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Impact of spatial organization on a novel auxotrophic interaction among soil microbes.

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Abstract

A key prerequisite to achieve a deeper understanding of microbial communities and to engineer synthetic ones is to identify the individual metabolic interactions among key species and how these interactions are affected by different environmental factors. Deciphering the physiological basis of species-species and species-environment interactions in spatially organized environment requires reductionist approaches using ecologically and functionally relevant species. To this end, we focus here on a specific defined system to study the metabolic interactions in a spatial context among a plant-beneficial endophytic fungus *Serendipita indica,* and the soil-dwelling model bacterium *Bacillus subtilis*. Focusing on the growth dynamics of *S. indica* under defined conditions, we identified an auxotrophy in this organism for thiamine, which is a key co-factor for essential reactions in the central carbon metabolism. We found that *S. indica* growth is restored in thiamine-free media, when co-cultured with *B. subtilis*. The success of this auxotrophic interaction, however, was dependent on the spatial and temporal organization of the system; the beneficial impact of *B. subtilis* was only visible when its inoculation was separated from that of *S. indica* either in time or space. These findings describe a key auxotrophic interaction in the soil among organisms that are shown to be important for plant ecosystem functioning, and point to the potential importance of spatial and temporal organization for the success of auxotrophic interactions. These points can be particularly important for engineering of minimal functional synthetic communities as plant-seed treatments and for vertical farming under defined conditions.

Introduction

Higher-level functions and population dynamics within microbial communities are underpinned by the interactions among the composing species within the community and their environment (Falkowski *et al*., 2008; Sañudo-Wilhelmy *et al*., 2014). Deciphering these interactions is a pre-requisite to understand and manage complex natural communities (Abreu and Taga, 2016; Widder *et al*., 2016) and to achieve community-level synthetic engineering (Großkopf and Soyer, 2014; Hays *et al*., 2015; Lindemann *et al*., 2016). To this end, increasing numbers of experimental studies and (meta)genomic surveys have shown that auxotrophic interactions, involving vitamins and amino acids, are wide-spread in many microbial natural communities (Sañudo-Wilhelmy *et al*., 2014; Morris *et al*., 2012; Helliwell *et al*., 2013) and can also be engineered genetically to create synthetic communities (Mee *et al*., 2014; Pande *et al*., 2014). Specific auxotrophic interactions among microbes are shown to influence ecosystem functioning; e.g. infection outcomes within higher organisms (Wargo and Hogan, 2006), ecological population dynamics in the oceans (Sañudo-Wilhelmy *et al*., 2014), and the level of biodegradation of organic matter under anoxic conditions (Schink, 1997; Embree *et al*., 2015).

It has been suggested that auxotrophies can result from reduced selective pressures for maintaining biosynthesis capabilities under stable metabolite availability due to abiotic or biotic sources (Morris *et al*., 2012; Helliwell *et al*., 2013). This proposition is supported by the observed independent evolution of vitamin and amino acid auxotrophies in different, unrelated taxa (Helliwell *et al*., 2011; Rodionova *et al*., 2015), and points to a direct linkage between ecological dynamics and evolution of auxotrophies (Embree *et al*., 2015). The possible fluctuations in a metabolite's availability in time and space would be expected to impact both the emergence of auxotrophies and the population dynamics of resulting auxotrophic species. For example, in the marine environment, where the observed auxotrophies relate mostly to the loss of biosynthesis capacity for vitamins and amino acids, population dynamics of auxotrophic species are believed to be directly linked to those of 'provider' species (Helliwell *et al*., 2013; Hom and Murray, 2014; Sañudo-Wilhelmy *et al*., 2014). The ecological influences of auxotrophic species on community structure and population dynamics can also be exerted by

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51 abiotic fluctuations or directly by the abundances and actions of higher organisms within the
52 system.
53
54 These ecological influences on microbial population dynamics can increase significantly in
55 spatially organized systems. Yet, the spatial context of microbial interactions is under-studied.
56 Considering that each species can display multiple metabolic actions that all affect a common
57 environment, it is not clear if auxotrophic interactions can always be successfully established
58 even if genetic/metabolic complementarity is present. For example, changes in environmental
59 pH upon growth of one species can affect its subsequent interactions with other microbes
60 (Vylkova, 2017; Ratzke and Gore, 2017). Similarly, extensive oxygen depletion upon
61 microbial colony growth plates (Peters and Wimpenny, 1987; Dietrich *et al*., 2013; Kempes
62 and Okegbe, 2014) can directly influence subsequent or simultaneous growth of different
63 species or their interactions. These possible interplays between species-species and species-
64 environment interactions are currently not well-understood and only explored in few studies,
65 which used either synthetically engineered interactions (Shou *et al*., 2007; Mee *et al*., 2014) or
66 enriched microbial communities (Embree *et al*., 2015). This lack of understanding, however,
67 causes a particular challenge for the engineering of novel applications of microbial
68 communities with inherent spatial organisation, such as seen in agriculture and involving for
69 example closed-ecosystem production, seed treatment and microbe-based biofertilisation
70 (Gòdia *et al*., 2002; Lucy *et al*., 2004; Richardson *et al*., 2011).
71
72 Towards addressing this challenge, we focus here on identifying potential metabolic
73 interactions among plant-beneficial endophytic fungus *Serendipita indica* (previously called
74 *Piriformospora indica* (Weiss *et al*., 2016)) and the common soil microbe *Bacillus subtilis*.
75 Identifying a defined media for *S. indica*, we found it to be auxotrophic for the vitamin B1,
76 thiamine. To study the potential auxotrophic interactions of *S. indica*, we then created a co-
77 culture system using *Bacillus subtilis*. We found that *S. indica* thiamine auxotrophy can be
78 satisfied and its growth is restored in the presence of *B. subtilis*. The success of this auxotrophic
79 interaction, however, is strongly dependent on temporal and spatial organization in the system.
80 These findings and the established synthetic co-culture can act as a basis to develop a more
81 complete functional synthetic community, as advocated for biotechnological applications and
82 for gaining insights into community function (Mee and Wang, 2012; Großkopf and Soyer, 2014;
83 Widder *et al*., 2016; Lindemann *et al*., 2016). With the inclusion of a plant, such a synthetic
84 community can allow further insights into microbe-microbe, and microbe-plant interactions
85 and development of new agricultural technologies such as in seed coating and vertical farming
86 in controlled environments.
87
88 **Results**
89 ***S. indica* is auxotrophic for thiamine.** *S. indica* is an endophytic fungus that can colonize
90 roots of a wide range of plants and can confer a range of beneficial effects, including enhancing
91 plant growth, resistance to biotic and abiotic stresses (Waller *et al*., 2005; Sherameti *et al*.,
92 2008; Vadassery *et al*., 2009), promotion of adventitious root formation in cuttings (Druege *et*
93 *al*., 2007), and assisting phosphate assimilation (Yadav *et al*., 2010). Despite its broad host
94 range, *S. indica* also has the ability to grow in the absence of host plants (Kumar *et al*., 2011).
95 Exploiting this ability, we attempted to create a fully defined growth medium that was based
96 on previous physiological studies on *S. indica* (Zuccaro *et al*., 2011; Kumar *et al*., 2011; Jacobs
97 *et al*., 2011; Varma *et al*., 2012; Qiang *et al*., 2012). Using our defined media, we tested the
98 effect of the vitamins on growth by cultivating *S. indica* in a series of vitamin-free media each
99 supplemented by a specific vitamin (Figure 1). The analyses showed that *S. indica* is
100 auxotrophic for thiamine; while none of the other individual vitamin additions supported

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101 growth, thiamine and full vitamin addition did. This finding was further confirmed by growing
102 *S. indica* on plates supplemented with an additional agar block only containing a defined
103 amount of thiamine. In this case, growth of *S. indica* resulted in expansion towards the thiamine
104 agar block, suggesting that growth occurs on a thiamine gradient or is linked with an active
105 chemotaxis towards thiamine source (Figure S1). We also quantified the growth of *S. indica*
106 with different concentrations of thiamine and found that hyphae growth and spore formation
107 showed a positive, but saturating, dependency on thiamine levels (Figure S2). At zero
108 concentration of thiamine in the media, we still observe germination and very little hyphal
109 growth (Figure S2), possibly supported by thiamine stored in spores. Besides these
110 measurements, we also observed thiamine effect on *S. indica* growth using time-lapse
111 microscopy (see further discussion below and supplementary videos 1 and 2).
112
113 ***S. indica* auxotrophy is reflected in its genomic enzyme content.** To support and better
114 understand these physiological results, we analyzed the *S. indica* genome for the presence of
115 genes associated with thiamine use, biosynthesis, and transport. Bioinformatics analysis
116 revealed that *S. indica* lacks most of the genes of the thiamine-biosynthesis pathway (Table S1,
117 Figure 2a). In particular, we were not able to find any homologs of the genes *thi5* and *thi6*. The
118 former encodes the enzyme involved in the synthesis of the thiamine-precursor
119 hydroxymethylpyrimidine (HMP), while the later encodes the bifunctional enzyme acting as
120 thiamine phosphate pyrophosphorylase and hydroxyethylthiazole kinase. A homolog of the
121 gene *thi4*, the product of which also relates to mitochondrial DNA damage tolerance (Machado
122 *et al*., 1997; Hohmann and Meacock, 1998; Wightman, 2003), is present. We also found that
123 *S. indica* contains a homolog of the *thi7* (or alternative name *thi10*) that encodes a thiamine
124 transporter, and a homolog of the *pho3* gene, whose product catalyzes dephosphorylation of
125 thiamine phosphate to thiamine, thereby increasing its uptake (Wightman, 2003). These
126 findings suggest that *S. indica* is unable to synthesize thiamine, but can acquire thiamine from
127 the environment, as also suggested for other microbes that encode thiamine salvage pathways
128 (Jenkins *et al*., 2007) . The utilization of thiamine in physiology is evidenced by the presence
129 of homologs of the *thi80* gene, which encodes a thiamine pyrophosphokinase involved in the
130 catalysis of thiamine into thiamine pyrophosphate (ThPP), the presence of at least one gene
131 encoding a ThPP binding domain-containing protein, and the role of ThPP as a key co-factor
132 involved in central metabolic reactions (see Figure 2b). The last point involves key metabolic
133 reactions such as pyruvate fermentation and conversion for entry into the citric acid cycle
134 (TCA), oxo-glutarate to succinyl-CoA conversion in the TCA cycle, transketolase reactions in
135 the pentose phosphate pathway, and biosynthesis reactions for leucine, isoleucine and valine
136 (Michal and Schomburg, 1999)
137
138 ***B. subtilis* complements *S. indica's* auxotrophy for thiamine and promotes its growth.**
139 Given the crucial role of thiamine-derived co-factors in central metabolism, *S. indica* growth
140 in nature apparently depends on environment-derived thiamine. Indeed, thiamine can be
141 synthesized by various bacteria, fungi and plants (Begley *et al.*, 1999; Jenkins *et al.*, 2007;
142 Jurgenson *et al.*, 2009). Among these, *B. subtilis*, a bacterium commonly found in soil (Hong
143 *et al*., 2009), is an established model organism (Mader *et al*., 2011), and well-studied for
144 thiamine biosynthesis (Schyns *et al*., 2005; Begley *et al*., 1999). Combined with the fact that
145 *B. subtilis* is normally a plant-beneficial microbe (Castillo *et al*., 2013), this motivated us to
146 explore the possibility that the identified *S. indica* auxotrophy for thiamine could be satisfied
147 upon co-culturing with *B. subtilis*. We created co-cultures of these two species on agar plates
148 using our defined media and two common nitrogen sources to evaluate possible auxotrophic
149 interaction under these conditions. We found that *B. subtilis* could indeed stimulate *S. indica*

