

Supplementary Material

Evaluating the biocontrol potential of a commercial yeast starter against fuel-ethanol fermentation contaminants

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Table S1: List of microorganisms used in the present study and their origin.

Species	Strain	origin
<i>Brettanomyces bruxellensis</i>	ISA 1649	beer
<i>B. bruxellensis</i>	ISA 1700	wine
<i>B. bruxellensis</i>	ISA 1791	wine
<i>B. bruxellensis</i>	ISA 2104	wine
<i>B. bruxellensis</i>	ISA 2116	wine
<i>B. bruxellensis</i>	ISA 2211	wine
<i>Hanseniaspora guilliermondii</i>	NCYC 2380	grape must
<i>Levilactobacillus brevis</i>	ISA 4385	faeces
<i>Lentilactobacillus hilgardii</i>	ISA 4387	wine
<i>Lactiplantibacillus plantarum</i>	ISA 4395	wine
<i>Leuconostoc mesenteroides</i> subsp. <i>cremoris</i>	ISA 4383	Hansen's dried starter powder
<i>Pediococcus parvulus</i>	ISA 4401	wine
<i>Pediococcus pentosaceus</i>	ISA 4379	cheese
<i>Saccharomyces cerevisiae</i>	Ethanol Red	fuel-ethanol starter

Table S2: Comparison of malic acid consumption during growth assays of *Levilactobacillus brevis*, *Lentilactobacillus hilgardii*, *Lactiplantibacillus plantarum*, *Leuconostoc mesenteroides* subsp. *cremoris*, *Pediococcus parvulus* and *Pediococcus pentosaceus*, inoculated in a cell-free supernatant pre-fermented by *S. cerevisiae* ER, without any treatment (Control-Assay) and after a pepsin treatment (Pepsin-assay). Data represented correspond to means $\pm$ SD (error bars) of two independent biological assays.

Time (days)	Malic acid concentration (g/L) in lactic acid bacteria growth assays											
	<i>L. brevis</i>		<i>L. hilgardii</i>		<i>L. plantarum</i>		<i>L. mesenteroides</i> subsp. <i>cremoris</i>		<i>P. parvulus</i>		<i>P. pentosaceus</i>	
	Biocontrol- assay	Pepsin- assay	Biocontrol- assay	Pepsin- assay	Biocontrol- assay	Pepsin- assay	Biocontrol- assay	Pepsin- assay	Biocontrol- assay	Pepsin- assay	Biocontrol- assay	Pepsin- assay
0	2.88 $\pm$ 0.44 ^a	2.40 $\pm$ 0.27 ^b	2.62 $\pm$ 0.01 ^a	2.64 $\pm$ 0.27 ^a	2.62 $\pm$ 0.01 ^a	2.64 $\pm$ 0.27 ^a	2.64 $\pm$ 0.01 ^a	2.65 $\pm$ 0.27 ^a	2.62 $\pm$ 0.00 ^a	2.69 $\pm$ 0.27 ^a	2.64 $\pm$ 0.01 ^a	2.40 $\pm$ 0.27 ^a
1	2.37 $\pm$ 0.11 ^c	2.40 $\pm$ 0.01 ^c	2.08 $\pm$ 0.11 ^d	2.61 $\pm$ 0.22 ^a	2.44 $\pm$ 0.58 ^b	2.43 $\pm$ 0.57 ^b	2.43 $\pm$ 0.01 ^b	2.43 $\pm$ 0.00 ^b	2.38 $\pm$ 0.05 ^b	2.05 $\pm$ 0.57 ^b	2.15 $\pm$ 0.50 ^b	2.01 $\pm$ 0.40 ^b
2	2.25 $\pm$ 0.15 ^d	2.26 $\pm$ 0.11 ^d	2.02 $\pm$ 0.05 ^d	2.40 $\pm$ 0.19 ^e	1.97 $\pm$ 0.04 ^c	2.11 $\pm$ 0.01 ^d	2.18 $\pm$ 0.11 ^c	2.40 $\pm$ 0.10 ^d	2.24 $\pm$ 0.20 ^d	2.18 $\pm$ 0.01 ^d	1.96 $\pm$ 0.19 ^c	1.97 $\pm$ 0.06 ^c
3	2.22 $\pm$ 0.07 ^e	2.18 $\pm$ 0.16 ^e	2.11 $\pm$ 0.08 ^f	2.11 $\pm$ 0.25 ^f	1.90 $\pm$ 0.23 ^e	2.11 $\pm$ 0.48 ^e	2.01 $\pm$ 0.25 ^e	1.84 $\pm$ 0.11 ^e	1.71 $\pm$ 0.08 ^c	2.15 $\pm$ 0.05 ^d	1.88 $\pm$ 0.06 ^d	1.90 $\pm$ 0.11 ^d
6	2.15 $\pm$ 0.13 ^f	1.84 $\pm$ 0.45 ^f	2.01 $\pm$ 0.10 ^g	1.94 $\pm$ 0.25 ^g	1.85 $\pm$ 0.16 ^g	1.96 $\pm$ 0.07 ^g	2.00 $\pm$ 0.08 ^f	1.80 $\pm$ 0.37 ^f	1.80 $\pm$ 0.03 ^e	2.00 $\pm$ 0.16 ^e	1.79 $\pm$ 0.20 ^e	1.80 $\pm$ 0.12 ^e
9	2.01 $\pm$ 0.00 ^g	1.51 $\pm$ 0.47 ^g	1.87 $\pm$ 0.25 ^h	1.75 $\pm$ 0.06 ^h	1.77 $\pm$ 0.04 ^h	1.71 $\pm$ 0.07 ^h	1.84 $\pm$ 0.07 ^f	1.71 $\pm$ 0.80 ^f	1.82 $\pm$ 0.11 ^e	1.65 $\pm$ 0.83 ^e	1.35 $\pm$ 0.02 ^f	1.77 $\pm$ 0.01 ^g
10	1.56 $\pm$ 0.06 ^h	1.48 $\pm$ 0.40 ^h	1.78 $\pm$ 0.33 ⁱ	1.60 $\pm$ 0.21 ⁱ	1.65 $\pm$ 0.16 ^k	1.49 $\pm$ 0.53 ^l	1.88 $\pm$ 0.57 ^f	1.50 $\pm$ 0.64 ^f	1.80 $\pm$ 0.03 ^f	1.32 $\pm$ 0.76 ^f	1.07 $\pm$ 0.38 ^h	1.70 $\pm$ 0.13 ⁱ

