

Low-Carbohydrate Tolerant LAB Strains Identified from Rumen Fluid: Investigation of Probiotic Activity and Legume Silage Fermentation

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Table S1. Carbohydrate-fermentation of RJ1 and S22 using API 50 CHL test kit.

| S. No | Carbohydrates | RJ1 | S22 |
|-------|--------------------------|-----|-----|
| 1 | Control | - | - |
| 2 | Glycerol | - | - |
| 3 | Erythritol | - | - |
| 4 | D-Arabinose | - | - |
| 5 | L.Arabinose | + | + |
| 6 | D-Ribose | + | + |
| 7 | D-Xylose | - | - |
| 8 | L-Xylose | - | - |
| 9 | D-Adonitol | - | - |
| 10 | Methyl-D-Xylopyranoside | - | - |
| 11 | D-Galactose | + | + |
| 12 | D-Glucose | + | + |
| 13 | D-Fructose | + | + |
| 14 | D-Mannose | + | + |
| 15 | L-Sorbose | + | - |
| 16 | L-Rhamnose | + | - |
| 17 | Dulcitol | - | - |
| 18 | Inositol | - | - |
| 19 | D-Mannitol | + | + |
| 20 | D-Sorbitol | + | - |
| 21 | Methyl-D-mannopyranoside | + | + |
| 22 | Methyl-D-glucopyranoside | + | - |
| 23 | N-Acetylglucosamine | + | + |
| 24 | Amygdalin | + | + |
| 25 | Arbutin | + | + |
| 26 | Esculin ferric citrate | + | + |
| 27 | Salicin | + | + |
| 28 | D-Cellobiose | + | + |
| 29 | D-Maltose | + | + |
| 30 | D-Lactose | + | + |
| 31 | D-Melibiose | + | + |
| 32 | D-Saccharose | + | + |
| 33 | D-Trehalose | + | - |
| 34 | Inulin | - | - |
| 35 | D-Mezitose | + | + |
| 36 | D-Raffinose | - | - |
| 37 | Amidon | - | - |
| 38 | Glycogen | - | - |
| 39 | Xylitol | - | - |
| 40 | Gentiobiose | + | - |
| 41 | D-Turanose | + | - |
| 42 | D-Lyxose | - | - |
| 43 | D-Tagatose | - | - |
| 44 | D-Fucose | - | - |
| 45 | L-Fucose | - | - |
| 46 | D-Arabitol | - | - |

| | | | |
|----|-------------------------------------|---|---|
| 47 | L-Arabinol | - | - |
| 48 | Potassium gluconate | + | + |
| 49 | Potassium gluconate 2-ketogluconate | - | - |
| 50 | Potassium gluconate 5-ketogluconate | - | - |

+ -presence; -absence.

Table S2. Qualitative analysis of enzyme production of RJ1 and S22 using API-ZYM test kit.

| S. No | Extra Cellular Enzymes | RJ1 | S22 |
|-------|------------------------------------|-----|-----|
| 1 | Control | - | - |
| 2 | Alkaline phosphatase | + | + |
| 3 | Esterase (C4) | ++ | ++ |
| 4 | Esterase lipase (C8) | +++ | ++ |
| 5 | Lipase (C14) | ++ | +++ |
| 6 | Leucine arylamidase | ++ | +++ |
| 7 | Valine arylamidase | ++ | +++ |
| 8 | Cystine arylamidase | ++ | ++ |
| 9 | Trypsin | ++ | + |
| 10 | α -Chymotrypsin | +++ | ++ |
| 11 | Acid phosphatase | +++ | ++ |
| 12 | Naphthol-AS-biphosphohydrolase | +++ | ++ |
| 13 | α -Galactosidase | +++ | +++ |
| 14 | β -Galactosidase | +++ | +++ |
| 15 | β -Glucuronidase | +++ | +++ |
| 16 | α -Glucosidase | +++ | ++ |
| 17 | β -Glucosidase | +++ | +++ |
| 18 | N-acetyl- β -glucosaminidase | ++ | ++ |
| 19 | α -Mannosidase | - | + |
| 20 | α -Fucosidase | + | + |

+++ -high; ++ - moderate; - no activity.