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150 growth under thiamine-free conditions and that *S. indica* growth followed a spatial pattern with
151 significant growth in the vicinity of the *B. subtilis* colony (Figure 3a).
152 We used time-lapse microscopy to quantify this spatial growth pattern of *S. indica* and
153 found that growth (as approximated by image density) happened faster at the side closer to the
154 *B. subtilis* colony compared to the far side of the plate (see Figure 3b and Supplementary
155 Videos 3 and 4). This could be explained by the presence of an increasing thiamine gradient
156 towards the *B. subtilis* colony that facilitates *S. indica* hyphal growth. While these findings
157 strongly suggest a *B. subtilis*–linked thiamine provision, which then promotes *S. indica* growth,
158 our attempts to quantify thiamine from agar plate co-cultures has failed, presumably due to a
159 combination of thiamine consumption and sensitivity limitations of available thiamine assays
160 (50 µg/L) (Lu and Frank, 2008). We were, however, able to detect thiamine from concentrated
161 *B. subtilis* and found the concentration in liquid culture supernatants to be approximately 7.56
162 µg/l.
163 While the above findings strongly suggest that the growth enabling of *S. indica* by *B.*
164 *subtilis* is due to thiamine supply, another theoretical possibility is that *B. subtilis* provides
165 metabolites other than thiamine, that allow bypassing of central reactions requiring thiamine
166 as a co-factor. In other words, provision of metabolites that are downstream of pyruvate in the
167 TCA cycle (Figure 2b). To rule out this possibility, we have analyzed growth of *S. indica* in
168 the absence of thiamine but supplemented with organic and amino acids that link to the central
169 carbon metabolism. We found that none of the 17 amino acids or 8 organic acids tested or their
170 combinations allowed for *S. indica* growth in the absence of thiamine (Figure S3). This finding
171 further confirmed that *B. subtilis* facilitated growth of *S. indica* in thiamine-free medium is
172 linked directly to thiamine.
173
174 **Metabolic profiling shows additional metabolic interactions between *S. indica* and *B.***
175 ***subtilis.*** To analyse the basis of metabolic interactions between the two organisms and to
176 collect more evidence for thiamine-based auxotrophy, we grew each organism in liquid culture
177 on its own and then cross-cultured the other organism on the supernatant of the first one. As
178 with agar plates, we found that in the absence of thiamine, the *S. indica* growth was limited to
179 spore germination (Figure 4). When supplemented with *B. subtilis* supernatant, however, *S.*
180 *indica* showed significantly increased growth in liquid culture (Figure 4). Consistent with this,
181 there was also a growth enhancement of *S. indica* by the *B. subtilis* supernatant when cultured
182 in the presence of thiamine.
183 To better understand the metabolic basis of these physiological observations, we
184 repeated these experiments and quantified the concentrations of the key organic acids linking
185 to the TCA cycle (lactate, acetate, pyruvate, and formate) in the supernatant of each organism
186 before and after cross-cultivation using ion chromatography. We found that the supernatant
187 from *B. subtilis* monoculture contained significantly higher amounts of acetate and some
188 formate and pyruvate, and that the extracellular levels for these compounds did not change in
189 the presence or absence of thiamine in the media (Figure S4). When *S. indica* was grown in the
190 *B. subtilis* supernatant and in the absence of thiamine, it consumed both acetate and formate
191 and produced pyruvate. In the presence of both thiamine and the *B. subtilis* supernatant, the
192 consumption of acetate and formate was also observed, but there was also production of lactate
193 in addition to pyruvate (Figure S4). These findings, in particular acetate and formate cross-
194 feeding from *B. subtilis* to *S. indica*, explain the positive impact of *B. subtilis* supernatant on
195 growth irrespective of thiamine availability. They also provide further support that the *B.*
196 *subtilis*-associated growth of *S. indica* relates to thiamine provision rather than organic acids,
197 since acetate and formate alone did not enable *S. indica* growth in thiamine-free media (Figure
198 S3). We further found that *S. indica* secreted an unidentified organic acid in thiamine-media,

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199 that showed an IC profile overlapping with that of glutamine, and that was consumed by *B.*
200 *subtilis* in the reverse experiment design (of growing *B. subtilis* in *S. indica* supernatant).
201
202 **The successful co-existence of *S. indica* and *B. subtilis* depends on spatiotemporal**
203 **organization in the system.** The above findings show that *B. subtilis* can support the growth
204 of *S. indica* in thiamine-free medium either through its supernatant or when co-cultured at a
205 distance on an agar plate. Both experimental setups were geared towards identifying possible
206 interactions among the two species through utilization of the excretions of one species by the
207 other, but did not necessarily consider the spatiotemporal factors on such interaction. Thus, a
208 remaining question was whether both species could still co-exist and establish a successful
209 interaction under different conditions regarding the spatial proximity or size of initial
210 inoculation, or the actual growth phase that the different species are in at the time of
211 introduction onto the agar. While addressing these questions is experimentally challenging, we
212 attempted here to analyse the impact of spatial-temporal factors on the outcome of the *S. indica*
213 – *B. subtilis* auxotrophic interaction by changing inoculation time and location on agar plates.
214 In particular, we separated the inoculation of *S. indica* spores from *B. subtilis* inoculation either
215 in time (by inoculating spores 3 days before *B. subtilis* colony inoculation) or space (by
216 inoculating *S. indica* and *B. subtilis* at certain distance to each other). Alternatively, we
217 inoculated *S. indica* spores after mixing with *B. subtilis*. These experiments mimic a scenario
218 commonly found in agri-technology practices when using pre-mixed cultures or spores of
219 different microbes as soil biofertilizers or plant seed pre-treatments (Turner, 1991). We found
220 that with temporal or spatial separation, both species could successfully grow in the absence of
221 thiamine, indicating a positive auxotrophic interaction (Figure 5). In contrast, direct co-
222 inoculation of *B. subtilis* with *S. indica* significantly hampered co-existence of the two species,
223 particularly reducing *S. indica* growth (Figure 5). These findings indicate the significance of
224 the spatiotemporal organization on microbial co-existence for the formation of stable
225 interactions and, hence, on higher order microbial community structures. Our observations
226 either reflect competition for resources upon co-inoculation, or alteration of the micro-
227 environment by one species so that the other cannot establish itself, as discussed further below.
228

229 **Conclusion**

230 Here, we report the discovery of a novel auxotrophy for thiamine in the endophytic fungus *S.*
231 *indica* and how this thiamine requirement can be satisfied by *B. subtilis*. Our findings suggest
232 that *S. indica* possesses thiamine transporters but lacks necessary thiamine biosynthesis genes,
233 and its growth in thiamine-free medium cannot be supported by any other vitamin or relevant
234 organic and amino acids. In contrast, co-culturing with *B. subtilis* in thiamine-free media allows
235 *S. indica* growth, indicating a successful auxotrophic interaction between the two organisms.
236 In addition, we have identified several cross-feeding interactions between the two organisms
237 involving overflow and consumption of organic acids. We found that the auxotrophic
238 interaction can only be achieved under conditions where the inoculation (and germination) of
239 the two species is separated in time or space.
240
241 These findings have implications both for the study of *S. indica*, as an important plant-
242 supporting soil fungus (Qiang *et al.*, 2012), and for the engineering and application of minimal
243 synthetic communities that aim to establish plant supporting soil communities. In the former
244 direction, future metabolic and physiological studies of *S. indica* will be enabled by the defined
245 media conditions and identified thiamine auxotrophy in this study. A key suggestion from a
246 biotechnological perspective, for example, is to consider thiamine as an important factor in the
247 commercial mass production of *S. indica* (Singhal *et al.*, 2017). In the latter direction, the
248 presented results point to the importance of the role of spatiotemporal dynamics on the outcome

