

# Supplementary Materials: Effect of $\beta$ -Estradiol on Mono- and Mixed-Species Biofilms of Human Commensal Bacteria *Lactobacillus Paracasei* AK508 and *Micrococcus Luteus* C01 on Different Model Surfaces

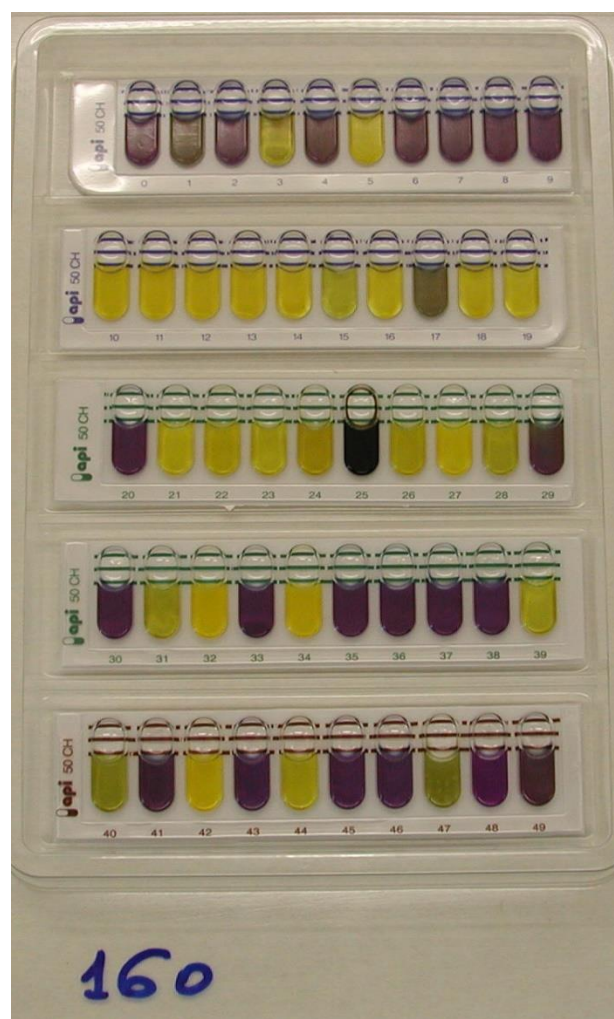
Anastasia A. Kiseleva <sup>1</sup>, Tatiana V. Solovyeva <sup>1</sup>, Maria A. Ovcharova <sup>1</sup>, Olga V. Geras'kina <sup>2</sup>, Sergey V. Mart'yanov <sup>1</sup>, Tatiana A. Cherdyntseva <sup>2</sup>, Natalya D. Danilova <sup>1</sup>, Marina V. Zhurina <sup>1</sup>, Ekaterina A. Botchkova <sup>1</sup>, Alexey V. Feofanov <sup>2,3</sup>, Vladimir K. Plakunov <sup>1</sup> and Andrei V. Gannesen <sup>1,\*</sup>

<sup>1</sup> Federal Research Center "Fundamentals of Biotechnology", Russian Academy of Sciences, Moscow 117312, Russia; anastkslv99@mail.ru (A.A.K.); solovyeva1944@mail.ru (T.V.S.); masha\_ovcharova\_97@mail.ru (M.A.O.); semartyan@inbox.ru (S.V.M.); leo\_523@mail.ru (N.D.D.); mzhurik@gmail.com (M.V.Z.); botchkovaekat@gmail.com (E.A.B.); plakunov@inmi.ru (V.K.P.)

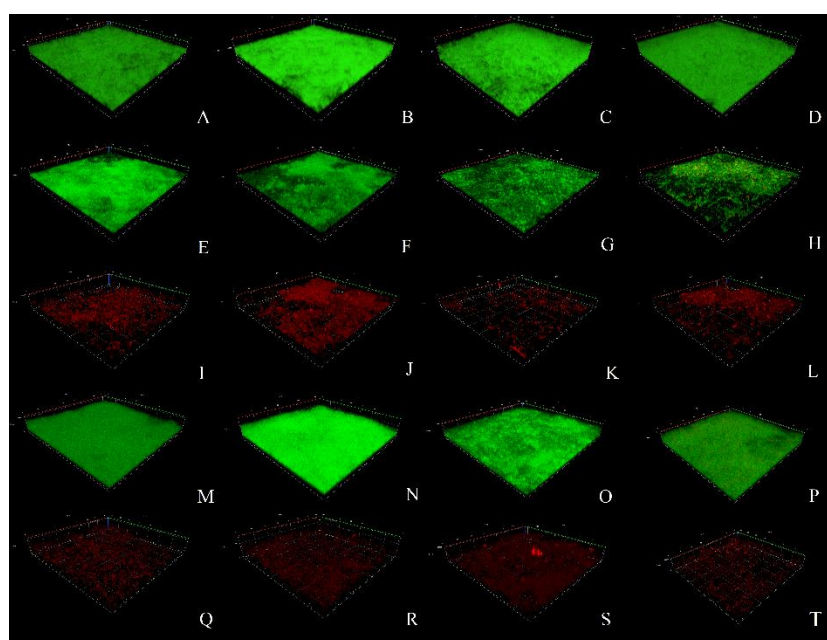
<sup>2</sup> Faculty of Biology, Lomonosov Moscow State University, Moscow 119234, Russia; olgsamsonova@yandex.ru (O.V.G.); taniacherd@yandex.ru (T.A.C.); avfeofanov@yandex.ru (A.V.F.)

