

Supplementary Material

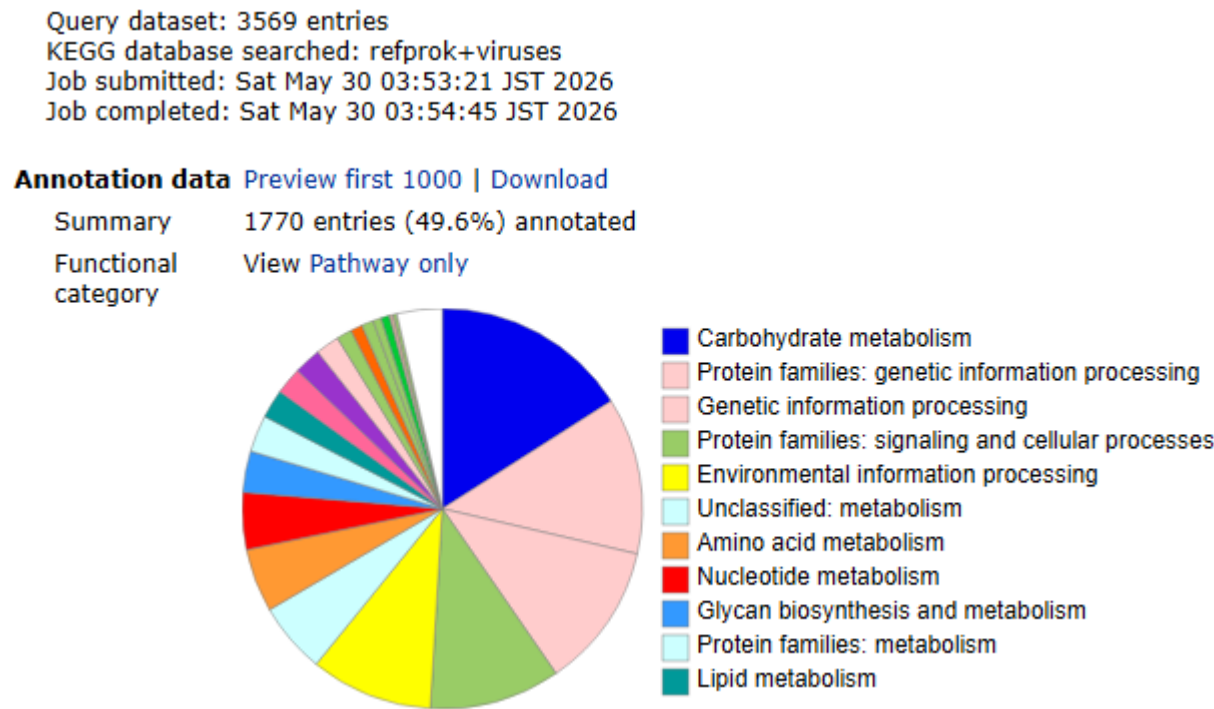


Figure S1 Schematic representation of the main metabolic pathways of the *L. rhamnosus* proteome, derived using KEGG GhostKOALA.

A Database hit with Enterocin_X_chain_beta (Bit Score=50.0618)	
Query	MTKLNE---AELSKISGGIGFLAIFVAAILGFLATDAWSHADELVAGVKQWE M K NE EL +I GGI P+ + A LG+L DAW H+D++++G R+GW
Enterocin_X_chain_beta	MKKYNELSKKELLQIOGGIAP---IVAGLGVLKDAWDHSDQIISGFKKGWNGGRRK
Organism	Enterococcus faeciumKU-85
Literature	Reference
B Database hit with LSEI_2386 (Bit Score=37.7354)	
Query	MKKFKDEEMPMTDLELSSFVGG-MLRDEGFTFNKFNWLTISF MK+F +++M M+D EL F+GG +RD TFNK + W +F
LSEI_2386	MKQFDEQKMVNMSDEELLGFIGGDSIRDVSPTFNKIRRFDFGLFK
Organism	Lactobacillus casei ATCC334
Literature	Reference
C Database hit with Carnocin_CP52 (Bit Score=73.559)	
Query	MVAGSIVLGGTVMSDRKQALFEMLSKAYSSPAVKADAKLKQIIESNAKQLEDAEDKTAIVAVVTQLSHDISKYYLIH-HTIPAEMIAVFNAIKADVFPADQVNAKRYRDQALAGLIAPIVW M K Q L+ LS+AY P VKA+ L +++ A +L + + +Y+ V++ LS IS YYL H IP+ M+ ++ I+ D+ ++ ++ R +A GL+++P ++
Carnocin_CP52	MDIRSQTLVNLNLEAYKDPEVKANEFSLKLVQCAGKLTASNSENSEYIEVISLLSRGISSYYLSHKRIIPSSMLTIYTIQKIDIKNGNIDTEKLKRYEIAKGLMSVFPYIY
Subclass	A
Organism	Carnobacterium piscicola
UniProt	P38582.1

Figure S2 BLAST hits for the Enterocin-X, LSEI_2386 and Carnocin_CP52 bacteriocin sequences against the KEGG GENES database (a non-redundant subset of KEGG gene/protein sequences).

