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Supplementary material

2 Multi-omics reveals the mechanism of *B. longum* in promoting the 3 formation of mixed-species biofilms

4 Tao Xu ^{a,b}, Yue Xiao ^{a,b}, Hongchao Wang ^{a,b}, Jinlin Zhu ^{a,b}, Wenwei Lu ^{a,b,c,d,*} and
5 Wei Chen ^{a,b,f}

6 a. State Key Laboratory of Food Science and Resources, Jiangnan University, Wuxi
7 214122, China; 6200113219@stu.jiangnan.edu.cn (T.X.); xiaoyue@jiangnan.edu.cn
8 (Y.X.); hcwang@jiangnan.edu.cn (H.W.); wx_zjl@jiangnan.edu.cn (J.Z.);
9 chenwei66@jiangnan.edu.cn (W.C.)

10 b. School of Food Science and Technology, Jiangnan University, Wuxi 214122,
11 China

12 c. (Yangzhou) Institute of Food Biotechnology, Jiangnan University, Yangzhou
13 225004, China

14 d. National Engineering Research Center for Functional Food, Jiangnan University,
15 Wuxi 214122, China

16 * Correspondence: luwenwei@jiangnan.edu.cn; Tel.: +86-510-85912155

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Table S1. Detail of 2216E medium/L

compound	content	compound	content
peptone	5 g	potassium bromide	0.08 g
yeast	1 g	strontium chloride	0.034 g
ferric citrate	0.1 g	boric acid	0.022 g
sodium chloride	19.45 g	sodium silicate	0.004 g
magnesium chloride	5.98 g	Sodium fluoride	0.0024 g
sodium sulphate	3.24 g	ammonium nitrate	0.0016 g
calcium chloride	1.8 g	disodium hydrogen phosphate	0.008 g
potassium chloride	0.55 g	sodium carbonate	0.16 g
pH 7.6 ± 0.2			

Table S2. Detail of AB medium/L

compound	content
sodium chloride	17.5 g
magnesium sulphate	12.3 g
acid-hydrolyzed casein	2 g
pH 7.5 ± 0.2	

Table S3. Additive of AB medium/L

compound	content
1 M potassium phosphate buffer	10 mL
0.1 M arginine solution	10 mL
50% glycerol	20 mL
pH 7.5 ± 0.2	

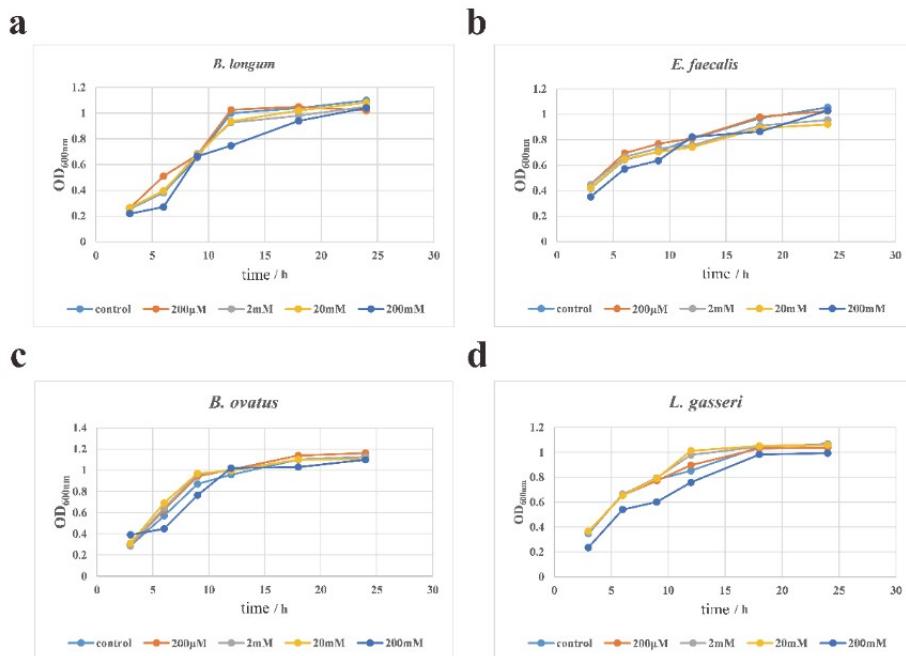
Tip: AB medium needs to be mixed with additives

Table S4: VIP value of differential metabolites between biofilm groups

Compound	VIP_score
Acetophenone	7.3333
L-Tyrosine	6.2038
3-(3,4-dihydroxyphenyl) propanoic acid	5.7281
2,6-Xylidine	4.2349
Isoleucine	3.118
Succinic acid	2.9781
Tyramine	2.958
Methylmalonic acid	2.8992
N-Benzylformamide	2.5283
DL-Arginine	2.3854
Adenine	2.2785
L-Glutamic acid	2.1248
L-Pyroglutamic acid	2.113
D-(+)-Tryptophan	1.9657

