Supplementary materials

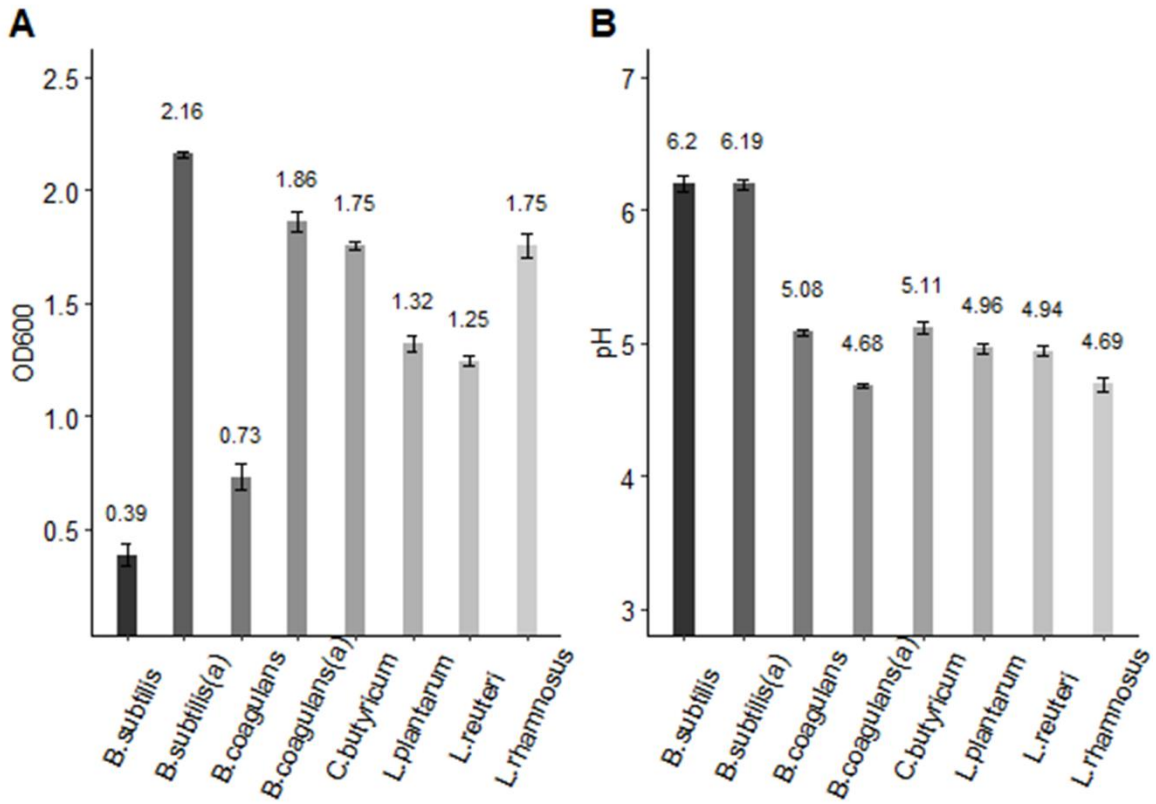

Fig. S1. Growth status (OD_{600}) (A) and pH value (B) of all probiotics used in this study. All probiotics were cultured with M9B medium at 37 °C for 24 hours. All *Lactobacillus* spp. and *C. butyricum* MIYAIRI 588 were cultured under anaerobic conditions. *B. coagulans* and *B. subtilis* natto NTU-18 were cultured under both anaerobic and aerobic conditions. "(a)" indicates aerobic culture. As shown in this figure, *B. subtilis* natto and *B. coagulans* grew much slower under anaerobic conditions than under aerobic conditions. The OD_{600} of anaerobically cultured *B. subtilis* natto was 0.39±0.05, and the OD_{600} of anaerobically cultured *B. coagulans* was

0.74±0.06.

