

1 **Determinants of synergistic cell-cell interactions in bacteria**

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13 **Running title**

14 Determinants of synergistic bacterial interactions

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22 **Abstract**

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

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42 **Keywords**

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

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46 **Introduction**

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

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

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

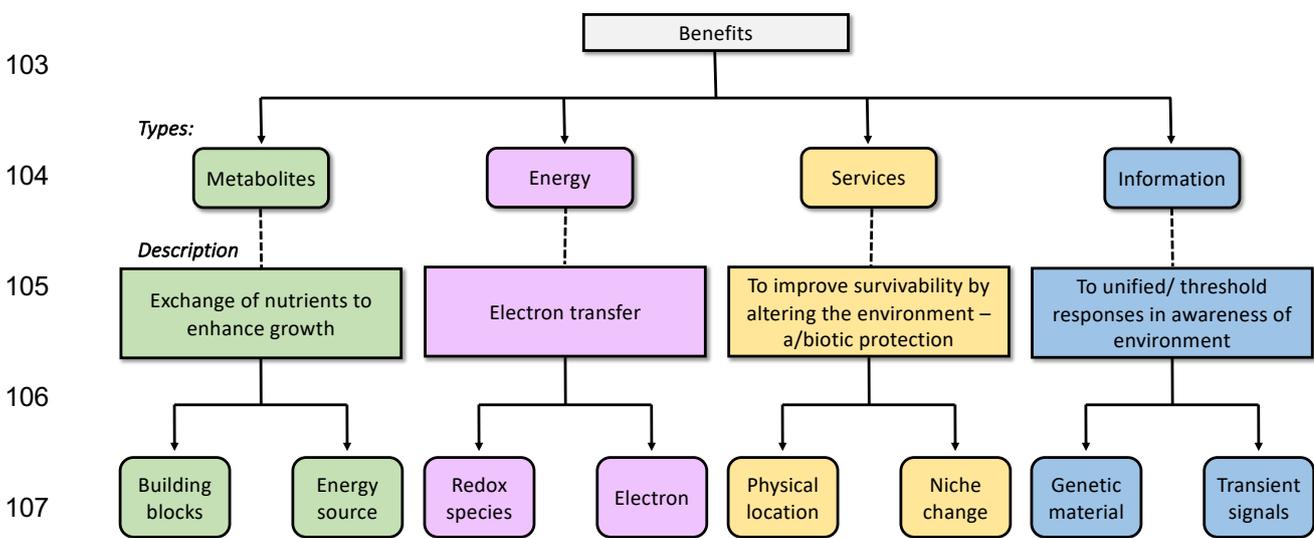
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82 **Benefits of synergistic cell-cell interaction**

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

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

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



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

109
 110 Besides metabolites, bacteria can also exchange electrons as a form of non-
 111 material energy between cells (Figure 1, Table 1, Supplementary Table 1). This can
 112 be achieved by shuttling redox compounds such as H₂ or formate via diffusion through
 113 the extracellular environment from electron-donating to electron-accepting cells
 114 (Stams et al. 2006; Stams and Plugge 2009; Shi et al. 2016). Alternatively, a transfer
 115 of electrons can be mediated by a direct physical contact of cell surfaces between
 116 interacting cells (Jiang et al. 2018; Meysman et al. 2019) or via long (µm-cm range)
 117 electrical connections between cells (Summers et al. 2010; Shi et al. 2016; Walker et
 118 al. 2020). The latter includes (i) electrically conductive nanowires that are formed by
 119

120 **Table 1: Mechanisms bacteria use to transfer synergistic benefits between bacterial cells.** Both
 121 the commodity (i.e. M = metabolites, E = electrons, S = services, and I = information) that has been
 122 shown to be exchanged using the different mechanisms and whether the exchange has been observed
 123 within members of the same or different species is indicated (- = no example found, + = few cases found
 124 (< 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; Viroille 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; Hogley et al. 2015

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 126 unicellular bacteria (e.g. *Geobacter* species) (Summers et al. 2010; Wang et al. 2019),
 127 (ii) conductive conduits that consist of multicellular, filamentous bacteria (Pfeffer et al.
 128 2012; Shi et al. 2016; Lovley 2017; Meysman 2018), or (iii) extracellular mineral
 129 particles that connect redox reactions that are catalysed by different microbial species
 130 (Reguera et al. 2005; Kato et al. 2012; Shi et al. 2016). In all of these cases, the