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249 of microbial interactions. The failure of establishment of auxotrophic interactions between *S.*
250 *indica* and *B. subtilis*, when co-cultured in the same place and time suggest that additional
251 ecological factors can override naïve expectations from complementary metabolic interactions.
252 This interplay between ecological forces, abundance, and interactions of auxotrophic species
253 is currently not well-understood and only explored in a few studies, which used either
254 synthetically engineered interactions (Shou *et al*., 2007; Mee *et al*., 2014) or enriched microbial
255 communities (Embree *et al*., 2015). Thus, our defined synthetic co-culture represents a
256 powerful system towards understanding this new emerging axis of the molecular ecology of
257 species interactions.
258
259 The observed failure to establish a successful auxotrophic interaction when co-cultured at the
260 same time and space could be related to several factors. At the simplest level of explanation, *S.*
261 *indica* and *B. subtilis* could be competing for and depleting the local carbon source. This is an
262 unlikely explanation for our experiments, where carbon levels were relatively high. An
263 alternative explanation would be that changes in ecological conditions caused by one species,
264 would prevent the other species from establishing itself. In this ecological explanation, both
265 the changes in local pH and in oxygen availability could be relevant. Changes in pH upon
266 microbial growth were highlighted recently as affecting the subsequent microbial interactions
267 and growth (Vylkova, 2017; Ratzke and Gore, 2017). Similarly, extensive oxygen depletion
268 upon microbial colony growth on agar plates has been shown in several studies (Peters and
269 Wimpenny, 1987; Dietrich *et al*., 2013; Kempes and Okegbe, 2014) and can directly influence
270 subsequent or simultaneous growth of different species or their interactions. This explanation
271 could be particularly relevant in our experiments, where we expect *S. indica* germination and
272 initial growth to require substantial oxygen (Tacon *et al*., 1983).
273
274 The finding that the success of auxotrophic interactions relates to spatiotemporal effects
275 suggest that consideration should be given to inoculation timing when designing or applying
276 biofertilizers or bio-control agents to the soil. Indeed, microbial interactions and synergisms
277 are suggested to be crucial for soil fertility, bioproductivity and ecosystem functioning (Perotto
278 and Bonfante, 1997; Bulgarelli *et al.*, 2013; Pérez-Jaramillo *et al.*, 2016). Plants significantly
279 benefit from symbioses with soil microbes, with benefits ranging from nutrient supply, growth
280 promotion to elevating plant stress resistance (Vessey, 2003; Esser, 2013; Davison, 1988;
281 Castillo *et al*., 2013; Yurgel *et al*., 2014). At the same time, soil microbes can interact among
282 themselves or alter each other's interactions with the plants (Veresoglou *et al*., 2012; Lareen
283 *et al*., 2016) (Fitter and Garbaye, 1994; Kohlmeier *et al*., 2005). The biochemical basis of these
284 potential multi-level interactions in the soil has remained mostly elusive to date, with few
285 documented cases of amino acid auxotrophies in specific soil bacteria and vitamin-provision
286 from plants relating to their root colonization (Nagae *et al*., 2016; diCenzo *et al*., 2015; Streit
287 *et al*., 1996). The presented synthetic community of *S. indica* and *B. subtilis* shows for the first
288 time that metabolic auxotrophy can directly underpin microbial interactions and growth, and
289 that the success of interaction can be determined by spatiotemporal organization in the system.
290 In to the future, this synthetic system allows controlled investigation (and potential
291 optimization) of fungal-bacteria interactions and can be further extended with additional
292 microbes and a plant. The resulting minimalist synthetic eco-system can provide a platform to
293 analyze and control cross-kingdom relationships between plants and their growth promoting
294 fungi and bacteria, and enable new applications for vertical farming and crop production in the
295 field.
296
297 **Materials and methods**

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298 ***S. indica* cultures, growth media and conditions.** The defined basic medium for testing *S.*
299 *indica* growth with ammonium as nitrogen source contained per liter; 15g agar, 20g glucose,
300 1.32 g $(NH_4)_2SO_4$, 0.89 g $Na_2HPO_4{\cdot}2H_2O$, 0.68 g KH_2PO_4, 35 mg $Na_2MoO_7{\cdot}2H_2O$, 5.2 mg
301 $MgSO_4$, 2.5 mg $FeSO_4{\cdot}7H_2O$, 0.74 mg $CaCl_2{\cdot}2H_2O$, 0.0043 mg $ZnSO_4{\cdot}7H_2O$, 0. 004 mg
302 $CuSO_4{\cdot}5H_2O$. Growth experiments for testing effects of different vitamins were performed in
303 24-well plates (Ref: 353226, Falcon), where each well contained 2 ml of the basic medium,
304 supplemented with either 200 µg/l of single vitamin solution, 1 g/l yeast extract, 200 µg/l
305 mixture of all eight vitamins tested, or equivalent amount of water. Each well was then
306 inoculated with 1 µl of *S. indica* spore suspension (approximately 500,000 spores/ml, counted
307 with Neubauer counting chamber), where spores were harvested from 6-8 weeks old *S. indica*
308 agar plates. In the 'non-inoculated' control treatment, 1 µl of water was used instead. Each
309 treatment condition was prepared in three technical replicates. 24-well plates were then sealed
310 with parafilm and placed in a 30 °C static incubator for 2 weeks. Images were taken with a gel
311 doc system (Syngene) at the end of this period.
312
313 **Experiments on agar plates, growth media and conditions.** The defined (basic) medium for
314 testing *S. indica* growth on agar plates contained per liter; 15 g agar, 20 g glucose, 0.89 g
315 $Na_2HPO_4{\cdot}2H_2O$, 0.68 g KH_2PO_4, 35 mg $Na_2MoO_7{\cdot}2H_2O$, 5.2 mg $MgSO_4$, 2.5 mg $FeSO_4{\cdot}7H_2O$,
316 0.74 mg $CaCl_2{\cdot}2H_2O$, 0.0043 mg $ZnSO_4{\cdot}7H_2O$, 0.004 mg $CuSO_4{\cdot}5H_2O$. When the chosen
317 nitrogen source was ammonium, 1.32 g/l $(NH_4)_2SO_4$ was added to this basic recipe. When the
318 chosen nitrogen source was glutamine, 1.46 g/l glutamine was added to this basic recipe. For
319 experiments with thiamine, 150 µg/l thiamine was added to the basic media after autoclaving.
320 Experiments were carried out on 60 mm dishes, filled with 6ml of agar medium given
321 above. A 500,000 spores/ml *S. indica* spore suspension, with spores harvested from 6-8 weeks
322 old *S. indica* agar plates, was inoculated with 2 µl (on the left side of the plates). At
323 approximately 2 cm distance to the right of the inoculum, either a 'mock' solution, a thiamine
324 block, or a *B. subtilis* inoculum were placed. The 'mock' solution was 2 µl of sterile water. The
325 thiamine blocks were made by pouring 6 ml of 1.5% agar solution containing150µg/l thiamine
326 into a 60 mm plate, and then punching a block out using a sterile pipette tip with a diameter of
327 4.7 mm. Therefore, each agar block used contained approximately 5.6 ng thiamine. The *B.*
328 *subtilis* inoculum was a 2 µl sample harvested from a culture, grown in 5ml liquid Lysogeny
329 broth (LB) (Bertani, 1951) to an $OD_{600} \approx 0.5$ measured by spectrophotometer (Spectronic 200,
330 Thermo Fisher Scientific), then washed and re-suspend to OD_{600} 0.5 with 10 mM $MgCl_2$. The
331 plates were incubated in 30 °C for 2 weeks. Images were taken with gel doc system (Syngene)
332 at the end of this period.
333
334 **Time-lapse microscopy and image analysis**. Time-lapse microscopy was performed on agar-
335 medium cultures that were prepared using the same basic medium described above. A 6-well
336 tissue culture plate (Ref: 353046, Falcon) was used and 1 µl of *S. indica*, *B. subtilis* or mock
337 solution prepared as described above were inoculated on each well accordingly to experiment
338 design. An Olympus IX83 microscope, UPlanFLN 4× objective and cellSens software were
339 used for recording the growth. Okolab stage top incubator (H301-T-UNIT-BL-Plus system,
340 and H301-EC chamber) were used for incubation, with a temperature sensor and lens heater
341 set to 30 °C and stabilized for at least 2 hours prior to the experiment. Different fields of view
342 were chosen at interior and periphery of each colony and images from those fields were
343 recorded using the automated microscope stage and Olympus cellSens software. Images were
344 taken in 1 hour intervals and put together as image series. ImageJ (Fiji) (Schindelin *et al.*, 2012)
345 was used for measuring the mean intensity on each field of view over time, normalized against
346 the intensity value of the first frame from each view point (as shown in Figure 3).
347

