

APPENDICES

APPENDICES

Appendix 1. High Performance Thin Layer Chromatography – Qualitative Analysis of LAB Bioconversion Activity

The results of qualitative analysis of the ability of LAB to convert oleic acid in to 10-HSA are summarised in subsection 3.1.2., and recorded in detail in Table A1 below.

Table A1. Qualitative Analysis of bioconversion activity by HPTLC.

Strain	Activity: ++, +, +(-), -(+), -
GK1	+(-)
GK2	-
GK3	+
GK4	+(-)
GK5	+
GK6	-
GK7	+(-)
GK8	-(+)
AF1	-
AF2	-
AF3	+
AF4	+(-)
AF5	-
AF6	+(-)
AF7	-(+)
CD1	-
CD2	-
CD3	+(-)
CD4	-(+)
CD5	+
CD6	+(-)
CD7	-
CD8	-
CD9	-
CD10	+(-)
CD11	-
CE1	-
CE2	+(-)
CE3	+(-)
CE4	+(-)
CE5	+(-)
CE6	-
CE7	-
CE8	-
CE9	-
CY1	-
CY2	+(-)
CY3	-
CY4	-

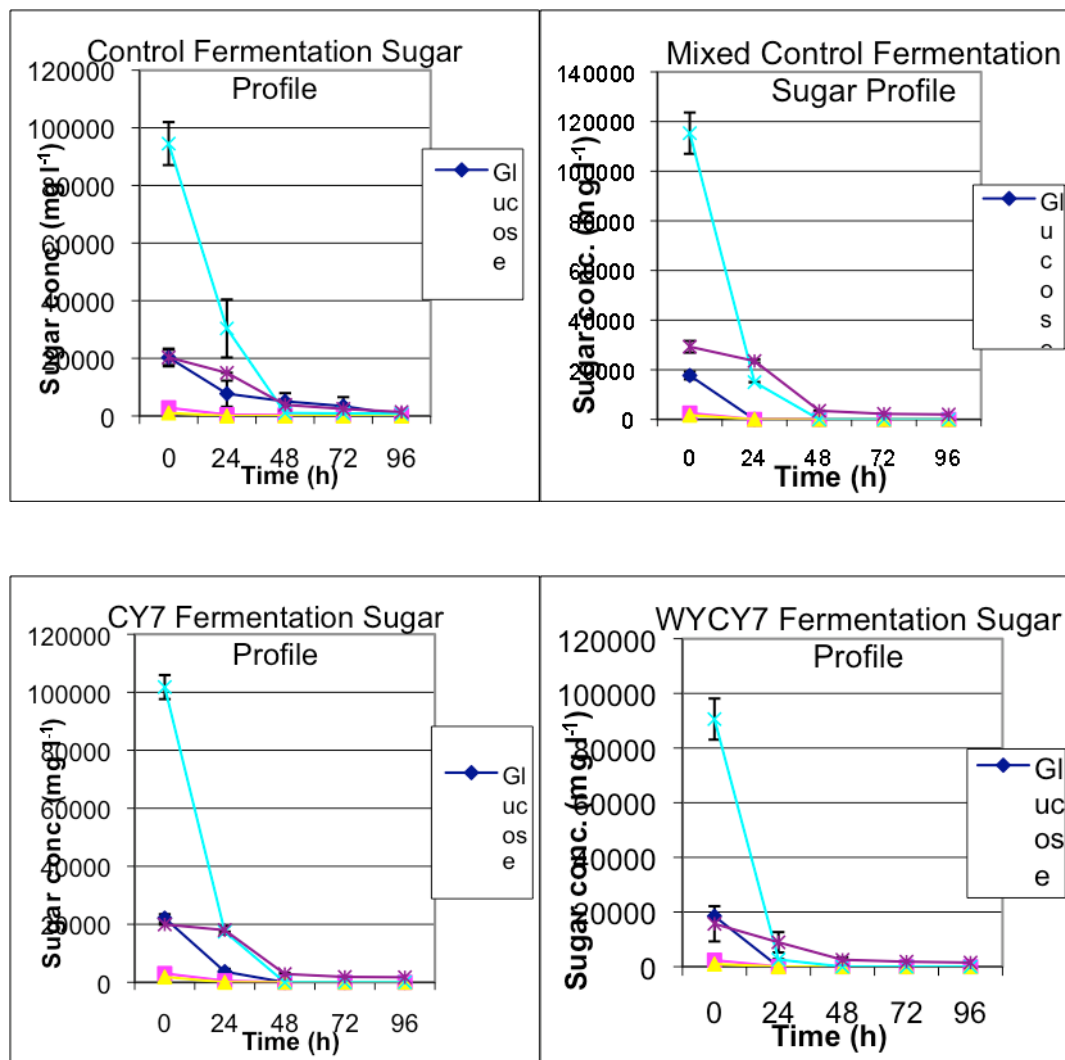
CY5	-
CY6	-(+)
CY7	++
CY8	-(+)
TK1	++
TK2	-(+)
TK3	-(+)
TK4	-(+)
TK5	-(+)
TK6	-
TK7	-
TK8	-
AL1	-
AL2	-
AL3	-(+)
AL4	-(+)
AL5	-
AL6	-
AL7	-
AL8	-
AL9	-(+)
AL10	-
ST1	-
ST2	-(+)
ST3	+(-)
ST4	-(+)
ST5	-
ST6	-
ST7	-
ST8	-(+)
ST9	-
ST10	+(-)
ST11	-(+)
ST12	-(+)
MA1	-(+)
MA2	-
MA3	-(+)
MA4	-
MA5	-
MA6	++
MA7	-
MA8	++
MA9	+
MA10	-
PL1	+
PL2	-
PL3	+
PL4	-
PL5	-
PL6	+(-)
PL7	-(+)
PL8	+(-)
KD1	++
KD2	+(-)
KD3	+(-)
KD4	-(+)
KD5	-
KD6	+