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

136 The third type of benefit bacteria exchange with each other are services (Figure
137 1, Table 1, Supplementary Table 1). A service results from the behaviour one
138 bacterium performs, typically to enhance its own Darwinian fitness, and which benefits
139 another bacterium in its immediate vicinity. Such services can include a range of
140 different traits such as transport (e.g. hitchhiking of a non-motile bacteria by attaching
141 to the flagellum of another motile bacterium (Muok et al. 2021)), detoxification (e.g.
142 degradation of an antibiotic (Sorg et al. 2016; Cubillos-Ruiz et al. 2022), or niche
143 construction (e.g. changing pH in a favourable direction (Aranda-Díaz et al. 2020)).
144 Other processes falling into this category are the production of biofilms (Madsen et al.
145 2016; Dragoš et al. 2018), the release of digestive enzymes (e.g. proteases, chitinases
146 (Drescher et al. 2014; Smith and Schuster 2019)) to degrade complex molecules or of
147 siderophores to sequester iron (Amin et al. 2009).

148 The final commodity that is exchanged between bacteria is information (Figure 1,
149 Table 1, Supplementary Table 1). Information can be transferred between cells in the
150 form of genetic material, which encodes a certain biochemical capability that is
151 beneficial to the receiver (e.g. a gene conferring antibiotic resistance (Kent et al.
152 2020)). Genetic information such as chromosomal or plasmid DNA can be passed from
153 one bacterium to another one using a variety of mechanisms including conjugation
154 (Lederberg and Tatum 1946), transformation (Griffith 1928), or transduction
155 (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).

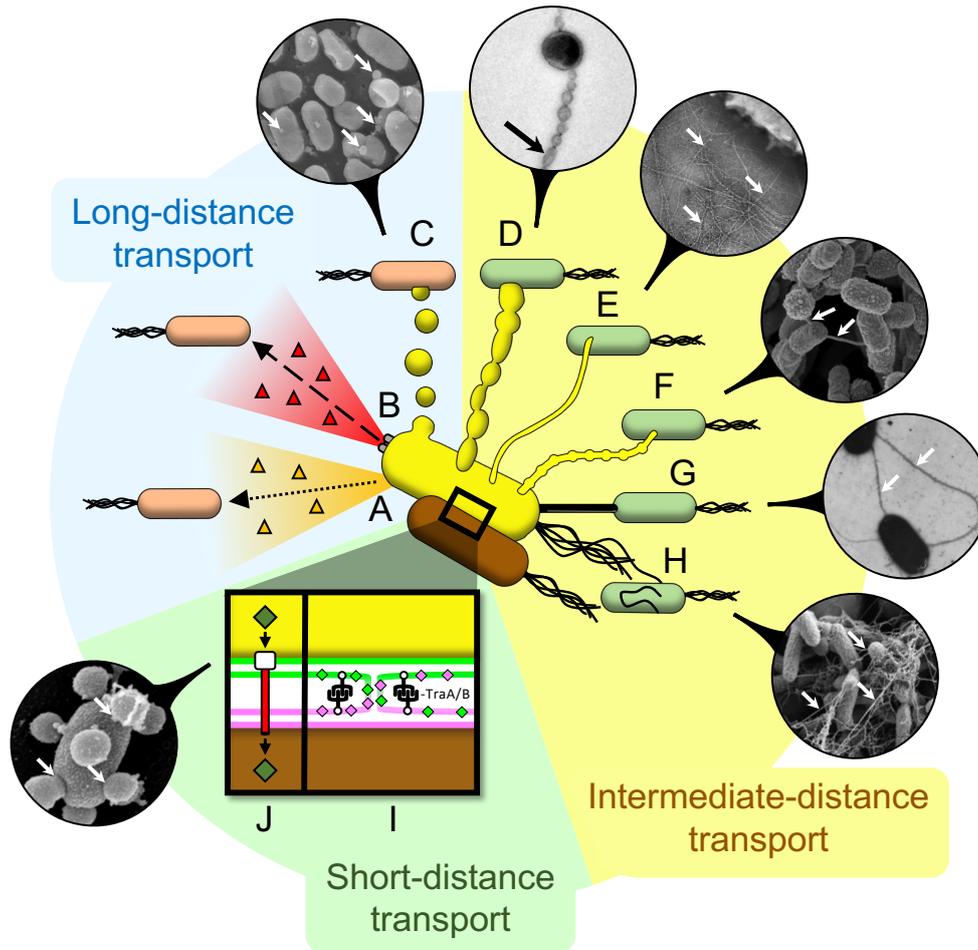
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181 **Mechanisms of metabolite transfer between bacterial cells**