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348 **Spatial and temporal separation experiments**. *S. indica* (500,000 spores/ml, determined by
349 counting with Neubauer counting chamber) and *B. subtilis* ($OD_{600}\approx$ 0.5, determined by
350 spectrophotometer (Spectronic 200, Thermo Fisher Scientific)) were cultivated on thiamine-
351 free synthetic medium containing ammonium as sole nitrogen source. 6-well tissue culture
352 plates (Ref: 353046, Falcon). On each plate, 1 µl of *S. indica* and 1µl of *B. subtilis* were
353 inoculated on 5 of the wells; one well was intentionally left non-inoculated as a blank. In the
354 "no separation" case, *S. indica* and *B. subtilis* were pre-mixed at 1:1 volume ration, and
355 inoculated on the center of each well. For "spatial separation" case, *S. indica* was inoculated
356 7.5 mm left to the center of a well and *B. subtilis* 7.5mm right to the center, leaving 15 mm
357 distance in between. For "temporal separation" case, *S. indica* was inoculated on each well, the
358 plates were then incubated for 3 days and *B. subtilis* was inoculated after this time. All the
359 plates were incubated in 30°C for 2 weeks (starting from the time of *S. indica* inoculation).
360 Images were taken by scanning each well under a microscope (Olympus IX83) using the same
361 exposure time under bright field.
362 ImageJ was used for measuring the biomass by integration of the total colony density.
363 An image of each colony was manually outlined using the selection tool. The selected area was
364 compared with the same location on a blank well from the same plate. The area and relative
365 intensity were recorded (using "measure" function) and used for calculating the colony growth.
366
367 **Supernatant cross-feeding experiments**. Axenic cultures of *S. indica* and *B. subtilis* were
368 cultivated in 50 ml basic medium described above, with or without thiamine. For *S. indica* an
369 inoculum of 50 µl of a 500,000 spores/ml spore suspension, harvested from 6-8 weeks old *S.*
370 *indica* agar plates, was used. For *B. subtilis*, an inoculum of 50µl, sampled from a culture grown
371 in LB to an $OD_{600}\approx$ 0.5 determined by spectrophotometer (Spectronic 200, Thermo Fisher
372 Scientific), and washed with 10 mM $MgCl_2$, was used. After one week incubation in 30°C and
373 at 150 rpm, *S. indica* cells were harvested by centrifugation at 18,000 g for 20 minutes. The
374 supernatant was collected and filtered through a 0.2 µm polyethersulfone (PES) filter (Ref:
375 WHA67802502, Whatman), while biomass was washed with 40 ml MilliQ water, dried using
376 a centrifugal evaporator (EZ-2 Elite, Genevac), and then weighted. The growth of *B. subtilis*
377 liquid cultures was monitored daily by taking 1 ml samples and measuring OD_{600} by
378 spectrophotometer (Spectronic 200, Thermo Fisher Scientific). At the end of 1 week, the
379 remaining liquid culture was centrifuged at 18,000 g for 10 min. The supernatant was collected
380 and filtered through a 0.2 µm PES filter, while biomass was discarded.
381 Both supernatants were mixed with fresh basic medium in a 1:1 ratio to set up new
382 axenic cultures of *S. indica* and *B. subtilis*. 1 ml liquid samples were collected from these
383 cultures after one week by filtering through a 0.2 µm nylon membrane (Ref: WHA7402004,
384 Whatman). Samples were transferred into polypropylene vials (Ref: 079812, Thermo Fisher
385 Scientific) for Ion Chromatography, which was performed using Dionex ICS-500^{+} and column
386 Dionex IonPac AS11-HC-4 µm (2 x 250 mm).
387
388 **Sequence analysis and BLAST search.** Sequences of key thiamine biosynthesis enzyme
389 genes (Table S1) from *Saccharomyces cerevisiae* S288c were compared with available *S.*
390 *indica* genome homologues using Position-Specific Initiated BLAST
391 (https://blast.ncbi.nlm.nih.gov/Blast.cgi), to identify the putative functions of the
392 corresponding genes. An e-value of 1e-6 was chosen as cut-off to identify homologous
393 sequences (Altschul, 1997). The results of the analysis using blastx are shown in Table S1,
394 while blastn and tblastx did not return any results with the chosen cut-off.
395
396 **Thiamine measurements on *B. subtilis* liquid cultures.** Axenic cultures of *B. subtilis* were
397 cultivated in 50ml synthetic medium containing glutamine as sole nitrogen source without

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398 thiamine. *B. subtilis* inoculum of 50 µl, sampled from a culture grown in LB to $OD_{600} \approx 0.5$
399 determined by spectrophotometer (Spectronic 200, Thermo Fisher Scientific), and washed with
400 10 mM $MgCl_2$ was used. After one week of incubation in 30 °C and at 150 rpm, *B. subtilis*
401 cultures were harvested by centrifugation at 18,000 g for 10 minutes. The supernatant was
402 collected and filtered through a 0.2 nm PES membrane filter (Ref: WHA67802502, Whatman).
403 The supernatant of 250 µl from each culture was transferred to a clean 1.5 ml Eppendorf tube,
404 followed by sequentially adding 10 µl 1% $K_3[Fe(CN)_6]$, 150µl 15% NaOH solution and 150µl
405 isobutanol. The tubes were shaken vigorously for 1 minute, followed by 2 minutes of
406 centrifugation at 13,000 g. The upper isobutanol layer of each tube was transferred to a new
407 1.5 ml Eppendorf tube, containing approximate 0.2g Na_2SO_4. The tubes were mixed well and
408 centrifuged for 1 minute at 13,000 g for solids to settle. 100 µl of supernatant from each tube
409 were transferred to 96-well plates (Ref: 3916, black flat bottom, Corning) and the fluorescence
410 was measured using a plate reader (CLARIOstar, BMG Labtech) at 365 nm excitation and 450
411 emission. The concentration of thiamine was determined with a series of known concentration
412 standard thiamine solutions under the same treatment.
413
414 ***S. indica* growth under different thiamine concentrations.** Synthetic medium containing
415 ammonium as sole nitrogen source was used for testing *S. indica* growth in different thiamine
416 concentrations. Media containing final thiamine concentrations of 0 µg/l, 1.5 µg/l, 15 µg/l, 150
417 µg/l and 1500 µg/l were prepared, and distributed in a 6-well tissue culture plate (Ref: 353046,
418 Falcon). Each 6-well plate contained one concentration condition, with 3ml medium in each
419 well. On each plate, 1µl of *S. indica* (500,000 spores/ml) were inoculated on the center of five
420 wells, while one well was intentionally left non-inoculated as a blank. Plates were incubated at
421 30 °C for 2 weeks. Afterwards, lids were removed and OD_{600} and fluorescence at 365 nm for
422 excitation and 450 nm for emission were measured using a plate reader (CLARIOstar, BMG
423 Labtech) and the plate scan function to get an overall reading of each well. Images of plates
424 were taken using a gel doc system (Syngene).
425

426 **References**

427 Abreu NA, Taga ME. (2016). Decoding molecular interactions in microbial communities.
428 Shen A (ed). *FEMS Microbiol Rev* **40**: 648–663.

429 Altschul S. (1997). Gapped BLAST and PSI-BLAST: a new generation of protein database
430 search programs. *Nucleic Acids Research* **25**: 3389–3402.

431 Begley TP, Downs DM, Ealick SE, McLafferty FW, Van Loon APGM, Taylor S, *et al*.
432 (1999). Thiamin biosynthesis in prokaryotes. *Arch Microbiol* **171**: 293–300.

433 Bertani G. (1951). Studies on lysogenesis. I. The mode of phage liberation by lysogenic
434 Escherichia coli. *J Bacteriol* **62**: 293–300.

435 Bulgarelli D, Schlaeppi K, Spaepen S, van Themaat EVL, Schulze-Lefert P. (2013). Structure
436 and Functions of the Bacterial Microbiota of Plants. *Annu Rev Plant Biol* **64**: 807–838.

437 Castillo H, Aguilar C, Reyes CF, Morales GG. (2013). Biological control of root pathogens
438 by plant-growth promoting Bacillus spp.

439 Davison J. (1988). Plant beneficial bacteria. *Nature Biotechnology*. e-pub ahead of print, doi:
440 10.1038/nbt0388-282.

441 diCenzo GC, Zamani M, Cowie A, Finan TM. (2015). Proline auxotrophy in Sinorhizobium

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442 meliloti results in a plant-specific symbiotic phenotype. *Microbiology (Reading, Engl)* **161**:
443 2341–2351.

444 Dietrich LEP, Okegbe C, Price-Whelan A, Sakhtah H, Hunter RC, Newman DK. (2013).
445 Bacterial community morphogenesis is intimately linked to the intracellular redox state. *J*
446 *Bacteriol* **195**: 1371–1380.

447 Druege U, Baltruschat H, Franken P. (2007). Piriformospora indica promotes adventitious
448 root formation in cuttings. *Scientia Horticulturae* **112**: 422–426.

449 Embree M, Liu JK, Al-Bassam MM, Zengler K. (2015). Networks of energetic and metabolic
450 interactions define dynamics in microbial communities. *Proc Natl Acad Sci USA* **112**: 15450–
451 15455.

452 Esser K. (2013). The Mycota: A Comprehensive Treatise on Fungi as Experimental Systems
453 for Basic and Applied Research. Agricultural Applications. XI.

454 Falkowski PG, Fenchel T, Delong EF. (2008). The microbial engines that drive Earth's
455 biogeochemical cycles. *Science*.

456 Fitter AH, Garbaye J. (1994). Interactions between mycorrhizal fungi and other soil
457 organisms. *Plant and Soil* **159**: 123–132.

458 Gòdia F, Albiol J, Montesinos JL, Pérez J, Creus N, Cabello F, *et al*. (2002). MELISSA: a
459 loop of interconnected bioreactors to develop life support in space. *J Biotechnol* **99**: 319–330.

460 Großkopf T, Soyer OS. (2014). Synthetic microbial communities. *Current Opinion in*
461 *Microbiology*. e-pub ahead of print, doi: 10.1016/j.mib.2014.02.002.

462 Hays SG, Patrick WG, Ziesack M, Oxman N, Silver PA. (2015). Better together: engineering
463 and application of microbial symbioses. *Current Opinion in Biotechnology* **36**: 40–49.

464 Helliwell KE, Wheeler GL, Leptos KC, Goldstein RE, Smith AG. (2011). Insights into the
465 evolution of vitamin B12 auxotrophy from sequenced algal genomes. *Mol Biol Evol* **28**:
466 2921–2933.

467 Helliwell KE, Wheeler GL, Smith AG. (2013). Widespread decay of vitamin-related
468 pathways: coincidence or consequence? *Trends Genet* **29**: 469–478.

469 Hohmann S, Meacock PA. (1998). Thiamin metabolism and thiamin diphosphate-dependent
470 enzymes in the yeast Saccharomyces cerevisiae: genetic regulation. *Biochim Biophys Acta*
471 **1385**: 201–219.

472 Hom EFY, Murray AW. (2014). Plant-fungal ecology. Niche engineering demonstrates a
473 latent capacity for fungal-algal mutualism. *Science* **345**: 94–98.

474 Hong HA, Khaneja R, Tam NMK, Cazzato A, Tan S, Urdaci M, *et al*. (2009). Bacillus
475 subtilis isolated from the human gastrointestinal tract. *Res Microbiol* **160**: 134–143.

476 Jacobs S, Zechmann B, Molitor A, Trujillo M, Petutschnig E, Lipka V, *et al*. (2011). Broad-
477 spectrum suppression of innate immunity is required for colonization of Arabidopsis roots by
478 the fungus Piriformospora indica. *Plant Physiol* **156**: 726–740.

bioRxiv preprint first posted online Sep. 28, 2017; doi: http://dx.doi.org/10.1101/195339. The copyright holder for this preprint (which was not peer-reviewed) is the author/funder. It is made available under a CC-BY-NC-ND 4.0 International license.

