

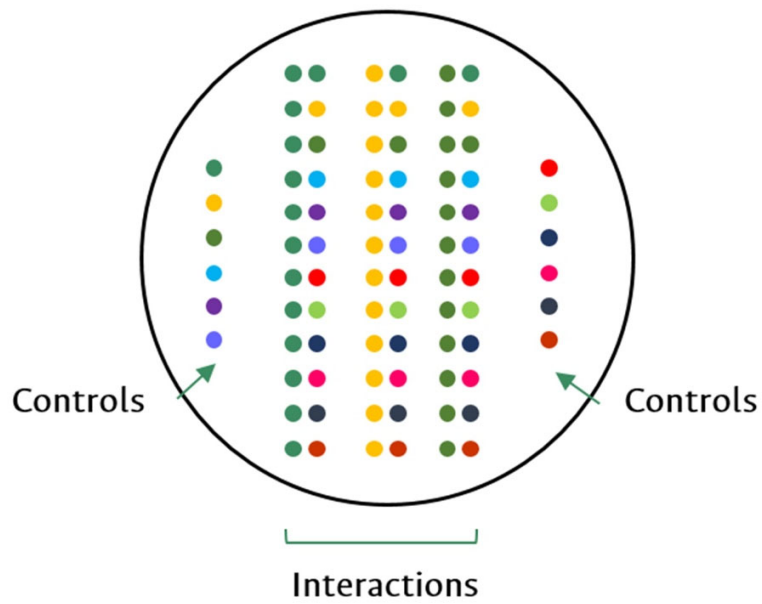
## SUPPLEMENTARY MATERIAL

### **Binary co-culture selection from marine-derived microorganisms for differential production of specialized metabolites**

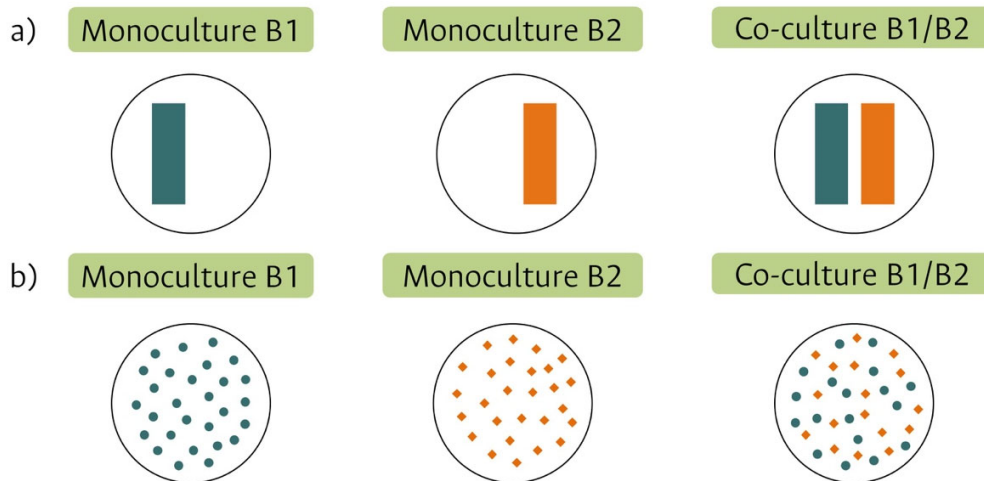
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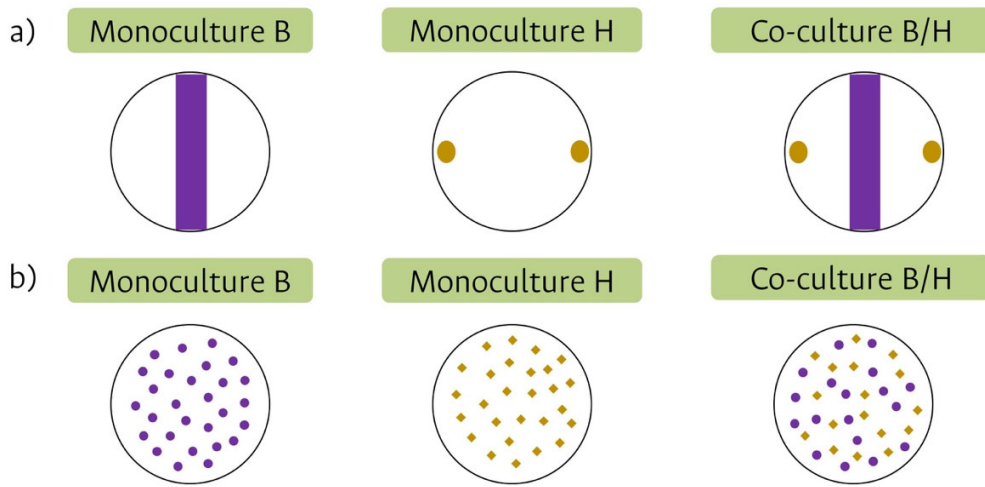
\*e-mail: lcastellanosh@unal.edu.co



