

# Supplement

Adejumo, I., & Adebisi, O. (2025). Peptides from hypothetical proteins of *Lactobacillus acidophilus* induce IL-4 and IL-10. *Biomedical Research and Therapy*, 12(3), 7191-7206. <https://doi.org/10.15419/bmrat.v12i3.963>

**Supplementary Table 1** Protein features of the reference genome

<b>Features</b>	<b><i>Number</i></b>
Hypothetical proteins	412
Proteins with functional assignments	1,497
Proteins with EC number assignments	481
Proteins with GO assignments	400
Proteins with pathway assignments	326
Proteins with PATRIC genus-specific family (PLfam) assignments	1,891
Proteins with PATRIC cross-genus family (PGfam) assignments	1,894

EC = Enzyme Commission; GO = Gene Ontology

**Supplementary Table 2** Specialty genes of the reference genome

	<b>Source</b>	<b>Genes</b>
Antibiotic resistance	PATRIC	19
Drug target	DrugBank	2
Transporter	TCDB	12

TCBD = The transporter classification database

**Supplementary Table 3 Antimicrobial** resistance genes associated with this reference genome

<b>AMR Mechanism</b>	<b>Genes</b>
Antibiotic inactivation enzyme	
Antibiotic target in susceptible species	Alr, Ddl, EF-G, EF-Tu, folA, Dfr, gyrA, gyrB, Iso-tRNA, MurA, rpoB, rpoC, s10p, s12p
Gene conferring resistance via absence	gidB
Protein altering cell wall charge conferring antibiotic resistance	GdpD, PgsA
AMR = antimicrobial resistance	



**Supplementary Table 4** Features of selected similar genomes to query reference genome of *Lactobacillus acidophilus*

	Genomes						
General information	LA1	ATCC 53544	FSI4	LA-G80-111	NCFM	DSM 20079	La-14
Genome ID	1579.80	1579.81	1579.28	1579.364	272621.13	1579.223	1314884.3
Genome Name	<i>Lactobacillus acidophilus</i> strain LA1	<i>Lactobacillus acidophilus</i> strain ATCC 53544	<i>Lactobacillus acidophilus</i> strain FSI4	<i>Lactobacillus acidophilus</i> strain LA-G80-111	<i>Lactobacillus acidophilus</i> NCFM	<i>Lactobacillus acidophilus</i> strain DSM 20079	<i>Lactobacillus acidophilus</i> La-14
Taxonomy information							
Taxon ID	1579	1579	1579	1579	272621	1579	1314884
Superkingdom	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria	Bacteria
Phylum	Bacillota	Bacillota	Bacillota	Bacillota	Bacillota	Bacillota	Bacillota
Class	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli	Bacilli
Order	Lactobacillales	Lactobacillales	Lactobacillales	Lactobacillales	Lactobacillales	Lactobacillales	Lactobacillales
Family	Lactobacillaceae	Lactobacillaceae	Lactobacillaceae	Lactobacillaceae	Lactobacillaceae	Lactobacillaceae	Lactobacillaceae
Genus	<i>Lactobacillus</i>	<i>Lactobacillus</i>	<i>Lactobacillus</i>	<i>Lactobacillus</i>	<i>Lactobacillus</i>	<i>Lactobacillus</i>	<i>Lactobacillus</i>

Species	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus acidophilus</i>	<i>Lactobacillus acidophilus</i>
Genome status	Complete	Complete	Complete	Complete	Complete	Complete	Complete

**Type information**

Strain	LA1	ATCC 53544	FSI4	LA-G80-111	NCFM	DSM 20079	La-14
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**Database cross reference (Accession)**

BioProject	PRJNA340059	PRJNA394684	PRJNA271341	PRJNA638040	15671160	PRJNA379350	PRJNA196176
BioSample	SAMN0563105	SAMN07357495	SAMN0327400	SAMN15165794	PRJNA82	SAMN06606133	SAMN0260321
	2		4				6
Assembly	GCA_00228621	GCA_002224305	GCF_00093462	GCA_013342945.1	SAMN02603047	GCA_003047065	GCA_00038967
	5.1	.1	5.1			.1	5.2
Gene	CP017062	CP022449	CP010432	CP054559	GCA_000011985.	CP020620	CP005926.1
					1		