KD7	+(-)
KD8	+(-)
BR1	+
BR2	-(+)
BR3	-
BR4	-(+)
BR5	+
BR6	-(+)
DL1	-(+)
DL2	-(+)
DL3	-(+)
DL4	+(-)
DL5	+
DL6	+
DL7	+(-)
DL8	+(-)
GO1	-(+)
GO2	+
GO3	+(-)
GO4	-(+)
GO5	+
GO6	+
GO7	+(-)
GO8	+(-)
GE1	-(+)
GE2	+(-)
GE3	-
GE4	+(-)
GE5	-(+)
GE6	-(+)
GE7	-
MT1	+(-)
MT2	+
MT3	+
MT4	-(+)
MT5	-(+)
MT6	-(+)
MT7	-
MT8	-
CG1	+(-)
CG2	-(+)
CG3	-
CG4	-
CG5	-
CG6	+(-)
CG7	++
CG8	+
CG9	-
CG10	-

KEY: ++ = strong positive activity
+ = positive activity
+(-) = weak positive activity
-(+) = very weak positive activity
- = negative activity

Appendix 2. Sugar Profiling of Fermentation Wash

The concentrations of glucose, fructose, sucrose, maltose, and maltotriose at the conclusion of all laboratory-scale fermentations are shown in Table 3.11 in subsection 3.2.9. The profiles of these sugars throughout the course of the fermentations are shown in Figure A1 a-j.



