

BSocial Tutorial

Deciphering Behaviour in Social Interactions

Updated: 10th April 2014

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PURPOSE OF THE WEBTOOL

Social interactions are hard to decipher in mixed microbial populations. The BSocial website offers a simple tool for deciphering these interactions, especially in situations where fluorescent tagging of multiple microbes gets complicated. Also, the tools offered by BSocial evaluate the effect of increasing diversity on function, and propose a sequential addition of strains for biofilm purposes. Specialists with different backgrounds like environmental sciences, pharmaceutical researches or microbial evolutionists can benefit from these tools.

ARTICLE ABSTRACT

Ecosystem functionality depends on interactions among population members, of the same or different taxa, and these are not the sum of pairwise member interactions. Know-how of the social interactions occurring in mixed-population biofilms are unknown due to the limitations posed in tagging each population. Our work elucidates the social effects each strain may have on a general community. Using 7 strains, we analyzed all combinatory strain mixes in a high

throughput approach, observing their growth on xenobiotic carbon sources (e.g. MTBE, since strains were selected on degradation/tolerance of this compound). The novelty arises in being able to determine which bacterial strains act as cooperators (increase community fitness), cheaters (decrease community fitness) or defectors (no effect on community fitness). Other analyses included the effect of increasing diversity on function, as well as recommending a possible sequence for adding strains for biofilm establishment without hindering the effects on No. generations or growth rate as each strain is added. From the 7 strains analyzed, we determined that *Rhodococcus ruber* EE6, *Agrobacterium* sp. MS2 and *Paenibacillus* sp. SH7 were appropriate candidates for an MTBE bioremediation biofilm-based technology, and suggest that these be added in the following ordered sequence: EE6, SH7 and MS2. Overall, the work appoints social behaviours on individual strains in mixed-populations in biofilm-like states, offering the analytical tools for higher number of individuals in mixed-population biofilms on an online-based webtool (BSocial <http://m4m.ugr.es/BSocial.html>) thus opening the fields of microbial ecology and engineering, in studying social interactions in natural and synthetic habitats.

Citation

Purswani Jessica, Romero-Zaliz Rocío C., Martín-Platero Antonio M., Guisado Isabel M., González-López Jesús, Pozo Clementina, BSocial: Deciphering Social Behaviors within Mixed Microbial Populations, *Frontiers in Microbiology* 8, Article 919, 2017 (10.3389/fmicb.2017.00919).

CONTACT US

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We welcome your feedback. Please send your comments/suggestions via e-mail.

BROWSER REQUIREMENTS

The webtool can be used with Mozilla Firefox, Google Chrome and Internet Explorer (except IE8).

DATA INPUT

The user requires a .csv file (comma separated values) with the following format (see Table 1 as an example). A sample dataset can be downloaded from <http://m4m.ugr.es/Data/MTBE.csv>.

Consortia	Strain_1	Strain_2	Strain_3	Log phase (h)	No. Generations	Growth rate
X1	1			10	5.2	0.52
X2		2		15	5.3	0.3533

X3			3	24	10	0.4167
X4	1	2		12	4.2	0.35
X5	1		3	20	12	0.6
X6		2	3	12	3	0.25
X7	1	2	3	12	3	0.25

Table 1. Example of data input for three strains.

- The first column should contain the name of the consortia (e.g., X6).
- The next X columns correspond to each of the X individual strains. This means that if you are working with mixed populations with 3 strains, the second, third and fourth column will contain the names of each strain (as seen in Table 1). The user needs to assign a number to each strain. Each number will be used to specify the presence of the strain for each consortia (row). For example, consortia X5 in Table 1 contains strain 1 (Strain_1) and strain 3 (Strain_3). Empty spaces will mean that the strain of that column is not present in the consortia, i.e. in consortia X5, strain 2 (Strain_2) is absent. BSocial will only accept an input from 2 of up to 10 strains (1023 consortia). If you would like to use the service for a larger number of strains, please contact us.
- The last three columns will contain the log phase (h), No. generations and the growth rate, in that order.

	No. Strains	No. Consortia
	1	1
	2	3
	3	7
	4	15
	5	31
	6	63
	7	127
	8	255
	9	511
	10	1023

If the number of strains does not coincide with the number of maximum combinations (2^n-1), the program will not run.