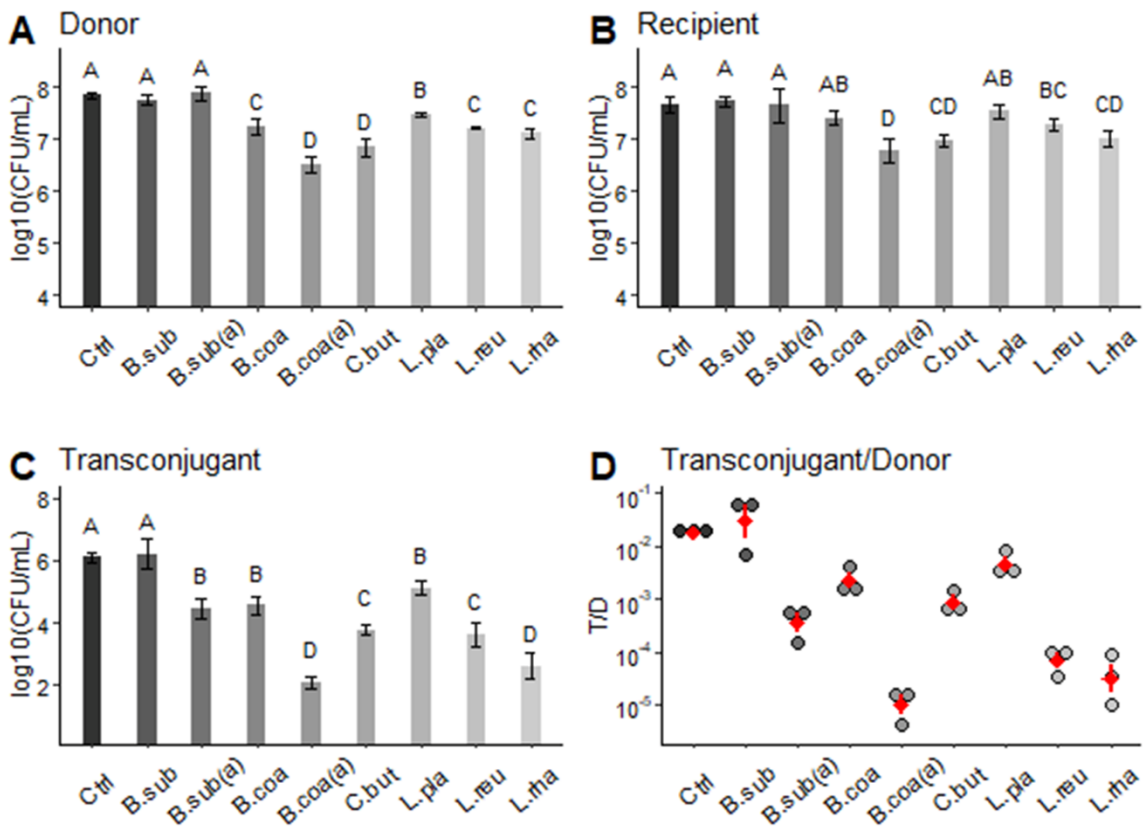

Fig. S2. Concentrations of donors (A), recipients (B), and transconjugants (C) and frequency of pCF10 transfer (transconjugants per donor, T/D) (D) after 4-hr mating assays with different probiotic SCS treatments. (A-C) The Y axis shows the concentrations of donors, recipients and transconjugants as $\log_{10}$(CFU/mL). Data are presented as the means±SDs (n=3). Multiple comparisons were evaluated using Duncan's multiple range test. The different uppercase letters above the bars or dots indicate the significance of differences between groups ($p<0.05$). (D) The

Y axis shows the number of transconjugants/donor (T/D). Each dot in the figure represents a replicate (n=3 for each group). Red diamonds with red lines represent the means±SDs (n=3). Ctrl: the group treated with fresh M9B medium (no SCS treatment), *B. sub*: *B. subtilis* natto, *B. coa*: *B. coagulans*, *C. but*: *C. butyricum*, *L. pla*: *L. plantarum*, *L. reu*: *L. reuteri*, *L. rha*: *L. rhamnosus*. "(a)" indicates aerobic culture.