182 Bacteria use a wide array of different mechanisms to exchange metabolites or services
183 between cells. These mechanisms are generally subdivided into two main categories,
184 depending on whether the transfer of materials depends on a direct physical contact
185 between bacterial cells (i.e. *contact-dependent* exchange) or not (i.e. *contact-*
186 *independent* exchange) (D'Souza et al. 2018; Pacheco et al. 2019; van Tatenhove-Pel
187 et al. 2021; Figure 2). In addition, these mechanisms differ with regard to the spatial
188 distance that is covered during the transfer process. While interactions that require a
189 close contact of cell surfaces for the interaction to occur result in a direct transfer of
190 metabolites between cells (distance: $< 1 \mu\text{m}$) (Benomar et al. 2015; Sah and Wall 2020;
191 López-García and Moreira 2021), other mechanisms that depend on the formation of,
192 for example, cell membrane protrusions (e.g. nanowires, pili, nanotubes) can cover
193 intermediate distances between cells (i.e. $\sim 1\text{-}60 \mu\text{m}$) (Remis et al. 2014; Dubey et al.
194 2016; Fischer et al. 2019). In contrast, contact independent interactions that for
195 example rely on an exchange of materials via diffusion through the extracellular
196 environment, can cover gaps between cells that range from direct cell proximity up to
197 very large distances (i.e. $> 100 \mu\text{m}$) (Be'Er et al. 2009; Cordero and Datta 2016; van
198 Tatenhove-Pel et al. 2021). In the following, we will provide an overview over the
199 different kinds of mechanisms bacteria use to transfer materials between bacterial cells
200 (Figure 2, Table 1, Supplementary Table 1).

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

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217 **Figure 2:** Mechanisms bacteria use to exchange synergistic benefits between bacterial cells. The
218 different transfer mechanisms are subdivided into three categories based on the distance they can
219 bridge. (A-C) Contact-independent transport mechanism that can cover longer distances (blue
220 background) include (A) passive diffusion, (B) active transport, and (C) vesicle-mediated exchange. (D-
221 H) Contact-dependent transport mechanisms covering short to intermediate distances between bacteria
222 (yellow background) comprise (D) vesicle chains, (E) nanowires, (F) nanotubes, (G) pili, and (H) flagella.
223 (I-J) The last category of contact-dependent processes involves mechanisms that facilitate transport
224 over relatively short-distances (green background) such as the (I) TraA/B-mediated fusion of outer
225 membranes and (J) type-III, -IV or -VI secretion systems. Electron micrographs show the corresponding
226 structures (arrows). Photos reproduced with permission from (C) Prof. Dr. Steven Biller, (D) (Fischer et
227 al. 2019), (E) (Dahl et al. 2022), (G) (Curtiss III, Roy, et al. 1969), (H) (Friedlander et al. 2013), (I,J)
228 (López-García and Moreira 2021).
229

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

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

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

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

261 the transferred commodity, thereby not only protecting it from environmental
262 influences, but also preventing its dilution into the surrounding medium (Woith et al.
263 2019).

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

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

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

286 et al. 2010; Wegener et al. 2015; Lovley 2017) are so-called *nanowires* (Figure 2E).
287 These membrane protrusions facilitate the exchange of electrons by directly shuttling
288 electrons or substitute metabolites like H₂ or formate, thus enabling further redox
289 reactions. The formation of nanowires seems to be species-specific and currently two
290 main models exist. In *Geobacter sulfurreducens*, nanowires have been suggested to
291 be a micrometer-long polymerization of the hexaheme cytochrome OmcS (Wang et al.
292 2019), while in *Shewanella oneidensis* MR-1, nanowires are considered to be outer
293 membrane extensions containing soluble periplasmic components together with
294 multiheme cytochromes (Pirbadian et al. 2014). Generally, a broad variety of bacterial
295 and archaeal species are known to use different cytochromes to facilitate the
296 extracellular transport of electrons (Cheng and Call 2016; Shi et al. 2016).

