

Supplementary files of

# The initial inoculation ratio regulates bacterial coculture interactions and metabolic capacity

Chun-Hui Gao<sup>1#</sup>, Hui Cao<sup>1#</sup>, Peng Cai<sup>1\*</sup>, Søren J. Sørensen<sup>2</sup>

<sup>1</sup>State Key Laboratory of Agricultural Microbiology, College of Resources and Environment, Huazhong Agricultural University, Wuhan 430070, China. <sup>2</sup>Section of Microbiology, Department of Biology, University of Copenhagen, Copenhagen, Denmark. \*Corresponding author: Peng Cai, cp@mail.hzau.edu.cn

#These authors contributed equally to this article.

The authors declare no competing financial interests.

**Table S1 List of 71 carbon sources**

<b>Id</b>	<b>Name</b>	<b>Usage<sup>1</sup></b>	<b>Prefer<sup>2</sup></b>
<b>2</b>	Dextrin	U1	EC
<b>3</b>	D-Maltose	U2	EC
<b>4</b>	D-Trehalose	U2	EC
<b>5</b>	D-Cellobiose	U1	None
<b>6</b>	Gentiobiosse	U1	EC
<b>7</b>	Sucrose	U1	None
<b>8</b>	D-Turanose	U1	None
<b>9</b>	Stachyose	U1	None
<b>10</b>	D-Raffinose	U1	None
<b>11</b>	$\alpha$ -D-Lactose	U1	None
<b>12</b>	D-Melibiose	U1	EC
<b>13</b>	$\beta$ -Methyl-D-Glucoside	U1	None
<b>14</b>	D-Salicin	U1	None
<b>15</b>	N-Acetyl-D-Glucosamine	U2	None
<b>16</b>	N-Acetyl- $\beta$ -Dmannosamine	U1	None
<b>17</b>	N-Acetyl-D-Galactosamine	U1	None
<b>18</b>	N-AcetylNeuraminic Acid	U2	EC
<b>19</b>	$\alpha$ -D-Glucose	U3	PP
<b>20</b>	D-Mannose	U2	PP
<b>21</b>	D-Fructose	U2	EC
<b>22</b>	D-Galactose	U1	EC
<b>23</b>	3-MethylGlucose	U1	None
<b>24</b>	D-Fucose	U1	PP
<b>25</b>	L-Fucose	U2	EC

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<b>26</b>	L-Rhamnose	U1	EC
<b>27</b>	Inosine	U2	None
<b>28</b>	D-Sorbitol	U1	EC
<b>29</b>	D-Mannitol	U2	EC
<b>30</b>	D-Arabitol	U1	None
<b>31</b>	myo-Inositol	U1	None
<b>32</b>	Glycerol	U1	None
<b>33</b>	D-Glucose-6-PO4	U2	EC
<b>34</b>	D-Fructose-6-PO4	U2	EC
<b>35</b>	D-Aspartic Acid	U1	None
<b>36</b>	D-Serine	U2	EC
<b>37</b>	Gelatin	U1	None
<b>38</b>	Glycyl-L-Proline	U1	EC
<b>39</b>	L-Alanine	U3	PP
<b>40</b>	L-Arginine	U3	PP
<b>41</b>	L-Aspartic Acid	U3	PP
<b>42</b>	L-Glutamic	U3	PP
<b>43</b>	L-Histidine	U3	PP
<b>44</b>	L-Pyroglutamic	U3	PP
<b>45</b>	L-Serine	U1	None
<b>46</b>	Pectin	U1	EC
<b>47</b>	D-Galacturonic Acid	U3	PP
<b>48</b>	L-Galactonic Acid Lactone	U2	EC
<b>49</b>	D-Gluconic	U2	PP
<b>50</b>	D-Glucuronic	U3	PP
<b>51</b>	Glucuronamid	U1	PP
<b>52</b>	Mucic Acid	U3	PP

