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### **Streptococcus salivarius MS-oral-D6 promotes gingival re-epithelialization in vitro through a secreted serine protease**

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## Supplementary Information

### ***Streptococcus salivarius* MS-oral-D6 promotes gingival re-epithelialization *in vitro* through a secreted serine protease**

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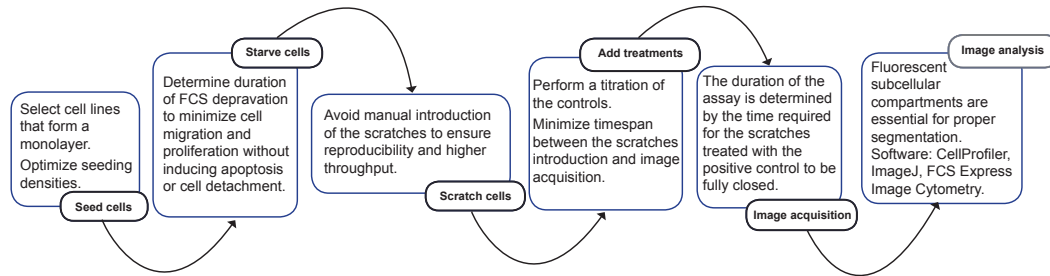
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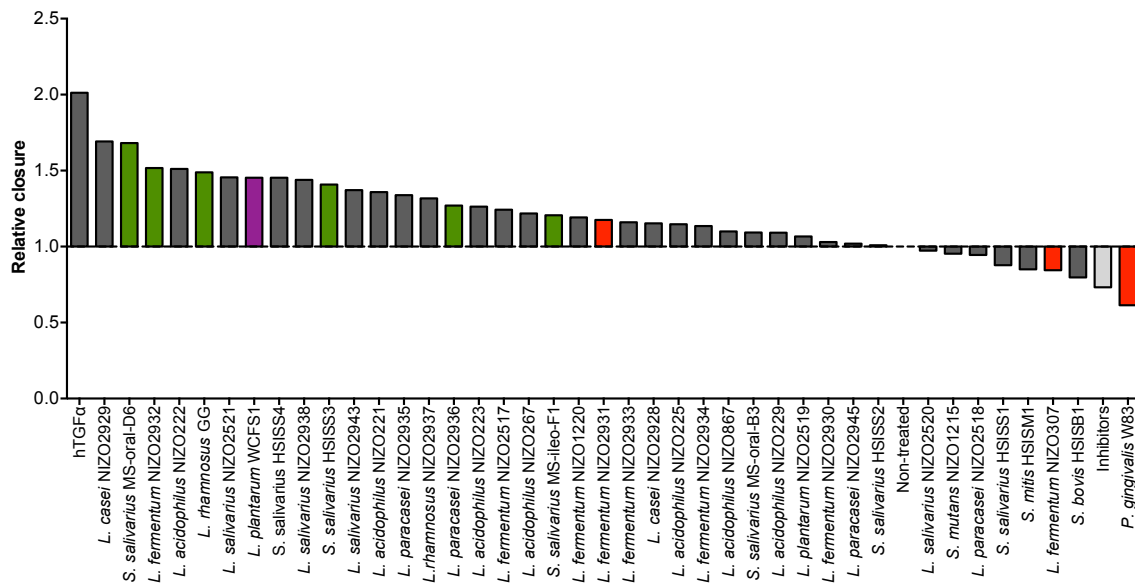
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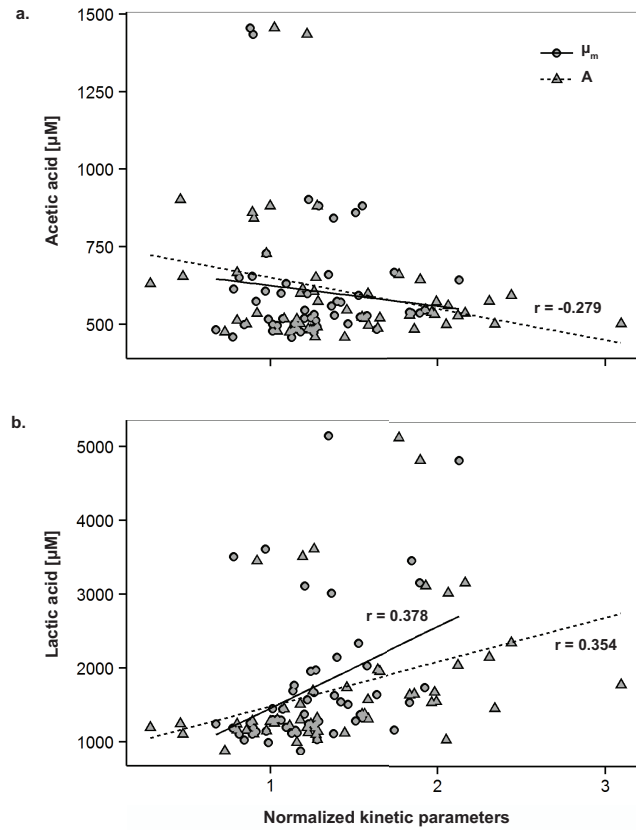
## Supplementary Figures