**Genomic statistics**

Chromosomes	1	1	1	1	1	1	1
Contigs	1	1	1	1	1	1	1

Genome	1991195	1991906	1991969	1991976	1993560	2009973	1991830
Length							
GC Content	34.7	34.71	34.71	34.70639	34.7	34.717083	34.7
Contig L50	1	1	1	1	1	1	1
Contig N50	1991195	1991906	1991969	1991976	1993560	2009973	1991830

### Annotation

### Statistics

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tRNA	61	61	61	61	61	60	61
rRNA	4	10	45	4	9	10	12
CDS	1917	1942	1908	1908	1882	1996	1894
CDS Ratio	0.96	0.97	0.96	0.9578429	0.94	0.99	0.95
Hypothetical	455	473	450	411	414	470	428
CDS							
Hypothetical	0.24	0.24	0.24	0.2154088	0.22	0.24	0.23
CDS Ratio							
PLFAM CDS	1915	1932	1908	1882	1881	1987	1894
PLFAM CDS	1	0.99	1	0.9863732	1	1	1
Ratio							

**Genome****Quality**

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Coarse	99.7	99.7	99.7	98.4	99.8	99.7	99.7
Consistency							
Fine	99.3	99.3	99.5	98.2	99.4	98.3	99.2
Consistency							
CheckM	100	100	100		100	100	100
Completeness							
Genome	Good	Good	Good	Good	Good	Good	Good
Quality							
<b>Isolation</b>	Commercial	Isolated from a	Yogurt	Human, Homo	Human, Homo	Human, Homo	
<b>Source</b>	dietary	rectal swab of an		sapiens	sapiens	sapiens	
	supplement	infant					

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**Supplementary Table 5** Characteristics of selected ARG-associated proteins

<b>BRC ID</b>	<b>Genes</b>	<b>Status</b>	<b>Property</b>	<b>Classification</b>	<b>Antibiotics Class</b>	<b>Product/Function</b>
fig 1579.814.peg.642	PgsA	Active	Antibiotic Resistance	daptomycin	Peptide antibiotics	CDP-diacylglycerol-- glycerol-3-phosphate 3- phosphatidyltransferase (EC 2.7.8.5)
fig 1579.814.peg.483	GdpD	Active	Antibiotic Resistance	daptomycin	Peptide antibiotics	Glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46)
fig 1579.814.peg.484	GdpD	Active	Antibiotic Resistance	daptomycin	Peptide antibiotics	Glycerophosphoryl diester phosphodiesterase (EC 3.1.4.46)

ARGs = antibiotic resistance genes

**Supplementary Table 6** Protein family identities, descriptions and statistics of selected hypothetical proteins of the reference genome

Family ID	Denoted	Descriptions	Min AA Length	Max AA Length	Mean	Std Dev
PLF_1578_00004147	QHP1	FIG015094: hypothetical protein	79	79	79	0
PLF_1578_00004054	QHP2	Hypothetical NagD-like phosphatase	258	258	258	0
PLF_1578_00006981	QHP3	Hypothetical protein VC0266 (sugar utilization related)	195	195	195	0
PLF_1578_00005637	QHP4	Hypothetical protein SAV1845	116	116	116	0
PLF_1578_00004553	QHP5	Hypothetical protein SAV1839	111	111	111	0
PLF_1578_00003962	QHP6	Distant homolog of hypothetical protein SA_21	274	274	274	0
PLF_1578_00007617	QHP7	Hypothetical DUF1027 domain protein	202	202	202	0

**Supplementary Table 7** Secondary structure results for selected hypothetical proteins

Proteins	Secondary structure elements (%)									
	AH	3 <sub>10</sub> helix	Pi hel	BB	ES	BT	BR	RC	AS	OS
QHP1	73.42	0.00	0.00	0.00	2.53	2.53	0.00	21.52	0.00	0.00
QHP2	36.82	0.00	0.00	0.00	18.60	8.53	0.00	36.05	0.00	0.00
QHP3	33.85	0.00	0.00	0.00	16.92	4.62	0.00	44.62	0.00	0.00
QHP4	91.38	0.00	0.00	0.00	0.00	1.72	0.00	6.90	0.00	0.00
QHP5	76.58	0.00	0.00	0.00	10.81	2.70	0.00	9.91	0.00	0.00
QHP6	33.21	0.00	0.00	0.00	26.64	7.66	0.00	32.48	0.00	0.00
QHP7	34.65	0.00	0.00	0.00	15.35	3.96	0.00	46.04	0.00	0.00

