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1 **Inhibitory effects of extracellular self-DNA: a general biological process?**

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17

Summary

- Self-inhibition of growth has been observed in different organisms, but an underlying common mechanism has not been proposed so far. Recently, extracellular DNA has been reported as species-specific growth inhibitor in plants and proposed as an explanation of negative plant-soil feedback. In this work the effect of exDNA was tested on different species to assess the occurrence of such inhibition in organisms other than plants.
- Bioassays were performed on six species of different taxonomic groups, including bacteria, fungi, algae, plants, protozoa and insects. Treatments consisted in the addition to the growth substrate of conspecific and heterologous DNA at different concentration levels.
- Results showed that treatments with conspecific DNA always produced a concentration dependent growth inhibition, which instead was not observed in the case of heterologous DNA.
- Reported evidence suggests the generality of the observed phenomenon which opens new perspectives in the context of self-inhibition processes. Moreover, the existence of a general species-specific biological effect of exDNA raises interesting questions on its possible involvement in self-recognition mechanisms. Further investigation at molecular level will be required to unravel the specific functioning of the observed inhibitory effects.

Key words: autotoxicity, exDNA, self-recognition, exDNA functions, heterologous DNA.

42 **Introduction**

43 Self-inhibition or autotoxicity has been reported for several organisms including bacteria
44 (Andersen et al. 1974; Trinick and Parker 1982), fungi (Bottone et al. 2011), algae (Inderjit
45 and Dakshini 1994), plants (Singh et al. 1999) and animals (Akin 1966).

46 The mechanism has been mostly ascribed to the release and accumulation of different toxic
47 compounds in the growth environment, but a specific class of chemicals accounting for both
48 toxicity and species-specificity has never been identified. On the other hand, theoretical and
49 modelling studies on species coexistence have suggested the involvement of a general
50 mechanism to explain species-specific inhibition (Freitas and Fredrickson 1978; Bever 1994;
51 Mazzoleni et al. 2010).

52 The recent observations by Mazzoleni et al. (2014) of inhibitory effects by extracellular self-
53 DNA in plants provided new perspectives for understanding litter autotoxicity and negative
54 plant-soil feedbacks. The authors reported significant evidence that fragmented extracellular
55 DNA (exDNA) has a concentration dependent and species-specific inhibitory effect on
56 plants' growth. These findings suggested an unexpected functional role of exDNA in intra-
57 and inter-specific plant interactions at ecosystem level.

58 While the molecular mechanisms behind these phenomena certainly deserve in-depth
59 investigations, more basic questions arise: does extracellular self-DNA act as inhibitor on
60 biological systems other than plants? Could this be the general mechanism behind the
61 observed phenomena of self-inhibition and autotoxicity?

62

Materials and Methods

In order to test the occurrence of species-specific inhibition by exDNA, a set of laboratory experiments was performed on six species selected across different taxonomic groups. Systematic experiments included exposures to self DNA and to heterologous DNA from *Arabidopsis thaliana* as a model organism, plus a control with distilled water. Extraction of genomic DNA from each species was performed using standard Qiagen® (Valencia, CA, USA) extraction kits and DNA purity was spectrophotometrically assessed at 260 nm on a NanoDrop™ 1000 (Thermo Scientific, Wilmington, DE, USA) and visually verified on 1.5% agarose gel using Sybr® Safe (Invitrogen). The extracted DNA was fragmented by sonication according to Mazzoleni et al. (2014) in order to obtain fragments mainly distributed in the range between 50 and 1000 bp, with similar size distribution for all DNA samples. The organisms were exposed to increasing concentrations of self-DNA while heterologous DNA was applied at the maximum concentration tested for self-DNA. Other experiments were preliminary performed to assess possible different effects from different sources of heterologous DNA. The specific experimental settings and treatment concentrations were adapted to the growth requirements of the different species as reported below. *Bacillus subtilis* was selected as target Gram-positive bacterium. It was pre-grown on Luria Broth (LB) at 37 °C with agitation (200 rpm). An inoculum was prepared with 10 ml of preculture and 4 ml of LB. Treatments included self-DNA at three concentration levels (40, 200, and 400 µg/ml) and heterologous DNA (400 µg/ml) from *A. thaliana*, *Aspergillus niger*, *Escherichia coli*, and *Sarcophaga carnaria*. All cultures were incubated with agitation (200 rpm) at 37 °C, with three replicates for each treatment and the control. After 24 hrs of incubation, 0.5 ml were taken from each tube and serial dilutions in LB were prepared, from which 100 µl were placed on LB agar plates. Plates were incubated at 37 °C until appearance of colony-forming units (CFU).