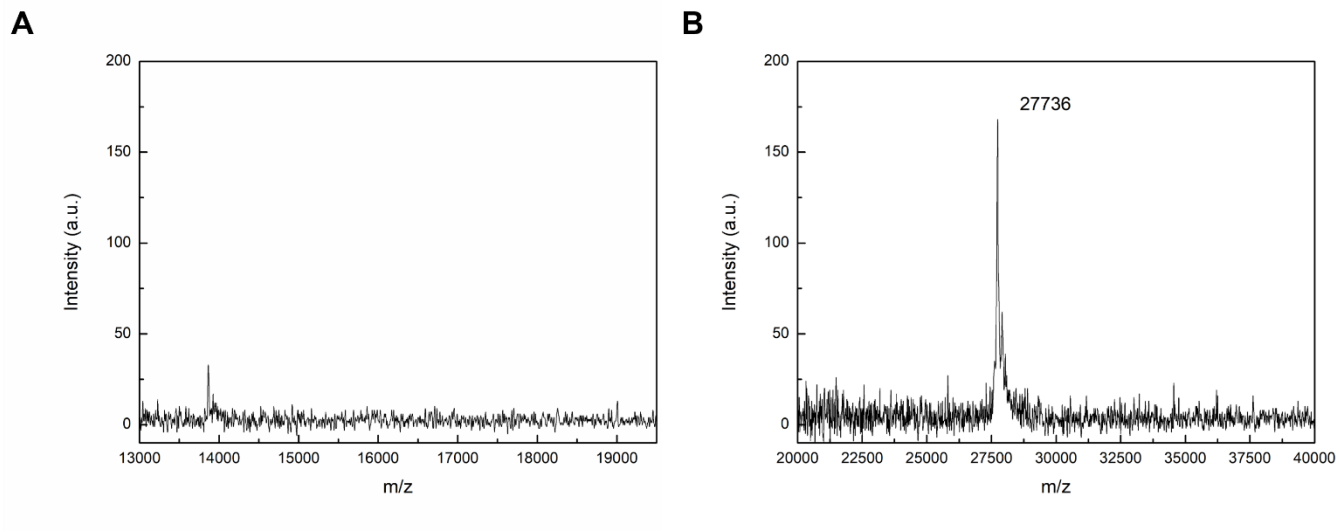

Fig. S3. Use of mass spectrometry to ensure the purity of nattokinase used in this study: (A) m/z between 13000 and 20000 and (B) m/z between 20000 and 40000. The m/z (27736) was similar to that of nattokinase (theoretical MW: 27698.69). The change in mass from 27736 to 27698.69 may result from modification of the protein with 3-chlorination (mass change: 34) or potassium (mass change: 36). In addition, as shown in the two mass spectra above, significant contamination with other proteins was not observed in our purified nattokinase.

TABLE S1 Mascot results for protein identification by peptide mass fingerprinting of the nattokinase tryptic digest analyzed using MALDI-TOF/TOF-MS

Mascot Search Results (Mascot: http://www.matrixscience.com/)

Protein View: ABM97611.1

Nattokinase, partial [*Bacillus subtilis*]

Database:	NCBIprot
Score:	1702
Monoisotopic mass (Mr):	27682
Calculated pI:	6.30
Taxonomy:	*Bacillus subtilis*
Search parameters	
Enzyme:	Trypsin
Variable modifications:	Carbamidomethyl (C), Oxidation (M)

Protein sequence coverage: 46%

Matched peptides are shown in ***bold red***.