AH = Alpha helix; BB = Beta bridge; ES = Extended strand; BT = Beta turn; BR = Bend region; RC = Random coil; AS = Ambiguous states; OS = Other states

**Supplementary Table 8** MolProbity, QMEAN, Solvation energy and Torsion angle energy scores for query hypothetical proteins

<b>Proteins</b>	<b>Transmembrane segment prediction</b>	<b>QMEAN score</b>	<b>C_b interaction energy</b>	<b>All atoms pairwise energy</b>	<b>Solvation energy</b>	<b>Torsion angle energy</b>	<b>Ramachandran favoured (%)</b>	<b>Ramachandran outliers (%)</b>
QHP1	Not predicted	0.58	0.79	2.77	1.93	-0.53	98.7	0.00
QHP2	Not predicted	1.18	0.42	0.60	1.31	0.69	98.05	0.00
QHP3	Not predicted	1.35	-0.82	0.10	0.86	1.22	97.93	0.00
QHP4	Not predicted	1.90	3.96	4.80	3.24	-0.47	100	0.00
QHP5	Not predicted	-0.96	0.26	3.21	1.11	-1.81	95.41	0.92, A33 LEU
QHP6	Predicted	-0.36	-0.60	1.48	0.45	-0.49	94.49	0.37, A30 VAL
QHP7	Not predicted	-4.33	0.08	0.83	-0.64	-4.30	81.82	5.45, A157 ILE, A209 ARG, A173 LYS, A178 ARG, A166 ASN, A159 GLU, A172 ARG, A177 SER, A175 LYS, A167 ASP, A174 ARG, A207 VAL

**Supplementary Table 9** Immunoogenicity, allergenicity and toxicity results of query hypothetical proteins

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<b>Proteins</b>	<b>Immunogenicity</b>	<b>Allergenicity</b>	<b>Toxicity</b>
QHP1	Nonantigenic	Nonallergenic	Nontoxic
QHP2	Antigenic	Nonallergenic	Nontoxic
QHP3	Nonantigenic	Nonallergenic	Nontoxic
QHP4	Nonantigenic	Nonallergenic	Nontoxic
QHP5	Antigenic	Nonallergenic	Nontoxic
QHP6	Nonantigenic	Nonallergenic	Nontoxic
QHP7	Nonantigenic	Nonallergenic	Nontoxic