88 *Trichoderma harzianum* was used as target fungus in a bioassay on spore germination.
89 Fungal spores were produced by pure cultures on potato dextrose agar (PDA). Spores were
90 diluted to a concentration of $1 \times 10^6 \text{ ml}^{-1}$. Treatments included extracellular self-DNA (8, 80,
91 and 800 $\mu\text{g/ml}$) and heterologous DNA (800 $\mu\text{g/ml}$) from *A. thaliana*, *Aspergillus niger*,
92 *Bacillus subtilis* and *Sarcophaga carnaria*, with three replicates for each treatment. The
93 germination bioassay was performed in ELISA plates (96 wells, 100 μl each), each well
94 coated with 10 μl of liquid 10% PDB substrate, DNA at treatment concentration, fungal
95 spores, and sterile distilled water. Spore germination and germ tube elongation of the conidia
96 were assessed by spectrophotometric analysis and optical microscopy after 20 hrs of
97 incubation at 24 °C.

98 The green microalga *Scenedesmus obliquus* was maintained in Chu's n° 10 medium (Chu
99 1942). The cultures were incubated at 25°C under 270 $\mu\text{moles photons m}^{-2} \text{ sec}^{-1}$ light
100 intensity with 16:8 hrs light photoperiod. Treatments of *S. obliquus* were carried out with
101 self-DNA (50 and 500 $\mu\text{g/ml}$) in the culture medium and heterologous DNA (500 $\mu\text{g/ml}$)
102 from *A. thaliana*, with two replicates for each treatment. Algal growth was assessed by cell
103 counts at the optical microscope after serial dilutions, and growth curves were built for each
104 treatment, until reaching stationary phase (7 days).

105 *Acanthus mollis* seedlings were treated with self-DNA (2, 20, and 200 $\mu\text{g/ml}$) and
106 heterologous DNA (200 $\mu\text{g/ml}$) from *A. thaliana*, *Quercus ilex* and *Sarcophaga carnaria*,
107 with three replicates for each treatment. Bioassays were done in vitro by using surface sterile
108 seeds (n=20 in each plate) placed in 9 cm Petri dishes over sterile filter papers imbibed with 4
109 ml of test solutions. Seedling root length was measured.

110 Plasmodia of the ameboid protozoan *Physarum polycephalum*, a slime mold widely used in
111 bioassays were maintained in the dark at 24 °C on 1% agar plates and were fed with oat
112 flakes. Laboratory stocks were subcultured onto new 1% water agar plates and fed oat flakes.

113 Mature cultures (15 days) on Petri plates were used to produce slime mold biomass for total
114 DNA extraction. Tip portions ($17 \pm 5 \text{ mm}^2$) of the plasmodia were taken from stock cultures 8
115 hours after feeding time and placed on agar substrates at the conditions of maintenance, with
116 three replicated plates for each treatment and the untreated control. Extracted self-DNA (290,
117 580, and 1060 $\mu\text{g/ml}$) and heterologous DNA (1060 $\mu\text{g/ml}$) from *A. thaliana* were applied on
118 0.2 g of oat flakes placed at the centre of each plate. Pictures of plasmodial growth patterns
119 were taken from each plate every 24 hrs for 96 hrs and used to calculate spreading area size
120 following Takamatsu et al. (2009).

121 The dipteran *Sarcophaga carnaria* was grown in pure culture on 12 x 12 cm^2 plates (2 cm
122 height) at 10 °C, fed with ground meat. Treatments included self-DNA (10, 100, and 1000
123 $\mu\text{g/ml}$) and heterologous DNA (1000 $\mu\text{g/ml}$) from *A. thaliana* mixed with 1 g of food. Three
124 replicated plates, each containing 10 larvae, were prepared for each treatment, plus the
125 untreated control. All plates were incubated in the dark at 10 °C. Development, survival, and
126 time required for the formation of pupae were monitored every 3 days during a 21-days
127 incubation period.