479 Jenkins AH, Schyns G, Potot S, Sun G, Begley TP. (2007). A new thiamin salvage pathway.
480 *Nat Chem Biol* **3**: 492–497.

481 Jurgenson CT, Begley TP, Ealick SE. (2009). The structural and biochemical foundations of
482 thiamin biosynthesis. *Annu Rev Biochem* **78**: 569–603.

483 Kanehisa M, Goto S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic*
484 *Acids Research*.

485 Kempes CP, Okegbe C. (2014). Morphological optimization for access to dual oxidants in
486 biofilms. e-pub ahead of print, doi: 10.3410/f.718206741.793493885.

487 Kohlmeier S, Smits THM, Ford RM, Keel C, Harms H, Wick LY. (2005). Taking the fungal
488 highway: mobilization of pollutant-degrading bacteria by fungi. *Environ Sci Technol* **39**:
489 4640–4646.

490 Kumar V, Sahai V, Bisaria VS. (2011). High-density spore production of Piriformospora
491 indica, a plant growth-promoting endophyte, by optimization of nutritional and cultural
492 parameters. *Bioresource Technology* **102**: 3169–3175.

493 Lareen A, Burton F, Schäfer P. (2016). Plant root-microbe communication in shaping root
494 microbiomes. *Plant Mol Biol* **90**: 575–587.

495 Lindemann SR, Bernstein HC, Song H-S, Fredrickson JK, Fields MW, Shou W, *et al*. (2016).
496 Engineering microbial consortia for controllable outputs. **10**: 2077–2084.

497 Lu J, Frank EL. (2008). Rapid HPLC measurement of thiamine and its phosphate esters in
498 whole blood. *Clin Chem* **54**: 901–906.

499 Lucy M, Reed E, Glick BR. (2004). Applications of free living plant growth-promoting
500 rhizobacteria. *Antonie Van Leeuwenhoek* **86**: 1–25.

501 Machado CR, Praekelt UM, de Oliveira RC, Barbosa AC, Byrne KL, Meacock PA, *et al*.
502 (1997). Dual role for the yeast THI4 gene in thiamine biosynthesis and DNA damage
503 tolerance. *J Mol Biol* **273**: 114–121.

504 Mader U, Schmeisky AG, Florez LA. (2011). Subti Wiki—a comprehensive community
505 resource for the model organism Bacillus subtilis. *Nucleic acids* …. e-pub ahead of print, doi:
506 10.1093/nar/gkr923.

507 Mee MT, Collins JJ, Church GM, Wang HH. (2014). Syntrophic exchange in synthetic
508 microbial communities. *Proc Natl Acad Sci USA* **111**: E2149–56.

509 Michal G, Schomburg D. (1999). Biochemical pathways: an atlas of biochemistry and
510 molecular biology.

511 Morris JJ, Lenski RE, Zinser ER. (2012). The Black Queen Hypothesis: evolution of
512 dependencies through adaptive gene loss. *MBio* **3**: e00036–12–e00036–12.

513 Nagae M, Parniske M, Kawaguchi M, Takeda N. (2016). The relationship between thiamine
514 and two symbioses: Root nodule symbiosis and arbuscular mycorrhiza. *Plant Signaling &*
515 *Behavior* **11**: e1265723.

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516 Pande S, Merker H, Bohl K, Reichelt M, Schuster S, de Figueiredo LF, *et al*. (2014). Fitness
517 and stability of obligate cross-feeding interactions that emerge upon gene loss in bacteria.
518 *ISME J* **8**: 953–962.

519 Perotto S, Bonfante P. (1997). Bacterial associations with mycorrhizal fungi: close and
520 distant friends in the rhizosphere. *Trends Microbiol* **5**: 496–501.

521 Peters AC, Wimpenny J. (1987). Oxygen profiles in, and in the agar beneath, colonies of
522 Bacillus cereus, Staphylococcus albus and Escherichia coli. *Microbiology*. e-pub ahead of
523 print, doi: 10.1099/00221287-133-5-1257.

524 Pérez-Jaramillo JE, Mendes R, Raaijmakers JM. (2016). Impact of plant domestication on
525 rhizosphere microbiome assembly and functions. *Plant Mol Biol* **90**: 635–644.

526 Qiang X, Weiss M, Kogel K-H, Schäfer P. (2012). Piriformospora indica-a mutualistic
527 basidiomycete with an exceptionally large plant host range. *Mol Plant Pathol* **13**: 508–518.

528 Ratzke C, Gore J. (2017). Modifying And Reacting To The Environmental pH Drives
529 Bacterial Interactions. *bioRxiv*. e-pub ahead of print, doi: 10.1101/136838.

530 Richardson AE, Lynch JP, Ryan PR, Delhaize E, Smith FA, Smith SE, *et al*. (2011). Plant
531 and microbial strategies to improve the phosphorus efficiency of agriculture. *Plant and Soil*
532 **349**: 121–156.

533 Rodionova IA, Li X, Plymale AE, Motamedchaboki K, Konopka AE, Romine MF, *et al*.
534 (2015). Genomic distribution of B-vitamin auxotrophy and uptake transporters in
535 environmental bacteria from the Chloroflexi phylum. *Environ Microbiol Rep* **7**: 204–210.

536 Sañudo-Wilhelmy SA, Gómez-Consarnau L, Suffridge C, Webb EA. (2014). The role of B
537 vitamins in marine biogeochemistry. *Ann Rev Mar Sci* **6**: 339–367.

538 Schindelin J, Arganda-Carreras I, Frise E, Kaynig V, Longair M, Pietzsch T, *et al*. (2012).
539 Fiji: an open-source platform for biological-image analysis. *Nat Methods* **9**: 676–682.

540 Schink B. (1997). Energetics of syntrophic cooperation in methanogenic degradation.
541 *Microbiol Mol Biol Rev* **61**: 262–280.

542 Schyns G, Potot S, Geng Y, Barbosa TM, Henriques A, Perkins JB. (2005). Isolation and
543 characterization of new thiamine-deregulated mutants of Bacillus subtilis. *J Bacteriol* **187**:
544 8127–8136.

545 Sherameti I, Tripathi S, Varma A. (2008). The root-colonizing endophyte Pirifomospora
546 indica confers drought tolerance in Arabidopsis by stimulating the expression of drought
547 stress-related genes in leaves. *Molecular plant- …* **21**: 799–807.

548 Shou W, Ram S, Vilar JMG. (2007). Synthetic cooperation in engineered yeast populations.
549 *Proc Natl Acad Sci USA* **104**: 1877–1882.

550 Singhal U, Attri MK, Varma A. (2017). Mass Cultivation of Mycorrhiza-Like Fungus
551 Piriformospora indica (Serendipita indica) by Batch in Bioreactor. In: Vol. 12.
552 *MYCORRHIZA-FUNCTION, DIVERSITY, STATE OF THE ART*. Springer International
553 Publishing: Cham, pp 365–384.

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554 Streit WR, Joseph CM, Phillips DA. (1996). Biotin and other water-soluble vitamins are key
555 growth factors for alfalfa root colonization by Rhizobium meliloti 1021. *Mol Plant Microbe*
556 *Interact* **9**: 330–338.

557 Tacon FL, Skinner FA, Mosse B. (1983). Spore germination and hyphal growth of a
558 vesicular–arbuscular mycorrhizal fungus, Glomus mosseae (Gerdemann and Trappe), under
559 decreased oxygen and …. *Canadian Journal of* ….

560 Turner JT. (1991). Factors Relating to Peanut Yield Increases After Seed Treatment with
561 Bacillus subtilis. *Plant Dis* **75**: 347.

562 Vadassery J, Ranf S, Drzewiecki C, Mithöfer A, Mazars C, Scheel D, *et al*. (2009). A cell
563 wall extract from the endophytic fungus Piriformospora indicapromotes growth of
564 Arabidopsis seedlings and induces intracellular calcium elevation in roots. *The Plant Journal*
565 **59**: 193–206.

566 Varma A, Sherameti I, Tripathi S, Prasad R, Das A. (2012). 13 The Symbiotic Fungus
567 Piriformospora indica: Review. *Fungal Associations*. e-pub ahead of print, doi:
568 10.1242/jcs.189431.

569 Veresoglou SD, Chen B, Rillig MC. (2012). Arbuscular mycorrhiza and soil nitrogen cycling.
570 *Soil Biology and Biochemistry* **46**: 53–62.

571 Vessey JK. (2003). Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*
572 **255**: 571–586.

573 Vylkova S. (2017). Environmental pH modulation by pathogenic fungi as a strategy to
574 conquer the host Hogan DA (ed). *PLoS Pathog* **13**: e1006149.

575 Waller F, Achatz B, Baltruschat H, Fodor J, Becker K, Fischer M, *et al*. (2005). The
576 endophytic fungus Piriformospora indica reprograms barley to salt-stress tolerance, disease
577 resistance, and higher yield. *Proc Natl Acad Sci USA* **102**: 13386–13391.

578 Wargo MJ, Hogan DA. (2006). Fungal—bacterial interactions: a mixed bag of mingling
579 microbes. *Current Opinion in Microbiology* **9**: 359–364.

580 Weiss M, Waller F, Zuccaro A, Selosse M-A. (2016). Sebacinales - one thousand and one
581 interactions with land plants. *New Phytol* **211**: 20–40.

582 Widder S, Allen RJ, Pfeiffer T, Curtis TP, Wiuf C. (2016). Challenges in microbial ecology:
583 building predictive understanding of community function and dynamics. *The ISME* …. e-pub
584 ahead of print, doi: 10.21236/AD1008795.

585 Wightman R. (2003). The THI5 gene family of Saccharomyces cerevisiae: distribution of
586 homologues among the hemiascomycetes and functional redundancy in the aerobic
587 biosynthesis of thiamin from pyridoxine. *Microbiology* **149**: 1447–1460.

588 Yadav V, Kumar M, Deep DK, Kumar H, Sharma R, Tripathi T, *et al*. (2010). A phosphate
589 transporter from the root endophytic fungus Piriformospora indica plays a role in phosphate
590 transport to the host plant. *J Biol Chem* **285**: 26532–26544.

