramphenicol	Diffusion	●		*Streptococcus pneumoniae*	32
	Siderophore	Diffusion		●	*Marinobacter* sp., coccolithophores, dinoflagellates	33
	pH change	Diffusion		●	*Acetobacter* sp., *Lactobacillus plantarum*	34
	Cellulose degradation	Diffusion		●	*Cellulomonas* sp., *Rhodopseudomonas capsulata*	35
	Siderophore	Active transport		●	*Pseudomonas* sp.	36
	Hitchhiking	Flagella attachment		●	*Streptomyces* spores, gram-positive and negative bacteria	37
	Aggregate formation	Flagella attachment		●	*Pelotomaculum thermopropionicum, Methanothermobacter thermautotrophicus,* 21 methanogens genera	38
	Aggregate formation	Flagella attachment		●	*Pelotomaculum thermopropionicum, Methanothermobacter thermautotrophicus*	39
	Aggregate formation	Flagella attachment		●	*Pelotomaculum thermopropionicum, Methanothermobacter thermautotrophicus*	40
	Adhesion	Flagella attachment	●		*Escherichia coli*	41
Information	CAI-1 and AI-2	Diffusion	●		*Vibrio cholerae*	42
	N-hexadecanoyl-L-homoserine lactone	Vesicles	●		*Paracoccus* sp.	43
	Signalling molecules	Nanotubes		●	Anaerobic sludge microbiota	44

	Quorum sensing	Diffusion	●		*Vibrio fischeri*	45
	Quorum sensing	Diffusion	●		*Streptococci*	46
	Quorum sensing	Diffusion, active transport	●	●	Several bacterial species	47
	Quorum sensing	Diffusion, active transport	●	●	Several bacterial species	48
	Bacterial volatile ammonia	Diffusion		●	*Bacillus subtilis*, *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus*	49
	Chromosomal DNA	Conjugation pili	●		*Mycobacterium smegmatis*	50
	DNA	Vesicles		●	*Alteromonas* sp., *Halomonas* sp., *Prochlorococcus* sp.	13
	Plasmid	Conjugation pili		●	Gram-negative bacteria	51
	Plasmid	Nanotubes	●		*Bacillus subtilis, Staphylococcus aureus, Escherichia coli*	52

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