

Comparative genome analysis of the immunomodulatory ability of *Lactiplantibacillus plantarum* and *Lactiplantibacillus pentosus* from Japanese pickles

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Fig. S1. Taxonomy identification for two species. Strains' name in pink are local *L. plantarum* strains, and strains' name in green are local *L. pentosus* strain used in this study. Highly supported nodes are indicated with a closed circle and actual value (bootstrap support value $\geq 50\%$).

green: *L. plantarum* pink: *L. pentosus*

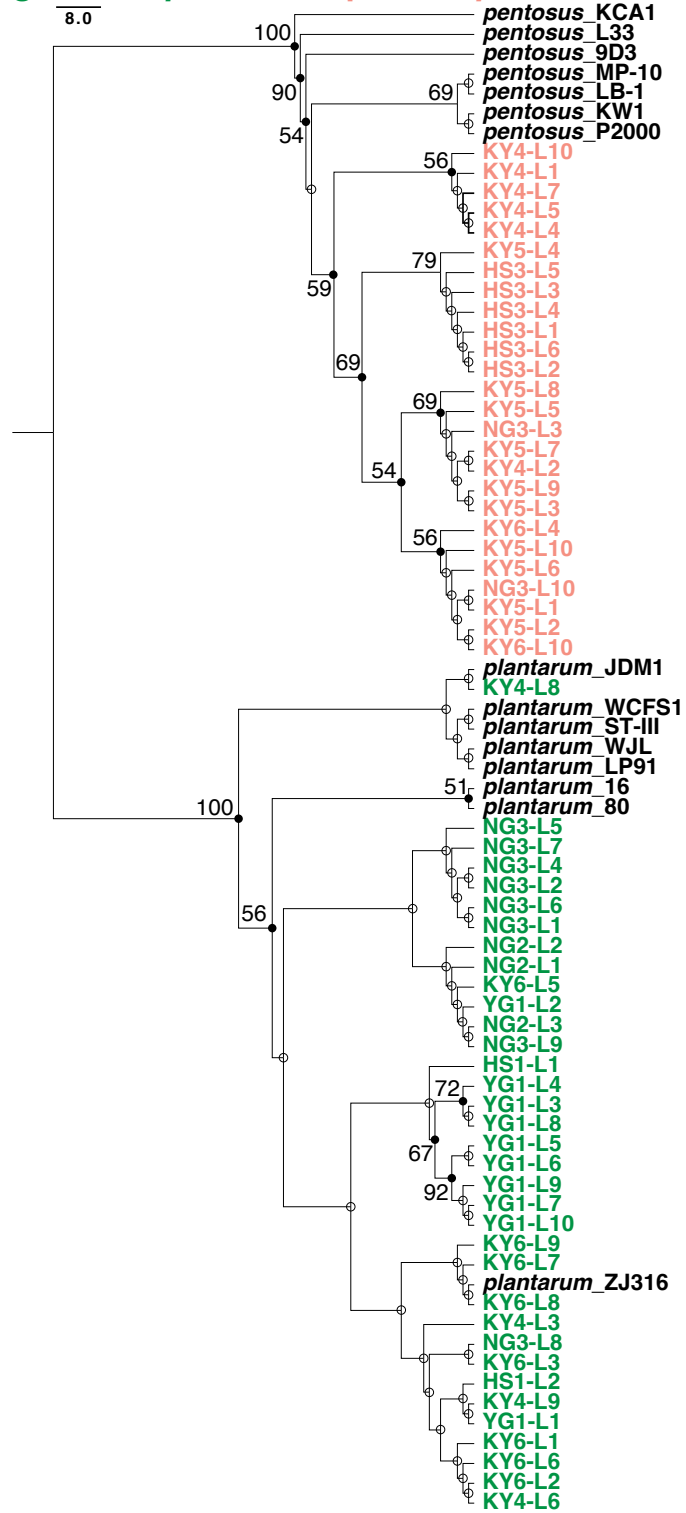


Fig. S2. Pairwise average nucleotide identity (ANI) and tetranucleotide frequency (TETRA). (A) and (C) ANI value computed for two species; (B) and (D) TETRA value computed for two species.