Different letters located over the error bars indicate significant differences ($p < 0.05$).

Table S3: Proteins identified in fraction 8 kDa by LC-MS/MS with Unused Protein Score above 1.3 and 95% confidence

% Coverage (95)	Unused Protein Score	^a Protein accession number	Name
8.1	25.16	P00360	Glyceraldehyde-3-phosphate dehydrogenase 1 OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=TDH1
9	20.17	P00359	Glyceraldehyde-3-phosphate dehydrogenase 3 OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=TDH3
2.6	2.56	P02994	Elongation factor 1-alpha OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=TEF1
3.5	2.35	P17076	60S ribosomal protein L8-A OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RPL8A
2	2	P14540	Fructose-bisphosphate aldolase OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=FBA1
2.1	2	P53334	Probable family 17 glucosidase SCW4 OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=SCW4
1	2	P05694	5-methyltetrahydropteroyltriglutamate-homocysteine methyltransferase OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=MET6
8.3	2	P0CX48	40S ribosomal protein S11-B OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RPS11B
2.5	2	P09938	Ribonucleoside-diphosphate reductase small chain 1 OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RNR2
6.9	2	P0CX30	40S ribosomal protein S23-B OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RPS23B
5.7	2	P38013	Peroxiredoxin AHP1 OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=AHP1
5.5	2	P0CX56	40S ribosomal protein S18-B OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=RPS18B
3.2	2	P00950	Phosphoglycerate mutase 1 OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=GPM1
1.4	2	P16467	Pyruvate decarboxylase isozyme 2 OS= <i>S. cerevisiae</i> (strain ATCC 204508 / S288c) OX=559292 GN=PDC5

^a Protein accession number in the UniProt protein database (<http://www.uniprot.org/>) for taxonomy *S. cerevisiae*

Table S4: Peptides identified by LC-MS/MS in fraction 8 kDa derived from the glyceraldehyde-3-phosphate dehydrogenase-1 protein TDH1 gene.

^a Peptide score is a count of the MS/MS peaks that match to a theoretical ion, for those ion types considered by

Confidence (%)	Sequence	Observed MW (Da)	^a Peptides score
99	ISWYDNEYGYSA	1466.5991	14
99	ISWYDNEYGYSAR	1622.6995	17
99	ISWYDNEYGYSAR	1638.6906	17
99	ISWYDNEYGYSARV	1721.7689	21
99	ISWYDNEYGYSARVV	1820.8391	22
99	ISWYDNEYGYSARVVD	1935.8632	19
99	LISWYDNEYGYSAR	1735.7845	17
99	VKLISWYDNEYGY	1648.7778	21
99	VKLISWYDNEYGYS	1735.8104	17
99	VKLISWYDNEYGYSA	1806.847	20
99	VKLISWYDNEYGYSAR	1962.9475	21

the Paragon™ algorithm.