128 A generalized linear mixed model (GLMM) was used to analyse the results of the bioassays.
129 Since different metrics were used to assess the performance of target species, data were
130 expressed as percent of untreated controls. Tested effects on species performance included
131 the target species (6 levels) as random effect, and treatment (3 levels: heterologous DNA,
132 self-DNA and untreated control) and 2nd order interaction as fixed effects. Since the
133 experimental design was not fully balanced with respect to concentration levels of DNA
134 treatment, a further GLMM was tested to assess the effect of DNA concentration, limited to
135 samples treated with self-DNA. Also in this model the target species (6 levels) and its
136 interaction with self-DNA concentration were included as random effects. In both GLMMs
137 pair-wise differences were tested for statistical significance using post-hoc Duncan tests.

138

139 **Results**

140 The experiments produced consistent results for all target species with evident effects of
141 inhibition by self-DNA (Figure 1). The effect of all treatments was highly significant with
142 different responses to either heterologous or self-DNA without differences between species
143 (Table 1a). The application of heterologous DNA did not produce any significant growth
144 reduction compared to control, with the exception of *B. subtilis* which showed some
145 inhibition also in this case (Table 2). This was consistent with results from preliminary tests
146 with different heterologous DNA sources, showing the absence of inhibitory effects in all
147 cases, with the exception of the tested bacterium, which was inhibited at variable levels by
148 heterologous DNA (Table 3).

149 On the contrary, treatments with conspecific DNA always resulted in a concentration
150 dependent growth reduction (Table 1b), showing an inhibitory effect on all tested species
151 (Table 2), consistent with the observations on plants by Mazzoleni et al. (2014). At lower
152 self-DNA concentration the inhibitory effect was reduced with different responses for
153 different species (see significant interactive term in Table 1b).

154

155 **Discussion**

156 Species-specific inhibitory effects of exDNA has been recently reported for higher plants
157 (Mazzoleni et al. 2014). Here we extend such results to a set of organisms from different
158 taxonomic groups.

159 Extracellular DNA has been found both in soil and marine sediments in large amounts
160 (Steffan et al. 1988). Its long persistence in soil has been related to chemical stability and
161 protection against enzymatic degradation by absorption to both mineral and organic
162 components (Levy-Booth et al. 2007). Such accumulation of DNA molecules mainly derives

163 from degradation of organic matter, though release by excretion from living cells is also
164 reported (Nielsen et al. 2007).

165 Extracellular DNA has been proposed to serve different functions (Vlassov et al. 2007). It has
166 been proposed to be a major source for the transfer of genetic information (Weinberg and
167 Stotzky 1972; Graham and Istock 1978; Nielsen et al. 2007). It has been reported to play a
168 role in the formation of microbial biofilms (Whitchurch et al. 2002; Steinberger and Holden
169 2005), in the protection from pathogen attack in root cap “slime” (Wen et al. 2009; Hawes et
170 al. 2011) and in extracellular traps (Brinkmann et al. 2004; Goldmann and Medina 2012).
171 Extracellular DNA has also been considered as a relevant source of nutrients for plants
172 (Paungfoo-Lonhienne et al. 2010) and microbes (Finkel and Kolter 2001; Palchevskiy and
173 Finkel 2006; Pinchuk et al. 2008).

174 The role of exDNA as species-specific inhibitor has been recently reported for higher plants
175 (Mazzoleni et al. 2014), providing a novel explanation for negative plant-soil feedbacks such
176 as inhibition of plant recruitment, growth and reproduction in soils previously occupied by
177 conspecifics (Bever et al. 1997, van der Putten 2003; Kulmatiski et al. 2008; Mangan et al.
178 2010). The same effect could be the explanation of the frequently reported interspecific
179 facilitation but rare occurrence of intraspecific facilitation in terrestrial ecosystems
180 (Bonanomi et al. 2010). Further studies are needed to clarify the interplay between DNA
181 persistence in the environment and related ecosystem diversity.