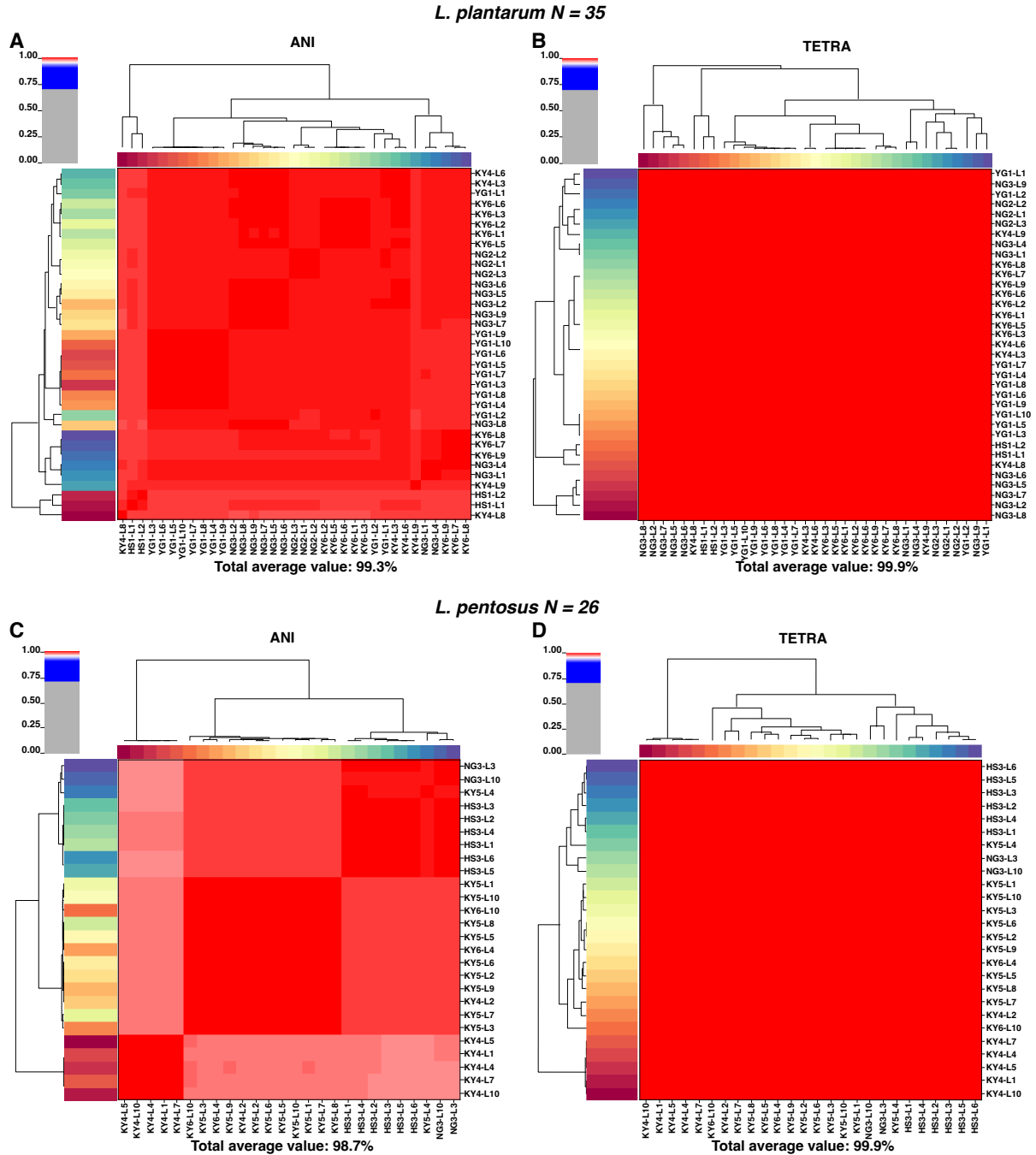


Fig. S3. Genomic statistics of two species. (A) *L. plantarum*; (B) *L. pentosus*.

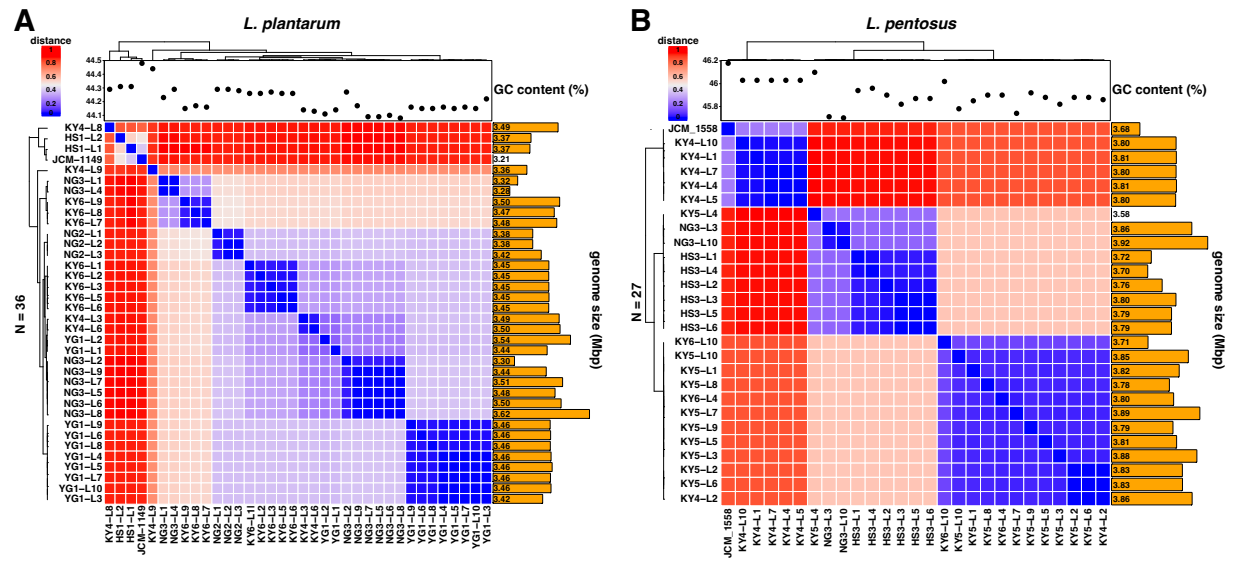


Fig. S4. Orthologous gene group composition varied across strains. (A) *L. plantarum*. (B) *L. pentosus*.