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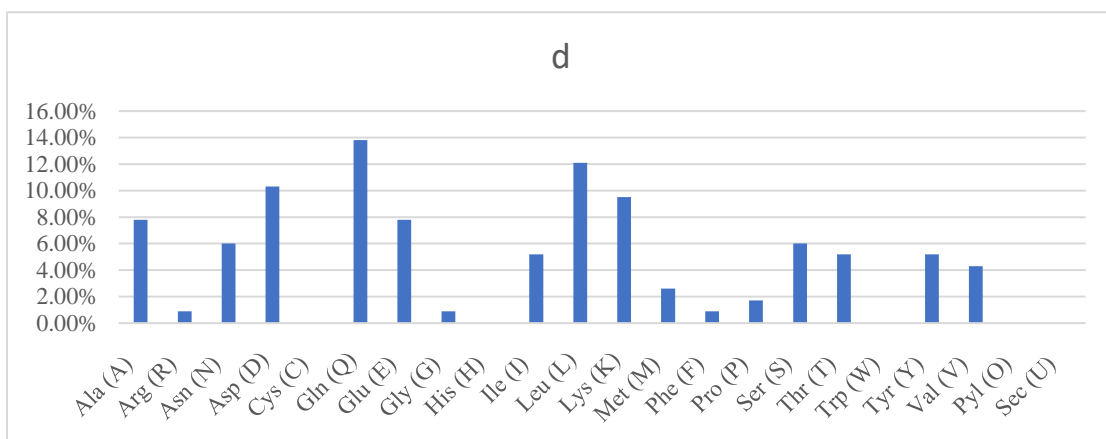
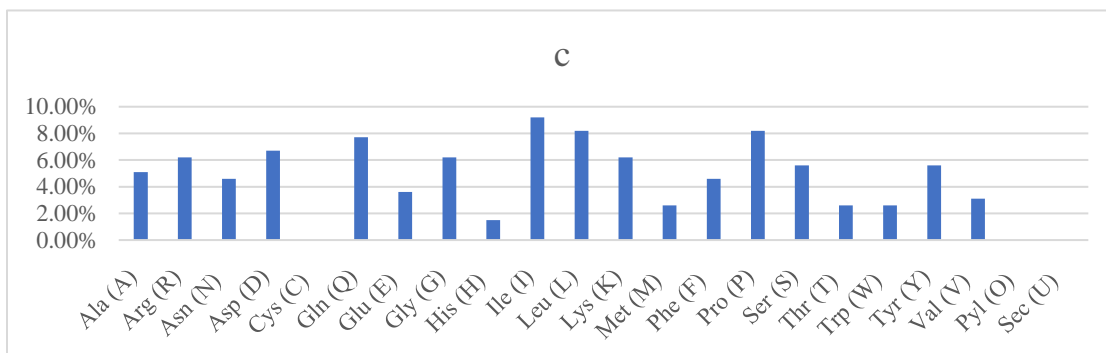
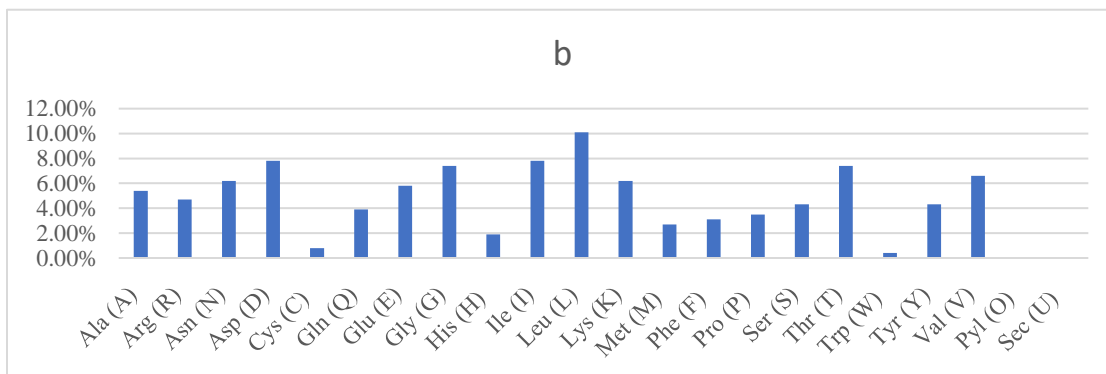
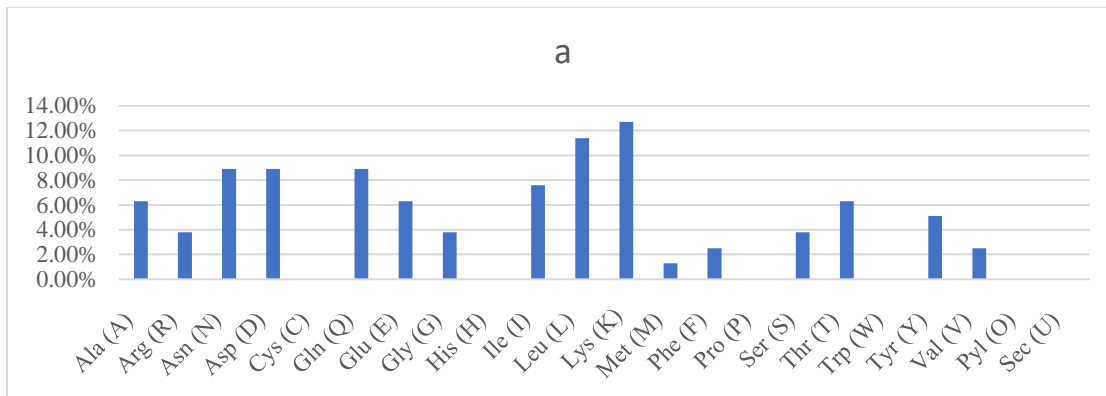
**Supplementary Table 10** Interleukins 4 and 10 predictions for derived peptides from hypothetical proteins of probiotic *Lactobacillus acidophilus* genome