<sup>3</sup> Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of sciences, Moscow 117997, Russia

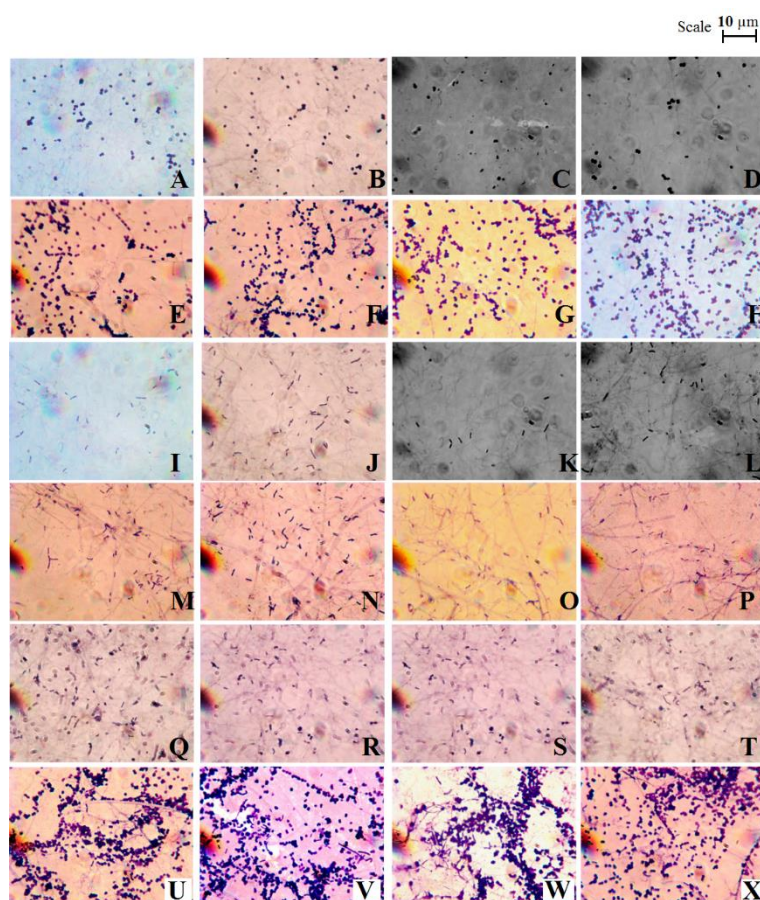
\* Correspondence: a.gannesen@fbras.ru or andrei.gannesen@gmail.com