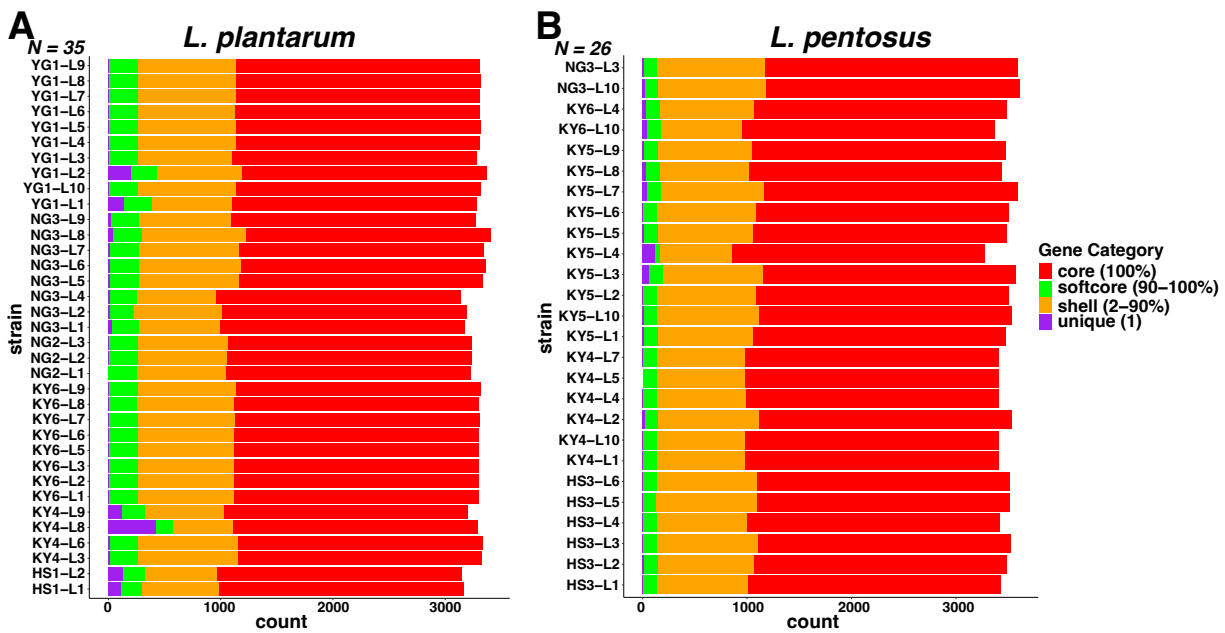


Fig. S5. Genetic distance analysis of sub-potential immunomodulation-related genes. (A) and (B) are genetic distance of *group_3159* shown in heatmaps and MDS analysis plot (n = 8); (D) and (E) are genetic distance of *group_511* shown in heatmaps and MDS analysis plot (n = 8). (C) and (F) are phylogenetic tree of *group_3159* and *group_511* based on their AA sequences, respectively. The number of strains that harbour the correspondent potential genes were indicated in 'n'. Highly supported nodes are indicated with a closed circle and actual value (bootstrap support value $\geq 50\%$). Red point marked the IL-10 stimulation active strain, and blue point marked the IL-12 stimulation active strain. Clusters were shown in red or blue color to represent IL-10 or IL-12 activity.

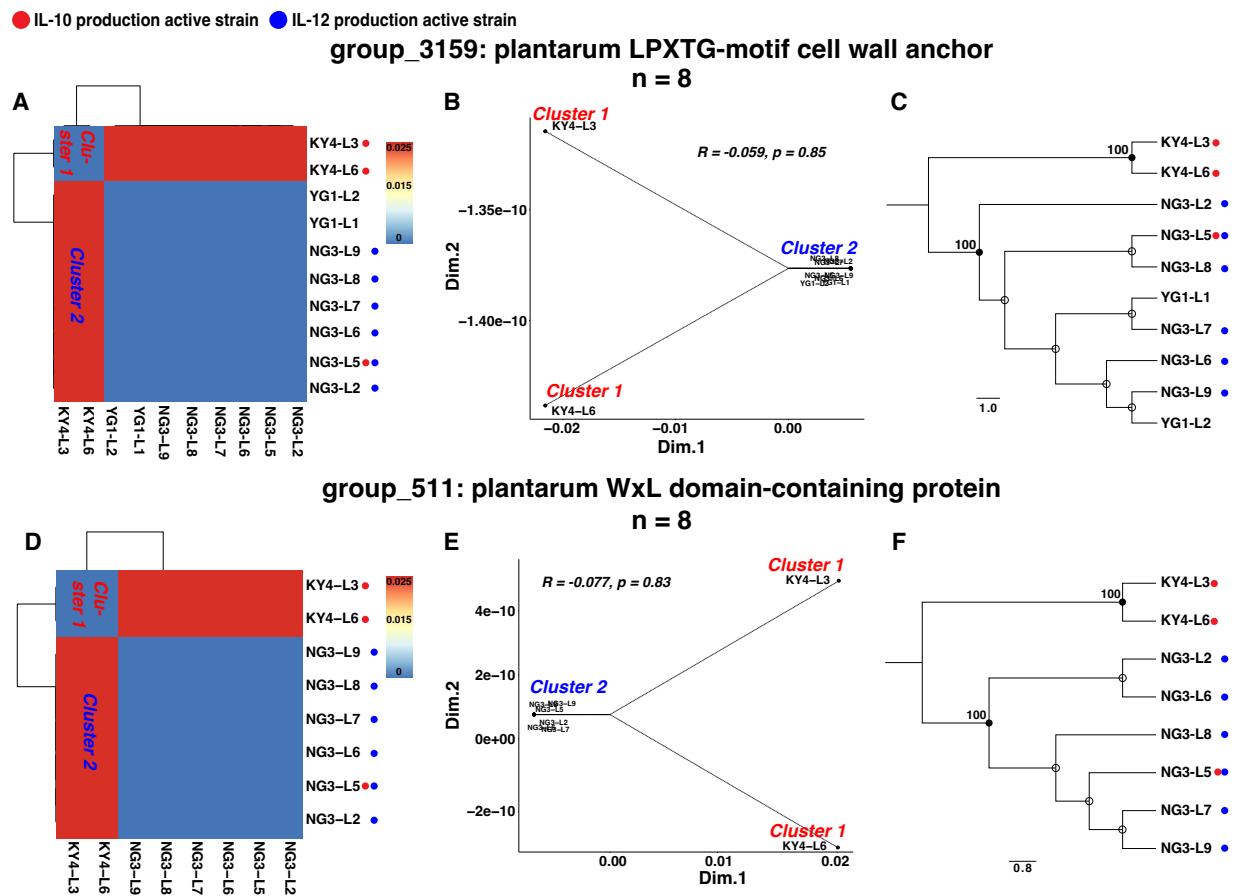


Fig. S6. *TagD-TagF1-TagF2* locus conserved by *group_1590*-possessing strains in cluster 2 (A) and cluster 1 (B) of *L. plantarum*.