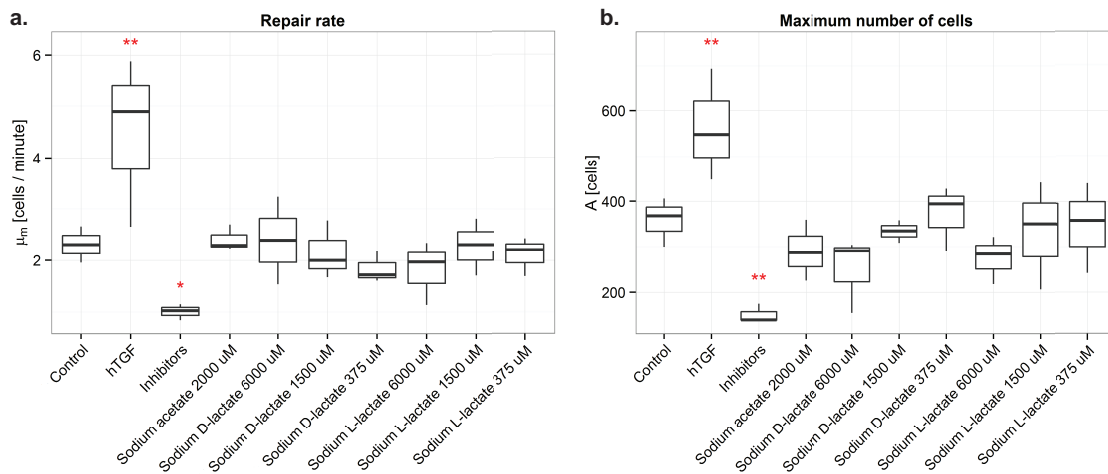
**Figure S1. Scratch assay workflow.** The assay workflow and crucial considerations are indicated in each step.



**Figure S2. Relative scratch closure after exposure to different bacterial treatments.** The closure of the scratches after stimulation of Ca9-22 cells with the bacterial treatments was calculated relative to that of the non-treated control. Green bars: potential stimulators; red bars: potential attenuators; purple bar: minor modulator. Human transforming growth factor alpha (hTGF, 4 ng/ml) and a combination of p38 (SB203580, 10  $\mu$ M) and MEK1/2 (U0126, 10  $\mu$ M) inhibitors were used as positive and negative controls respectively.



**Figure S3. Correlation between metabolic end products of bacteria and re-epithelialization kinetics.** Association between the concentrations of (a) acetic and (b) lactic acid with the kinetic parameters (A and  $\mu_m$ ) of re-epithelialization assessed by Pearson correlation (n = 54).



**Figure S4. Re-epithelialization kinetics of cells exposed to acetate, D-lactate and L-lactate.** (a) Repair rate. (b) Maximum number of cells within the scratched area after 5 hours.

**Table S1. Re-epithelialization parameter values obtained from the preliminary screening.**

Estimated parameters ( $\mu_m$ ,  $\lambda$ , A) and goodness of fit ( $R^2$ ) obtained after fitting the Gompertz function through the enumeration of infiltrating cells over time.