182 The experiments presented in this paper confirmed the occurrence and the concentration
183 dependency of the inhibition by extracellular self-DNA in bacteria, fungi, algae, plants,
184 protozoa and insects. The possible bias in these results by the presence of residual chemicals
185 from DNA extraction can be excluded because the heterologous DNA, not producing
186 inhibitory effects, was extracted with the same method and applied at the same high
187 concentration of self-DNA.

188 The range of target species, including prokaryotes and both unicellular and multicellular
 189 eukaryotes, highlights the widespread occurrence of self-DNA inhibitory effect. An
 190 interesting evidence of self-inhibition in vertebrates was reported on *Rana pipiens* (Richards
 191 1958, 1962), clearly showing a significant reduction of tadpoles growth in water previously
 192 occupied by conspecifics, unaffected by the presence of unrelated species and only slightly
 193 inhibited by phylogenetically related ones (Akin 1966). Richards (1958) suggested that "alga-
 194 like" pathogens could be the cause of the observed growth inhibition, but the involvement of
 195 such pathogens in small tadpoles inhibition was later falsified (West 1960). Akin (1966)
 196 suggested the involvement of an unknown self-inhibiting agent. Other works related this
 197 inhibition to the production of some "proteinaceous" compounds by large tadpoles (Rose and
 198 Rose 1961, Runkova et al. 1974, Stepanova 1974, Steinwascher 1978). Notably, Richards
 199 (1962) showed that growth inhibition could be removed after physical and chemical
 200 treatments like filtration, centrifugation, heating, sonication, freezing and thawing, ultraviolet
 201 light and low pH. We propose that all these observations can coherently be ascribed to the
 202 species-specific inhibitory effects of exDNA accumulated in the growth medium.

203 A distinct topic where the specificity of action of exDNA could play an important role is self-
 204 recognition. Callaway and Mahall (2007) reviewed the evidence regarding how plants are
 205 able to distinguish self from non-self conspecific individuals. In particular, Dudley and File
 206 (2007) demonstrated kin recognition at root level in *Cakile edentula* without proposing an
 207 explanatory mechanism. Considering the high specificity of the information stored in DNA,
 208 we speculate that it can potentially mediate recognition not only at species level, but also
 209 within species to distinguish kin from unrelated individuals.

210 In this work, we presented phenomenological evidence supporting the hypothesis of the
 211 general occurrence of an inhibitory effect of extracellular self-DNA and of its possible
 212 involvement in recognition signalling processes. Are these functions of exDNA going to be a

new paradigm? The reported findings certainly suggest intriguing questions and ideas, which may open new research scenarios. For example, in ecology, experiments can be planned to investigate the relevance of this effect in the regulation of species coexistence and competition, in the interactions with natural enemies, in relation with nutrient depletion and symbiont community changes, and its general occurrence in natural conditions. Moreover, a more comprehensive experimental design should address the relationship between inhibition and phylogenetic distance among target species and exDNA sources.

In a broader context of life sciences, other issues can be considered. The reported species-specificity of DNA inhibition seems consistent in eukaryotes (both unicellular and multicellular organisms), but this should be further investigated on a larger number of taxa. On the other hand, the effect on prokaryotes appears less certain considering that heterologous DNA also produced a performance reduction in the only observed case of *Bacillus subtilis*. This definitely requires further experimental work on more species.

Finally, the investigation of the molecular mechanisms behind the observed inhibitory phenomenon is certainly a major challenge to be faced. It has been widely demonstrated that exDNA can be uptaken by living cells in both prokaryotes and eukaryotes, such as higher plants (Paungfoo-Lonhienne et al. 2010) and mammalian (Groneberg et al. 1975) where it can be transported to the nucleus (Wienhues et al. 1987) and possibly integrated into the genome of the guest cell (Doerfler et al. 1995). Indeed, cells present mechanisms of protection from exDNA uptake. Bacterial restriction enzymes cleave foreign nucleic acids while protecting their own genome by methylation (Wilson 1988). More sophisticated processes of specific clearance of exDNA are found in vertebrates (e.g. Stenglein 2009). The above mentioned mechanisms refer to the recognition of exogenous DNA, whereas little is known about the processes involved in specific responses to self-DNA, for which the mechanisms of viral, retroviral transposons, or other types of parasitic DNA could be taken

238 into account. Future studies are needed to clarify the inhibitory effects of extracellular self-
239 DNA at both cellular and molecular levels, including the processes of recognition, uptake,
240 and transport in both prokaryotes and eukaryotes.