■ TagD-F1-F2 locus genes □ neighboring genes

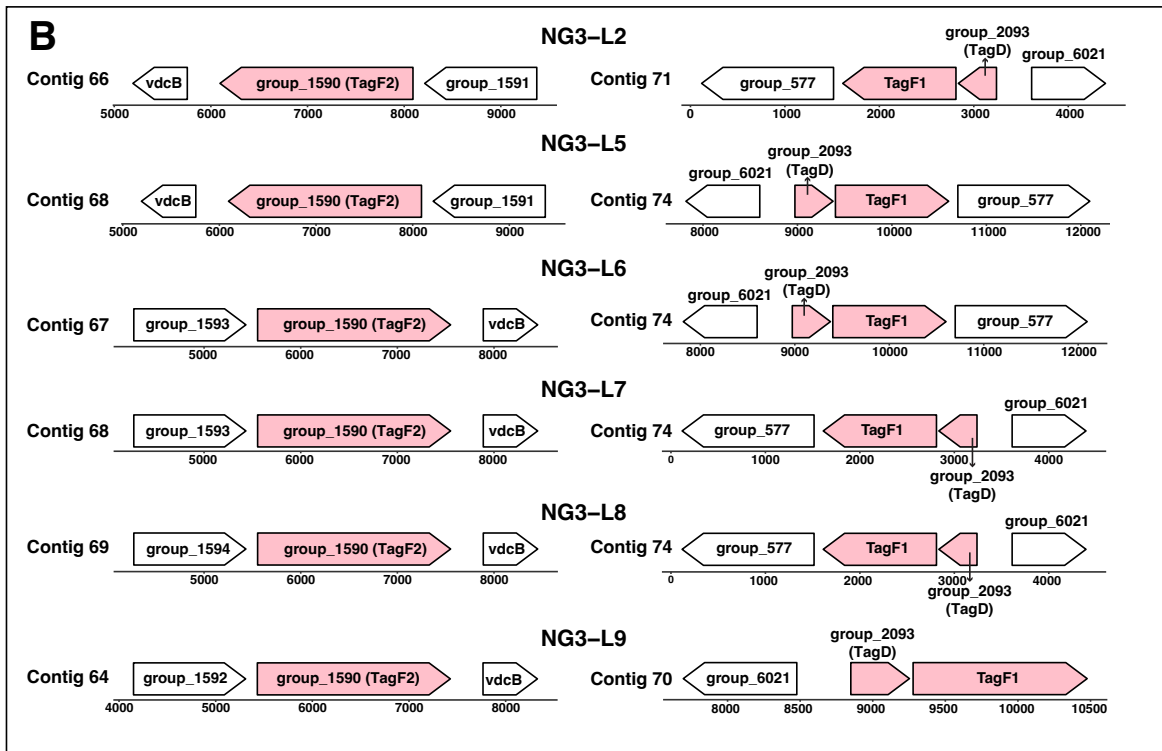
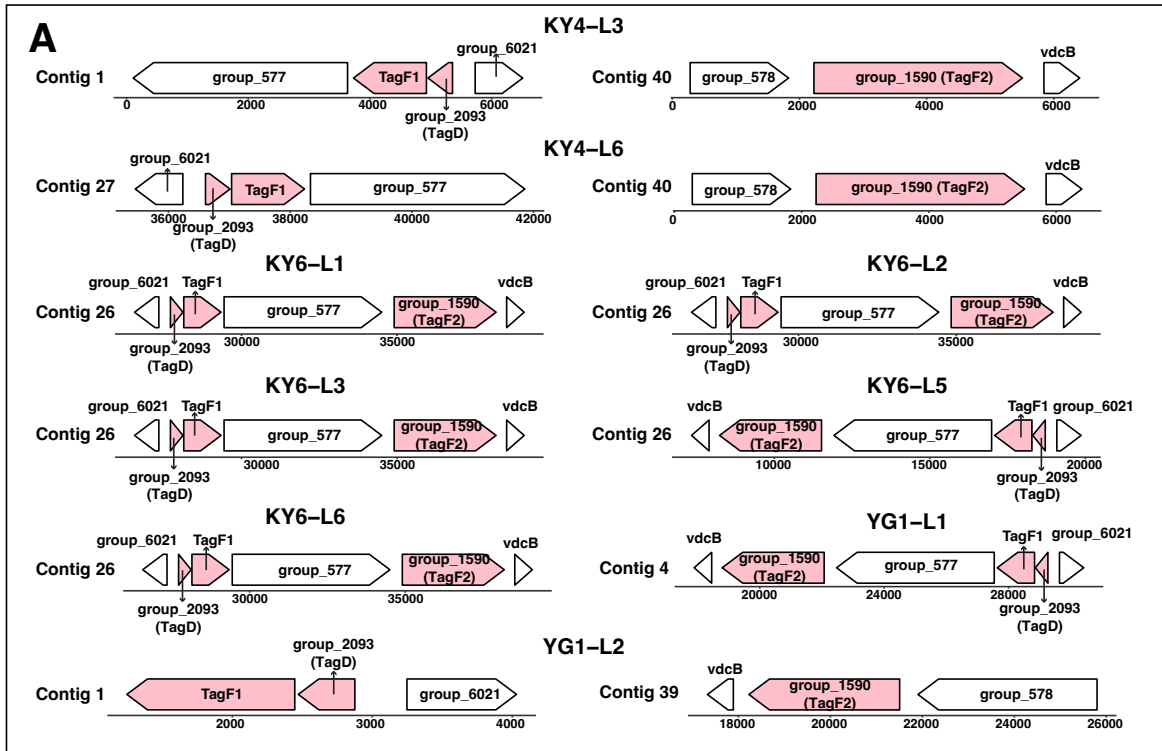
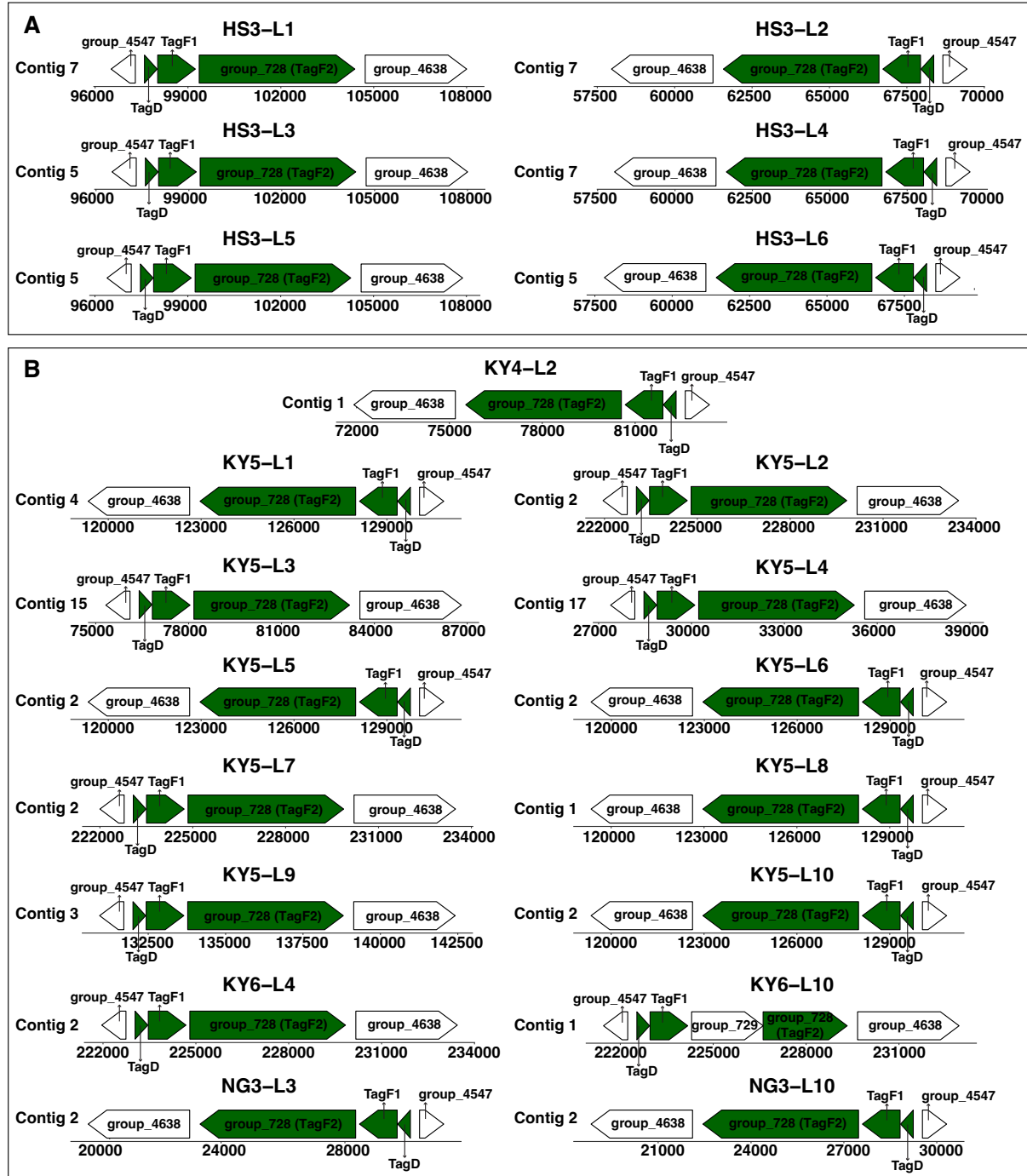