Treatment	$\mu_m$	SEM_ $\mu_m$	$\lambda$	SEM_ $\lambda$	A	SEM_A	RMSE	$R^2$
Non-treated	2.063	0.222	-1.705	9.229	373.044	16.060	19.489	0.973
Non-treated	3.387	0.088	27.853	1.542	396.658	2.604	4.742	0.999
Non-treated	3.279	0.098	35.843	2.116	483.291	5.235	6.837	0.999
Non-treated	3.141	0.187	-1.647	4.876	533.865	11.488	15.415	0.992
hTGF $\alpha$	6.758	0.398	2.752	3.418	757.562	9.661	20.526	0.993
hTGF $\alpha$	6.357	0.390	4.831	3.850	781.560	11.610	22.570	0.993
hTGF $\alpha$	12.769	1.106	25.065	2.927	802.766	9.705	25.753	0.992
hTGF $\alpha$	11.183	0.922	17.142	3.331	847.313	11.089	28.153	0.990
Inhibitors	1.005	0.138	1.545	10.916	166.001	8.021	11.053	0.959
Inhibitors	2.244	0.151	5.285	2.467	153.482	1.459	3.983	0.993
Inhibitors	1.942	0.096	32.367	2.852	220.714	2.711	4.964	0.996
Inhibitors	2.702	0.090	13.794	1.841	286.696	2.055	4.357	0.998
<i>L. acidophilus</i> NIZO221	2.202	0.153	-5.555	6.030	402.460	11.038	13.451	0.988
<i>L. acidophilus</i> NIZO221	4.684	0.268	5.744	4.251	709.642	12.944	19.716	0.993
<i>L. acidophilus</i> NIZO222	4.048	0.298	14.540	5.223	583.356	13.409	20.769	0.990
<i>L. acidophilus</i> NIZO222	5.546	0.340	18.293	2.718	464.329	4.932	11.914	0.995
<i>L. acidophilus</i> NIZO223	4.044	0.253	7.086	3.766	474.305	6.887	13.844	0.993
<i>L. acidophilus</i> NIZO223	3.726	0.184	7.507	3.606	551.548	8.509	13.240	0.995
<i>L. acidophilus</i> NIZO225	2.388	0.187	-5.197	6.776	436.145	13.482	16.409	0.986
<i>L. acidophilus</i> NIZO225	3.854	0.230	3.894	4.163	538.170	9.048	15.429	0.993
<i>L. acidophilus</i> NIZO229	3.767	0.326	-2.556	6.345	553.970	13.915	22.977	0.983
<i>L. acidophilus</i> NIZO229	2.816	0.219	1.524	6.060	450.367	11.786	17.029	0.987
<i>L. acidophilus</i> NIZO267	2.884	0.222	-8.883	7.369	603.444	22.685	22.026	0.985
<i>L. acidophilus</i> NIZO267	3.317	0.237	-4.034	5.339	497.914	10.522	17.046	0.988
<i>L. acidophilus</i> NIZO867	3.566	0.353	-4.087	6.740	477.394	12.031	22.299	0.978
<i>L. acidophilus</i> NIZO867	4.367	0.305	-0.739	4.232	513.221	8.004	16.578	0.990
<i>L. casei</i> NIZO2928	2.349	0.099	20.401	2.603	288.089	3.135	5.666	0.997
<i>L. casei</i> NIZO2928	4.172	0.188	14.882	2.543	455.897	4.560	9.405	0.996
<i>L. casei</i> NIZO2929	4.456	0.162	10.244	2.495	613.671	6.392	10.728	0.997
<i>L. casei</i> NIZO2929	4.102	0.233	8.360	3.868	558.495	8.866	15.228	0.994
<i>L. fermentum</i> NIZO1220	3.478	0.158	11.404	3.430	540.528	8.426	11.990	0.996
<i>L. fermentum</i> NIZO1220	3.694	0.255	-4.808	5.104	547.553	10.909	18.023	0.989
<i>L. fermentum</i> NIZO2517	4.367	0.271	8.418	3.420	462.678	6.019	13.060	0.993
<i>L. fermentum</i> NIZO2517	3.965	0.196	10.395	3.340	534.176	7.373	12.686	0.995
<i>L. fermentum</i> NIZO2930	3.034	0.223	-1.071	6.213	538.680	15.426	19.238	0.987
<i>L. fermentum</i> NIZO2930	3.858	0.143	11.431	2.245	458.286	4.070	7.932	0.997
<i>L. fermentum</i> NIZO2931	2.855	0.207	15.438	5.399	439.657	11.008	15.483	0.990
<i>L. fermentum</i> NIZO2931	2.200	0.206	-6.566	8.311	414.243	15.973	18.598	0.979
<i>L. fermentum</i> NIZO2932	4.703	0.354	7.576	4.850	598.438	11.513	21.365	0.989