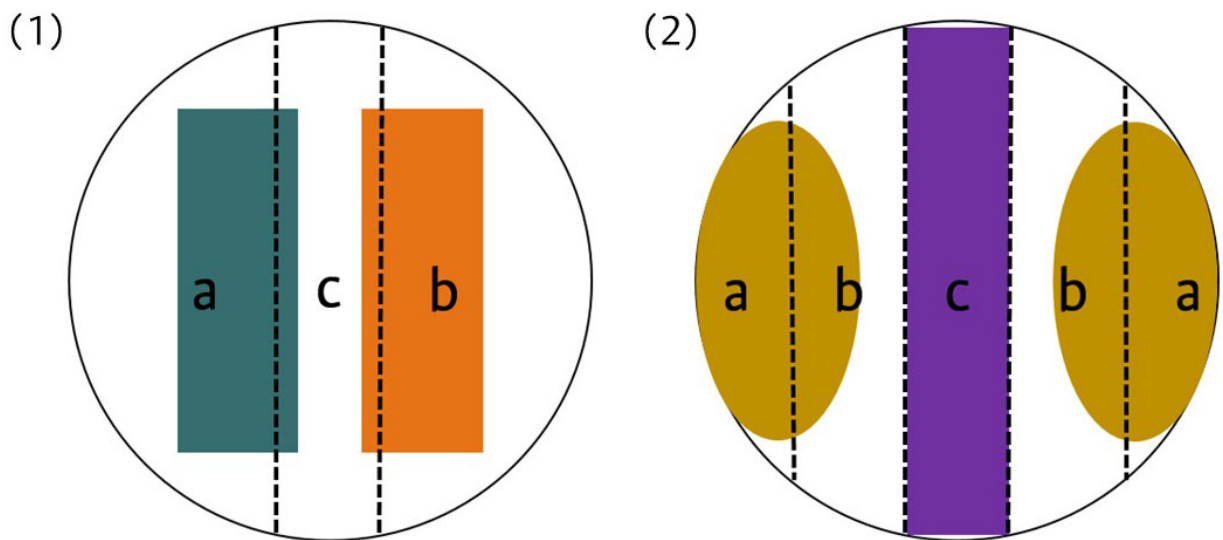
**Figure 1S.** Scheme of the binary interaction assay used a selection tool to evaluate bacteria-bacteria distance interactions. This methodology was adapted from the proposal by Seyedsayamdost and collaborators.<sup>29</sup>



**Figure 2S.** Bacteria-bacteria co-cultures: a) distance interaction, b) contact interaction.



**Figure 3S.** Bacteria-fungus co-cultures: a) distance interaction, b) contact interaction.



**Figure 4S.** Defined zones for obtaining organic extracts in distance assays between (1) bacteria-bacteria: (a) Zone of bacteria 1, (b) Zone of bacteria 2 and (c) Zone of interaction; and (2) bacteria-fungus: (a) Zone of fungus, (b) Zone of interaction, (c) Zone of bacteria.