Fig. S7. *TagD-TagF1-TagF2* locus conserved by *group_728*-possessing strains in cluster 2 (A) and cluster 1 (B) of *L. pentosus*.

■ **TagD–F1–F2 locus genes** □ **neighboring genes**



wta genes ▶ **neighboring genes** ◻

KY4-L3

Contig 11 group.1123 TagE bmrA
15000 14000 15000 16000 17000 18000
petS1.2
Contig 3 TagO kimA
68800 69000 70000 71000
Contig 33 pncB2 TagA nagR
17000 18000 19000
group.2107
Contig 4 TagU.1 ssuB.1
112000 113000 114000
Contig 72 TagO.3 group.3305
1000 1500 2000 2500 3000

KY4-L6

Contig 24 bluO.6 TagG group.1930
34000 35000 36000 37000
group.5760
Contig 11 group.1123 TagE bmrA
15000 14000 15000 16000 17000 18000
group.5760
Contig 2 TagU.3 eccA.2
128000 129000 130000 131000
Contig 34 pncB2 TagA nagR
17000 18000 19000
group.2107
Contig 4 TagU.1 ssuB.1
112000 113000 114000
Contig 73 TagO.3 group.3305
1000 1500 2000 2500 3000

KY6-L1

Contig 14 group.1930 TagO bluO.6
29000 30000 31000 32000
Contig 15 TagU.3 eccA.2
41000 42000 43000 44000
group.5760
Contig 15 pncB2 TagA nagR
67000 68000 69000 70000
group.2107
Contig 3 group.1029 TagU.1
32000 33000 34000 35000
Contig 5 bmrA TagE macB.2
61000 62000 63000 64000 65000 66000
Contig 8 TagU.2
84000 85000 86000 87000 88000 89000

