Supplementary Information for

Selection of carbohydrate-active probiotics from the gut of carnivorous fish fed plant-based diets

Cláudia R. Serra, Eduarda M. Almeida, Inês Guerreiro, Rafaela Santos, Daniel L. Merrifield, Fernando Tavares, Aires Oliva Teles, Paula Enes

Supplementary Table S1. Oligonucleotide primers used in this study

Target enzyme[a] / gene[b]	Primer Name	Primer Sequence (5´-3´)
β-glucanase (GH16 - EC 3.2.1.73) / *bglS*	BglS-339F	AGGGATCGTTTCATCGTTCT
	BglS-553R	TAATAGAGTTTGGCTGCCAATC
Levanase (β-D-fructofuranosidase) (GH32 - EC 3.2.1.80) / *sacC*	SacC-106F	CCTCAATATCACTTCACACCGGAG
	SacC-336R	ATCTACAACTGCGCTTCCAGAAAA
Mannan endo-1,4-β-mannosidase (GH26-EC 3.2.1.78) / *gmuG*	GmuG-563F	TCAGGCCGCTGCATGAAATGAACG
	GmuG-786R	AATATCCACGTAAGACGCGCCCGG
Endo-1,5-α-L-arabinanase (GH43 - EC:3.2.1.99) / *abnA*	AbnA-311F	GGGCGCCGGACATCCAATACTATA
	AbnA-564R	AGTCAGCTTAATGCCGCTCCAAAA
Arabinoxylan arabinofuranohydrolase (GH43 - EC:3.2.1.55) / *xynD*	XynD-361F	AAATGGGCAGGTGCGTCATGGGC
	XynD-591R	GTCGTCATCTACAAATACTGCCGG

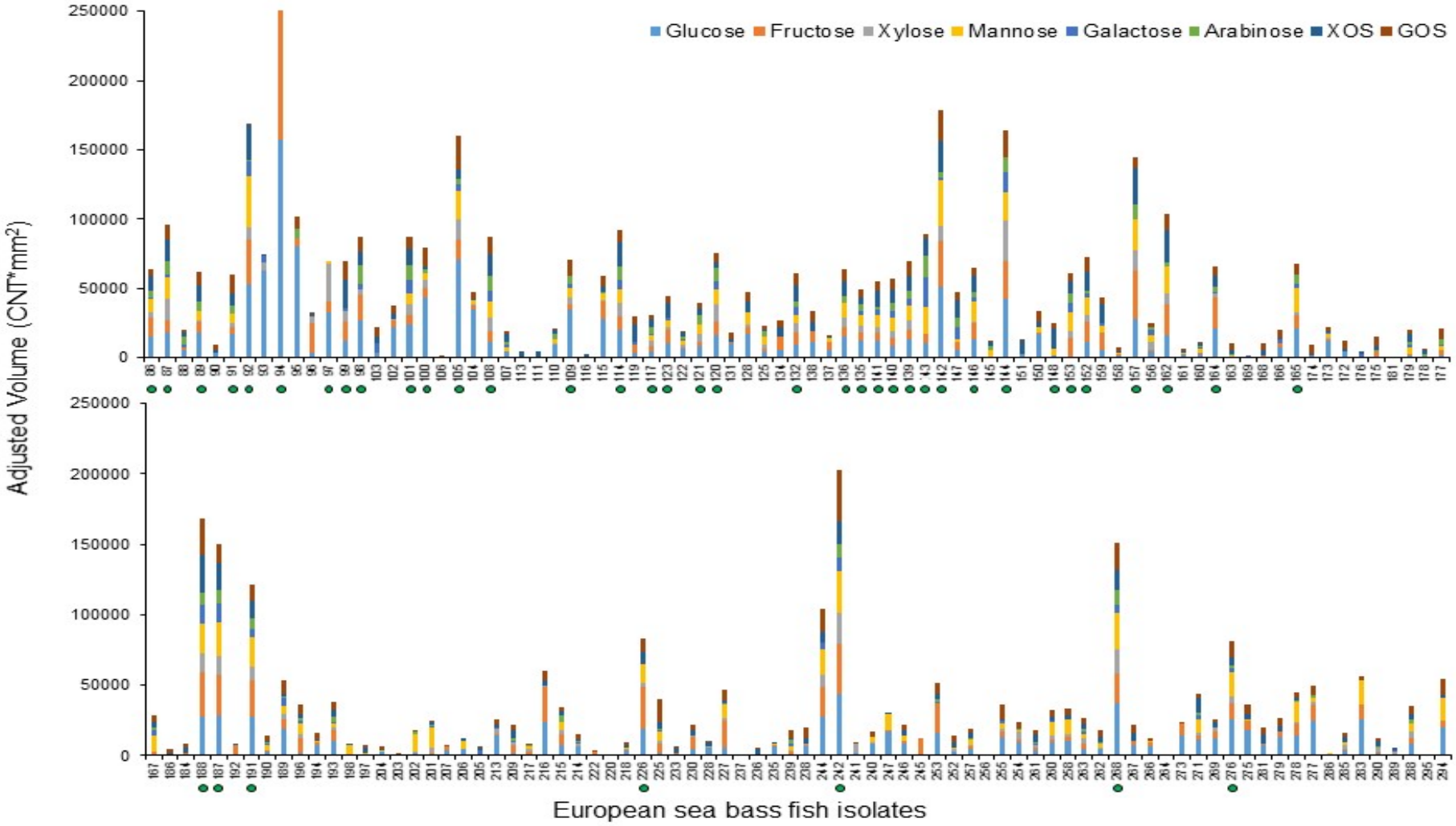
[a] the enzyme Glycoside Hydrolase Family (GH) number and the EC number are provide in brackets
[b] gene name in *B. subtilis* strain 168 genome, whose sequence was used to design the oligonucleotide primers