**Figure S1.** The API test of *L. paracasei* AK508. The list of compounds is presented in the Table S1.

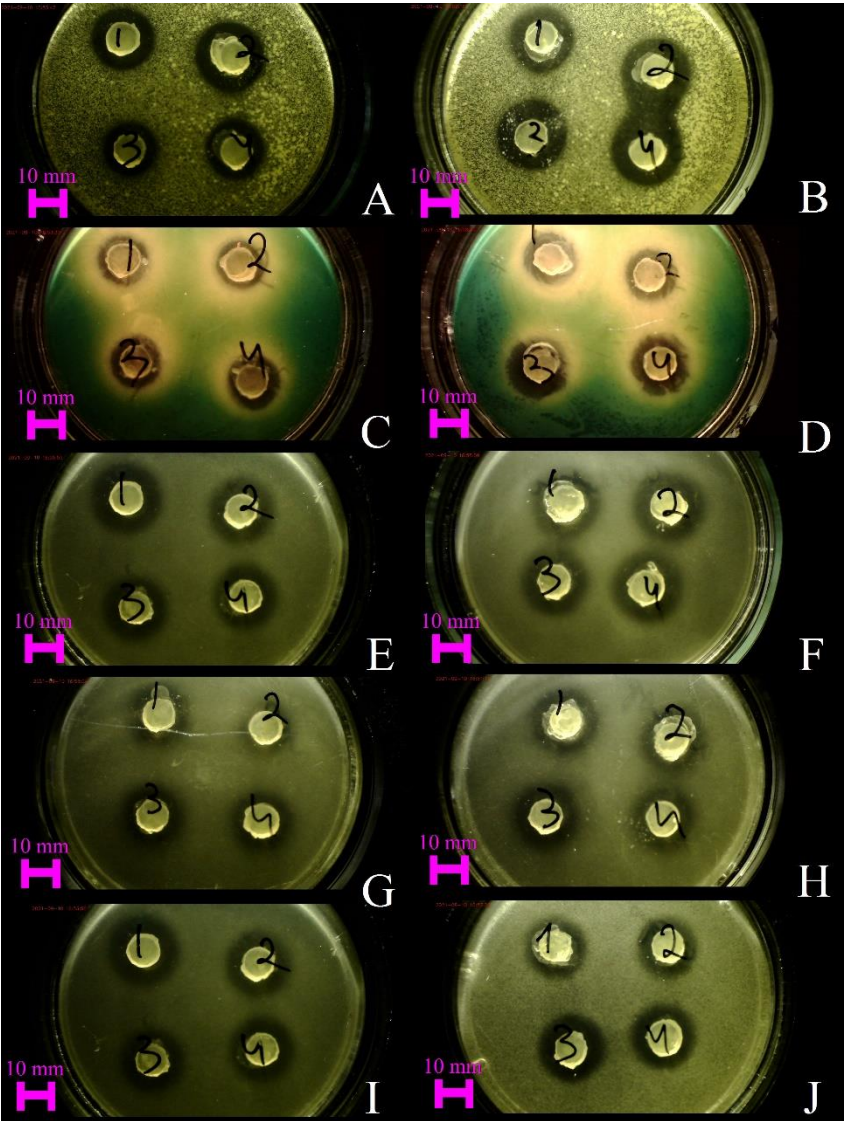


**Figure S2.** The CLSM images of monospecies (A–L) and mixed-species (M–T) biofilms of *M. luteus* and *L. paracasei*. A–D – monospecies biofilms of *M. luteus* stained with SYTO9, E–L – monospecies biofilms of *L. paracasei* stained with SYTO9 (E–H) and the FISH probe with R6G (I–L). M–T – mixed-species biofilms stained with SYTO9 (M–P) and the FISH probe with R6G (Q–T). A, E, I, M, Q – control samples, B, F, J, N, R – ethanol samples, C, G, K, O, S – estradiol  $2.2 \times 10^{-10}$  M; D, H, L, P, T –  $2.2 \times 10^{-8}$  M.



**Figure S3.** Representative images of the samples of monospecies *M. luteus* biofilms (A–H), monospecies *L. paracasei* biofilms (I–P) and mixed-species biofilms (Q–X) for aggregation assessment in the system with tubes (A–D, I–L, Q–T) and on the solid medium in Petri dishes (E–H, M–P, U–X).

A,E,I,M,Q,U – control samples, B,F,J,N,R,V – ethanol samples, C,G,K,O,S,W – estradiol  $2.2 \times 10^{-10}$  M, D,H,L,P,T,X – estradiol  $2.2 \times 10^{-8}$  M.



**Figure S4.** The images of inhibition zones around the agar blocks with *L. paracasei* grown areobically (A,C,E,G,I) and anaerobically (B,D,F,H,J). A,B – *M. luteus*, C,D – *P. aeruginosa* PAO1, E,F – *Escherichia coli* K12, G,H – *Staphylococcus aureus* 209P, I,J – *Staphylococcus epidermidis* ATCC14990. 1 – control, 2 – ethanol, 3 – estradiol  $2.2 \times 10^{-8}$  M, 4 – estradiol  $2.2 \times 10^{-10}$  M.

**Table S1.** API test of *L. paracasei* AK508. Red label is for differences between strain AK508 and type strain R094.

No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
<b>Time/</b>	24	-	-	-	+	-	+	-	-	-	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+
<b>h</b>	48	-	-	-	+	-	+	-	-	-	-	+	+	+	+	+	+	-	+	+	-	+	+	+	+
<b>Substrate</b>	O	Glycerol	Erythritol	D-ara binose	L-ara binose	D-ribose	D-xyl ose	L-xyl ose	D-ado nitol	Meth yl-β-D-xylopyranoside	D-galactose	D-glucose	D-fructose	D-mannose	L-sorbose	L-rhamnose	Dulcitol	Inositol	D-mannitol	D-sorbitol	Methyl-α-D-mannopyranoside	Met hyl-α-D-glucopyranoside	N-acetyl glucosamine	Amygdalin	Arbutin



No.	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49
Time/	24	+	+	+	+	-	-	-	+	-	+	-	-	-	+	-	-	+	-	+	-	-	?	-	-
h	48	+	+	+	+	-	-	+	+	-	+	-	-	-	+	?	-	+	-	+	-	-	?	-	-
Substrate	Esc uli n ferr ic citr ate	Sali cin	D- cell obi ose	D- mal tos e	D- lact (bo vin ose)	D- mel ybi ose	D- suc ros e	D- tre gal ose	Inul in	D- melez itose	D- raff ino	Ami don (star ch)	Gly cog en	Xyl itol	Ge ntio bio se	D- tur ano se	D- lyx ose	D- tag atos e	D- fuc ose	L- fuc ose	D- arabi tol	L- arab itol	Pota ssiu m gluc onat e	Pota ssiu m 2- keto gluc onat e	Pota ssiu m 5- keto gluc onat e