KY6-L2

Contig 14 group.1930 TagO bluO.6
29000 30000 31000 32000
Contig 15 pncB2 TagA nagR
67000 68000 69000 70000
group.2107
Contig 3 group.1029 TagU.1
32000 33000 34000 35000
Contig 5 bmrA TagE macB.2
61000 62000 63000 64000 65000 66000
Contig 8 TagU.2
84000 85000 86000 87000 88000 89000

KY6-L3

Contig 14 group.1930 TagO bluO.6
29000 30000 31000 32000
group.5760
Contig 15 nagR TagA pncB2
11000 12000 13000 14000
Contig 15 TagU.3
35000 36000 37000 38000
group.5760
Contig 3 TagU.1
107000 108000 109000 110000
Contig 5 bmrA TagE macB.2
61000 62000 63000 64000 65000 66000
Contig 8 TagU.2
84000 85000 86000 87000 88000 89000

KY6-L5

Contig 14 group.1930 TagO bluO.6
29000 30000 31000 32000
group.5760
Contig 15 nagR TagA pncB2
11000 12000 13000 14000
Contig 15 TagU.3
35000 36000 37000 38000
group.5760
Contig 3 group.1029 TagU.1
32000 33000 34000 35000
Contig 5 macB.2 TagE bmrA
71000 72000 73000 74000 75000
Contig 8 TagU.2
84000 85000 86000 87000 88000 89000

KY6-L6

Contig 14 group.1930 TagO bluO.6
29000 30000 31000 32000
Contig 15 nagR TagA pncB2
11000 12000 1300

Fig. S9. The rest genes involved in poly (grop) WTA biosynthesis conserved by group_728-possessing strains of *L. pentosus*.

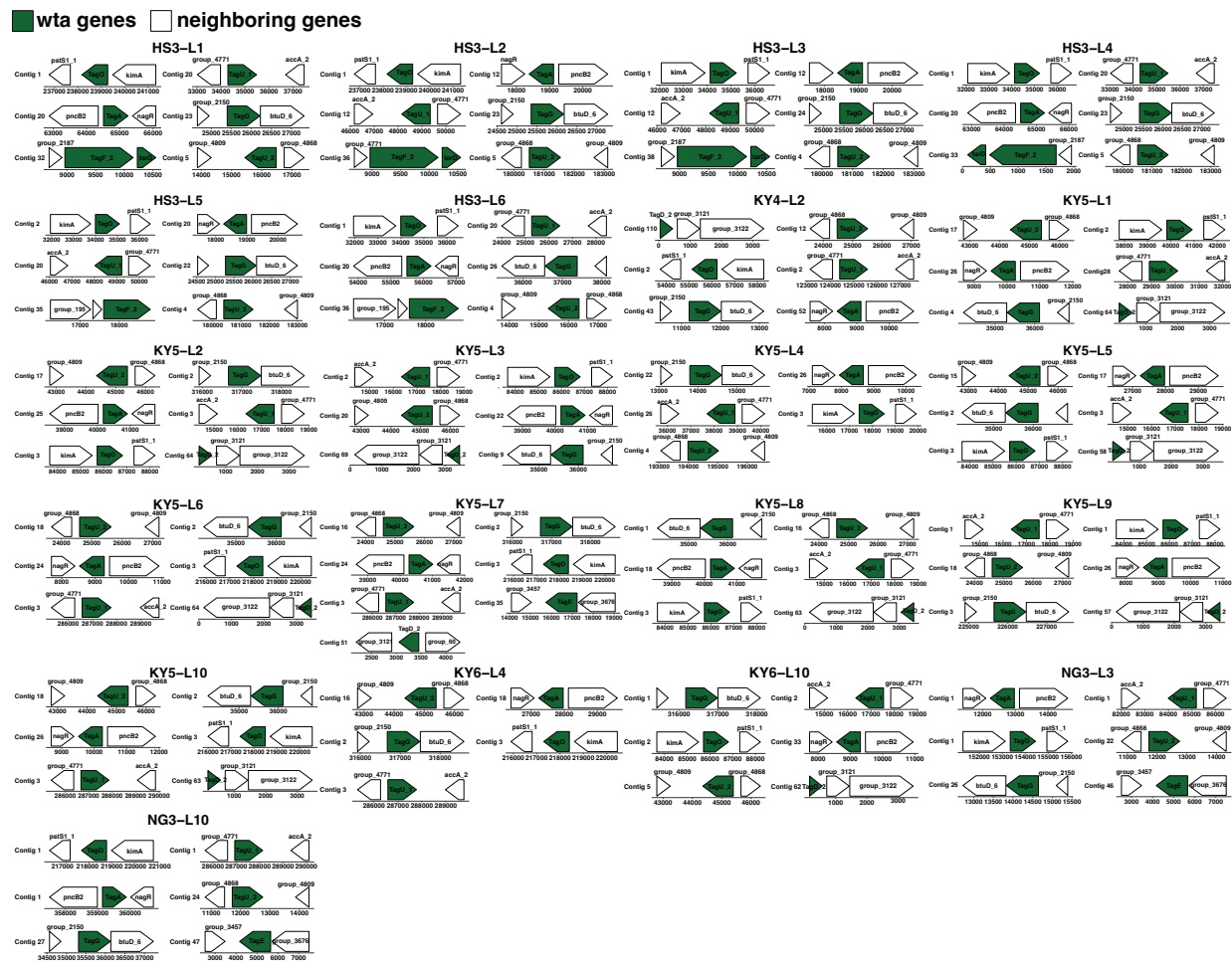


Fig. S10. Threshold-based filtration of OGs across two species. (A) IL-10-inducing comparative analysis of *L. plantarum*. (B) IL-12-inducing comparative analysis of *L. plantarum*. (C) IL-10-inducing comparative analysis of *L. pentosus*. (D) IL-12-inducing comparative analysis of *L. pentosus*. The threshold for number of active and silent strains in correspondent group was calculated as follows: $n(\text{active strains}) \geq 80\% * N(\text{active strains})$ & $n(\text{silent strains}) \leq 50\% * N(\text{silent strains})$. 'N (active strains)' and 'N (silent strains)' are total number of strains in active or silent group. 'n(active strains)' and 'n(silent strains)' are number of strains in each OG.