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<b>53</b>	Quinic Acid	U3	PP
<b>54</b>	D-Saccharic	U3	PP
<b>55</b>	p-Hydroxy-Phenylacetic Acid	U1	PP
<b>56</b>	Methyl Pyruvate	U1	PP
<b>57</b>	D-Lactic Acid Methyl Ester	U1	None
<b>58</b>	L-Lactic Acid	U3	PP
<b>59</b>	Citric Acid	U3	PP
<b>60</b>	$\alpha$ -Keto-Glutaric Acid	U1	PP
<b>61</b>	D-Malic Acid	U1	None
<b>62</b>	L-Malic Acid	U3	PP
<b>63</b>	Bromo-Succinic	U1	PP
<b>64</b>	Tween 40	U1	None
<b>65</b>	$\gamma$ -Amino-Butyric Acid	U3	PP
<b>66</b>	$\alpha$ -Hydroxy-Butyric Acid	U1	None
<b>67</b>	$\beta$ -Hydroxy-D,L-Butyric Acid	U1	PP
<b>68</b>	$\alpha$ -Keto-Butyric Acid	U1	None
<b>69</b>	Acetoacetic Acid	U1	PP
<b>70</b>	Propionic Acid	U1	PP
<b>71</b>	Acetic Acid	U1	None
<b>72</b>	Formic Acid	U1	None

Note: <sup>1</sup> Carbon usage groups were determined by hierarchical clustering of CUE profiles of all cultures (see Figure 4 for more information). <sup>2</sup> Carbon preferences were determined by comparing the CUE of monocultures as follows (see also Figure 3). EC, *Escherichia coli* K-12 preferred carbon source; PP, *Pseudomonas putida* KT2440 preferred carbon source; None: no preference.

## Figure S1

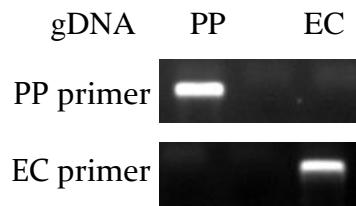


Figure S1. Specificity of species-specific primers. The PCR experiments were performed with *E. coli* and *P. putida* specific primers (left) and their genomic DNA (top), respectively. EC, *Escherichia coli* K-12; PP, *Pseudomonas putida* KT2440.

## Figure S2

Preference  None  *E. coli*  *P. putida*



Figure S2. Final ratio of all cocultures in 71 carbon sources (left to right, top to down, carbon id from 2 to 72). The adjusted p-value (ANOVA) were showed on the top of each subplot. The x-axis indicates coculture initial ratios (*E. coli/P. putida*), and the y-axis is the final ratio (log10-transformed). Green, none preferred carbon sources; Red, *E. coli* preferred carbon sources; Blue, *P. putida* preferred carbon sources.