241

References

- Akin G. 1966.** Self-inhibition of growth in *Rana pipiens* tadpoles. *Physiological Zoology* **39**: 341–356.
- Andersen RJ, Wolfe MS, Faulkner DJ. 1974.** Autotoxic antibiotic production by a marine *Chromobacterium*. *Marine Biology* **27**: 281–285.
- Bever JD. 1994.** Feedback between plants and their soil communities in an old field community. *Ecology* **75**: 1965–1977.
- Bever JD, Westover M, Antonovics J. 1997.** Incorporating the soil community into plant population dynamics: the utility of the feedback approach. *Journal of Ecology* **85**: 561–573.
- Bonanomi G, Incerti G, Capodilupo M, Mazzoleni S. 2010.** Rare self-facilitation in terrestrial plants as compared to aquatic sessile organisms: empirical evidences and causal mechanisms. *Community Ecology* **11**: 148–159.
- Bottone EJ, Nagarsheth N, Chiu K. 2011.** Evidence of self-inhibition by filamentous fungi accounts for unidirectional hyphal growth in colonies. *Canadian Journal of Microbiology* **44**: 390–393.
- Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. 2004.** Neutrophil extracellular traps kill bacteria. *Science* **303**: 1532–5.
- Callaway RM, Mahall BE. 2007.** Plant ecology: family roots. *Nature* **448**: 145–147.
- Chu S. 1942.** The influence of the mineral composition of the medium on the growth of planktonic algae: part I. Methods and culture media. *The Journal of Ecology* **30**: 284–325.
- Doerfler W, Orend G, Schubbert R, Fechteler K, Heller H, Wilgenbus P, Schröer J. 1995.** On the insertion of foreign DNA into mammalian genomes: mechanism and consequences. *Gene* **157**: 241–245.
- Dudley SA, File AL. 2007.** Kin recognition in an annual plant. *Biology Letters* **3**: 435–8.
- Finkel SE, Kolter R. 2001.** DNA as a nutrient: novel role for bacterial competence gene homologs. *Journal of Bacteriology* **183**: 6288–6293.
- Freitas M De, Fredrickson A. 1978.** Inhibition as a factor in the maintenance of the diversity of microbial ecosystems. *Journal of General Microbiology*: 307–320.
- Goldmann O, Medina E. 2012.** The expanding world of extracellular traps: not only neutrophils but much more. *Frontiers in Immunology* **3**: 420.
- Graham JB, Istock CA. 1978.** Genetic exchange in *Bacillus subtilis* in soil. *Molecular & General Genetics* **166**: 287–290.
- Groneberg J, Brown DT, Doerfler W. 1975.** Uptake and fate of the DNA of adenovirus type 2 in KB cells. *Virology* **64**: 115–131.