To use the webtool, first upload the .csv file, then choose the number of strains used in your file and give a name to your project. Finally, click RUN. The results should not take longer than a few minutes.

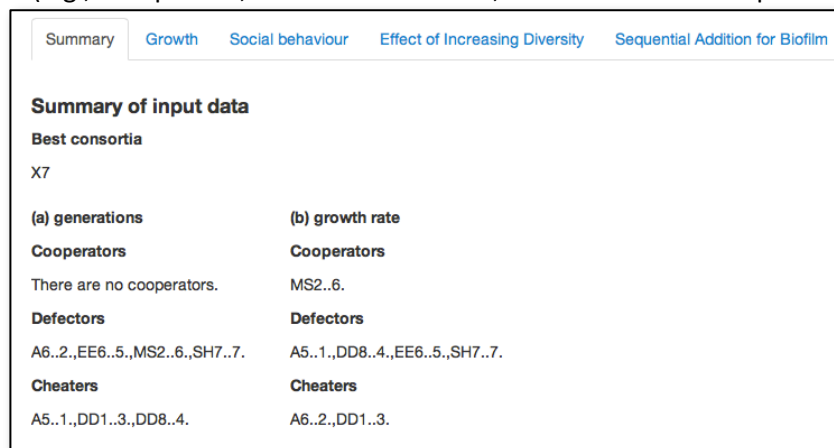
ANALYSING LESS NUMBER OF STRAINS WITH SAME DATA

Once the application has finished processing, the user can select a subset of the strains for further analysis. To do this, select or deselect the boxes beside each strain name and click the RUN button again.

OUTPUT INTERPRETATION

Summary tab

The output will show the name of the best consortia and will display the social behaviour of each strain (e.g., co-operator, cheater or defector, see article manuscript for details) for No.



generations (a) and growth rate (b) (Figure 1).

Figure 1. Summary tab.

Growth tab

The best 10 growing consortia will be shown in order, according to highest No. generations, and then lowest Log phase time (Figure 2 (b)).

Social behaviour tab

In this tab the user can download box plot representation of fitness data for each strain and for the assignation of cooperators, defectors and cheaters for a) No. generations and b) growth rate data (Figure 3). Three box plots are assigned to each strain: blue – data points of relative fitness of each consortia value normalized by the individual strain's value; turquoise – data points of relative fitness of each consortia where the strain is not present normalized by the individual strain's value; and white – data points of relative fitness of each consortia where the strain is present normalized by the individual strain's value.

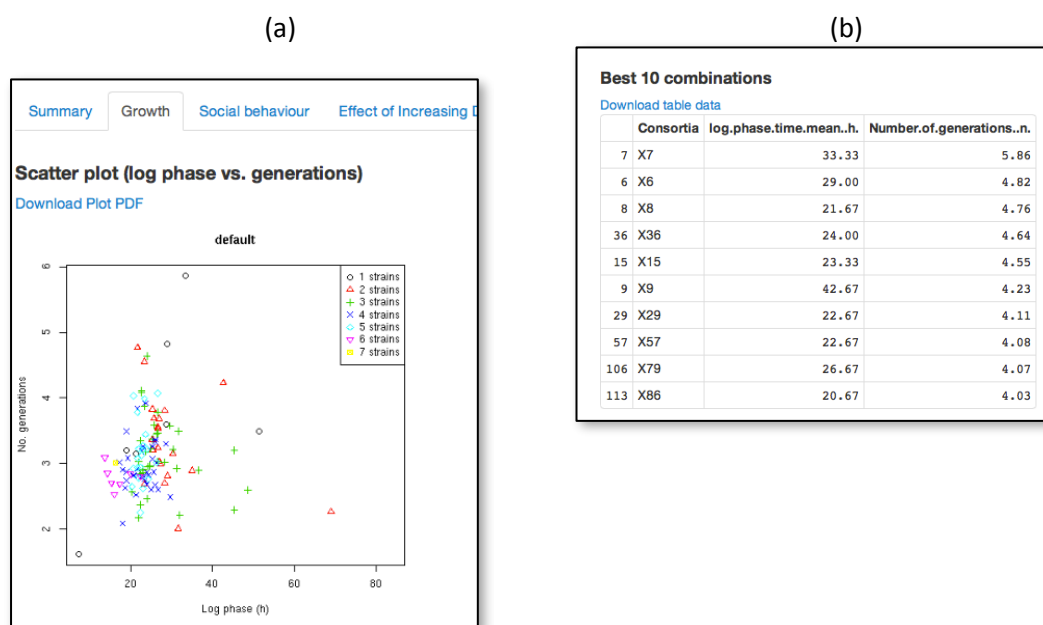


Figure 2. Growth tab. (a) Plot of No. generations x Log phase (h) for each consortia. (b) Best 10 consortia.