## Figure S3

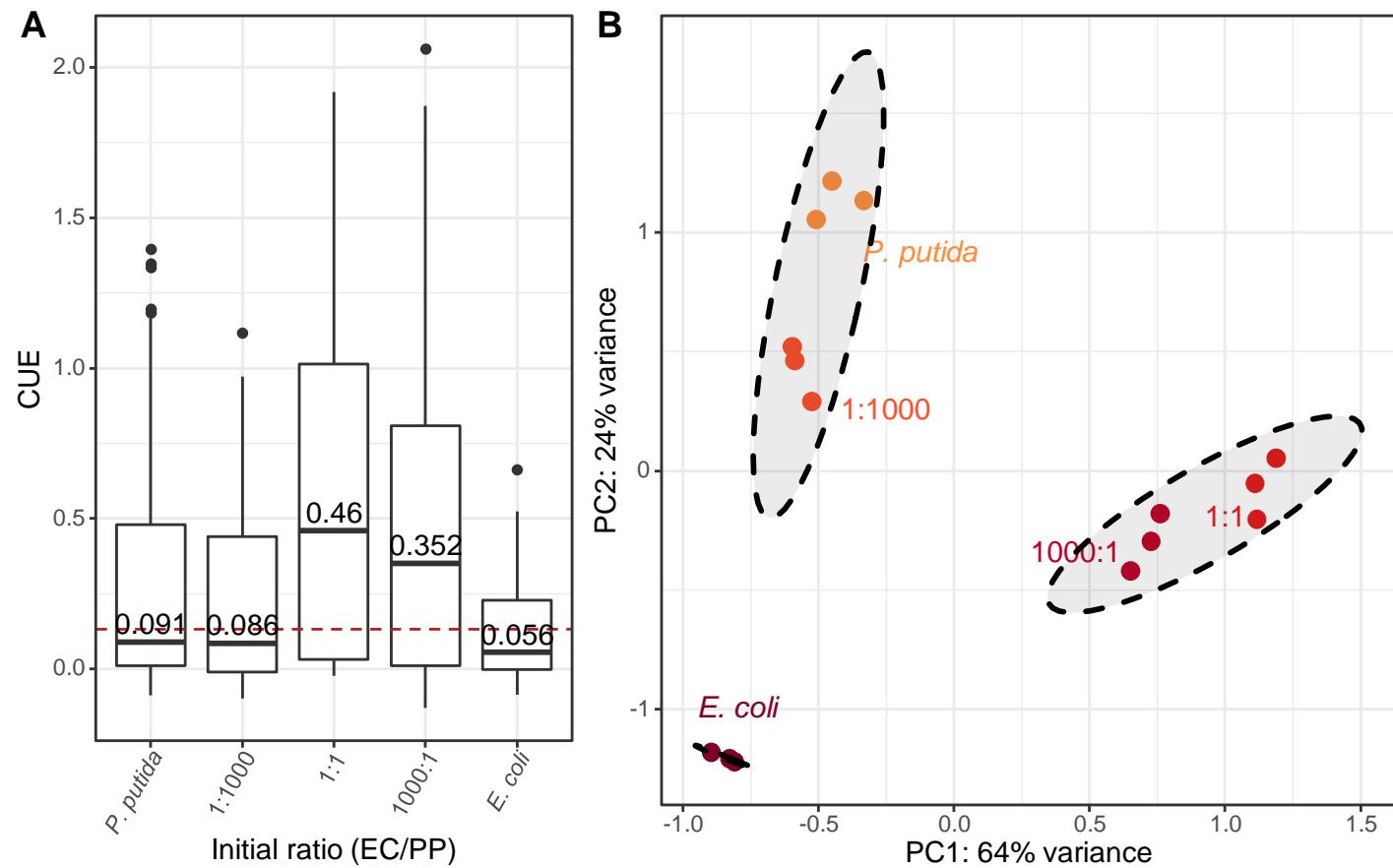


Figure S3. The carbon usage profiles of two monocultures (*E. coli* and *P. putida*) and three cocultures (1:1000, 1:1, and 1000:1). (A) boxplot. Number shows the median of each culture, and horizontal line shows the mean of all cultures. (B) PCA analysis of carbon usage profiles. Ellipses represent the 95% confidence interval of clustering.