Supplementary Table S2. Susceptibility of selected isolates to various antimicrobial agents

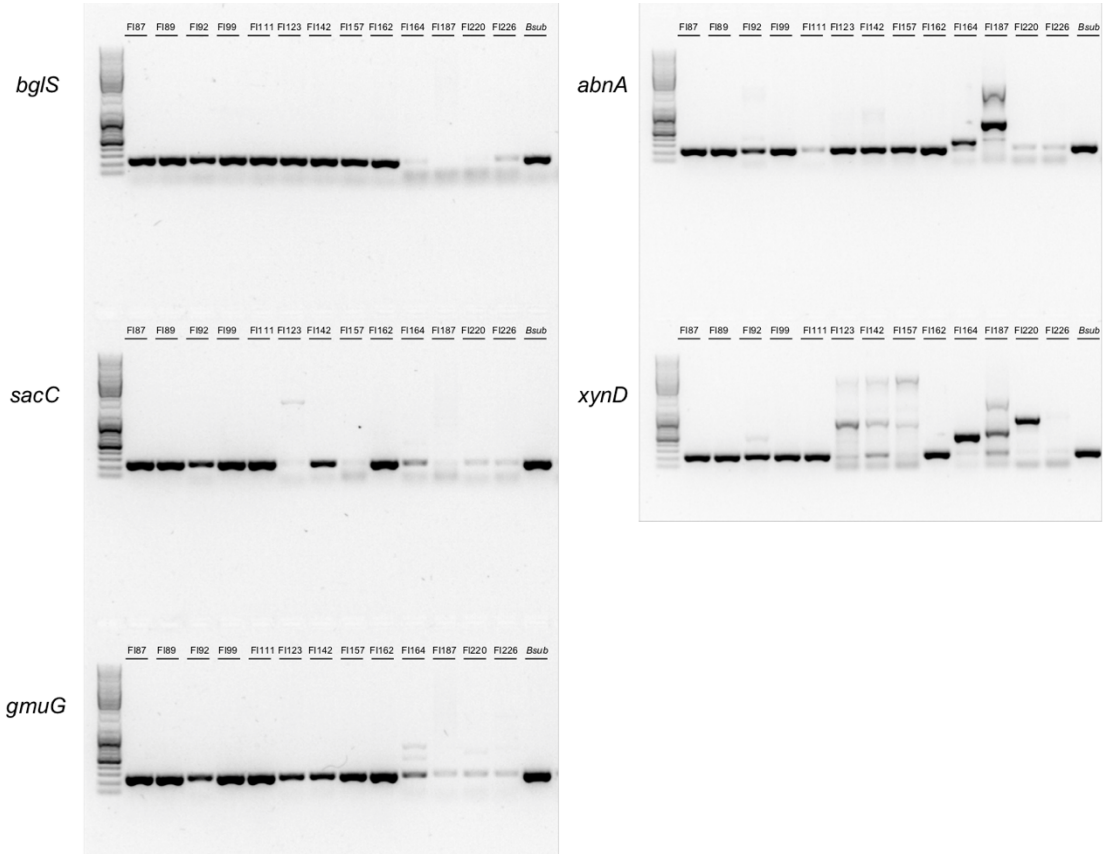
	MIC [a] (μg ml^{-1})						
Isolate [b]	CL	TC	EM	KM	VA	GM	SM
FI86	6	1.5	0.19	1.5	3	0.38	1,5-2
FI87	4	0.094	0.094	0.75	0.75	0.125	4.-6
FI89	4	0.125	0.094	0.75	1	0.125	4
FI91	4	0.75	0.125	3	2	0.75	0.75
FI92	5	0.75	0.142	0.625	0.375	0.094	0.75
FI94	> 256	4	> 256	3	8	0.75	24
FI97	4	0.38	0.125	2	2	0.28	0.38
FI98	1.5	0.047	0.032	0.25	0.094	0.094	0,75-1,0
FI99	3.5	0.22	0.0945	0.625	0.625	0.1095	1.875
FI100	4	0.75	0.125	3	8	0.75	1.5
FI101	8	0.5	0.094	1	0.25	0.38	1.5
FI105	> 256	4	> 256	2.-3	4	0.75	16-24
FI108	12	0.5	1	1.5	0.38	0.125	1.5
FI109	4	0.75	0.125	2	4	0.38	2
FI114	12	0.38	0.5	1.5	0.5	0.19	2,-3
FI117	6	4	0.19	1	1	0.064	4
FI120	> 256	4,-6	> 256	3	4	0.5	24
FI121	6	3.-4	0.125	0.75	1	0.125	4
FI123	3.5	1.75	0.1095	0.875	0.875	0.1095	2.5
FI132	> 256	2	> 256	1,5-4 (?)	6	0.75	32
FI135	14	0.315	> 256	1.75	2.75	0.565	3
FI136	> 256	2	> 256	4	6	0.75	32
FI139	> 256	2	> 256	12	4	0.5	19