591 Yurgel SN, Rice J, Domreis E, Lynch J, Sa N, Qamar Z, *et al*. (2014). Sinorhizobium

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592 melilotiFlavin Secretion and Bacteria-Host Interaction: Role of the Bifunctional RibBA
593 Protein. *Mol Plant Microbe Interact* **27**: 437–445.

594 Zuccaro A, Lahrmann U, Güldener U, Langen G, Pfiffi S, Biedenkopf D, *et al*. (2011).
595 Endophytic Life Strategies Decoded by Genome and Transcriptome Analyses of the
596 Mutualistic Root Symbiont Piriformospora indica Howlett BJ (ed). *PLoS Pathog* **7**:
597 e1002290–26.

598

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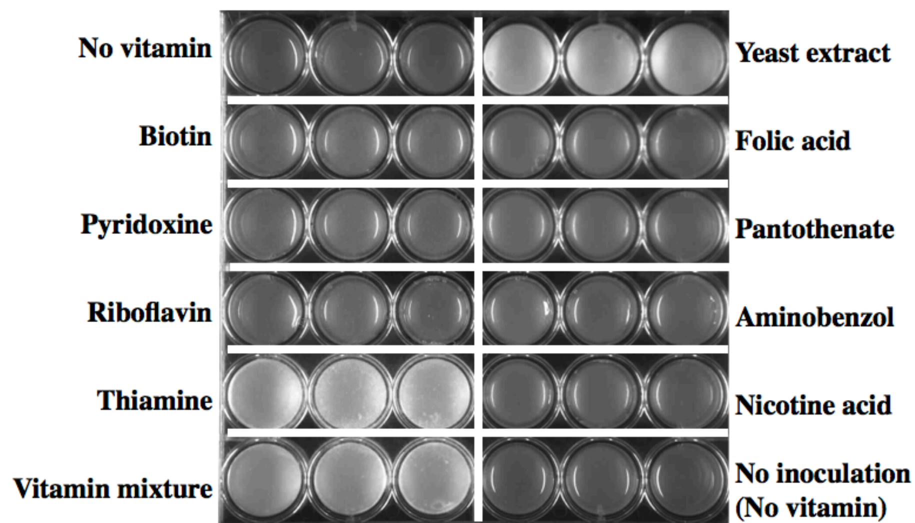
599 **FIGURES & TABLES**



600
601 **Figure 1. *S. indica* growth under different conditions.** Growth on agar plates with defined
602 medium supplemented with different vitamins as shown on each row and column. Each
603 treatment has three replicates presented in 3 adjacent wells. *S. indica* grows in white colonies.
604 Images were taken after two weeks of growth.
605

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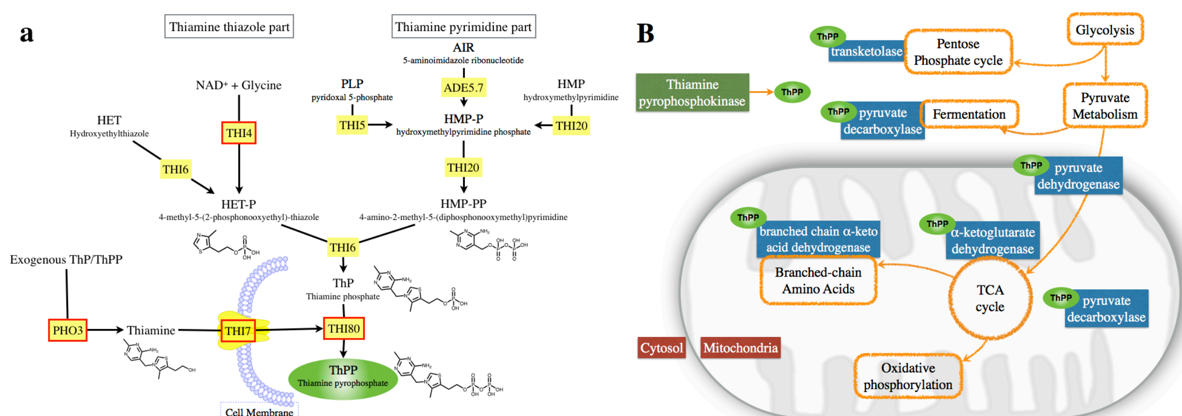

606
607 **Figure 2. Thiamine related genes and reactions. a.** Overview of the thiamine biosynthesis
608 pathway in *Saccharomyces cerevisiae* based on (Wightman, 2003), and as included in the
609 KEGG metabolic pathways (Pathway: sce00730) (Kanehisa and Goto, 2000). Yellow squares
610 indicate genes encoding for the enzymes in the corresponding reactions. Red borders indicate
611 genes for which there are *S. cerevisiae* homologues in *S. indica* (see *Methods*). **b.** Simplified
612 schematic of central metabolism in eukaryotic cells with cytosol and mitochondria
613 compartments indicated. Orange enclosures show reactions of the central metabolism, while
614 blue squares show essential enzymes catalyzing these reactions and requiring ThPP as a co-
615 factor (shown in green).
616
617

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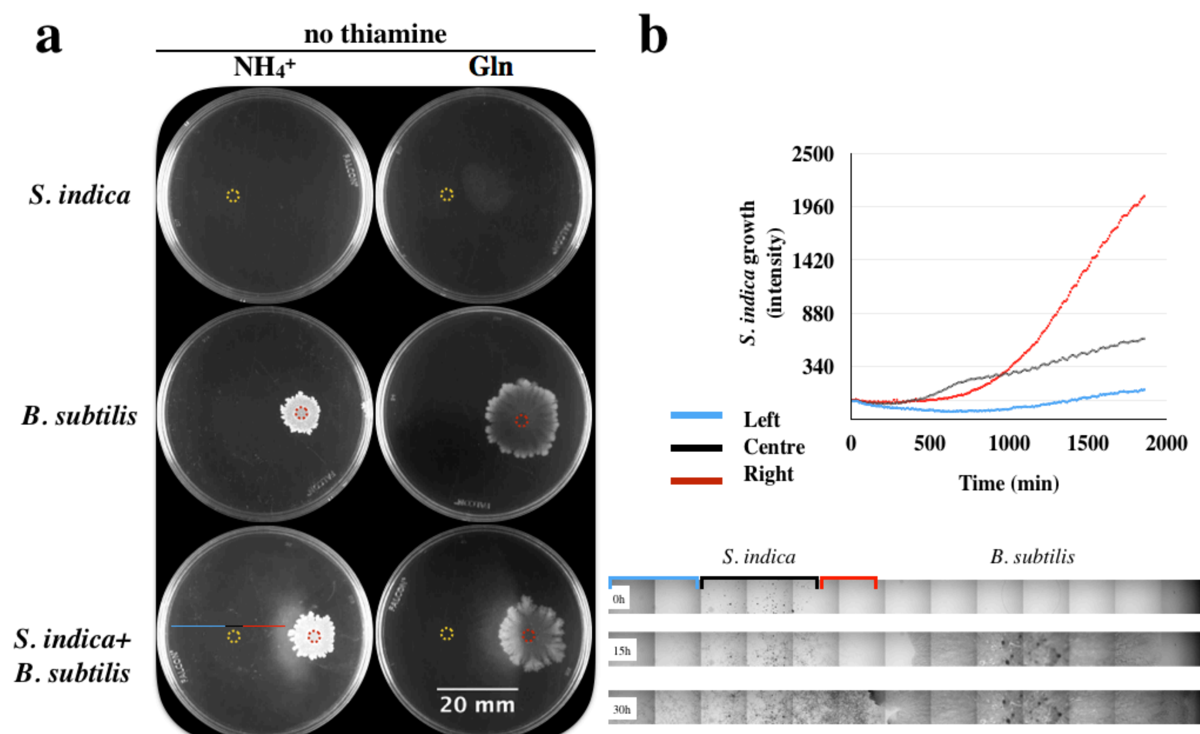

618
619 **Figure 3. *S. indica* and *B. subtilis* interactions on agar plates. a**. Rows from top to bottom
620 showed growth of monocultures of *S. indica*, *B. subtilis*, and their co-culture respectively under
621 the absence of thiamine. The yellow dotted circle on the images indicates the *S. indica*
622 inoculation point. Red dotted circle indicates *B. subtilis* inoculation point. The left and right
623 columns show growth on plates after two weeks, using ammonium and glutamine as nitrogen
624 sources respectively. When both organisms were cultured together (bottom row), *B. subtilis*
625 and *S. indica* were inoculated on the right and left of the plate respectively. Plates shown are
626 representative of at least 3 replicates for each condition. We performed 2 biological replicates
627 of this experiment, with qualitatively similar results. **b.** Bottom: Time lapse image series of *S.*
628 *indica* and *B. subtilis* growth on agar plates in the absence of thiamine and with ammonium as
629 nitrogen source. Each image strip is composed of microscopy images of the same horizontal
630 section from the middle of the plate through the inoculation point of *S. indica* and *B. subtilis*,
631 at 0 h, 15 h and 30 h after inoculation. *S. indica* growth across defined parts of this horizontal
632 section (blue, black, and red lines – also compare bottom left plate in 3a) was quantified by
633 measuring image density and plotted over time (upper chart). The sections correspond to the *S.*
634 *indica* colony side closer to *B. subtilis* (red), the middle of the colony (black), and the colony
635 side far from *B. subtilis* (blue).
636