297 The third mechanism bacteria employ to establish cell-cell connections are
298 *nanotubes*. These more recently discovered structures are composed of chains of
299 continuous membranous segments traversed by an uninterrupted lumen (Dubey et al.
300 2016; Figure 2F). As such, their structure shows a striking similarity to the previously
301 discussed vesicle chains. Two types of nanotubes have been described. First,
302 *intercellular nanotubes* that connect neighbouring cells and, second, *extending*
303 *nanotubes* that reach into the surrounding of the cell and seem to function like plant
304 roots by searching for nutrients and interaction partners (Dubey et al. 2016). In general,
305 nanotubes are known to facilitate cell-cell communication and mediate the exchange
306 of cytoplasmic metabolites and plasmid DNA between cells of the same or different
307 bacterial species (Dubey and Ben-Yehuda 2011; Pande et al. 2015; Dubey et al. 2016;
308 Stempler et al. 2017).

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

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

318 The fifth mechanism bacteria use to establish connections over intermediate
319 distances between cells is mediated by *flagella* (Figure 2H). These filaments, which
320 mainly consist of flagellin, serve the main purpose to propel bacteria forward
321 (Silverman and Simon 1977). However, more recent studies have revealed that flagella
322 can also be used by bacteria to bind interaction partners in order to reduce the spatial
323 distance for an otherwise diffusion-based exchange (Marx 2009; Ishii et al. 2005). One
324 study even suggested that the flagellar cap protein FliD was involved in synchronizing
325 the metabolism of interacting cells of *Pelotomaculum thermopropionicum* and
326 *Methanothermobacter thermautotrophicus*, thus pointing to an important role of flagella
327 for triggering the syntrophic interaction between interacting strains (Shimoyama et al.
328 2009).

329 The final way of how bacteria can transfer synergistic benefits between cells is
330 by interacting with each other via immediate cell surface connections (Benomar et al.
331 2015; Charubin et al. 2020; Figures 2I,J). For this, cells frequently produce an
332 extracellular matrix that mainly consists of polysaccharides, proteins, and DNA
333 (Flemming and Wingender 2010), thus leading to the formation of a free-floating
334 (Gilbertie et al. 2019) or surface-attached bacterial aggregate (Madsen et al. 2016).
335 Direct cell surface connections are the closest interaction two cells can form (Benomar

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

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

346

347 **Transfer mechanisms determine the specificity of synergistic interactions**

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

353 In the case of contact-independent interactions, goods are mostly unspecifically
354 released into the extracellular environment (e.g. biofilm production, enzymes to
355 degrade toxins, metabolites). These so-called *public goods* are equally accessible to
356 both the cells producing them and other cells that are present in the local environment.
357 Specificity with regards to the type of cells taking advantage of the traded good can
358 emerge based on the molecular nature of the secreted substance. For example, the
359 majority of quorum sensing signals can only be understood by members of the same
360 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.

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

395

396 **Detection and recognition mechanisms are often highly specific**

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

401 Bacteria feature highly sensitive olfactory systems that they use to detect and
402 localize suitable food sources (Yawata et al. 2020) or symbiotic partners (Taylor and
403 Stocker 2012). In this context, the successful localisation of the source of a chemical
404 gradient depends on properties of the individual cell (e.g. the sensitivity of a strain's
405 chemotaxis pathway or its swimming speed) as well as on environmental factors such
406 as the viscosity of the surrounding medium or the amount of chemoattractant that is
407 emitted from the source (Keegstra et al. 2022). To localize a certain target, bacteria
408 require sufficiently strong emission levels of the corresponding chemoattractant. Thus,
409 over longer distances, bacteria are more likely to be attracted to chemicals that are
410 produced by larger assemblages of bacteria (e.g. biofilms (Moore-Ott et al. 2022)) or
411 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

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

442 This kind of toxin-based cell-cell recognition mechanisms can be contact-dependent
443 or contact-independent. Contact-dependent recognition of other cells can be mediated
444 by receptors that specifically bind other cells (e.g. interactions between TraA and TraB
445 in myxobacteria (Cao and Wall 2017) or CdiA of the contact-dependent inhibition
446 system in *Escherichia coli* (Aoki et al. 2005)). In a second step, the toxin is then
447 delivered to target cells where it takes full effect. This can be achieved by a transfer of
448 outer membrane material that contains the toxic protein (Vassallo et al. 2018; Figure
449 2I) or via the delivery of the toxic protein into the cytoplasm of the target cell using type
450 III (Zhu et al. 2006), IV (Souza et al. 2015), VI (Crisan and Hammer 2020), or VII (Cao
451 et al. 2016) secretion systems (Figure 2J). Alternatively, other strains that try to invade
452 a local group of bacteria can also be eradicated by releasing toxins (e.g. bacteriocins)
453 into the extracellular environment (Tait and Sutherland 2002). Again, only bacteria that
454 are resistant to the toxin will be able to survive, thus representing a strong filter that
455 favours closely related kin. In the absence of other, potentially exploitative genotypes,
456 synergistic interactions among the members of the local communities can thrive
457 unrestrictedly.