## Figure S4

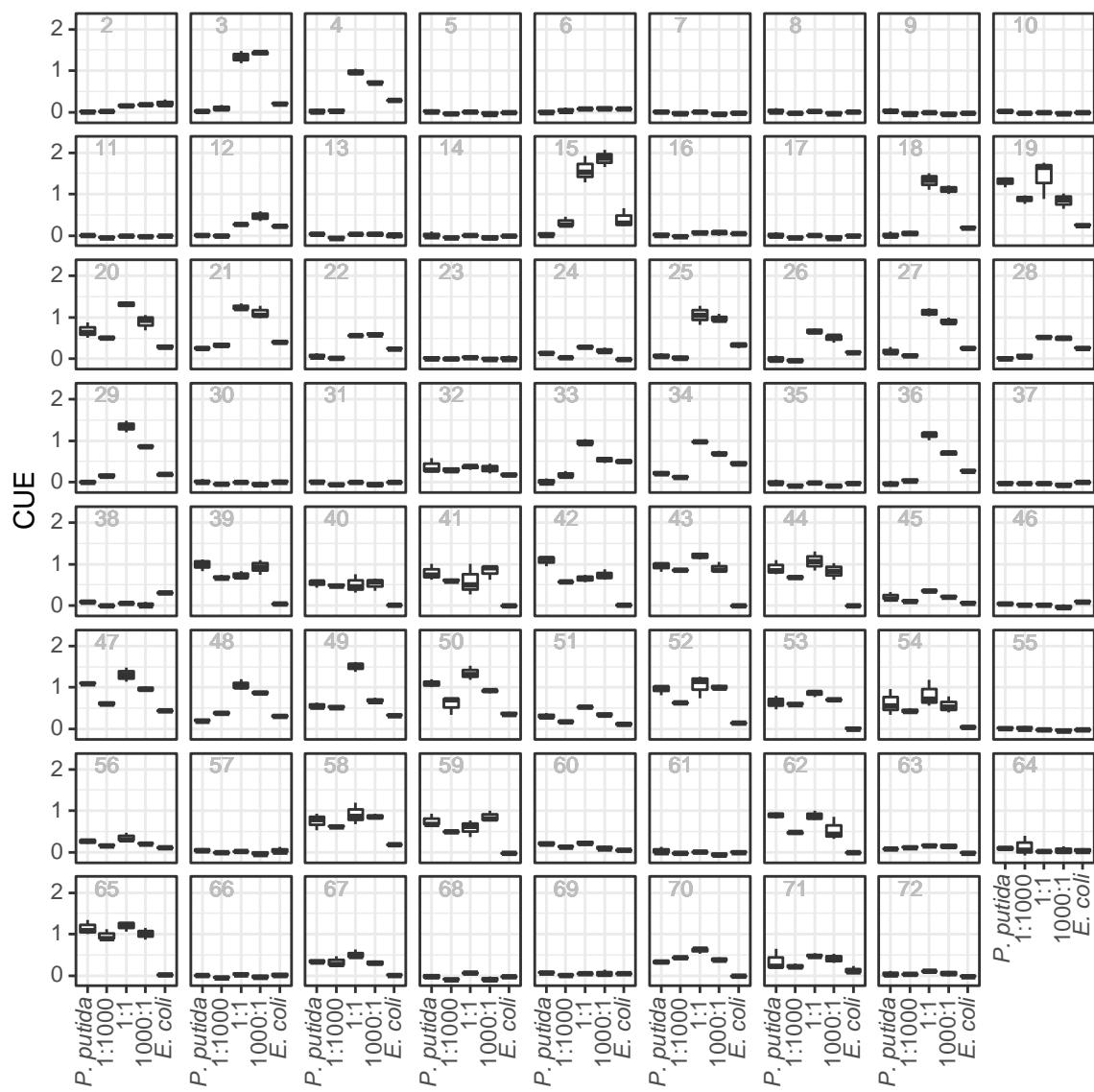


Figure S4. The CUE of mono- and cocultures in 71 carbon sources (left to right, top to down, carbon id from 2 to 72). The x-axis indicates culture conditions (there are a total of 5 culture conditions: *P. putida*, the “1:1000” coculture ; the “1:1” coculture; the “1000:1” coculture and *E. coli* ), and the y-axis indicates CUE.