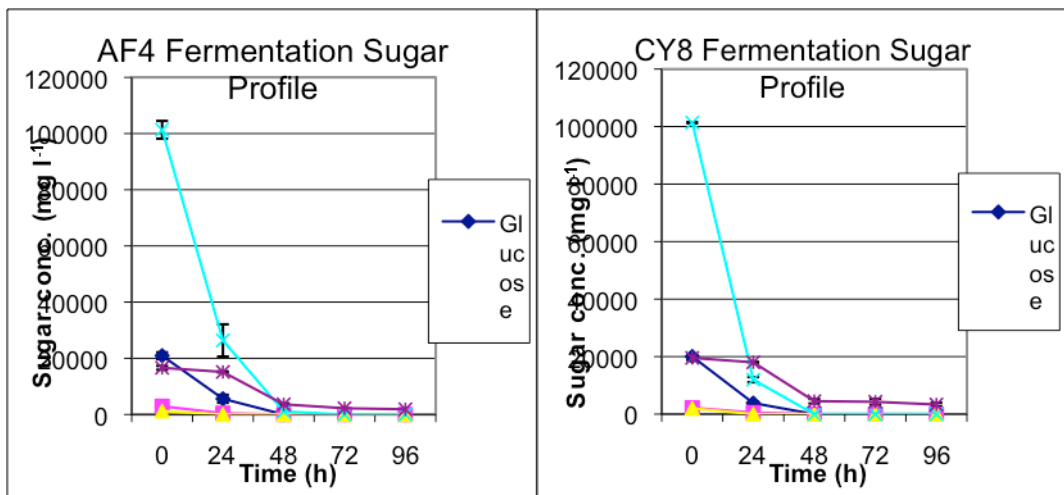
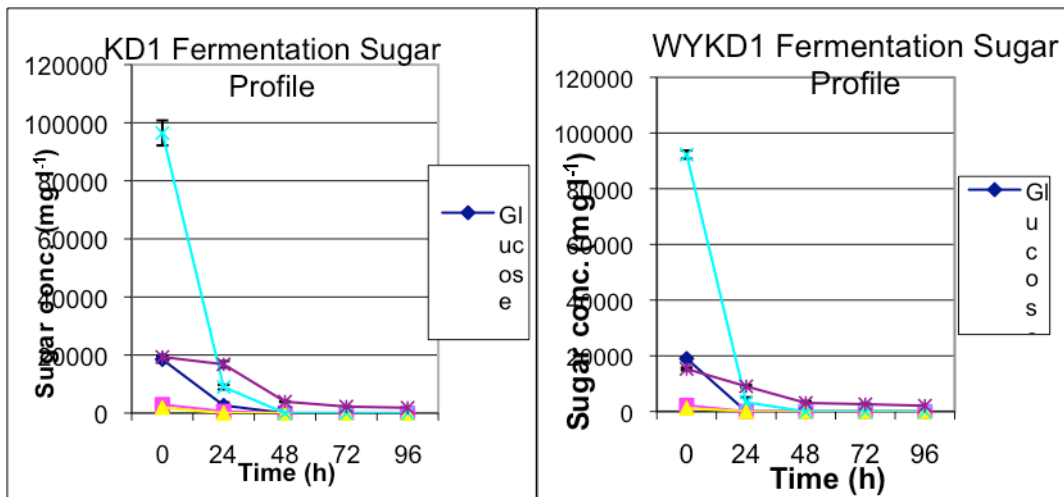
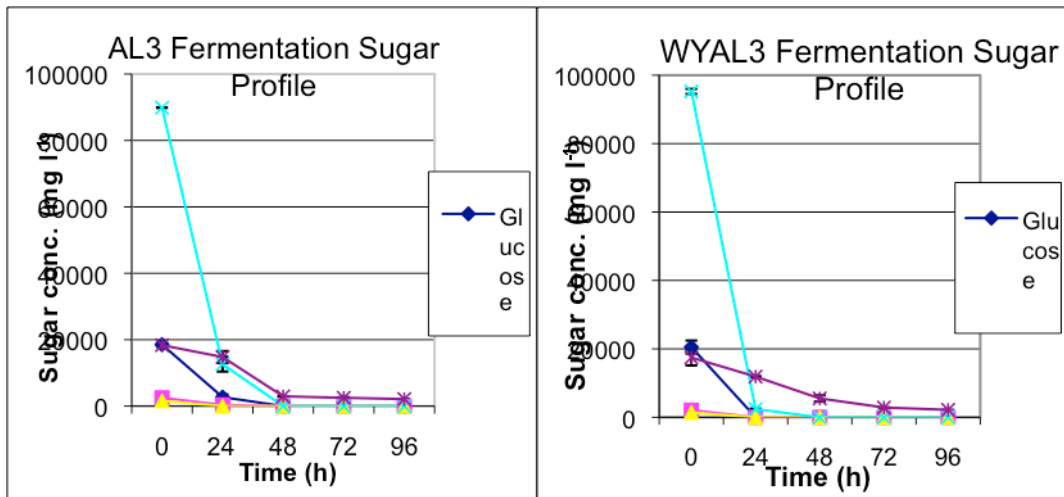