Table S3. Antibiotic resistance profile of *L. plantarum* RJ1 and *P. pentosaceus* S22 analyzed by the disc-diffusion method (S-susceptible; R-resistant; M-moderate).

| S. No | Antibiotics | Disc Potency (mcg) | RJ1 | S22 |
|-------|---------------------------|--------------------|-----|-----|
| 1 | Lomefloxacin (LOM) | 10 | R | S |
| 2 | Norfloxacin (NX) | 10 | M | M |
| 3 | Azithromycin (AZM) | 15 | S | S |
| 4 | Vancomycin (VA) | 30 | R | R |
| 5 | Doxycycline hydrochloride | | S | S |
| 6 | Co-Trimoxazole (COT) | 25 | S | S |
| 7 | Nitrofurantoin (NIT) | 300 | S | S |
| 8 | Gatifloxacin (GAT) | 5 | S | S |
| 9 | Carbenicillin (CB) | 100 | S | M |
| 10 | Cefoxitin (CX) | 30 | M | M |
| 11 | Clindamycin (CD) | 2 | M | S |
| 12 | Chloramphenicol (C) | 30 | S | S |
| 13 | Erythromycin (E) | 15 | S | M |
| 14 | Metronidazole (MT) | 5 | R | R |
| 15 | Penicillin (P) | 10 | M | R |
| 16 | Tetracycline (TE) | 30 | S | S |

| | | | | |
|----|------------------|-----|---|---|
| 17 | Bacitracin (B) | 10 | M | M |
| 18 | Polymyxin-B (PB) | 300 | M | R |
| 19 | Gentamycin (GEN) | 10 | S | M |
| 20 | Neomycin (N) | 30 | S | S |

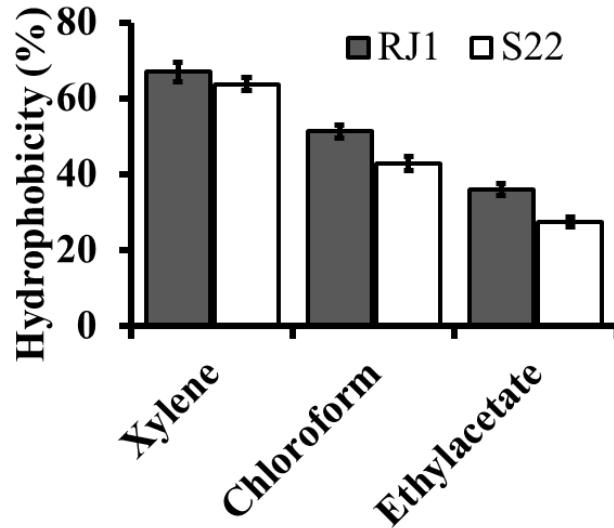


Figure S1. Assessment of cell-surface hydrophobicity of *L. plantarum* RJ1 and *P. pentosaceus* S22.

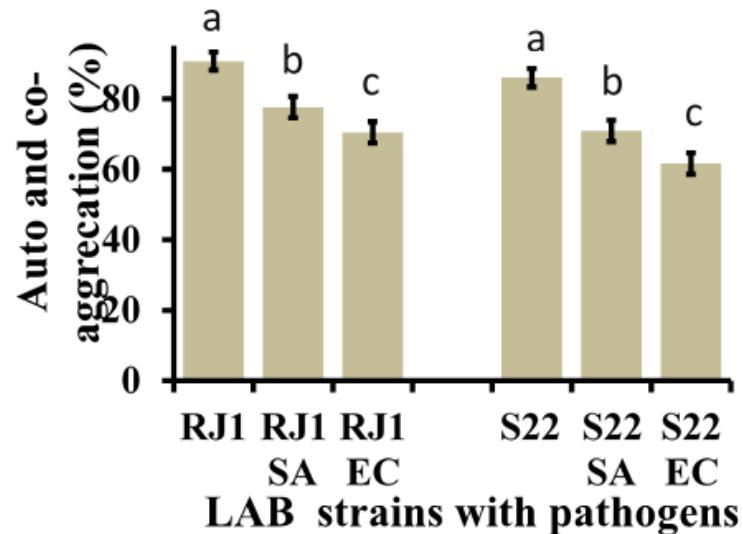


Figure S2. The auto and co-aggregation abilities of RJ1 and S22 (SA-*Staphylococcus aureus*; EC-*E.coli* pathogens).