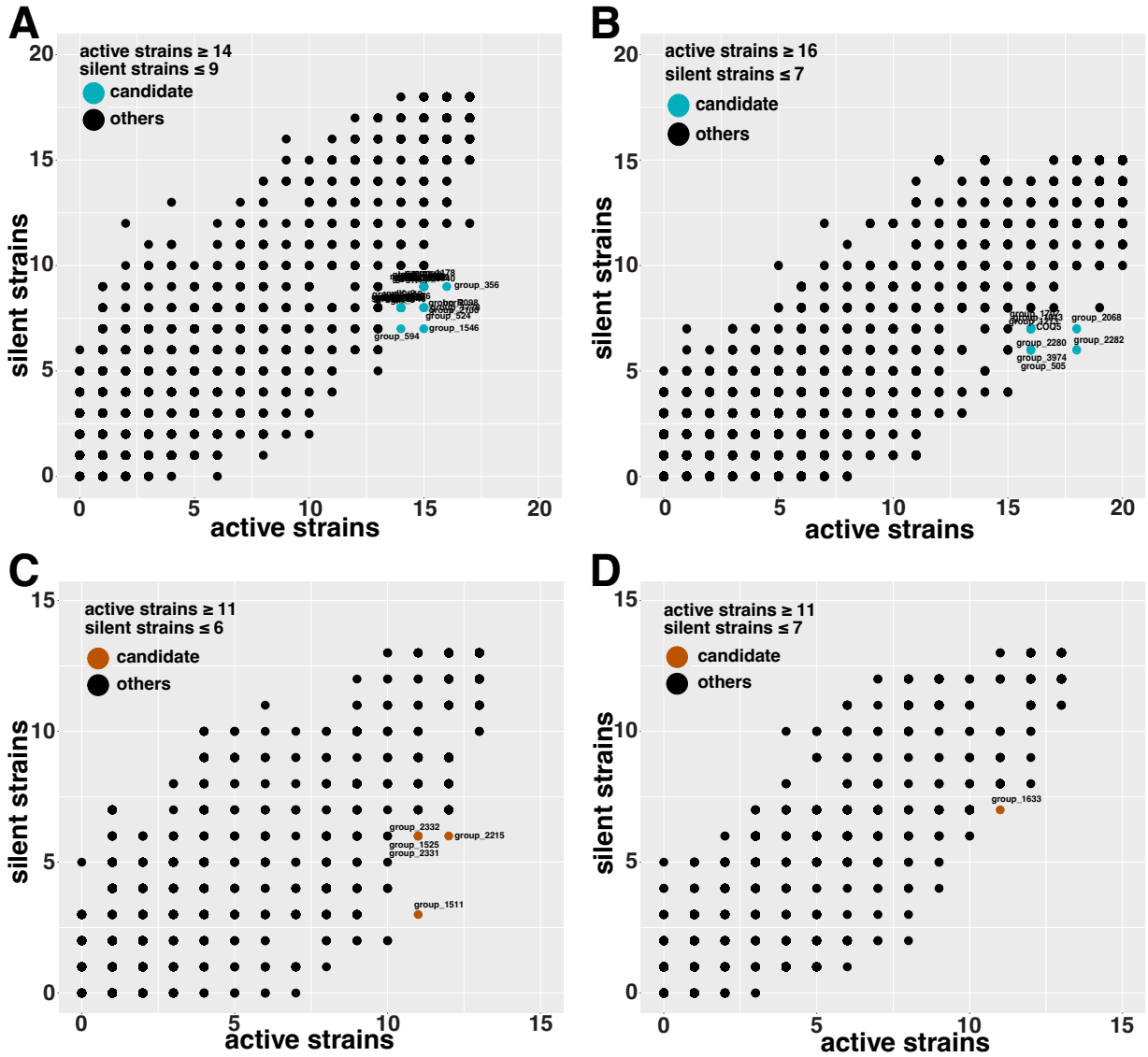


Table S1 IL-10 and IL-12 average production exhibited by active local strains across species.

	IL-10 active strains	active strains avg IL-10 concn (pg/ml)	IL-12 active strains	active strains avg IL-12 concn (pg/ml)
<i>L. plantarum</i>	17	7.38	20	43.43
<i>L. pentosus</i>	13	13.00	13	77.98

Table S2. *L. plantarum* IL-10 and IL-12 production exhibited by strains.

<i>L. plantarum</i>	IL-10 trend	IL-10 concn (pg/ml)	IL-12 trend	IL-12 concn (pg/ml)
HS1-L1	silent	0.00	silent	0.00
HS1-L2	silent	0.00	silent	0.00
KY4-L3	active	11.60	silent	0.00
KY4-L6	active	16.18	silent	0.00
KY4-L8	active	11.49	silent	0.00
KY4-L9	active	6.73	active	29.21
KY6-L1	silent	0.00	active	12.20
KY6-L2	active	4.17	silent	0.00
KY6-L3	active	8.06	active	5.41
KY6-L5	active	5.60	silent	0.00
KY6-L6	active	3.83	active	2.70
KY6-L7	active	4.59	silent	0.00
KY6-L8	active	4.07	active	2.75
KY6-L9	silent	0.00	active	71.18
NG2-L1	silent	0.00	silent	0.00
NG2-L2	silent	0.00	silent	0.00
NG2-L3	silent	0.00	active	21.16
NG3-L1	active	7.28	active	44.07
NG3-L2	silent	0.00	active	122.69
NG3-L4	silent	0.00	active	177.92
NG3-L5	active	4.90	active	23.11
NG3-L6	silent	0.00	active	37.74
NG3-L7	silent	0.00	active	84.51
NG3-L8	silent	0.00	active	171.49
NG3-L9	silent	0.00	active	38.71
YG1-L1	silent	0.00	silent	0.00
YG1-L2	silent	0.00	silent	0.00
YG1-L3	active	8.06	silent	0.00
YG1-L4	active	5.41	active	2.76
YG1-L5	active	6.81	silent	0.00
YG1-L6	active	4.50	active	3.68
YG1-L7	active	4.21	active	5.83
YG1-L8	silent	0.00	silent	0.00
YG1-L9	silent	0.00	active	5.53
YG1-L10	silent	0.00	active	5.83
JCM-1149	silent	0.00	active	3.81

Table S3. *L. pentosus* IL-10 and IL-12 production exhibited by strains.