- 276 **Hawes MC, Curlango-Rivera G, Wen F, White GJ, Vanetten HD, Xiong Z. 2011.**
 277 Extracellular DNA: the tip of root defenses? *Plant Science* **180**: 741–745.
- 278 **Inderjit, Dakshini KMM. 1994.** Algal allelopathy. *The Botanical Review* **60**: 182–196.
- 279 **Kulmatiski A, Beard KH, Stevens JR, Cobbold SM. 2008.** Plant-soil feedbacks: a meta-
 280 analytical review. *Ecology Letters* **11**: 980–992.
- 281 **Levy-Booth DJ, Campbell RG, Gulden RH, Hart MM, Powell JR, Klironomos JN,**
 282 **Pauls KP, Swanton CJ, Trevors JT, Dunfield KE. 2007.** Cycling of extracellular DNA in
 283 the soil environment. *Soil Biology and Biochemistry* **39**: 2977–2991.
- 284 **Mangan SA, Schnitzer SA, Herre EA, Mack KML, Valencia MC, Sanchez EI, Bever JD.**
 285 **2010.** Negative plant-soil feedback predicts tree-species relative abundance in a tropical
 286 forest. *Nature* **466**: 752–755.
- 287 **Mazzoleni S, Bonanomi G, Giannino F, Incerti G, Dekker SC, Rietkerk M. 2010.**
 288 Modelling the effects of litter decomposition on tree diversity patterns. *Ecological Modelling*
 289 **221**: 2784–2792.
- 290 **Mazzoleni S, Bonanomi G, Incerti G, Chiusano M, Termolino P, Mingo A, Senatore M,**
 291 **Giannino F, Cartenì F, Rietkerk M, Lanzotti V. 2014.** Inhibitory and toxic effects of
 292 extracellular self-DNA in litter: a mechanism for negative plant-soil feedbacks? *New*
 293 *Phytologist*. doi 10.1111/nph.13121
- 294 **Nielsen KM, Johnsen PJ, Bensasson D, Daffonchio D. 2007.** Release and persistence of
 295 extracellular DNA in the environment. *Environmental Biosafety Research* **6**: 37–53.
- 296 **Palchevskiy V, Finkel SE. 2006.** Escherichia coli competence gene homologs are essential
 297 for competitive fitness and the use of DNA as a nutrient. *Journal of Bacteriology* **188**: 3902–
 298 3910.
- 299 **Paungfoo-Lonhienne C, Lonhienne TGA, Mudge SR, Schenk PM, Christie M, Carroll**
 300 **BJ, Schmidt S. 2010.** DNA is taken up by root hairs and pollen, and stimulates root and
 301 pollen tube growth. *Plant Physiology* **153**: 799–805.
- 302 **Pinchuk GE, Ammons C, Culley DE, Li S-MW, McLean JS, Romine MF, Neilson KH,**
 303 **Fredrickson JK, Beliaev AS. 2008.** Utilization of DNA as a sole source of phosphorus,
 304 carbon, and energy by *Shewanella* spp.: ecological and physiological implications for
 305 dissimilatory metal reduction. *Applied and Environmental Microbiology* **74**: 1198–1208.
- 306 **Van der Putten WH. 2003.** Plant defense belowground and spatiotemporal processes in
 307 natural vegetation. *Ecology* **84**: 2269–2280.
- 308 **Richards C. 1958.** The inhibition of growth in crowded *Rana pipiens* tadpoles. *Physiological*
 309 *Zoology* **31**: 138–151.
- 310 **Richards C. 1962.** The control of tadpole growth by alga-like cells. *Physiological Zoology*
 311 **35**: 285–296.

- 312 **Rose SM, Rose FC. 1961.** Growth controlling exudates of tadpoles. *Symposia of the Society*
313 *for Experimental Biology* **15**: 207–218.
- 314 **Runkova GG, Stepanova ZL, Kovalchuk LA. 1974.** Organ specificity in the action of
315 metabolites of amphibian larvae on their endogenous metabolism under condition of
316 increased population density. The role of protein in the regulating influence of metabolites.
317 *Akademia Nauk SSSR Doklady Biological Sciences Section* **217**: 328–330.
- 318 **Singh HP, Batish DR, Kohli RK. 1999.** Autotoxicity: concept, organisms, and ecological
319 significance. *Critical Reviews in Plant Sciences* **18**: 757–772.
- 320 **Steffan R, Goksøyr J, Bej AK, Atlas RM. 1988.** Recovery of DNA from soils and
321 sediments. *Applied and Environmental Microbiology* **54**: 2908–2915.
- 322 **Steinberger RE, Holden PA. 2005.** Extracellular DNA in single- and multiple-species
323 unsaturated biofilms. *Applied and Environmental Microbiology* **71**: 5404–5410.
- 324 **Steinwascher K. 1978.** Interference and exploitation competition among tadpoles of *Rana*
325 *utricularia*. *Ecology*, 1039–1046.
- 326 **Stenglein MD, Burns MB, Li M, Lengyel J, Harris RS. 2010.** APOBEC3 proteins mediate
327 the clearance of foreign DNA from human cells. *Nature Structural & Molecular Biology* **17**:
328 222–229.
- 329 **Stepanova ZL.** The chemical nature of the products of metabolism of amphibian larvae,
330 excreted into the water. *Soviet Journal of Ecology* **5**: 148–149.
- 331 **Takamatsu A, Takaba E, Takizawa G. 2009.** Environment-dependent morphology in
332 plasmodium of true slime mold *Physarum polycephalum* and a network growth model.
333 *Journal of Theoretical Biology* **256**: 29–44.
- 334 **Trinick MJ, Parker CA. 1982.** Self-inhibition of rhizobial strains and the influence of
335 cultural conditions on microbial interactions. *Soil Biology and Biochemistry* **14**: 79–86.
- 336 **Vlassov VV, Laktionov PP, Rykova EY. 2007.** Extracellular nucleic acids. *BioEssays* **29**:
337 654–667.
- 338 **Weinberg SR, Stotzky G. 1972.** Conjugation and genetic recombination of *Escherichia coli*
339 in soil. *Soil Biology and Biochemistry* **4**: 171–180.
- 340 **Wen F, White GJ, VanEtten HD, Xiong Z, Hawes MC. 2009.** Extracellular DNA is
341 required for root tip resistance to fungal infection. *Plant Physiology* **151**: 820–829.
- 342 **West LB. 1960.** The nature of growth inhibitory material from crowded *Rana pipiens*
343 tadpoles. *Physiological Zoology*, 232–239.
- 344 **Whitchurch CB, Tolker-Nielsen T, Ragas PC, Mattick JS. 2002.** Extracellular DNA
345 required for bacterial biofilm formation. *Science* **295**: 1487.
- 346 **Wienhues U, Hosokawa K, Höveler A, Siegmann B, Doerfler W. 1987.** A novel method