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

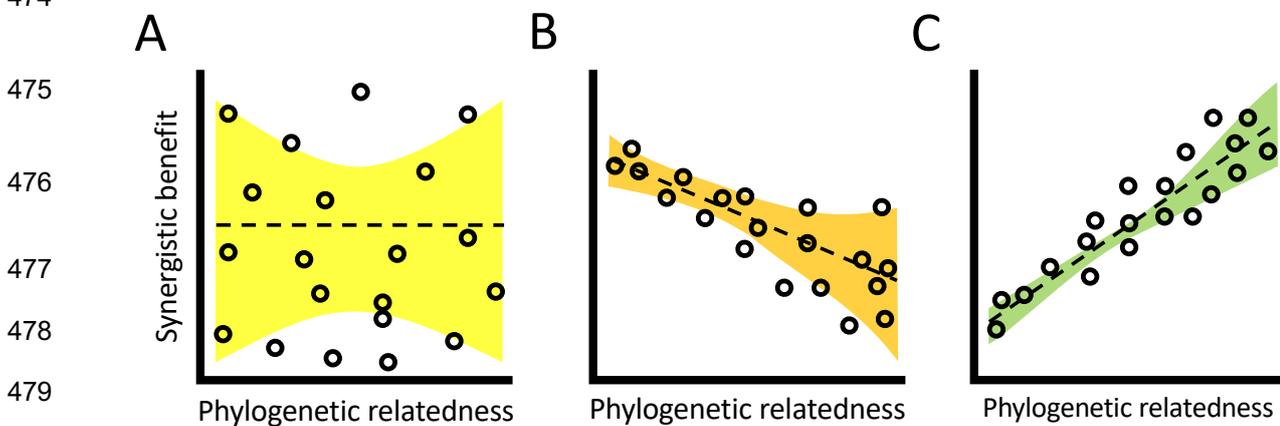
462 systems only allow the establishment of interactions among conspecific genotypes
463 (Aoki et al. 2005; Cao and Wall 2017), others also include interspecific interactions
464 (e.g. co-aggregation).

465

466 **Relatedness among partners determines the establishment of synergistic** 467 **interactions**

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

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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.

491 First, the phylogenetic relatedness between interacting partners might not
492 predictably influence the formation of synergistic interactions (Figure 3A). Not finding
493 a significant relationship between both parameters could be due to problems of the
494 experimental setup. For example, an imbalanced or biased phylogenetic distribution of
495 strains used to experimentally test this hypothesis could explain the inability to detect
496 a significant statistical relationship (Horner-Devine and Bohannan 2006; Cadotte et al.
497 2017; Mahon et al. 2021). Alternatively, high rates of horizontal gene transfer within
498 communities could erode the taxonomic specificity of synergistic interactions (Butaite
499 et al. 2021). Finally, also other factors such as the simultaneous operation of multiple
500 effects (e.g. different interactions) with opposing consequences or the confounding
501 effect of a previous coevolutionary history could distort the ability to detect a clear
502 relationship between both parameters (Fritschie et al. 2014; Venail et al. 2014).

503 The second possible outcome is a negative relationship between the
504 phylogenetic relatedness between partners and the propensity for the establishment
505 of a synergistic interaction (Figure 3B). In other words, synergistic interactions are
506 more likely to occur between close relatives. A causal explanation for this could be that
507 phylogenetically more closely related bacteria are also more similar in their metabolic
508 capabilities and therefore to occupy a similar ecological niche (Goberna et al. 2019).
509 This could enhance the ecological opportunity to establish synergistic interactions
510 among more closely related individuals (Horner-Devine and Bohannan 2006). In
511 addition, closely related species are also more likely to share the same mechanisms
512 to exchange materials, such as certain contact-dependent interactions, which could
513 introduce a taxonomic bias (Horner-Devine and Bohannan 2006; Sher et al. 2011).
514 Finally, so-called *kin selection mechanism* that promote interactions among closer
515 relatives (e.g. the production of toxins that kill non-resistant strains) could explain the
516 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

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

558

559 **Conclusion and future perspectives**

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

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

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

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

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

602

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