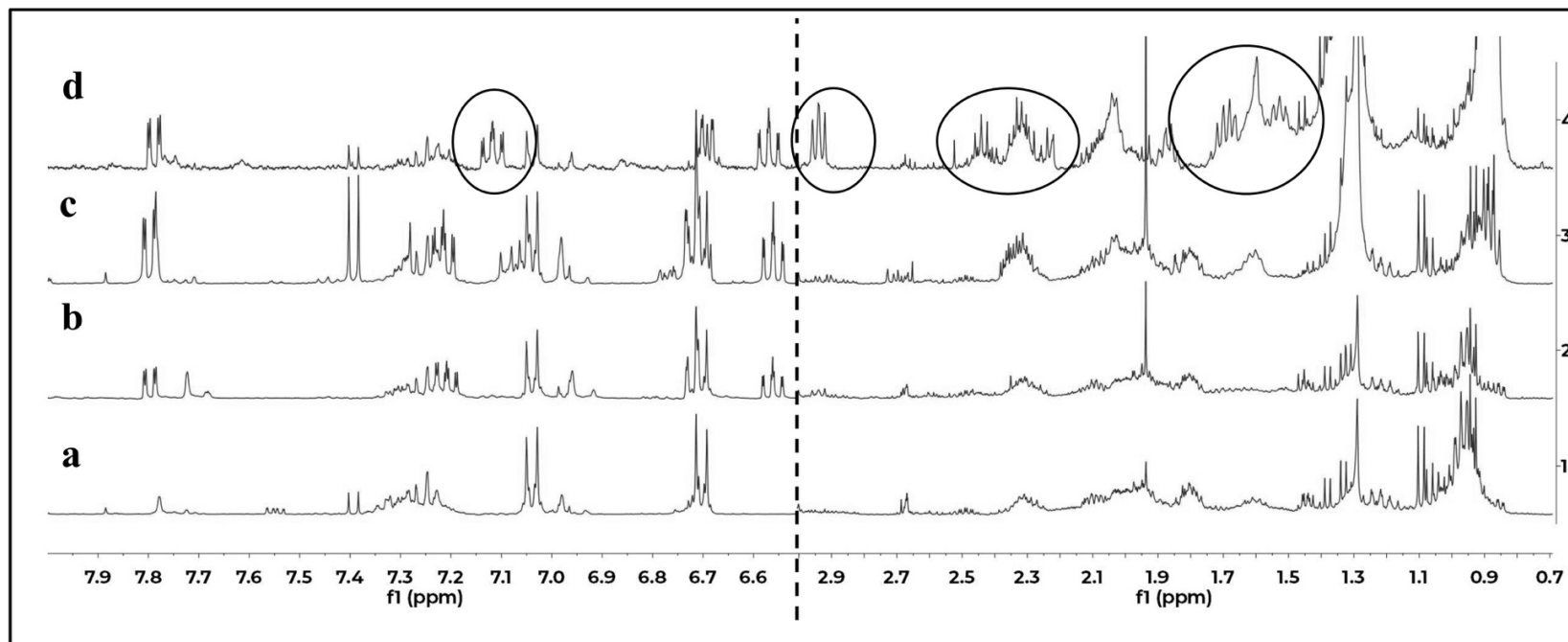
	IBUN-5.1	RKHC62b	RKHC59b	RKHC28	RKHC26	RKHC9	PNM-216	PNM-210	PNM-161a	PNM-157	PNM-123	PNM-115	PNM-25	PNM-9
PNM-9	■	■	■	■	■	■	■	■	■	■	■	■	■	■
PNM-25	■	■	■	■	■	■	■	■	■	■	■	■	■	■
PNM-115	■	■	■	■	■	■	■	■	■	■	■	■	■	■
PNM-123	■	■	■	■	■	■	■	■	■	■	■	■	■	■
PNM-157	■	■	■	■	■	■	■	■	■	■	■	■	■	■
PNM-161a	■	■	■	■	■	■	■	■	■	■	■	■	■	■
PNM-210	■	■	■	■	■	■	■	■	■	■	■	■	■	■
PNM-216	■	■	■	■	■	■	■	■	■	■	■	■	■	■
RKHC9	■	■	■	■	■	■	■	■	■	■	■	■	■	■
RKHC26	■	■	■	■	■	■	■	■	■	■	■	■	■	■
RKHC28	■	■	■	■	■	■	■	■	■	■	■	■	■	■
RKHC59b	■	■	■	■	■	■	■	■	■	■	■	■	■	■
RKHC62b	■	■	■	■	■	■	■	■	■	■	■	■	■	■
IBUN-5.1	■	■	■	■	■	■	■	■	■	■	■	■	■	■

**Figure 5S.** Summary of phenotypic changes observed in the 91 bacteria-bacteria interactions evaluated in a distance assay. (■): There was no visible interaction, (■): Growth inhibition, (■): Other types of interaction such as changes in pigmentation, sporulation and macroscopic morphology.

**Table 1S.** Summary of bacteria-bacteria co-cultures evaluated by contact assay. It includes the criterion of pairing and if there was a change in the metabolic profile with respect to the corresponding monocultures

Binary co-culture		Criterion of pairing	Phenotypic change	Change	
Isolate	Isolate			<sup>1</sup> H NMR	HPLC-DAD
<b>PNM-9</b>	<b>PNM-161a</b>	Genus <i>Streptomyces</i>	—	-	—
<b>PNM-9</b>	<b>PNM-216</b>	Ecological criteria	—	—	—
<b>PNM-9</b>	<b>RKHC-26</b>	Mycolic acid containing bacteria	—	—	—
<b>PNM-9</b>	<b>IBUN-5.1</b>	Genus <i>Streptomyces</i>	—	—	—
<b>PNM-161a</b>	<b>RKHC-26</b>	Mycolic acid containing bacteria	—	+	—
<b>PNM-161a</b>	<b>IBUN-5.1</b>	Genus <i>Streptomyces</i>	—	—	—
<b>RKHC-9</b>	<b>RKHC-26</b>	Ecological criteria	No evidence of RKHC-26 growth	—	—
<b>RKHC-9</b>	<b>RKHC-28</b>	Ecological criteria	No evidence of RKHC-26 growth	—	—
<b>RKHC-9</b>	<b>RKHC-62b</b>	Ecological criteria	—	—	—
<b>RKHC-26</b>	<b>RKHC-28</b>	Ecological criteria	—	—	—
<b>RKHC-26</b>	<b>RKHC-62b</b>	Ecological criteria	—	—	—
<b>RKHC-26</b>	<b>IBUN-5.1</b>	Mycolic acid containing bacteria	No evidence of RKHC-26 growth	—	—
<b>RKHC-28</b>	<b>RKHC-62b</b>	Ecological criteria	—	—	—

For each co-culture those isolates that survived until the end of the fermentation time are highlighted in bold. (+): A change was observed compared to the corresponding monocultures (-): No change was observed compared to the corresponding monocultures.



**Figure 6S.** Comparison of  $^1\text{H}$  NMR spectra for organic extracts of: (a) culture media LB, (b) monoculture of *Streptomyces* sp. PNM-161a, (c) monoculture of *Rhodococcus* sp. RKHC-26 and (d) co-culture



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