347 for transfection and expression of reconstituted DNA-protein complexes in eukaryotic cells.
348 *DNA* **6**: 81–89.

349 **Wilson GG. 1988.** Type II restriction-modification systems. *Trends in Genetics* **4**: 314–318.

350

Tables

Table 1. Summary of the general linear mixed model (GLMM) testing for main and interactive effects of target species and treatments on species performance in the bioassays.

a) Model I: self and heterologous DNA						
	Effect type	SS	df	MS	<i>F</i>	<i>P</i>
Target species	Random	2134.7	5	426.9	1.53	0.2656
Treatment	Fixed	88928.9	2	44464.4	159.60	< 0.0001
Target species x Treatment	Random	2822.9	10	282.3	7.66	< 0.0001
b) Model II: concentration of self-DNA						
	Effect type	SS	df	MS	<i>F</i>	<i>P</i>
Target species	Random	18277.5	5	3655.5	6.55	0.0077
Concentration	Fixed	21909.3	2	10954.7	20.13	0.0005
Target species x Concentration	Random	5095.7	9	566.2	14.91	< 0.0001

Table 2. Performance of target species exposed to extracellular heterologous DNA from *Arabidopsis thaliana* and self-DNA at different concentration levels. Data are mean \pm standard deviations of different growth metrics for different species, expressed as % of untreated controls. Within each target species, asterisks indicate significant difference between exposure to heterologous and self-DNA at high concentration (Duncan post-hoc tests for the effect of treatment from GLMM model I in Table 1). Different letters indicates significantly different groups for the effect of self-DNA concentration (Duncan post-hoc tests from GLMM model II in Table 1). Values not significantly different from the controls are reported in italic fonts.

Target species	H DNA		self-DNA	
	high	high	mid	low
<i>Bacillus subtilis</i>	58.2 \pm 7.4 *	7.7 \pm 5.6 <i>a</i>	6.0 \pm 2.6 <i>a</i>	41.4 \pm 6.5 <i>b</i>
<i>Physarum polycephalum</i>	93.9 \pm 7.5 *	0.7 \pm 0.2 <i>a</i>	18.4 \pm 3.9 <i>b</i>	44.7 \pm 7.5 <i>c</i>
<i>Scenedesmus obliquus</i>	95.8 \pm 6.7 *	14.1 \pm 6.7 <i>a</i>	-	60.6 \pm 3.4 <i>b</i>
<i>Trichoderma harzianum</i>	93.3 \pm 9.0 *	9.1 \pm 3.0 <i>a</i>	53.0 \pm 10.0 <i>b</i>	67.0 \pm 16.0 <i>c</i>
<i>Acanthus mollis</i>	94.8 \pm 8.7 *	26.8 \pm 1.4 <i>a</i>	81.7 \pm 3.7 <i>b</i>	98.1 \pm 5.4 <i>c</i>
<i>Sarcophaga carnaria</i>	96.1 \pm 4.0 *	12.5 \pm 4.0 <i>a</i>	11.7 \pm 3.0 <i>a</i>	44.2 \pm 8.0 <i>b</i>