<i>L. fermentum</i> NIZO2932	4.431	0.198	12.777	2.945	582.075	7.096	12.405	0.996
<i>L. fermentum</i> NIZO2933	3.382	0.238	11.479	5.377	535.058	13.260	18.378	0.990
<i>L. fermentum</i> NIZO2933	3.288	0.245	1.827	5.134	449.916	9.110	16.059	0.988
<i>L. fermentum</i> NIZO2934	3.578	0.306	-3.048	6.062	504.246	11.787	20.542	0.984
<i>L. fermentum</i> NIZO2934	2.021	0.181	-9.869	7.565	351.634	11.102	14.991	0.981
<i>L. fermentum</i> NIZO307	1.909	0.193	-4.612	7.800	299.344	9.452	14.491	0.977
<i>L. fermentum</i> NIZO307	3.365	0.255	4.495	4.566	394.486	6.847	13.920	0.989
<i>L. paracasei</i> NIZO2518	2.951	0.149	11.733	3.984	486.020	9.243	12.033	0.995
<i>L. paracasei</i> NIZO2518	2.695	0.300	-11.068	8.910	435.511	15.107	22.893	0.971
<i>L. paracasei</i> NIZO2935	2.984	0.140	14.742	3.263	421.068	5.975	9.496	0.996
<i>L. paracasei</i> NIZO2935	2.956	0.225	0.404	6.429	525.502	15.794	19.486	0.987
<i>L. paracasei</i> NIZO2936	4.664	0.333	6.884	4.388	559.395	9.511	18.753	0.990
<i>L. paracasei</i> NIZO2936	5.237	0.281	3.572	3.200	604.986	7.298	15.081	0.994
<i>L. paracasei</i> NIZO2945	2.020	0.197	-11.301	8.221	350.321	11.820	16.150	0.977
<i>L. paracasei</i> NIZO2945	4.291	0.327	1.973	4.622	504.729	8.746	17.912	0.988
<i>L. plantarum</i> NIZO2519	5.781	0.307	6.491	2.383	488.376	4.373	10.937	0.995
<i>L. plantarum</i> NIZO2519	3.779	0.275	-0.584	5.666	603.011	14.480	21.230	0.988
<i>L. plantarum</i> WCFS1	3.870	0.340	10.364	5.778	505.312	11.892	21.186	0.985
<i>L. plantarum</i> WCFS1	3.851	0.282	3.946	5.052	529.654	10.730	18.617	0.989
<i>L. rhamnosus</i> GG	5.115	0.336	2.611	4.214	644.435	10.420	19.977	0.991
<i>L. rhamnosus</i> GG	3.807	0.206	13.318	3.665	517.701	8.015	13.430	0.995
<i>L. rhamnosus</i> NIZO2937	2.576	0.151	16.406	4.352	394.780	7.990	11.248	0.993
<i>L. rhamnosus</i> NIZO2937	3.240	0.269	11.024	5.931	469.145	12.014	18.869	0.986
<i>L. salivarius</i> NIZO2520	2.871	0.163	3.814	4.867	524.758	12.629	14.560	0.993
<i>L. salivarius</i> NIZO2520	4.735	0.334	3.665	3.763	482.040	6.723	15.273	0.990
<i>L. salivarius</i> NIZO2521	2.236	0.190	13.953	6.274	339.745	9.693	14.019	0.986
<i>L. salivarius</i> NIZO2521	4.776	0.350	1.970	4.745	606.738	11.032	21.034	0.989
<i>L. salivarius</i> NIZO2938	4.117	0.253	5.968	4.937	692.678	15.925	20.778	0.992
<i>L. salivarius</i> NIZO2938	3.796	0.251	8.229	4.650	537.134	10.452	17.157	0.991
<i>L. salivarius</i> NIZO2943	5.505	0.910	8.431	6.017	373.175	8.753	23.754	0.959
<i>L. salivarius</i> NIZO2943	1.489	0.197	-12.067	12.057	286.851	15.560	17.918	0.957
<i>P. gingivalis</i> W83	3.886	0.268	1.745	3.233	343.523	4.085	10.138	0.991
<i>P. gingivalis</i> W83	4.017	0.260	13.458	2.741	319.152	3.380	8.504	0.993
<i>P. gingivalis</i> W83	3.857	0.380	20.001	3.873	284.127	4.375	11.137	0.987
<i>P. gingivalis</i> W83	2.208	0.212	-0.592	3.639	155.689	2.135	5.860	0.983
<i>S. bovis</i> HSISB1	2.658	0.212	-2.583	5.328	347.927	6.954	13.112	0.986
<i>S. bovis</i> HSISB1	2.428	0.161	-4.680	5.386	405.702	9.261	12.962	0.990
<i>S. mitis</i> HSISM1	3.007	0.268	-0.414	6.680	455.614	12.476	19.619	0.983
<i>S. mitis</i> HSISM1	4.852	0.230	10.980	2.925	586.425	6.806	13.078	0.996
<i>S. mutans</i> NIZO1215	2.456	0.280	-1.911	7.780	330.646	9.805	17.897	0.972
<i>S. mutans</i> NIZO1215	2.728	0.166	-5.230	4.546	410.563	7.324	11.891	0.991
<i>S. salivarius</i> HSISS1	2.562	0.165	-0.300	4.766	383.315	7.435	11.886	0.991
<i>S. salivarius</i> HSISS1	2.496	0.239	-12.955	8.164	436.620	14.495	19.691	0.978
<i>S. salivarius</i> HSISS2	2.794	0.162	5.061	4.356	430.180	8.089	12.096	0.993