FI140	16	0.5	0.75	1.5	0.5	0.125	3
FI141	6	1.5	0.125	0.75	1	0.125	2
FI142	3	1.5	0.125	0.875	1.125	0.172	2.5
FI143	6	0.5	0.19	2	4	0.38	0,75-2
FI144	> 256	4	> 256	3	6	0.5	16
FI146	6	0.75	0.125	2	6	0.38	1.5
FI148	3	0.094	0.125	0.75	1.5	0.25	0.5
FI152	12	0.25	0.5	2	3	0.5	8
FI153	8	0.625	0.315	0.875	0.315	0.1095	0.625
FI157	3	4.5	0.1095	0.315	1	0.079	1.75
FI162	3.5	6	0.1095	0.75	1.125	0.094	7
FI164	5.5	20.625	2.032	0.3125	1	0.0585	4.25
FI165	4	0.094	0.094	0.38	0.75	0.094	1
FI187	4.5	0.1875	0.0705	0.875	0.22	0.094	2.5
FI188	32	4.-6	> 256	2	2.-3	0.38	48
FI191	8	0.047	0.064	0.38	0.125	0.016	0.75
FI226	2	0.625	0.1095	3	3	0.315	0.565
FI242	3	0.22	0.25	1.5	4	0.845	2.5
FI268	48	0.19	0.25	1.5	1.5	0.38	4
FI276	4	0.5	0.125	4	3	0.38	1.5

[a] MICs were determined by the Etest® method and in grey boxes are the MIC values above the reference breakpoint (EFSA-FEEDAP, 2012). CL, Cloramphenicol; TC, Tetracyclin, EM, Erythromycin; KM, Kanamycin; VA, Vancomycin; GM, Gentamycin; SM, Streptomycin.