1 **AQSVPYGISQ IKAPALHSQG YTGSNVKVAV IDSGIDSSHP DLNVR**GGASF

51 VPSETNPYQD GSSHGTHAAG TIAALNNSIG VLGVAPSASL YAVKVLDSTG

101 SGQYSWIING IEWAISNNMD VINMSLGGPT GSTALKTVVD K**AVSSGIVVA**

151 **AAAGNEGSSG STSTVGYPAK YPSTIAVGAV NSSNQR**ASFS SVGSELDVMA

201 PGVSIQSTLP GGTYGAYNGT SMATPHVAGA AALILSK**HPT WTNAQVRDRL**

251 **ESTATYLGNS FYYGKGLINV QAAAQ**

Query	Start – End	Observed	Mr (expt)	Mr (calc)	ppm	M	Score	Expect	Rank	U	Peptide
1851	1–12	645.8546	1289.6945	1289.6979	-2.63	0	57	9.2e-05	1	U	-.AQSVPYGISQIK.A
3191	13–27	765.3864	1528.7581	1528.7634	-3.43	0	85	2.5e-08	1		K.APALHSQGYTGSNVK.V
3194	13–27	510.5940	1528.7601	1528.7634	-2.19	0	28	0.0027	1		K.APALHSQGYTGSNVK.V
3196	13–27	765.3880	1528.7614	1528.7634	-1.27	0	54	1.8e-05	1		K.APALHSQGYTGSNVK.V
4966	28–45	947.4820	1892.9494	1892.9592	-5.17	0	45	6e-05	1		K.VAVIDSGIDSSHPDLNVR.G

4968	28–45	631.9916	1892.9531	1892.9592	-3.23	0	26	0.0059	1	K.VAVIDSGIDSSHPDLNVR.G
4969	28–45	947.4839	1892.9532	1892.9592	-3.16	0	106	1.1e-10	1	K.VAVIDSGIDSSHPDLNVR.G
4972	28–45	631.9919	1892.9538	1892.9592	-2.85	0	43	0.00042	1	K.VAVIDSGIDSSHPDLNVR.G
4973	28–45	631.9920	1892.9542	1892.9592	-2.66	0	29	0.0019	1	K.VAVIDSGIDSSHPDLNVR.G
4975	28–45	631.9922	1892.9549	1892.9592	-2.27	0	26	0.0038	1	K.VAVIDSGIDSSHPDLNVR.G
4977	28–45	631.9924	1892.9553	1892.9592	-2.08	0	40	0.00059	1	K.VAVIDSGIDSSHPDLNVR.G
4979	28–45	631.9924	1892.9555	1892.9592	-1.98	0	41	0.00056	1	K.VAVIDSGIDSSHPDLNVR.G
4980	28–45	947.4852	1892.9558	1892.9592	-1.81	0	32	0.00091	1	K.VAVIDSGIDSSHPDLNVR.G
4981	28–45	631.9926	1892.9560	1892.9592	-1.70	0	52	2.3e-05	1	K.VAVIDSGIDSSHPDLNVR.G
4982	28–45	631.9927	1892.9564	1892.9592	-1.49	0	32	0.0039	1	K.VAVIDSGIDSSHPDLNVR.G
4984	28–45	631.9931	1892.9575	1892.9592	-0.92	0	30	0.0045	1	K.VAVIDSGIDSSHPDLNVR.G
4985	28–45	631.9931	1892.9575	1892.9592	-0.92	0	30	0.0016	1	K.VAVIDSGIDSSHPDLNVR.G
4988	28–45	631.9935	1892.9588	1892.9592	-0.24	0	32	0.0033	1	K.VAVIDSGIDSSHPDLNVR.G
4989	28–45	631.9938	1892.9597	1892.9592	0.25	0	49	3.5e-05	1	K.VAVIDSGIDSSHPDLNVR.G
7063	142 – 170	1298.1433	2594.2721	2594.2824	-3.98	0	120	1.3e-10	1	K.AVSSGIVVAAAAGNEGSSGSTSTV GYPAK.Y
7064	142 – 170	1298.1434	2594.2723	2594.2824	-3.89	0	76	3.5e-06	1	K.AVSSGIVVAAAAGNEGSSGSTST VGYPAK.Y
7066	142 – 170	865.7648	2594.2727	2594.2824	-3.75	0	67	2.8e-05	1	K.AVSSGIVVAAAAGNEGSSGSTST VGYPAK.Y
7067	142 – 170	1298.1438	2594.2730	2594.2824	-3.60	0	83	6.8e-07	1	K.AVSSGIVVAAAAGNEGSSGSTST VGYPAK.Y
7069	142 – 170	649.5759	2594.2744	2594.2824	-3.09	0	37	0.00078	2	K.AVSSGIVVAAAAGNEGSSGSTST VGYPAK.Y
7071	142 – 170	865.7654	2594.2745	2594.2824	-3.05	0	53	0.00067	1	K.AVSSGIVVAAAAGNEGSSGSTST VGYPAK.Y
7072	142 – 170	865.7654	2594.2745	2594.2824	-3.05	0	54	0.00052	1	K.AVSSGIVVAAAAGNEGSSGSTSTV GYPAK.Y
7078	142 – 170	865.7659	2594.2758	2594.2824	-2.55	0	47	0.0028	1	K.AVSSGIVVAAAAGNEGSSGSTST VGYPAK.Y
7081	142 – 170	865.7664	2594.2773	2594.2824	-1.98	0	68	2.4e-05	1	K.AVSSGIVVAAAAGNEGSSGSTSTV GYPAK.Y
7082	142 – 170	865.7664	2594.2774	2594.2824	-1.91	0	46	0.0039	1	K.AVSSGIVVAAAAGNEGSSGSTSTV