Peptides	IL-4				IL-10					
	MP	SVM	Prediction	pI	Mol wt	SVM	Prediction	Immunogenicity	Allergenicity	Toxicity
		Score				Score				
QHP2a										
<a href="#">MKDYRLFLIDLDGTVYRG</a>	0	0.25	Inducer	6.38	2175.8	0.84	Inducer	Antigenic	Nonallergenic	Nontoxic
CKDYRLFLIDLDGTVYRG	1	0.28	Inducer	6.35	2147.74	0.78	Inducer	Antigenic	Nonallergenic	Nontoxic
DKDYRLFLIDLDGTVYRG	1	0.22	Inducer	4.58	2159.69	0.95	Inducer	Antigenic	Nonallergenic	Nontoxic
EKDYRLFLIDLDGTVYRG	1	0.23	Inducer	4.69	2173.72	0.93	Inducer	Antigenic	Nonallergenic	Nontoxic
FKDYRLFLIDLDGTVYRG	1	0.28	Inducer	6.38	2191.78	0.89	Inducer	Antigenic	Nonallergenic	Nontoxic
NKDYRLFLIDLDGTVYRG	1	0.31	Inducer	6.38	2158.71	0.91	Inducer	Antigenic	Nonallergenic	Nontoxic
MCDYRLFLIDLDGTVYRG	2	0.29	Inducer	4.43	2150.76	0.88	Inducer	Antigenic	Nonallergenic	Nontoxic
MKQYRLFLIDLDGTVYRG	3	0.34	Inducer	8.83	2188.85	0.89	Inducer	Antigenic	Nonallergenic	Nontoxic
MKDYRVFLIDLDGTVYRG	6	0.38	Inducer	6.38	2161.77	0.85	Inducer	Antigenic	Nonallergenic	Nontoxic
MKDYRLELIDLDGTVYRG	7	0.33	Inducer	4.69	2157.74	0.92	Inducer	Antigenic	Nonallergenic	Nontoxic
MKDYRLFLIDLDGTVYRV	18	0.38	Inducer	6.38	2217.88	0.73	Inducer	Antigenic	Nonallergenic	Nontoxic
MKDYRLFLIDLDGTVYRW	18	0.27	Inducer	6.38	2304.96	0.63	Inducer	Antigenic	Nonallergenic	Nontoxic
QHP2b										
QNKRQPTVLVNSLDEFEL	0	0.26	Inducer	4.68	2130.66	0.81	Inducer	Antigenic	Nonallergenic	Nontoxic
ANKRQPTVLVNSLDEFEL	1	0.27	Inducer	4.68	2073.6	0.53	Inducer	Antigenic	Nonallergenic	Nontoxic