<i>S. salivarius</i> HSISS2	3.552	0.200	-1.664	4.415	571.413	10.662	15.536	0.993
<i>S. salivarius</i> HSISS3	3.765	0.320	-8.290	6.604	590.106	15.259	23.816	0.983
<i>S. salivarius</i> HSISS3	3.951	0.249	3.196	4.133	510.647	8.200	15.284	0.992
<i>S. salivarius</i> HSISS4	3.036	0.167	1.082	4.539	526.667	10.990	14.060	0.993
<i>S. salivarius</i> HSISS4	2.676	0.233	-15.673	9.055	623.015	30.786	25.030	0.980
<i>S. salivarius</i> MS-ileo-F1	4.238	0.149	13.609	2.729	686.391	8.935	11.797	0.998
<i>S. salivarius</i> MS-ileo-F1	2.761	0.167	14.701	5.259	525.992	15.674	15.573	0.992
<i>S. salivarius</i> MS-oral-B3	3.089	0.138	17.773	3.442	497.342	8.364	10.844	0.996
<i>S. salivarius</i> MS-oral-B3	2.960	0.183	4.652	4.461	430.211	7.964	12.881	0.992
<i>S. salivarius</i> MS-oral-D6	4.710	0.357	5.496	5.878	753.939	19.739	27.863	0.988
<i>S. salivarius</i> MS-oral-D6	5.018	0.228	12.373	3.065	678.156	8.703	14.765	0.996

**Table S2. Lactic and acetic acid quantification.** The concentration of accumulated lactic and acetic acid in the supernatant of Ca9-22 cells treated with lactic acid bacteria for 5 hours.