Table S5: Peptides identified by by LC-MS/MS in fraction 8 kDa derived from the glyceraldehyde-3-phosphate dehydrogenase protein-3 TDH2/3 gene.

Confidence (%)	Sequence	Observed MW	^a Peptides score
99	LVSWYDNEYGYSTR	1751.7799	21
99	LVSWYDNEYGYSTRV	1850.8474	17
99	PANLPWGSS	927.4459	12
99	VKLVSWYDNEYGY	1634.7628	18
99	VKLVSWYDNEYGYSTR	1978.9414	19
99	VSWYDNEYGYSTR	1654.691	19
99	VSWYDNEYGYSTR	1638.6947	19
99	VSWYDNEYGYSTRV	1737.7631	20
99	VSWYDNEYGYSTRVV	1836.8304	21
99	VSWYDNEYGYSTRVVD	1951.8558	20
98.9	VSWYDNEYGYST	1482.593	12
98.8	RDPANLPW	967.4885	9

count of the MS/MS peaks that match to a theoretical ion, for those ion types considered by the Paragon™ algorithm.

^a Peptide score is a

Fig. S1

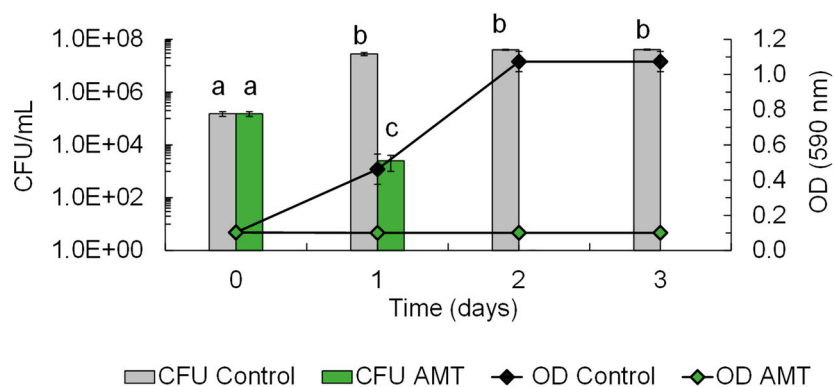


Figure S1. Culturable cells (CFU/mL) and optical density (OD) of *Hanseniaspora guilliermondii* in the antimicrobial tests (AMT) without addition of Fraction 8 kDa (Control) and with addition of 1 mg/mL of Fraction 8 kDa (AMT). Data represented correspond to mean values of triplicate independent assays $\pm$ SD (error bars). Different letters located over the error bars indicate significant differences ($p < 0.05$).

Fig. S2

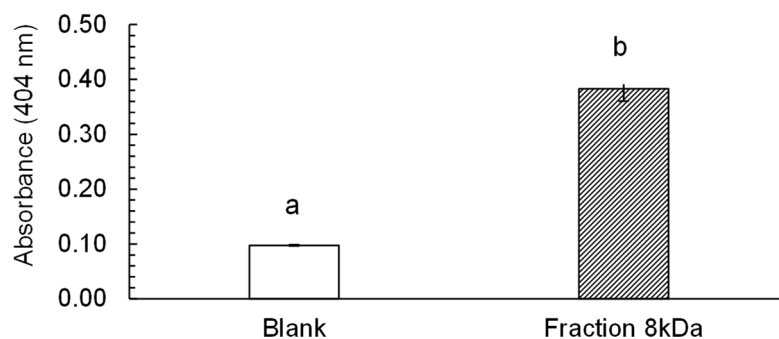


Figure S2. Absorbance (404 nm) comparison of the blank with the GAPDH-derived AMPs presents in the fraction 8 kDa determined by indirect ELISA. Values represented correspond to the means $\pm$ SD (error bars) of three absorbance measurements. Different letters located over the error bars indicate significant differences ($p < 0.05$).