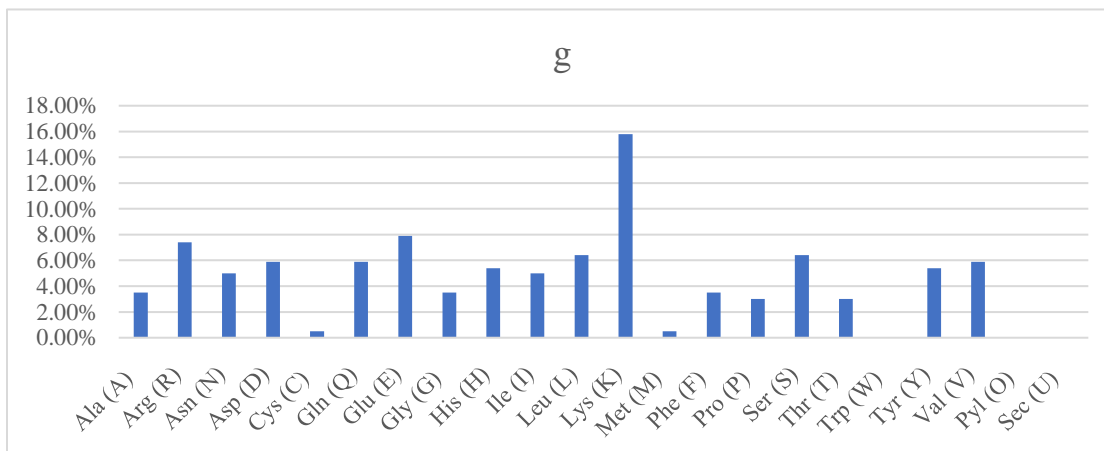
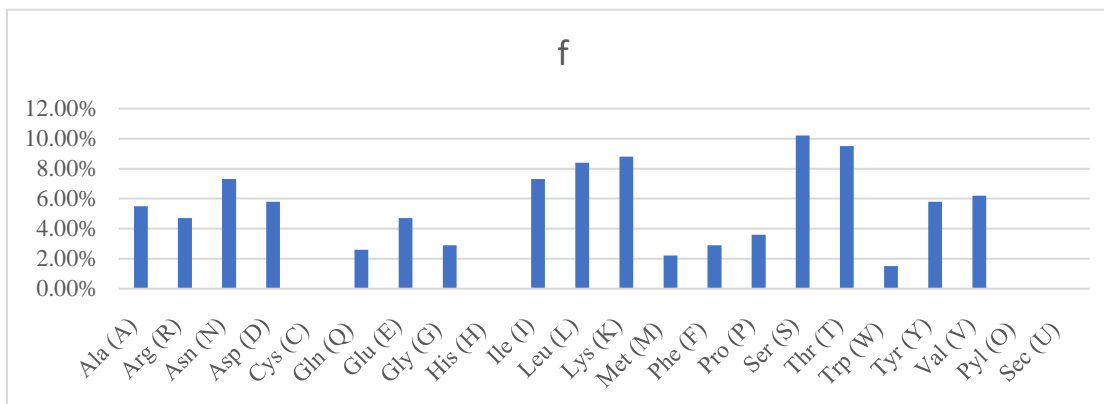
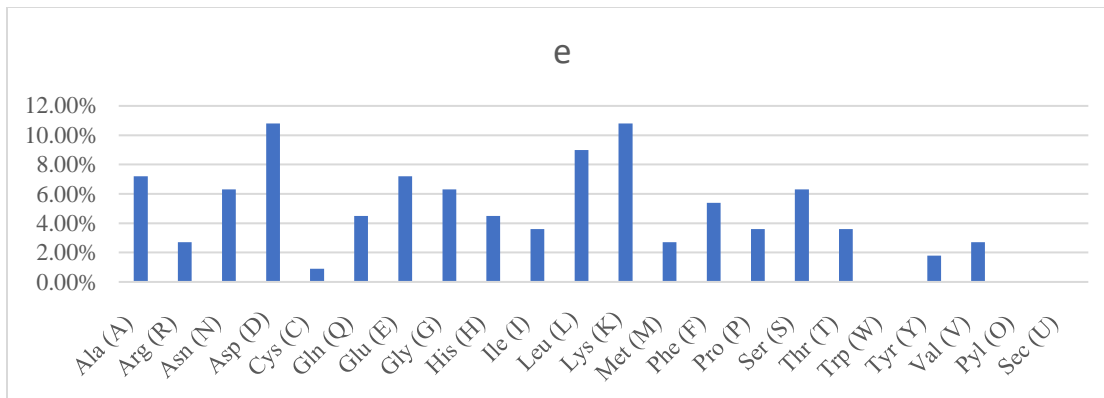
CNKRQPTVLVNSLDEFEL	1	0.24	Inducer	4.68	2105.66	0.47	Inducer	Antigenic	Nonallergenic	Nontoxic
DNKRQPTVLVNSLDEFEL	1	0.25	Inducer	4.32	2117.61	0.67	Inducer	Antigenic	Nonallergenic	Nontoxic
QCKRQPTVLVNSLDEFEL	2	0.3	Inducer	4.68	2119.69	0.33	Inducer	Antigenic	Nonallergenic	Nontoxic
QDKRQPTVLVNSLDEFEL	2	0.2	Inducer	4.32	2131.64	0.85	Inducer	Antigenic	Nonallergenic	Nontoxic
QNKFQPTVLVNSLDEFEL	4	0.35	Inducer	4.14	2121.65	0.54	Inducer	Antigenic	Nonallergenic	Nontoxic
QNKKQPTVLVNSLDEFEL	4	0.3	Inducer	4.68	2102.65	0.58	Inducer	Antigenic	Nonallergenic	Nontoxic
QHP5a										
MKHFLGTVFGAAAGFVF	0	0.39	Inducer	9.11	1913.58	0.65	Inducer	Antigenic	Nonallergenic	Nontoxic
QKSDDELNQNLNNMDTKD	14	0.36	Inducer	4.23	2135.52	0.31	Inducer	Antigenic	Nonallergenic	Nontoxic