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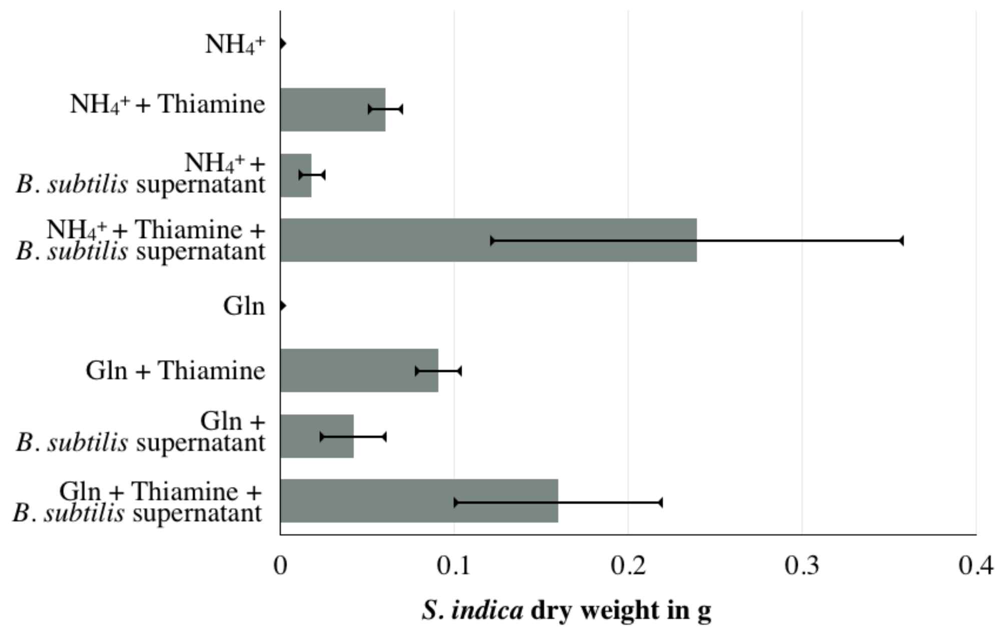

637
638 **Figure 4. *S. indica* growth under different media compositions.** Growth using either
639 ammonia or glutamine and supplemented with thiamine or *B. subtilis* supernatant, as indicated
640 on the y-axis. The x-axis shows *S. indica* growth approximated by total dry weight after one
641 week of growth (see *Methods*).
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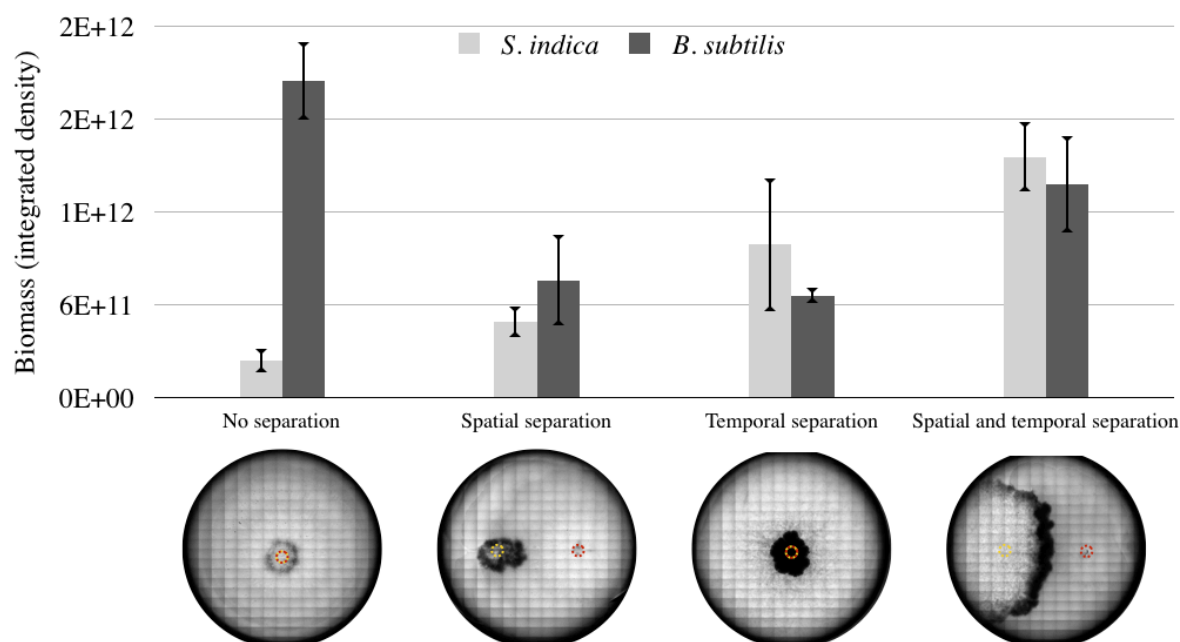

646
647 **Figure 5. Biomass of *B. subtilis* and *S. indica* under different spatiotemporal culturing**
648 **cases**. "No separation" refers to *B. subtilis* culture and *S. indica spores* being pre-mixed at 1:1
649 volume ratio, and then inoculated as a single solution. "Spatial separation" refers to
650 approximately 1.5 cm separation of *S. indica* (left) and *B. subtilis* (right) inoculation points.
651 "Temporal separation" refers to inoculation of *S. indica* 3 days prior to *B. subtilis* inoculation.
652 The yellow dotted circle on the images indicates the *S. indica* inoculation point. Red dotted
653 circle indicates *B. subtilis* inoculation point. Growth of the different species was approximated
654 by tracing their respective colonies on the plate and measuring the image intensity from the
655 engulfing areas 2 weeks after *S. indica* inoculation. Measurements are from 3 replicate agar
656 plates, with a representative plate image shown at the bottom. These images show microscopic
657 scans of each plate at 2 weeks of growth. For the "no separation" case, there was no observable
658 *S. indica* colony expansion after 1 week. We performed 2 biological repetitions of this
659 experiment, with qualitatively similar results.
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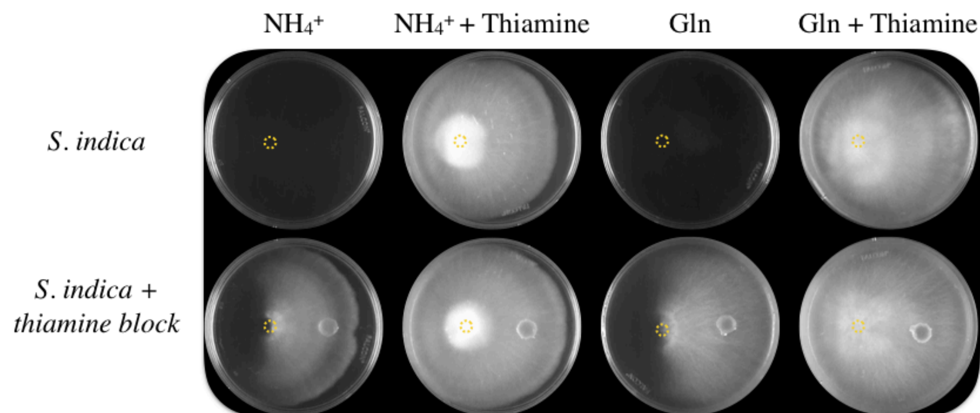
663 **SUPPLEMENTARY FIGURES**
664



665
666 **Supplementary Figure 1.** *S. indica* growth on agar plates after two weeks. Columns show
667 different media containing thiamine or not and using ammonium and glutamine as nitrogen
668 sources (shown as "NH_4^+" or "Gln" on the panels). The top and bottom rows show the images
669 of agar plates without or with an additional agar block containing thiamine, placed ~1.5 cm to
670 the right side of *S. indica* inoculation point, which is indicated with a yellow circle.
671
672

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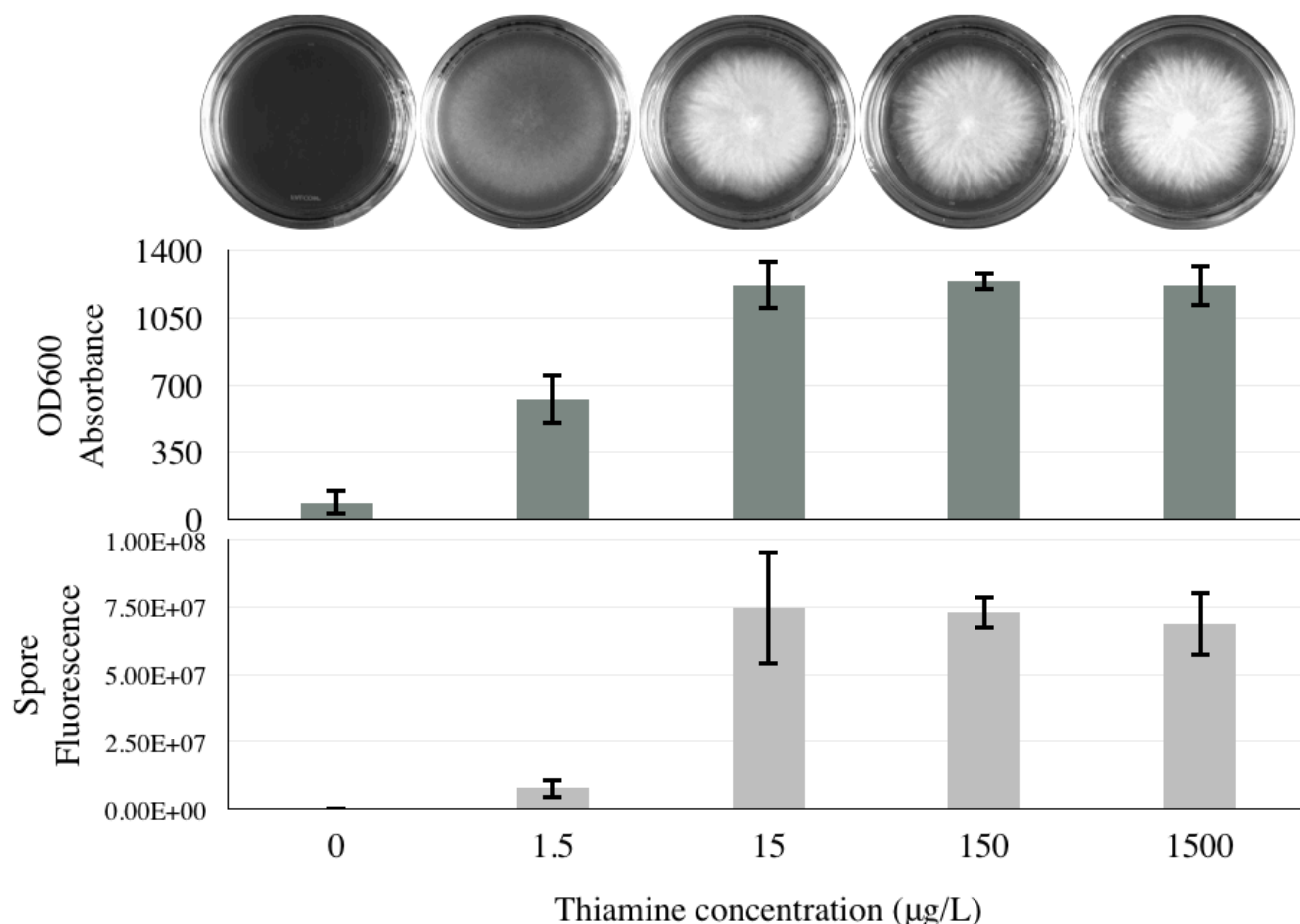