## Figure S5

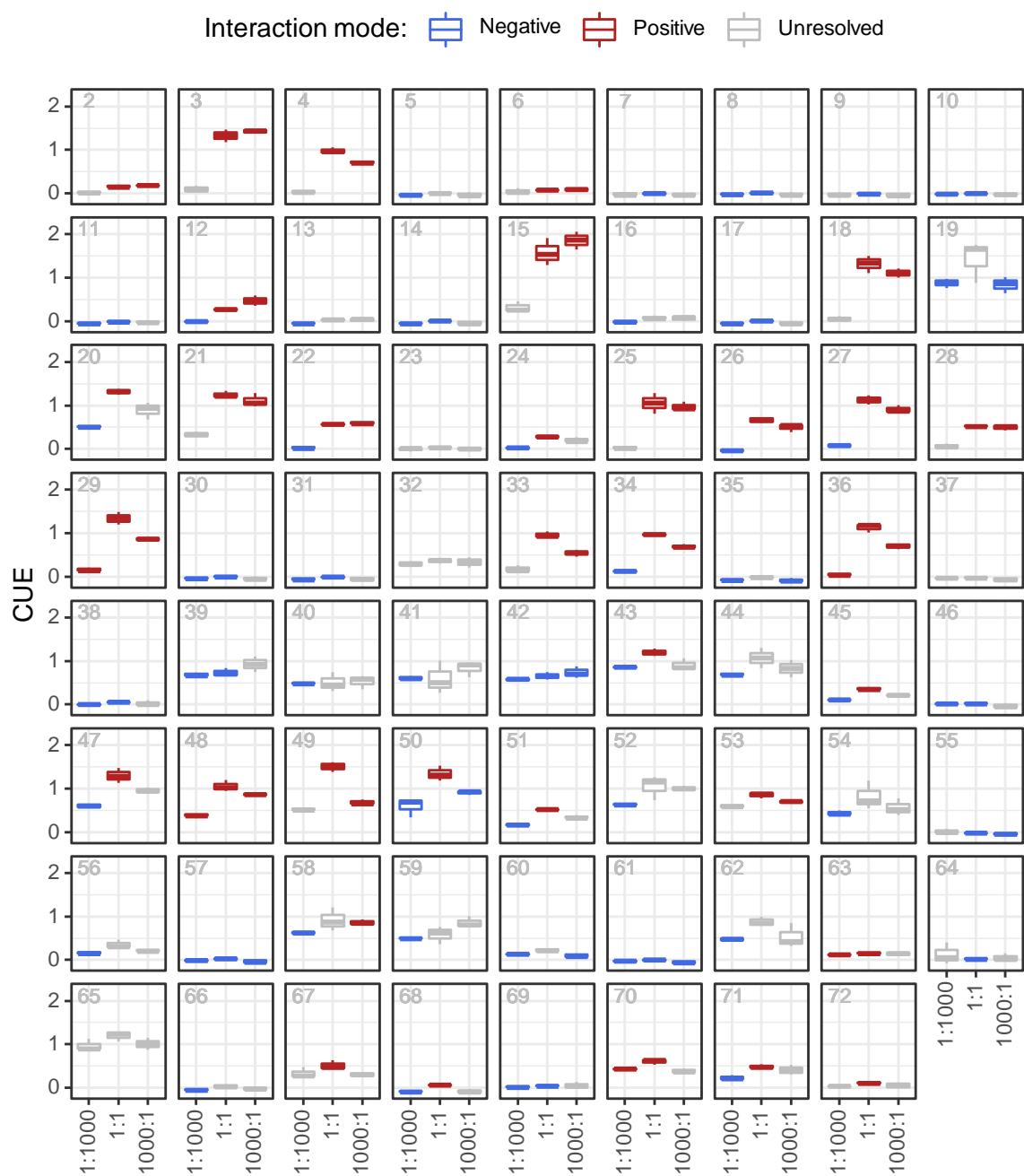


Figure S5. The interaction mode of all combinations in 71 carbon sources (left to right, top to down, carbon id from 2 to 72). The x-axis indicates coculture initial ratios (*E. coli/P. putida*), and the y-axis indicates CUE. Blue, negative interaction; Red, positive interaction; Gray, unresolved interaction.