365 **Table 3.** Performance of target species exposed to extracellular heterologous DNA from
366 different sources. Data are mean \pm standard deviations of different growth metrics for
367 different species, expressed as % of untreated controls. Values not significantly different
368 from the controls are reported in italic fonts.

Target species	Source of heterologous DNA				
	<i>Escherichia coli</i>	<i>Bacillus subtilis</i>	<i>Aspergillus niger</i>	<i>Sarcophaga carnaria</i>	<i>Quercus ilex</i>
<i>Bacillus subtilis</i>	51 \pm 13%	-	62 \pm 24%,	42 \pm 13%	-
<i>Trichoderma hartianum</i>	-	108 \pm 14%	91 \pm 11%	98 \pm 9%	-
<i>Acanthus mollis</i>	-	-	-	102 \pm 11%	94 \pm 19%

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Figure Legends

Figure 1. Effects of exposure to heterologous DNA from *Arabidopsis thaliana* and self-DNA on different organisms. All species show significant concentration dependent inhibitory effects by self-DNA. See Materials and Methods for details on experimental conditions.

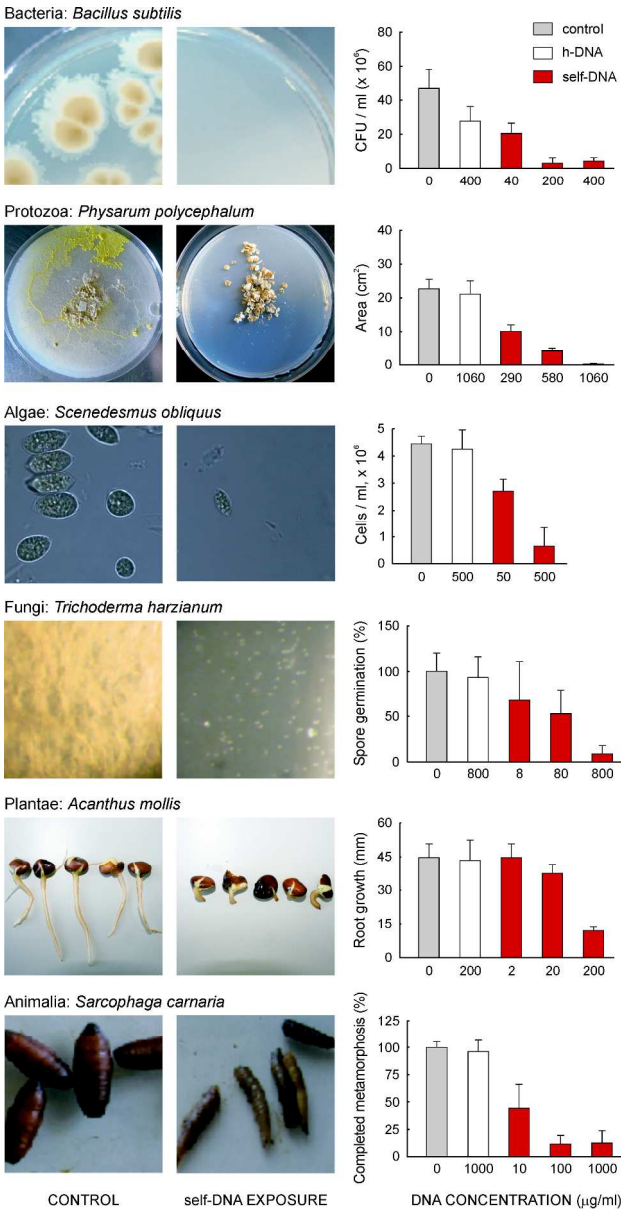


Figure 1