674
675 **Supplementary Figure 2.** *S. indica* growth on medium containing different concentrations of
676 thiamine and ammonium as nitrogen source. Images at top show two weeks growth of *S. indica*
677 on agar plates, and at different concentrations of thiamine as shown below on the bottom *x*-
678 axis. Each condition was repeated 6 times and images shown here are representatives for each
679 condition. Upper and lower bar-plots show plate absorbance at OD_{600} and fluorescence
680 intensity (measured at 390 excitation and 470 emission for detection of *S. indica* spores)
681 respectively.
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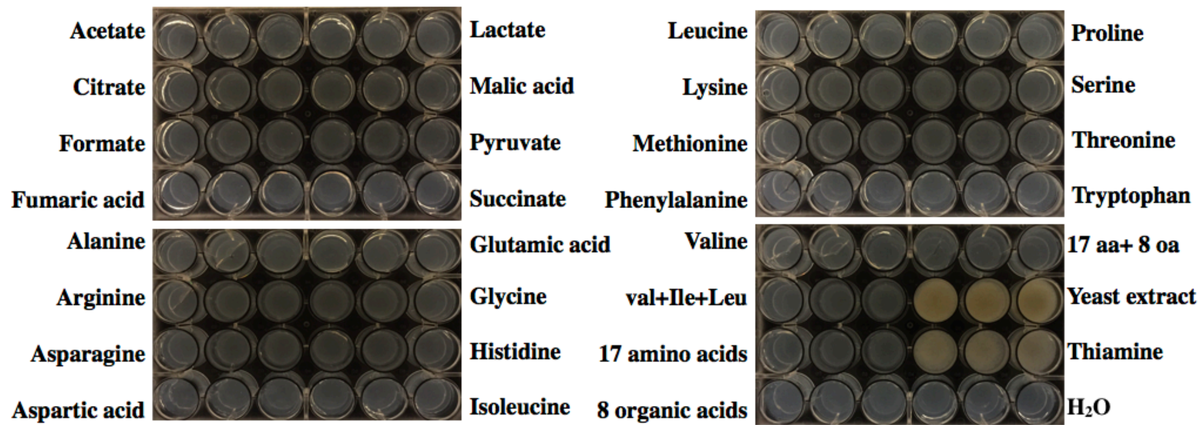

686
687 **Supplementary Figure 3.** *S. indica* amino acid and organic acid screen. *S. indica* growth on
688 the media containing different amino acid or organic acid as supplement. Each treatment has
689 three replicates presented in 3 adjacent wells. *S. indica* growth is visible as white-yellow
690 colonies on the surface of a well. Images were taken after two weeks of growth.
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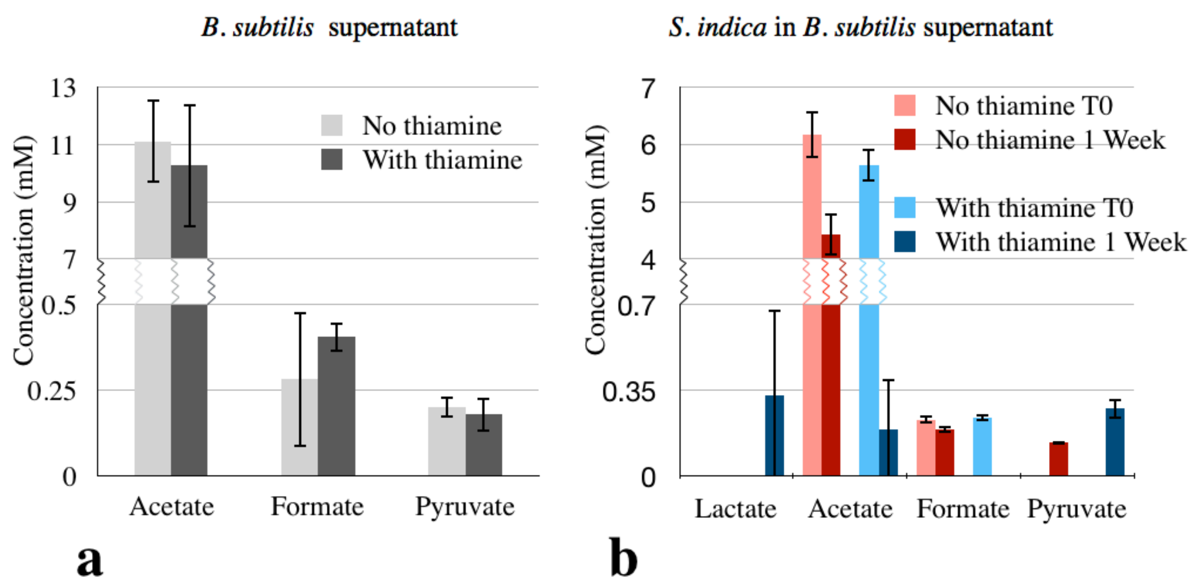

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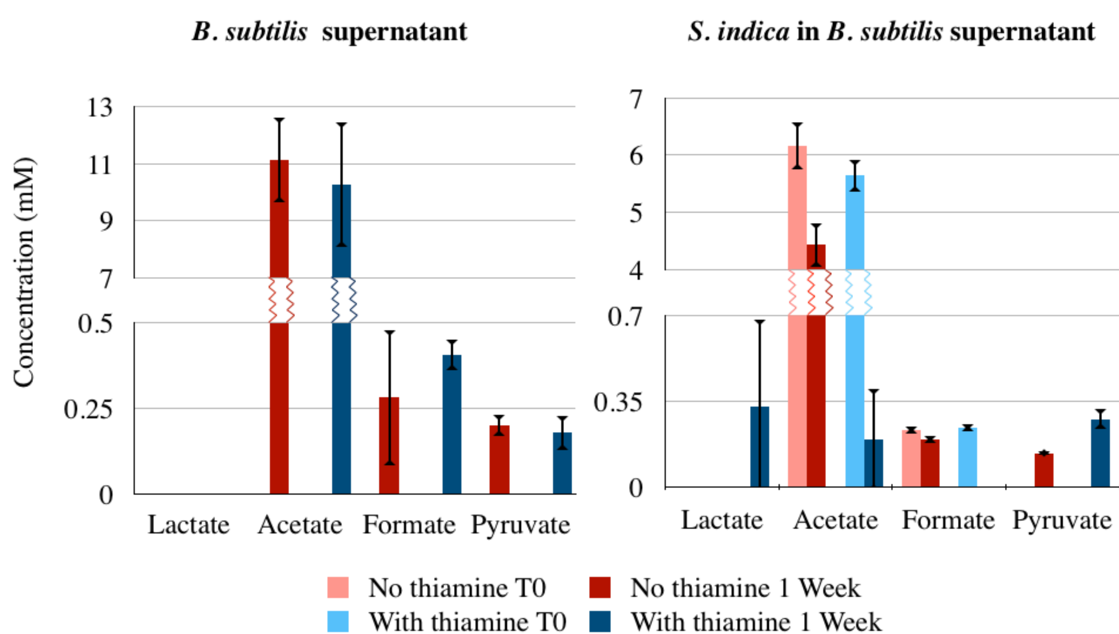

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696
697 **Supplementary Figure 4.** Concentrations of key organic acids in the supernatant of *B. subtilis*
698 growth media in the presence of absence of *S. indica*. The left chart shows the supernatant after
699 one week of *B. subtilis* cultivation in liquid medium containing glutamine as sole nitrogen
700 source. The right chart shows the supernatant after one week of cultivation of *S. indica* in *B.*
701 *subtilis* supernatant mixed with the same volume of fresh medium. T0 and T1 indicate the
702 initial condition and after 1 week of growth respectively. Note that T0 concentrations in the
703 right chart correspond to concentrations in the left chart diluted with fresh media.
704

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705 **Supplementary Table 1.**
706 Comparison of the thiamine-related genes/proteins of *S. indica* with those of *S. cerevisiae*.

Gene name	Function	Source organism for sequence	Blastx result using *S.indica* genome Max score/Query covery/E value
THI6	Bifunctional hydroxyethylthiazole kinase/thiamine-phosphate diphosphorylase	*Saccharomyces cerevisiae* S288c	No
THI80	Thiamine diphosphokinase		104/84%/4e^{-26}
Thi4	1. Thiamine thiazole synthase 2. Mitochondrial DNA damage tolerance		47.8/15%/2e^{-06}
THI5	Pyrimidine precursor biosynthesis		No
THI11	Pyrimidine precursor biosynthesis		No
THI12	Pyrimidine precursor biosynthesis		No
THI13	Pyrimidine precursor biosynthesis		No
PHO3	Thiamin-repressible acid phosphatase		114/86%/3e^{-27}
THI10 (THI7)	Thiamin transporter		176/92%/5e^{-48}
PDC2	Pyruvate decarboxylase, transcriptional activator in response to thiamin starvation.		No
RPI1	Transcriptional regulator		No

707
708
709 **Supplementary video 1.** *S. indica* monoculture on synthetic medium without thiamine.
710
711 **Supplementary video 2.** *S. indica* monoculture on synthetic medium with thiamine.
712
713 **Supplementary video 3.** *S. indica* and *B. subtilis* co-culture on synthetic medium without
714 thiamine.
715
716 **Supplementary video 4.** *S. indica* and *B. subtilis* co-culture on synthetic medium with
717 thiamine.
718