Table S1 Condensed gene table of the BAGEL4-identified Class II bacteriocin gene cluster in *Lactobacillus rhamnosus* 20-12 (AOI: contig_58.14.AOI_01).

Name	Function	Motifs
orf00001		
orf00003	hypothetical protein pMRC01_047	
orf00006		
orf00008		
orf00012		
orf00014	NADH-specific methylglyoxal reductase OS=Escherichia coli (strain K12) OX=83333 GN=ydjG PE=1 SV=1	
orf00016	Aldo-keto reductase IolS OS=Bacillus subtilis (strain 168) OX=224308 GN=iolS PE=1 SV=1	
orf00017		
orf00018	Inner membrane protein YjjP OS=Shigella flexneri OX=623 GN=yjjP PE=3 SV=1	
orf00021		
HlyD	Accessory factor for ABC-transporter PlnH	PF13437
HlyD	AbpD bacteriocin export accessory protein	PF13437
LanT	Transport/processing ATP-binding protein ComA OS=Streptococcus pneumoniae (strain ATCC BAA-255 / R6) OX=171101 GN=comA PE=3 SV=1	PF00005;PF03412
143.2;LSEI_2386	143.2;LSEI_2386	
orf00029	Bacteriocin production related histidine kinase	
orf00030	response regulator PlnD	PF00072
orf00031		
orf00033		
orf00035		
orf00037		
orf00040		
EntA Immun	Putative bacteriocin immunity protein	PF08951
97.2;Enterocin_X_chain_beta	Bacteriocin_Ilc; 97.2;Enterocin_X_chain_beta	PF10439;
orf00047		
orf00050	ggmotif; Bacteriocin_Ilc;	; PF10439;
orf00053		
orf00058		
orf00059	P71468_LACPL PlnI (Immunity protein PlnI, membrane-bound protease CAAX family)	
orf00062	Putative HTH-type transcriptional regulator slr0846 OS=Synechocystis sp. (strain PCC 6803 / Kazusa) OX=1111708 GN=slr0846 PE=4 SV=2	
orf00064	Uncharacterized MFS-type transporter Rv1250 OS=Mycobacterium tuberculosis (strain ATCC 25618 / H37Rv) OX=83332 GN=Rv1250 PE=3 SV=1	
orf00065		
orf00066		
51.2;Carnocin_CP52	51.2;Carnocin_CP52	

orf00070	Oxidoreductase YdhF OS=Escherichia coli (strain K12) OX=83333 GN=ydhF PE=1 SV=2
orf00071	
orf00074	Uncharacterized oxidoreductase SSP1627 OS=Staphylococcus saprophyticus subsp. saprophyticus (strain ATCC 15305 / DSM 20229 / NCIMB 8711 / NCTC 7292 / S-41) OX=342451 GN=SSP1627 PE=3 SV=1
orf00075	Probable NADH-dependent flavin oxidoreductase YqiG OS=Bacillus subtilis (strain 168) OX=224308 GN=yqiG PE=3 SV=1
orf00076	
orf00078	Fumarate hydratase class II OS=Listeria innocua serovar 6a (strain ATCC BAA-680 / CLIP 11262) OX=272626 GN=fumC PE=3 SV=1
orf00079	
orf00082	
orf00086	
orf00090	Divalent metal cation transporter MntH OS=Lactobacillus sakei subsp. sakei (strain 23K) OX=314315 GN=mntH PE=3 SV=1
orf00092	

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class IIb bacteriocin, lactobin A/cerein 7B famil Enterocin X |
>Prokka
MTKLNEAELSKISGGIGPLAIPVAAILGFLATDAWSHADELVAGVKQGWERS
>BAGEL4
MTKLNEAELSKISGGIGPLAIPVAAILGFLATDAWSHADELVAGVKQGWERS

bacteriocin-like peptide, LSEI_2386
>Prokka
MKKFKDEEMPMMTDLELSSFVGGMLRDEGFTFNKFKNWLTSIFG
>BAGEL4
MKKFKDEEMPMMTDLELSSFVGGMLRDEGFTFNKFKNWLTSIFG

Bacteriocin_IIc
>Prokka
MDHLKTLNTADLASISGGDNGNLWTFIGKAIGSTARSWAEGAMFAPAIGPAKEIVDKLNGN
>BAGEL4
MDHLKTLNTADLASISGGDNGNLWTFIGKAIGSTARSWAEGAMFAPAIGPAKEIVDKLNG

Carnocin_CP52
>Prokka
MSDKKQALFEMLSKAYSSPAVKADAKLKQIIIESNAKQLEDAEDKTAYVAVVTQLSHDISKYYLIHHTIPAEMI
AVFNAIKADV PADQVNAKRYRDQALAAGLIAIPIVWGGGH
>BAGEL4
MVAGSIVLG GTVMSDKKQALFEMLSKAYSSPAVKADAKLKQIIIESNAKQLEDAEDKTAYVAVVTQLSHDISKY
YLIHHTIPAEMIAVFNAIKADV PADQVNAKRYRDQALAAGLIAIPIVW

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Figure S3 Amino acid sequence variants of BAGEL4-identified *Lactobacillus rhamnosus* 20-12 bacteriocins as annotated by Prokka and BAGEL4.