Compound	VIP_score
trans-3-Indoleacrylic acid	1.6582
Leucylproline	1.6441
5,6-diphenyl-2,3-dihydropyrazine	1.6287
carbamate	1.5963
4-Hydroxybenzaldehyde	1.594
Hypoxanthine	1.5057
Guanine	1.4819
Y-Aminobutyric acid (GABA)	1.3981
L-Phenylalanine	1.3482
Dipropylene glycol dimethyl ether	1.3299
Palmitic Acid	1.1818
Valylproline	1.115
Betaine	1.1127
D-(+)-Proline	1.0941
N'-hydroxy-2,5-dimethoxybenzenecarboximidamide	1.0887
Carnosine	1.0344
Cordycepin	1.0216
L-(-)-Methionine	1.0154

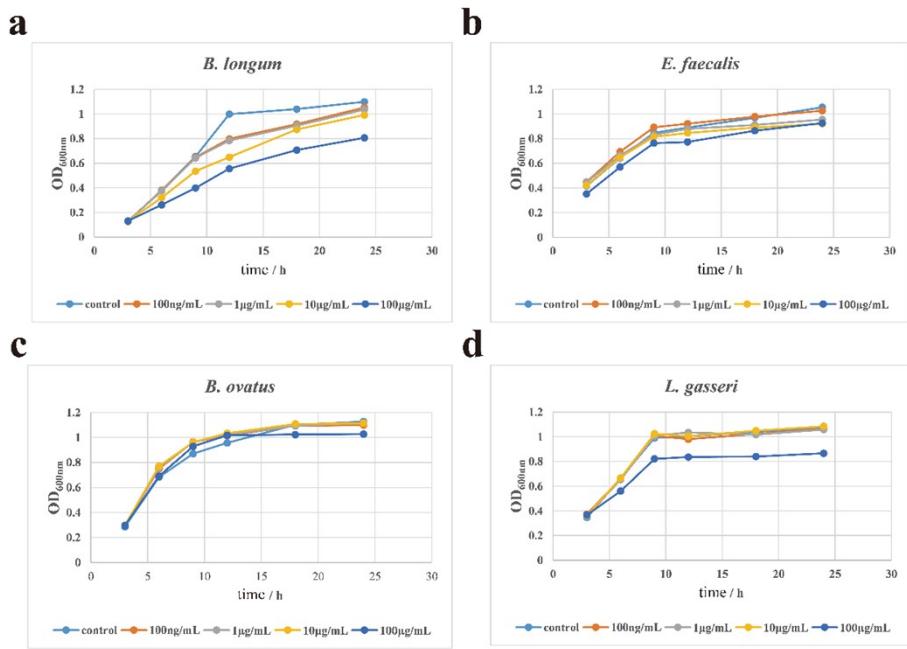
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29 **Fig S1.** Effect of AI-2 inhibitor on the growth of each strain. (a) *B. longum*, (b) *E. faecalis*, (c) *B.*
30 *ovatus*, and (d) *L. gasseri*.

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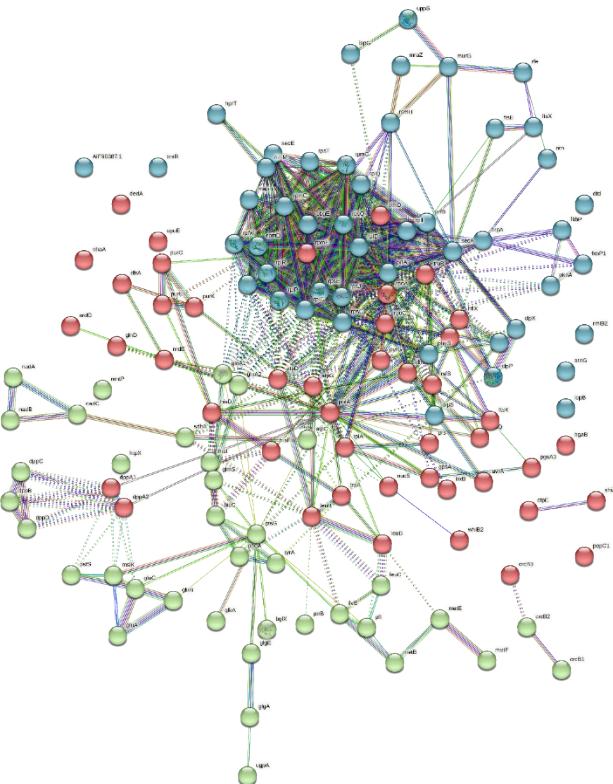


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33 **Fig S2.** Effect of AHLs inhibitor on the growth of each strain. (a) *B. longum*, (b)

34 *E. faecalis*, (c) *B. ovatus*, and (d) *L. gasseri*.

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37 **Fig S3.** Protein-protein interaction (PPI) network of DEGs.