										GYPAK.Y
3852	171 – 186	832.4206	1662.8266	1662.8325	-3.55	0	71	3.6e-07	1	K.YPSTIAVGAVNSSNQR.A
3853	171 – 186	555.2828	1662.8267	1662.8325	-3.51	0	54	0.0002	1	K.YPSTIAVGAVNSSNQR.A
3854	171 – 186	832.4206	1662.8267	1662.8325	-3.48	0	108	1.7e-10	1	K.YPSTIAVGAVNSSNQR.A
3857	171 – 186	832.4209	1662.8272	1662.8325	-3.18	0	81	1.2e-07	1	K.YPSTIAVGAVNSSNQR.A
3858	171 – 186	832.4209	1662.8272	1662.8325	-3.18	0	97	1.2e-08	1	K.YPSTIAVGAVNSSNQR.A
3861	171 – 186	832.4214	1662.8282	1662.8325	-2.59	0	58	1.1e-05	1	K.YPSTIAVGAVNSSNQR.A
3862	171 – 186	832.4214	1662.8282	1662.8325	-2.59	0	101	1.1e-09	1	K.YPSTIAVGAVNSSNQR.A
3865	171 – 186	555.2835	1662.8287	1662.8325	-2.31	0	38	0.0051	1	K.YPSTIAVGAVNSSNQR.A
3866	171 – 186	555.2836	1662.8289	1662.8325	-2.20	0	39	0.0019	1	K.YPSTIAVGAVNSSNQR.A
3867	171 – 186	832.4218	1662.8291	1662.8325	-2.08	0	60	2.3e-06	1	K.YPSTIAVGAVNSSNQR.A
3868	171 – 186	832.4219	1662.8292	1662.8325	-2.00	0	69	2.8e-06	1	K.YPSTIAVGAVNSSNQR.A
3869	171 – 186	832.4219	1662.8292	1662.8325	-2.00	0	69	5.1e-07	1	K.YPSTIAVGAVNSSNQR.A
3871	171 – 186	832.4220	1662.8294	1662.8325	-1.86	0	83	6.7e-08	1	K.YPSTIAVGAVNSSNQR.A
3872	171 – 186	832.4221	1662.8296	1662.8325	-1.78	0	83	3.4e-07	1	K.YPSTIAVGAVNSSNQR.A
3874	171 – 186	832.4221	1662.8297	1662.8325	-1.71	0	89	1.3e-08	1	K.YPSTIAVGAVNSSNQR.A
3875	171 – 186	832.4221	1662.8297	1662.8325	-1.71	0	76	3.2e-07	1	K.YPSTIAVGAVNSSNQR.A
3876	171 – 186	832.4223	1662.8300	1662.8325	-1.50	0	70	2.7e-07	1	K.YPSTIAVGAVNSSNQR.A
3880	171 – 186	832.4228	1662.8310	1662.8325	-0.91	0	55	1.3e-05	1	K.YPSTIAVGAVNSSNQR.A
3881	171 – 186	832.4228	1662.8310	1662.8325	-0.91	0	77	6.4e-08	1	K.YPSTIAVGAVNSSNQR.A
3884	171 – 186	832.4240	1662.8335	1662.8325	0.56	0	73	7.3e-06	1	K.YPSTIAVGAVNSSNQR.A
1459	238 – 247	605.3079	1208.6012	1208.6051	-3.22	0	46	0.0004	1	K.HPTWTNAQVR.D
5653	248 – 265	1042.9965	2083.9784	2083.9851	-3.21	1	78	4.7e-08	1	R.DRLESTATYLGNSFYYGK.G
5655	248 – 265	695.6673	2083.9801	2083.9851	-2.39	1	28	0.0026	1	R.DRLESTATYLGNSFYYGK.G
5656	248 – 265	695.6673	2083.9801	2083.9851	-2.39	1	31	0.0014	1	R.DRLESTATYLGNSFYYGK.G
5657	248 – 265	695.6675	2083.9806	2083.9851	-2.14	1	39	0.00083	1	R.DRLESTATYLGNSFYYGK.G
5658	248 – 265	695.6675	2083.9806	2083.9851	-2.14	1	57	5.4e-06	1	R.DRLESTATYLGNSFYYGK.G
5661	248 – 265	695.6676	2083.9810	2083.9851	-1.96	1	50	2.2e-05	1	R.DRLESTATYLGNSFYYGK.G
5662	248 – 265	695.6677	2083.9812	2083.9851	-1.88	1	36	0.00038	1	R.DRLESTATYLGNSFYYGK.G
5664	248 – 265	695.6678	2083.9815	2083.9851	-1.69	1	25	0.0047	1	R.DRLESTATYLGNSFYYGK.G