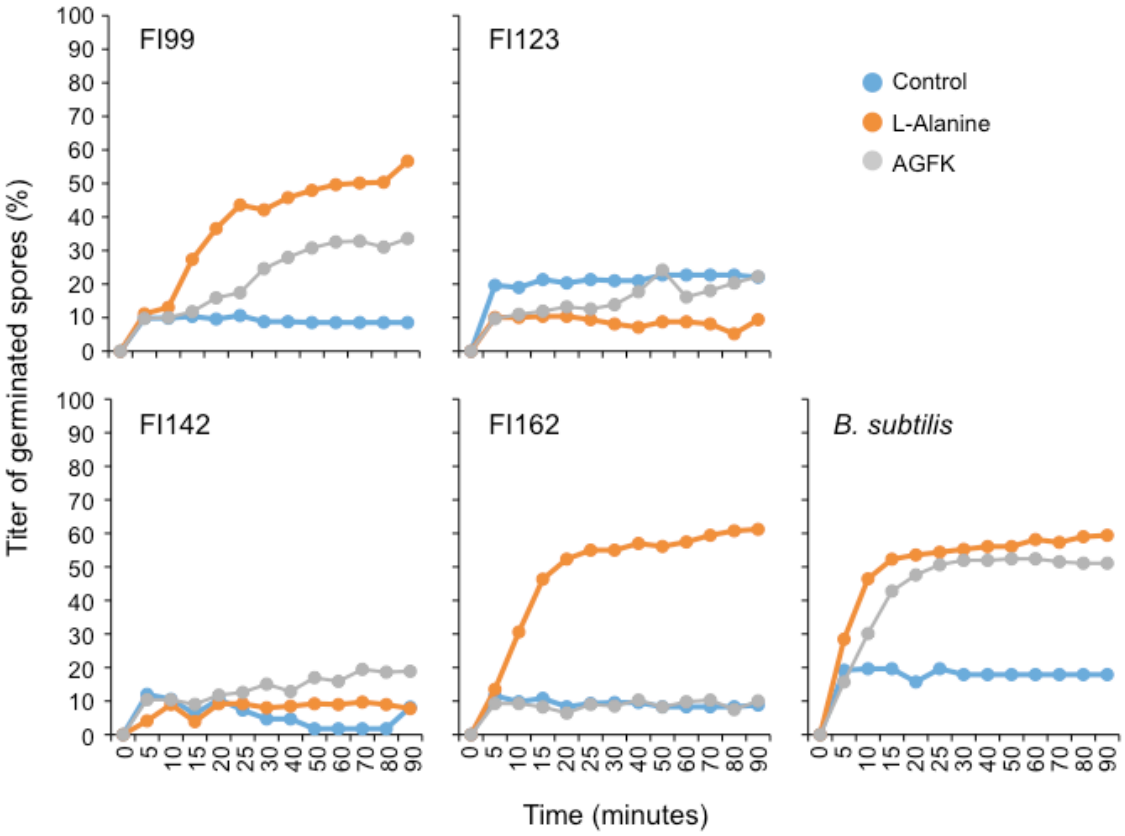
[b] Highlighted in underlined lettering are the isolates showing resistance to 2 or more antimicrobials. All isolates showing any antimicrobial resistance were discarded from the rest of the study.



Supplementary Figure S1. Carbohydrolitic profile of the 161 sporeformers (number in the x axis) isolated from the gut of European sea bass, when cultured on solid minimal medium supplemented with D-glucose, D-fructose, D-xylose, L-arabinose, D-galactose, D-mannose, Xylooligosaccharides (XOS) and Galactooligosaccharides (GOS). Growth was quantified by measuring the colonies volume from images like the ones presented in Figure 3 using Quantity One software V.4.6.9 (Bio-Rad) with local background subtraction method (adjusted volume=[CNT*mm^2] data counts/mm^2).



Supplementary Figure S2. Full-length agarose gels used for resolution of PCR products from genes coding for β-glucanase (*bglS),* levanase or β-D-fructofuranosidase (*sacC),* mannan endo-1,4-β-mannosidase (*gmuG),* endo-1,5-α-L-arabinanase (*abnA)* and arabinoxylan arabinofuranohydrolase (*xynD)* carbohydrases, in the genome of 13 fish isolates (FI numbers on top of the figure). Parts of gels showing the amplicons of isolates FI87, FI89, FI92, FI99, FI123, FI142, FI157, FI162, FI164, FI187, FI226 and of *B. subtilis* 168 (*Bsub*) were used in the construction of Figure 4.



Supplementary Figure S3. Germination of populations of purified spores of sporeformers fish isolates FI99, FI123, FI142 and FI162 at 37ºC in 50mM Tris-HCl, pH7.5 (control, blue circles) or in response to the addition of 100mM L-alanine (orange circles) or a mixture of 100mM KCl, 56mM glucose, 56mM fructose and 33mM L-asparagine (AGFK, grey circles). *Bacillus subtilis* 168 was used as control.