Figure A1. a (control), b (mixed control), c (CY7), d (WYCY7), e (AL3), f (WYAL3), g (KD1), h (WYKD1), i (AF4), and j (CY8). Sugar profiles of fermentations (each point is an average of duplicate samples taken from two separate fermentations, error bars indicate SD).

Appendix 3. Sensory Analysis of New-Make Sprits

The results of the sensory analysis of new-make spirits are shown in Figures 3.20 and 3.21 of subsection 3.3.4. These charts represent the average panel scores, shown in Tables A2 a-d and A3 a-d.

Table A2 a. Average sensory panel scores.

	Pungent	Peaty	Feinty	Cereal	Green/grassy
Control	1.27	0.16	0.96	0.67	0.67
KD1	1.08	0.26	0.93	0.74	0.59
AL3	1.12	0.19	0.95	0.75	0.87
AF4	1.19	0.22	0.99	0.73	0.63
CY7	1.21	0.16	1.02	0.74	0.86
CY8	1.14	0.17	1.04	0.81	0.68
ANOVA	NS	NS	NS	NS	P=0.0022

NS = no statistically significant difference; P = probability of samples being different in terms of this attribute

Table A2 b. Average sensory panel scores.

	Floral	Fruity/estery	Solventy	Soapy	Sweet
Control	0.61	0.64	0.60	0.50	0.43
KD1	0.56	0.65	0.64	0.57	0.79
AL3	0.57	0.77	0.65	0.56	0.42
AF4	0.48	0.78	0.61	0.54	0.49
CY7	0.69	0.66	0.76	0.51	0.42
CY8	0.51	0.64	0.63	0.66	0.36
ANOVA	NS	NS	NS	NS	P=0.0027

NS = no statistically significant difference; P = probability of samples being different in terms of this attribute

Table A2 c. Average sensory panel scores.

	Oily	Sour	Sulfury	Meaty	Stale
Control	0.57	0.39	0.36	0.23	0.28
KD1	0.65	0.46	0.77	0.47	0.39
AL3	0.61	0.40	0.52	0.29	0.29
AF4	0.58	0.41	0.49	0.33	0.25
CY7	0.50	0.57	0.51	0.31	0.31
CY8	0.43	0.26	0.57	0.32	0.33

ANOVA	NS	P=0.0479	P=0.0036	P=0.0116	NS
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NS = no statistically significant difference; P = probability of samples being different in terms of this attribute

Table A2 d. Average sensory panel scores.

	Clean
Control	1.35
KD1	1.07
AL3	1.29
AF4	1.29
CY7	1.25
CY8	1.36
ANOVA	NS

NS = no statistically significant difference; P = probability of samples being different in terms of this attribute

Table A3 a. Average sensory panel scores.

	Pungent	Peaty	Feinty	Cereal	Green/Grassy
Control	1.14	0.19	0.59	0.56	0.69
WYC	1.13	0.27	1.03	0.85	0.75
WYCY7	1.26	0.37	1.33	0.77	0.91
WYAL3	1.21	0.41	1.08	0.79	0.93
WYKD1	0.99	0.25	0.89	0.77	0.85
ANOVA	NS	P=0.0157	P=0.0000	NS	P=0.0088

NS = no statistically significant difference; P = probability of samples being different in terms of this attribute

Table A3 b. Average sensory panel scores.