MP= mutation position; pI = isoelectric point; MW = molecular weight

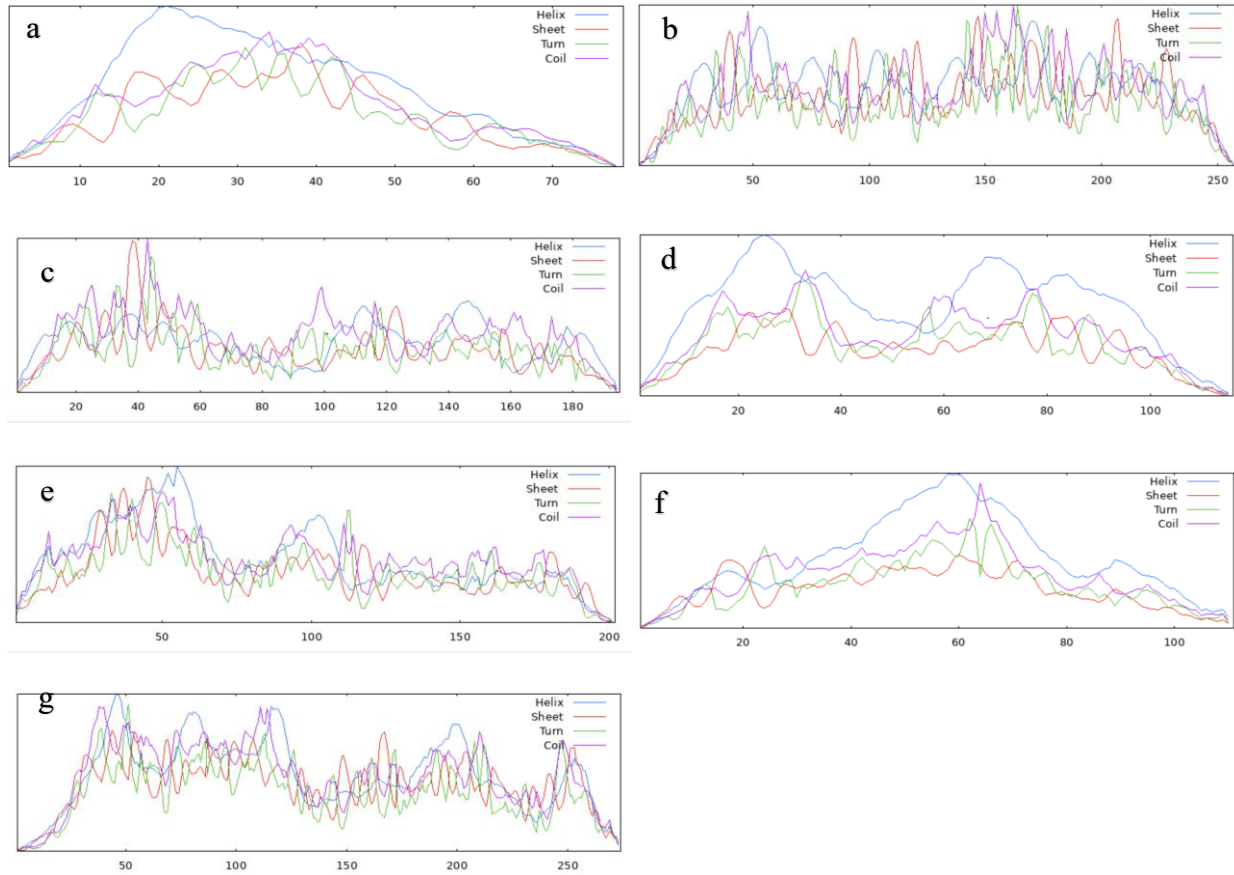
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**Supplementary Fig. 1a-g** Amino acid compositions of QHP1 (a), QHP2 (b), QHP3 (c), QHP4 (d), QHP5 (e), QHP6 (f) and QHP7 (g), showing different level of each amino acids according to the composition of the hypothetical protein in question



**Supplementary Fig. 2a-g** Pictorial representation of helices, sheets, turns and coils of QHP1 (a), QHP2 (b), QHP3 (c), QHP4 (d), QHP5 (e), QHP6 (f) and QHP7 (g)