5666	248 – 265	695.6678	2083.9817	2083.9851	-1.60	1	44	6.9e-05	1	R.DRLESTATYLGNSFYYGK.G
5668	248 – 265	695.6680	2083.9821	2083.9851	-1.43	1	44	7.1e-05	1	R.DRLESTATYLGNSFYYGK.G
5669	248 – 265	695.6681	2083.9824	2083.9851	-1.26	1	24	0.0053	1	R.DRLESTATYLGNSFYYGK.G
5670	248 – 265	1042.9985	2083.9825	2083.9851	-1.22	1	51	1.6e-05	1	R.DRLESTATYLGNSFYYGK.G
4614	250 – 265	907.4330	1812.8515	1812.8570	-3.02	0	80	3.3e-08	1	R.LESTATYLGNSFYYGK.G
4615	250 – 265	605.2918	1812.8536	1812.8570	-1.88	0	24	0.0055	1	R.LESTATYLGNSFYYGK.G
519	266 – 275	492.7756	983.5366	983.5400	-3.39	0	37	0.0037	1	U K.GLINVQAAAQ.-
524	266 – 275	492.7761	983.5377	983.5400	-2.33	0	49	0.00021	1	U K.GLINVQAAAQ.-
526	266 – 275	492.7761	983.5377	983.5400	-2.33	0	40	0.0021	1	U K.GLINVQAAAQ.-
528	266 – 275	492.7762	983.5379	983.5400	-2.06	0	36	0.0057	1	U K.GLINVQAAAQ.-
530	266 – 275	492.7763	983.5381	983.5400	-1.88	0	34	0.0056	1	U K.GLINVQAAAQ.-
532	266 – 275	492.7765	983.5384	983.5400	-1.64	0	31	0.0041	1	U K.GLINVQAAAQ.-

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