	Floral	Fruity/Estery	Solventy	Soapy	Sweet
Control	0.75	0.83	0.57	0.56	0.51
WYC	0.56	0.56	0.77	0.61	0.51
WYCY7	0.59	0.73	0.72	0.70	0.45
WYAL3	0.69	0.69	0.56	0.69	0.61
WYKD1	0.54	0.76	0.64	0.51	0.99
ANOVA	NS	NS	NS	NS	P=0.0000

NS = no statistically significant difference; P = probability of samples being different in terms of this attribute

Table A3 c. Average sensory panel scores.

	Oily	Sour	Sulfury	Meaty	Stale
Control	0.51	0.56	0.43	0.34	0.34
WYC	0.65	0.47	0.60	0.37	0.43
WYCY7	0.73	0.66	0.71	0.29	0.65
WYAL3	0.62	0.60	0.58	0.53	0.58
WYKD1	0.81	0.37	0.48	0.23	0.41
ANOVA	NS	NS	P=0.0000	P=0.0003	P=0.0131

NS = no statistically significant difference; P = probability of samples being different in terms of this attribute

Table A3 d. Average sensory panel scores.

	Clean
Control	1.67
WYC	1.55
WYCY7	1.40
WYAL3	1.45
WYKD1	1.62
ANOVA	P=0.0042

NS = no statistically significant difference; P = probability of samples being different in terms of this attribute

Appendix 4. Reassignment of LAB strain CY7 from *L. fermentum* to *L. paracasei*

CLUSTAL W alignments of *L. fermentum* and *L. paracasei* 16S rRNA gene sequences, and of the partial and full 16S rRNA gene sequences from LAB strain CY7 can be seen in Figures 4.1 and 4.2 respectively, in subsection 4.1.1. The partial and full 16S rRNA gene sequences that lead to the initial incorrect identification and subsequent reassignment are shown in Figures 5.2 and 5.3 respectively.

```
>gb|FJ211396.1| Lactobacillus fermentum strain IMAU60078 16S ribosomal RNA gene,
partial sequence
Length=1449
```

```
Score = 763 bits (413), Expect = 0.0
Identities = 413/413 (100%), Gaps = 0/413 (0%)
Strand=Plus/Minus
```

```
Query 3 GATGCACCTTCTCCGGTTAAGCCGAAGGCTTTCACATCAGACTTAGAAAAACCGCCTGCACT 62
      |||
Sbjct 615 GATGCACCTTCTCCGGTTAAGCCGAAGGCTTTCACATCAGACTTAGAAAAACCGCCTGCACT 556

Query 63 CTCTTTACGCCCAATAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGG 122
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Sbjct 555 |||CTCTTTACGCCCAATAAAATCCGGATAACGCTTGCCACCTACGTATTACCGCGGCTGCTGG 496
Query 123 CACGTAGTTAGCCGTGACTTTCTGGTTAAATACCGTCAACGTATGAACAGTTACTCTCAT 182
Sbjct 495 |||CACGTAGTTAGCCGTGACTTTCTGGTTAAATACCGTCAACGTATGAACAGTTACTCTCAT 436
Query 183 ACGTGTCTTCTTTTAAACAACAGAGCTTTACGAGCCGAAACCCTTCTTCACTCACGCGGTG 242
Sbjct 435 |||ACGTGTCTTCTTTTAAACAACAGAGCTTTACGAGCCGAAACCCTTCTTCACTCACGCGGTG 376
Query 243 TTGCTCCATCAGGCTTGCGCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGT 302
Sbjct 375 |||TTGCTCCATCAGGCTTGCGCCATTGTGGAAGATTCCCTACTGCTGCCTCCCGTAGGAGT 316
Query 303 ATGGGCCGTGTCTCAGTCCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATC 362
Sbjct 315 |||ATGGGCCGTGTCTCAGTCCCATTGTGGCCGATCAGTCTCTCAACTCGGCTATGCATCATC 256
Query 363 GCCTTGGTAGGCCGTTACCCACCAACAAGCTAATGCACCGCAGGTCCATCCA 415
Sbjct 255 |||GCCTTGGTAGGCCGTTACCCACCAACAAGCTAATGCACCGCAGGTCCATCCA 203

```

Figure A2 Partial 16S rRNA gene sequence of strain CY7, confirming identity as *L. fermentum*.