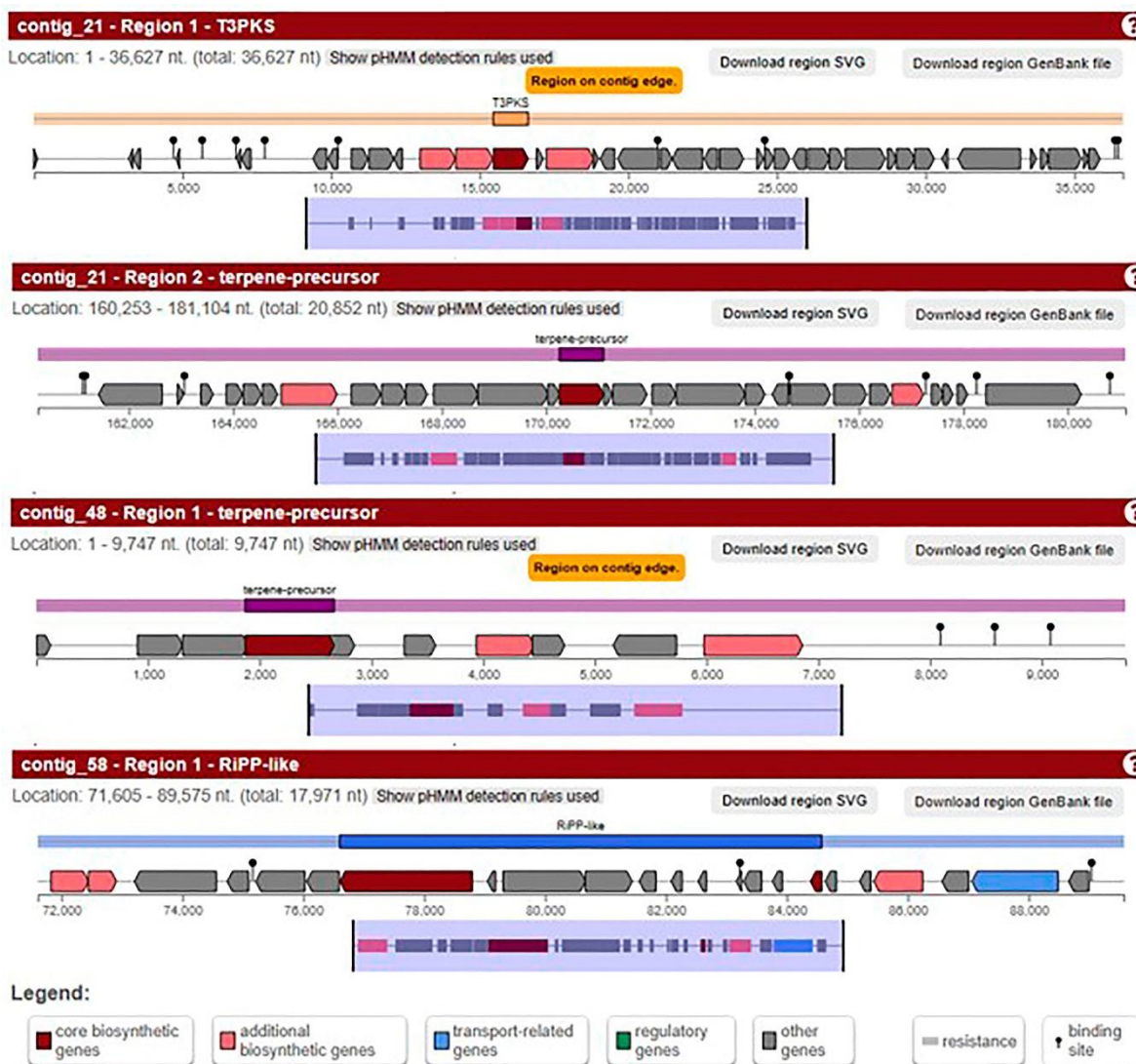


Figure S4 Secondary metabolite biosynthetic gene clusters (BGCs) of *L. rhamnosus* 20-12 identified by antiSMASH 8.0.4. The figure displays four distinct regions of interest:

1. Contig_21, Region 1: A Type III Polyketide Synthase (T3PKS) cluster (36.6 kb) involved in the production of phenolic antioxidants.
2. Contig_21, Region 2: A terpene precursor biosynthetic cluster (20.8 kb) encoding polyprenyl synthetase family proteins.
3. Contig_48, Region 1: An additional terpene precursor cluster (9.7 kb) likely involved in membrane stabilization.
4. Contig_58, Region 1: A ribosomally synthesized and post-translationally modified peptide-like (RiPP-like) cluster (17.9 kb) corresponding to the Class II bacteriocin assembly.

The gene color coding (Legend bottom left) indicates core biosynthetic genes (pink), transport-related genes (blue), and regulatory genes (green).