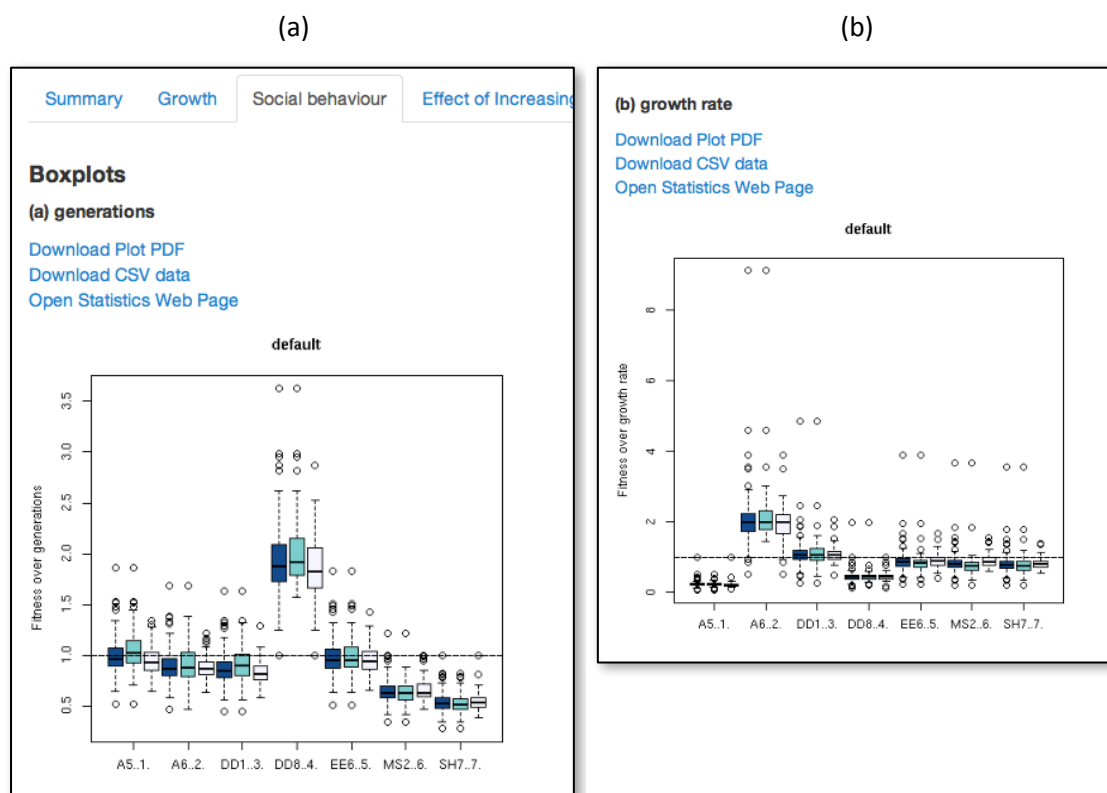


Figure 3. Social behaviour tab. (a) No. generations. (b) Growth rate.

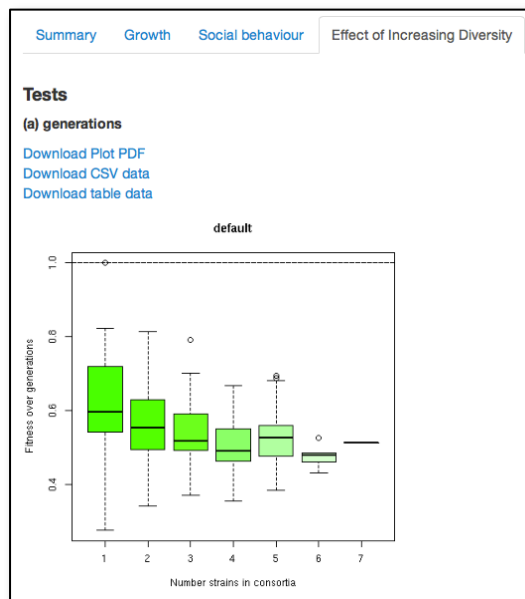
Effect of increasing Diversity tab

Graphs a) and c) describe the effect of increasing diversity on No. generations and growth rate respectively (Figure 4). This analysis allows the user to evaluate whether it was worth using high number of combinations in order to obtain a high growth rate/No. generations (consortia fitness is normalized by the highest value of all individual strain).