<i>L. pentosus</i>	IL-10 trend	IL-10 concn (pg/ml)	IL-12 trend	IL-12 concn (pg/ml)
HS3-L1	active	15.67	active	38.27
HS3-L2	active	19.58	silent	0.00
HS3-L3	silent	0.00	silent	0.00
HS3-L4	silent	0.00	silent	0.00
HS3-L5	silent	0.00	silent	0.00
HS3-L6	active	2.42	active	5.55
KY4-L1	silent	0.00	active	217.79
KY4-L2	silent	0.00	active	187.91
KY4-L4	active	6.81	active	176.69
KY4-L5	silent	0.00	active	176.41
KY4-L7	silent	0.00	active	20.55
KY4-L10	silent	0.00	silent	0.00
KY5-L1	active	3.70	active	5.59
KY5-L2	silent	0.00	active	3.44
KY5-L3	silent	0.00	active	32.30
KY5-L4	active	13.24	silent	0.00
KY5-L5	active	10.95	silent	0.00
KY5-L6	active	22.00	silent	0.00
KY5-L7	active	8.48	silent	0.00
KY5-L8	active	12.56	silent	0.00
KY5-L9	active	48.11	silent	0.00
KY5-L10	active	3.23	silent	0.00
KY6-L4	active	2.34	active	24.59
KY6-L10	silent	0.00	active	118.78
NG3-L3	silent	0.00	silent	0.00
NG3-L10	silent	0.00	active	5.94
JCM-1558	silent	0.00	silent	0.00

Table S4. *L. plantarum* IL-10 and IL-12 production exhibited by prefectures.

Prefecture	strain number	IL-10 active	active strain avg IL-10 concn (pg/ml)	IL-12 active	active strain avg IL-12 concn (pg/ml)
Hiroshima	2	0	0.00	0	0.00
Kyoto	12	10	7.63	6	20.58
Nagano	11	2	6.09	9	80.16
Yamagata	10	5	5.80	5	4.73

Table S5. *L. pentosus* IL-10 and IL-12 production exhibited by prefectures.

Prefecture	total number of strains	IL-10 active strain	avg IL-10 concn by active strain(pg/ml)	IL-12 active strain	avg IL-12 concn by active strain(pg/ml)
Hiroshima	6	3	12.56	2	21.91
Kyoto	18	10	13.14	10	96.40
Nagano	2	0	0.00	1	5.94

Table S10. 31 OGs filtered from IL-10-inducing group comparison of *L. plantarum*. OGs annotation colored in red are probiotic marker genes.

Gene	annotation
bcrR	helix-turn-helix domain-containing protein
bglA	3_6-phospho-beta-glucosidase
group_356	glycosyltransferase
group_524	LysM-peptidoglycan-binding domain-containing protein
group_594	TetR/AcrR family transcriptional regulator
group_1177	hypothetical protein
group_1178	hypothetical protein
group_1518	hypothetical protein
group_1546	terminase TerL endonuclease subunit
group_1778	phage portal protein
group_1781	HK97 gp10 family phage protein
group_1837	YxeA family protein
group_1840	WxL domain-containing protein
group_2076	PTS cellobiose transporter subunit IIC
group_2077	hypothetical protein
group_2098	hypothetical protein
group_2100	P27 family phage terminase small subunit
group_2485	major tail protein
group_2547	sodium:proton antiporter
group_2549	TetR/AcrR family transcriptional regulator
group_2940	hypothetical protein
group_2941	4-carboxymuconolactone decarboxylase
group_2942	MerR family transcriptional regulator
group_2944	helix-turn-helix domain-containing protein
group_3440	DUF805 domain-containing protein
hdfR_5	transcriptional regulator
lepA_2	translation elongation factor 4
nudC	NAD(+) diphosphatase
smc_8	CAP domain-containing protein
yknY	ABC transporter ATP-binding protein
yydK_3	GntR family transcriptional regulator

Table S11. 9 OGs filtered from IL-12-inducing group comparison of *L. plantarum*. OGs annotation colored in red are probiotic marker genes.

Gene	annotation
COQ5	class I SAM-dependent methyltransferase, partial
group_505	PTS transporter subunit EIIC
group_1013	Gp15 family bacteriophage protein
group_1273	restriction endonuclease
group_1707	hypothetical protein
group_2068	phage tail domain-containing protein
group_2280	putative minor capsid protein
group_2282	ImmA/IrrE family metallo-endopeptidase
group_3974	hypothetical protein

Table S12. 5 OGs filtered from IL-10-inducing group comparison of *L. pentosus*. OGs annotation colored in red are probiotic marker genes.

Gene	annotation
group_1511	hypothetical protein
group_1525	nicotinate phosphoribosyltransferase
group_2215	type II toxin-antitoxin system Phd/YefM family antitoxin
group_2331	hypothetical protein
group_2332	hypothetical protein

Table S13. One OGs filtered from IL-12-inducing group comparison of *L. pentosus*. OGs annotation colored in red are probiotic marker genes.

Gene	annotation
group_1633	hypothetical protein