Fig. S3

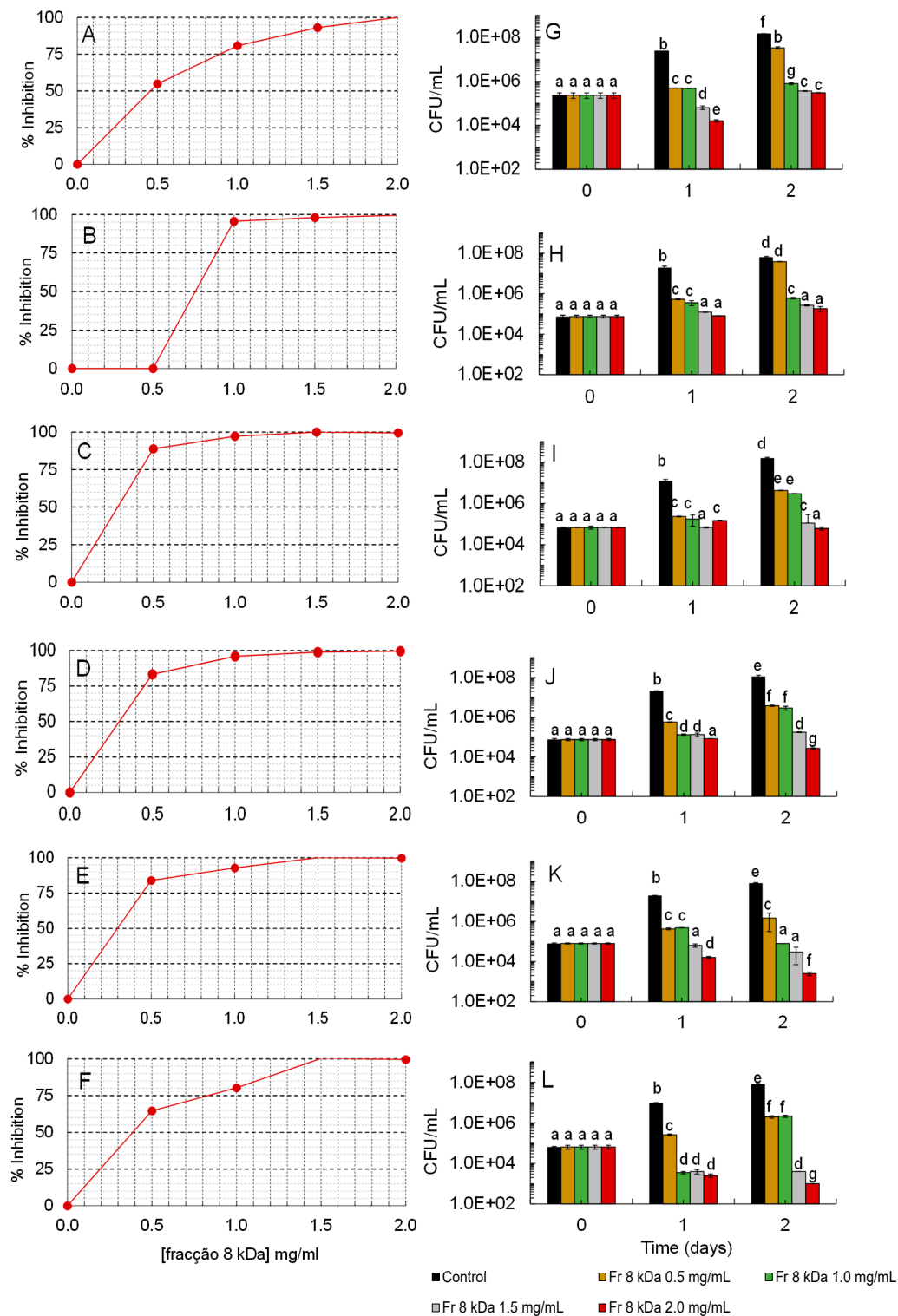


Figure S3. Growth inhibition (%) (A-F) and culturability (G-L) of *B. bruxellensis* strains in the absence of fraction 8 kDa (Control) and in the presence of 0.5, 1.0, 1.5 and 2.0 mg/mL of fraction 8 kDa (Fr 8 kDa). (A, G) strain ISA 1649; (B, H) ISA 1700; (C, I) ISA 1791; (D, J) ISA 2104; (E, K) ISA 2116; (F, L) ISA 2211. The percentage of growth inhibition (measured by absorbance) was determined when the respective control assay reached the stationary growth phase, ca 48 h. Data represented correspond to mean values of triplicate independent assays $\pm$ SD (error bars). Different letters located over the error bars indicate significant differences ($p < 0.05$).