Graphs b) and d) describe the effect of increasing diversity using the best strains on No. generations and growth rate respectively (Figure 4). This analysis allows the user to evaluate whether it was worth using just X number of best strains and their combinations to obtain a high growth rate/yield. This is a cumulative analysis. Individual strain values were ordered from minimum to maximum for growth rate or No. generations. Box plots containing data of fitness were plotted against increasing number of best strains in consortia (consortia fitness is normalized by the highest value of all individual strain).

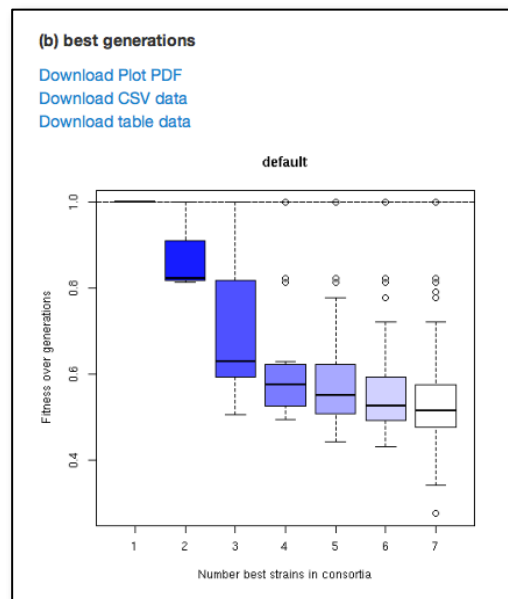
When fitness is greater than 1, a table showing the consortia reaching this fitness values are shown.

(a)



(c)

(b)



(d)

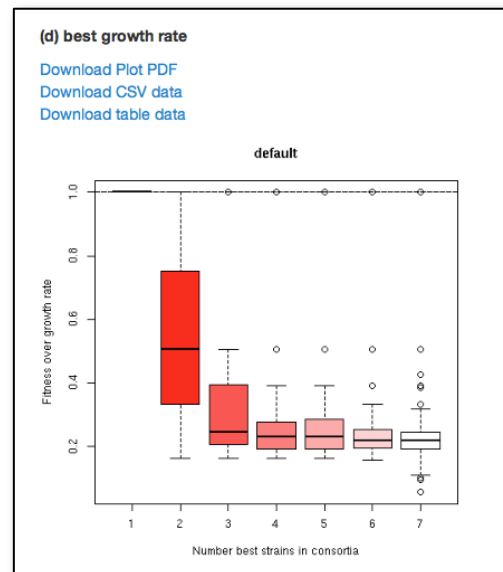
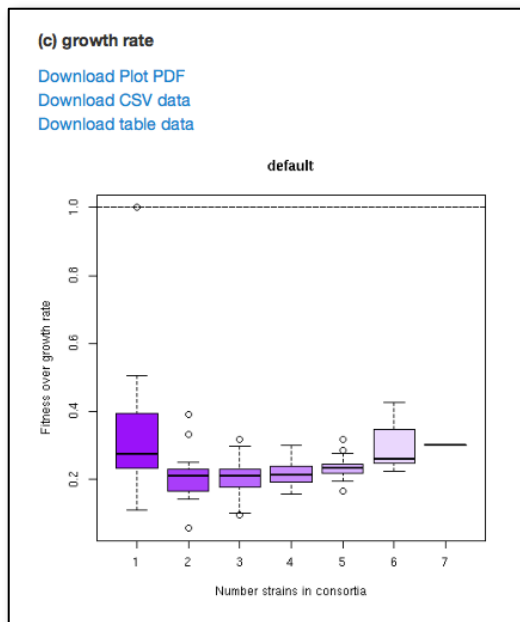


Figure 4. Effect of increasing diversity tab. (a) Increasing diversity on No. generations. (b) Increasing diversity using the best strains on No. generations. (c) Increasing diversity on growth rate. (d) Increasing diversity using the best strains on growth rate.