Sample	MOI	Average lactic acid ( $\mu\text{M}$ )	SD	Average acetic acid ( $\mu\text{M}$ )	SD
<i>S. salivarius</i> MS-ileo-F1	10	1132.70	109.26	489.66	8.48
<i>S. salivarius</i> MS-ileo-F1	50	1806.30	166.35	484.83	1.74
<i>S. salivarius</i> MS-ileo-F1	250	3297.59	150.40	534.78	0.08
<i>S. salivarius</i> HSISS3	10	1409.29	39.12	510.94	11.65
<i>S. salivarius</i> HSISS3	50	1854.83	480.18	555.26	36.59
<i>S. salivarius</i> HSISS3	250	1636.28	95.47	558.10	13.16
<i>L. paracasei</i> NIZO2936	10	1555.87	111.50	524.13	6.90
<i>L. paracasei</i> NIZO2936	50	2085.80	55.07	549.75	23.58
<i>L. paracasei</i> NIZO2936	250	4972.71	167.86	650.92	8.54
<i>L. plantarum</i> WCFS1	10	1467.63	160.46	511.91	16.32
<i>L. plantarum</i> WCFS1	50	1859.18	92.97	510.18	9.73
<i>L. plantarum</i> WCFS1	250	3058.46	49.31	551.83	6.80
<i>S. salivarius</i> MS oral D6	10	1088.16	98.32	487.01	28.41
<i>S. salivarius</i> MS oral D6	50	1119.90	2.27	470.87	13.56
<i>S. salivarius</i> MS oral D6	250	1548.31	21.41	567.48	30.34
<i>L. fermentum</i> NIZO1220	10	1135.79	105.26	501.61	10.37
<i>L. fermentum</i> NIZO1220	50	1217.28	77.94	586.34	12.89
<i>L. fermentum</i> NIZO1220	250	1299.28	20.45	870.57	10.60
<i>L. rhamnosus</i> GG	10	1256.33	41.33	475.82	1.76
<i>L. rhamnosus</i> GG	50	1597.45	89.39	490.92	10.09
<i>L. rhamnosus</i> GG	250	3557.57	51.46	609.40	3.86
<i>P. gingivalis</i> W83	10	1014.32	139.60	487.59	13.05
<i>P. gingivalis</i> W83	50	1130.31	27.70	659.93	6.20
<i>P. gingivalis</i> W83	250	1218.68	25.37	766.21	135.26
<i>L. fermentum</i> NIZO307	10	1123.77	22.25	689.05	38.72
<i>L. fermentum</i> NIZO307	50	1191.20	83.64	861.03	19.83
<i>L. fermentum</i> NIZO307	250	1221.19	30.63	1444.98	10.23



**Table S3. Conservation of the identified secreted proteins among *S. salivarius* sequenced genomes.** Presence (+) or absence (-) of the proteins identified in the spent culture medium of *Streptococcus salivarius* MS-oral-D6 among the sequenced strains of the same species.

Strain	Serine protease	Peptidase M26	Peptidoglycan hydrolase	Surface antigen
SK126	-	-	+	+
726_SSAL	-	-	+	-
39-09 S16	-	-	+	+
ATCC 7073	-	-	+	+
HSISS2	-	-	+	+
NCTC 8618	-	+	+	+
JIM8777	-	+	+	+
CCHSS3	-	+	+	+
57.l	-	+	+	+
JF	-	+	+	+
PS4	-	+	+	+
K12	-	+	+	+
20-02 S1	-	+	-	+
22-08 S7	-	+	+	+
34-19 S9	-	+	+	+
34-24 S10	-	+	+	+
84-12 S20	-	+	+	+
37-09 S13	-	+	-	+
39-07 S15	-	+	+	-
40-02 S18	-	+	+	+
85-04 S22	-	+	+	+
85-05 S23	-	+	+	+
140_SSAL	-	+	+	+
2202 S3	-	+	+	+
HSISS3	-	+	+	+
GED7778A	+	+	+	+
M18	+	-	+	+
1003_SOLI	+	-	+	+
HSISS4	+	+	+	+
NU10	+	+	+	+
YU10	+	+	+	+
UC3162	+	+	+	+
KB005	+	+	+	+
1270_SSAL	+	+	+	+
20-12 S2	+	+	-	+
37-08 S12	+	+	+	-
HSISS1	+	+	+	+