Fig. S4

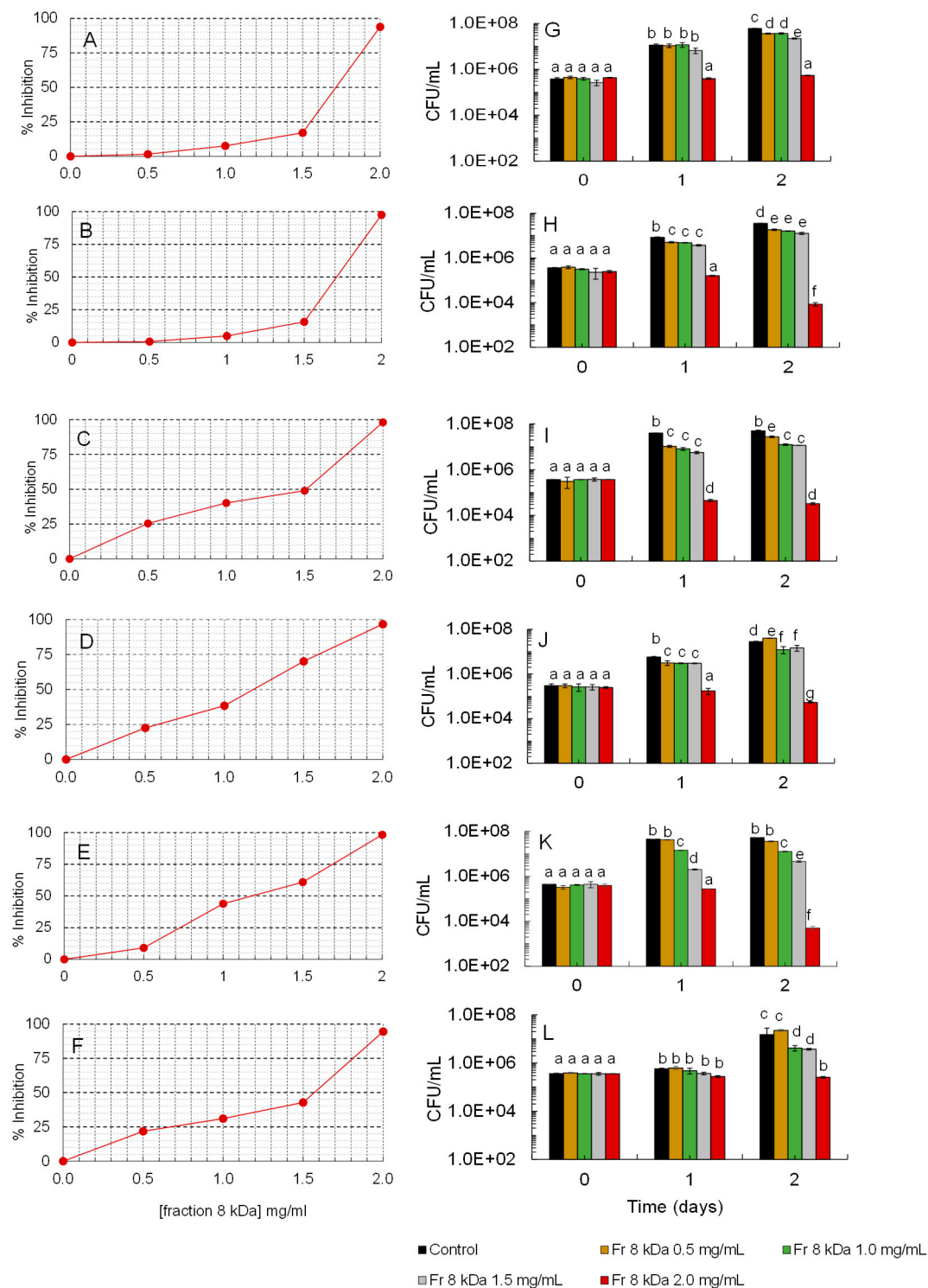


Figure S4. Growth inhibition percentage of *L. brevis* (A), *L. hilgardii* (B), *L. plantarum* (C), *L. mesenteroides* subsp. *cremoris* (D), *Pediococcus parvulus* (E), and *Pediococcus pentosaceus* (F) measured by OD 590 nm, in the presence of different concentrations of fraction 8 kDa (0.5, 1.0, 1.5 and 2.0 mg/mL), values determined when the respective control assay reached the stationary growth phase, ca 48 h. Culturability (CFU/mL) of *L. brevis* (G), *L. hilgardii* (H), *L. plantarum* (I), *L. mesenteroides* subsp. *cremoris* (J), *P. parvulus* (K), and *P. pentosaceus* (L) in the absence of fraction 8 kDa (Control) and in the presence of fraction 8 kDa (Fr 8 kDa) at the concentrations above-mentioned. Different letters located over the error bars indicate significantly different values ($p < 0.05$).