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>gb|EU187503.1| Lactobacillus paracasei strain IMPC 2.1 16S ribosomal RNA gene,
partial sequence
Length=1495

Score = 2680 bits (1451), Expect = 0.0
Identities = 1451/1451 (100%), Gaps = 0/1451 (0%)
Strand=Plus/Minus

Query 1 TAATCATTTGTCCACCTTAGACGGCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGG 60
Sbjct 1487 |||TAATCATTTGTCCACCTTAGACGGCTCGCTCCCTAAAAGGGTTACGCCACCGGCTTCGG 1428
Query 61 GTGTTACAAACTCTCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGAACGTATTACCC 120
Sbjct 1427 |||GTGTTACAAACTCTCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGAACGTATTACCC 1368
Query 121 GCGGCGTGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTA 180
Sbjct 1367 |||GCGGCGTGCTGATCCGCGATTACTAGCGATTCCGACTTCGTGTAGGCGAGTTGCAGCCTA 1308
Query 181 CAGTCCGAAC TGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTT 240
Sbjct 1307 |||CAGTCCGAAC TGAGAATGGCTTTAAGAGATTAGCTTGACCTCGCGGTCTCGCAACTCGTT 1248
Query 241 GTACCATCCATTGTAGCACGTGTGTAGCCAGGTCATAAGGGGCATGATGATTTGACGTC 300
Sbjct 1247 |||GTACCATCCATTGTAGCACGTGTGTAGCCAGGTCATAAGGGGCATGATGATTTGACGTC 1188
Query 301 ATCCCACCTTCCTCCGTTTGTACCCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTG 360
Sbjct 1187 |||ATCCCACCTTCCTCCGTTTGTACCCGGCAGTCTTACTAGAGTGCCCAACTAAATGCTG 1128
Query 361 GCAACTAGTCATAAGGGTTGCGCTCGTTGCGGGACTTAACCAACATCTCACGACACGAG 420
Sbjct 1127 |||GCAACTAGTCATAAGGGTTGCGCTCGTTGCGGGACTTAACCAACATCTCACGACACGAG 1068
Query 421 CTGACGACAACCATGCACCCTGTCAATTTGCCCCCGAAGGGGAAACCTGATCTCTCAG 480
Sbjct 1067 |||CTGACGACAACCATGCACCCTGTCAATTTGCCCCCGAAGGGGAAACCTGATCTCTCAG 1008
Query 481 GTGATCAAAAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCAATTAACACAT 540
Sbjct 1007 |||GTGATCAAAAAGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCAATTAACACAT 948
Query 541 GCTCCACCGCTTGTGCGGGCCCCGTC AATTCCTTTGAGTTTCAACCTTGCGGTGCTACT 600
Sbjct 947 |||GCTCCACCGCTTGTGCGGGCCCCGTC AATTCCTTTGAGTTTCAACCTTGCGGTGCTACT 888

```

Query 601 CCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACAC 660
 |||||
 Sbjct 887 CCCCAGGCGGAATGCTTAATGCGTTAGCTGCGGCACTGAAGGGCGGAAACCCTCCAACAC 828

Query 661 CTAGCATTCATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTTCGCTACCCATG 720
 |||||
 Sbjct 827 CTAGCATTCATCGTTTACGGCATGGACTACCAGGGTATCTAATCCTGTTTCGCTACCCATG 768

Query 721 CTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAGCCGCCCTTCGCCACTGGTGTCTTCC 780
 |||||
 Sbjct 767 CTTTCGAGCCTCAGCGTCAGTTACAGACCAGACAGCCGCCCTTCGCCACTGGTGTCTTCC 708

Query 781 ATATATCTACGCATTTACCAGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGT 840
 |||||
 Sbjct 707 ATATATCTACGCATTTACCAGCTACACATGGAGTTCCACTGTCCTCTTCTGCACTCAAGT 648

Query 841 TTCCAGTTTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAA 900
 |||||
 Sbjct 647 TTCCAGTTTCCGATGCGCTTCCTCGGTTAAGCCGAGGGCTTTCACATCAGACTTAAAAA 588

Query 901 ACCGCCTGCGCTCGCTTTACGCCAATAAAATCCGGATAACGCTTGCCACCTACGTATTAC 960
 |||||
 Sbjct 587 ACCGCCTGCGCTCGCTTTACGCCAATAAAATCCGGATAACGCTTGCCACCTACGTATTAC 528

Query 961 CGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTCTGGTTGGATACCGTCACGCCGACAAC 1020
 |||||
 Sbjct 527 CGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTCTGGTTGGATACCGTCACGCCGACAAC 468

Query 1021 AGTTACTCTGCCACCATTCTTCTCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTC 1080
 |||||
 Sbjct 467 AGTTACTCTGCCACCATTCTTCTCCAACAACAGAGTTTTACGACCCGAAAGCCTTCTTC 408

Query 1081 ACTCACGCGGCGTTGCTCCATCAGACTTGGCTCCATTGTGGGAAGATTCCCTACTGCTGCC 1140
 |||||
 Sbjct 407 ACTCACGCGGCGTTGCTCCATCAGACTTGGCTCCATTGTGGGAAGATTCCCTACTGCTGCC 348

Query 1141 TCCCCTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCAACCTCTCAGTTCCG 1200
 |||||
 Sbjct 347 TCCCCTAGGAGTTTGGGCCGTGTCTCAGTCCCAATGTGGCCGATCAACCTCTCAGTTCCG 288

Query 1201 CTACGTATCATCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATACGCCGCGGGTCC 1260
 |||||
 Sbjct 287 CTACGTATCATCGCCTTGGTGAGCCATTACCTCACCAACTAGCTAATACGCCGCGGGTCC 228

Query 1261 ATCCAAAAGCGATAGCTTACGCCATCTTTCAGCCAAGAACCATGCGGTTCTTGGATCTAT 1320
 |||||
 Sbjct 227 ATCCAAAAGCGATAGCTTACGCCATCTTTCAGCCAAGAACCATGCGGTTCTTGGATCTAT 168

Query 1321 GCGGTATTAGCATCTGTTTCCAAATGTTATCCCCACTTAAGGGCAGGTTACCCACGTGT 1380
 |||||
 Sbjct 167 GCGGTATTAGCATCTGTTTCCAAATGTTATCCCCACTTAAGGGCAGGTTACCCACGTGT 108

Query 1381 TACTCACCCGTCCGCCACTCGTTCCATGTTGAATCTCGGTGCAAGCACCGATCATCAACG 1440
 |||||
 Sbjct 107 TACTCACCCGTCCGCCACTCGTTCCATGTTGAATCTCGGTGCAAGCACCGATCATCAACG 48

Query 1441 AGAACTCGTTC 1451
 |||||
 Sbjct 47 AGAACTCGTTC 37

Figure A3 Full 16S rRNA gene sequence of strain CY7, resulting in the reassignment of this strain as *L. paracasei*.

Appendix 5. Internet Resources used in this Study

Bioinformatic links

<http://www.brenda-enzymes.org/>

<http://www.ebi.ac.uk/>

<http://www.eurofindna.com/>

<http://www.expasy.org/>

<http://www.genome.jp/>

<http://www.ncbi.nlm.nih.gov/>

Bibliographic links

<http://www.maltwhiskytrail.com/>

<http://scotchwhisky.net/>

<http://www.whisky-heritage.co.uk/>

