**Supplementary Material**

*Weissella cibaria* riboflavin-overproducing and dextran-producing strains useful for the development of functional bread

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**Supplementary Table S1.** Description and location of oligonucleotides used for DNA amplification and sequencing of *rib* operon and *dsr* genes

### Amplification and sequencing of the *rib* operon

| Amplicon size (bp) | Primers for amplification (5’-3’) Length (nt) | Primers for sequencing (5’-3’) Length (nt) |
|-------------------|---------------------------------------------|---------------------------------------------|
| 4045 bp           | For1: TGGCCTTCGCTGATATTTCG 20              | For2: TTTTGCCCCCTTTACGCAG 19               |
|                   | Rev1: AGCATTGTACATCCCCCTCAAA 21           | For3: TATCAAGCCGCACAAACG 19               |
|                   | For4: TCCCACACTACACACAAAC 21              | For5: CTAGTACGACGTTGTATTTGATT 23          |
|                   | Rev2: GTGGAATTTCCTGTCGTCGG 20             | Rev2: GTGGAATTTCCTGTCGTCGG 20             |
|                   | Rev3: CTTCAATACCCTGCTGGCT 19              | Rev3: CTTCAATACCCTGCTGGCT 19              |
|                   | Rev4: TCCACGTGCCATCCGACC 21              | Rev4: TCCACGTGCCATCCGACC 21              |

**Primer walking hybridization map**

![Primer walking hybridization map](image)

### Amplification and sequencing of the *dsr* gene

| Amplicon size (bp) | Primers for amplification (5’-3’) Length (nt) | Primers for sequencing (5’-3’) Length (nt) |
|-------------------|---------------------------------------------|---------------------------------------------|
| 4546 bp           | For1: GAAAGATTATGCCCGTTA 20                 | For2: TGGCGTGAAAAAGATGGTAA 20               |
|                   | Rev1: GCCATATAACAGACTCCTCAAA 22            | For3: TTGAAAAATACCGCGACACA 20              |
For1: TGGGTTAATGCCTACGGAAG  
For2: CCTGCCAAATGGTATTGCTT  
For3: AAAGCTTGATTGCGGACAAC  
For4: CGTTGCTTACCCGTTACCAT  
For5: CTACCGCACTTGCACTGTCA  

Primer walking hybridization map
Supplementary Table S2. Detection of LAB survival after dough fermentation and prior to the baking process

| W. cibaria strain | CFU/g   |
|------------------|---------|
| Uninoculated LAB | $4.37 \times 10^6$ |
| BAL3C-5          | $2.26 \times 10^9$ |
| BAL3C-5 B2       | $1.83 \times 10^9$ |
| BAL3C-7          | $1.82 \times 10^9$ |
| BAL3C-7 B2       | $9.15 \times 10^8$ |
| BAL3C-22         | $2.09 \times 10^9$ |
| BAL3C-22 B2      | $2.81 \times 10^9$ |

Doughs were independently inoculated with cells of each BAL3C strain at a concentration of $1 \times 10^9$ CFU/g, and after 16 h of fermentation prior baking, the level of LAB CFU/g for the inoculated doughs was determined by plating on MRS agar medium.

To that end, 10 g samples of dough (prepared as described in Materials and Methods) were homogenized in 90 mL of sterile peptone water (1 g/L peptone, 8.5 g/L NaCl) in 250 mL flasks and incubated at 28 °C for 1 h with shaking (200 rpm). For CFU/g quantification, samples of 1 mL were collected by centrifugation at 10 000 rpm and ten-fold dilutions of the supernatants were spread as 0.1 mL aliquots on MRS agar plates that were incubated at 30 °C for ~ 48 h.

In the spontaneously fermented uninoculated dough, low concentrations of LAB ($4.37 \times 10^6$ CFU/g), likely endogenous to the white wheat flour, were detected. In addition, values $>1 \times 10^9$ CFU/g were detected in all doughs inoculated with each BAL3C strain.
**Supplementary Table S3.** Comparison of flavin levels present in the experimental breads quantified by direct fluorescence measurement (direct determination) or after HPLC analysis (HPLC)

| W. cibaria strain | Direct determination (mg/100 g of bread) | HPLC (mg/100 g of bread) | Ratio Direct/HPLC |
|-------------------|------------------------------------------|---------------------------|------------------|
| Without BAL       | 0.20±0.01                                | 0.09±0.01                 | 2.17             |
| BAL3C-5           | 0.24±0.02                                | 0.08±0.02                 | 2.90             |
| BAL3C-5 B2        | 0.45±0.04                                | 0.56±0.02                 | 0.81             |
| BAL3C-7           | 0.25±0.00                                | 0.16±0.02                 | 1.50             |
| BAL3C-7 B2        | 0.48±0.01                                | 0.61±0.08                 | 0.78             |
| BAL3C-22          | 0.25±0.01                                | 0.13±0.02                 | 1.95             |
| BAL3C-22 B2       | 0.43±0.06                                | 0.46±0.06                 | 0.93             |

*Bread samples were subjected to acidic and thermal treatment to convert flavins into riboflavin prior to measurement of fluorescence.
Supplementary Figure S1. Dextran levels produced by *W. cibaria* strains grown in RAMS medium. Values are represented as mean ± standard deviation of three independent experiments. Statistical analyses were carried out by t-test to determine if parental and mutant dextran levels were significantly different (A), or by one-way Anova to establish differences in dextran production between groups (B). In both cases a *p* value ≤ 0.05 was considered significant.
Supplementary Figure S2. Riboflavin produced by *W. cibaria* strains grown in RAMS medium. Values are represented as mean ± standard deviation of three independent experiments. Statistical analyses were carried out by t-test to determine if parental and mutant riboflavin levels were significantly different (A), or by one-way Anova to establish differences in riboflavin production between groups (B), in both cases a *p* value ≤ 0.05 was considered significant.
Supplementary Figure S3. Riboflavin produced by *W. cibaria* strains growth in RAM medium. Values are represented as the mean ± standard deviation of three independent experiments. Statistical analyses were carried out by t-test to determine if parental and mutant riboflavin levels were significantly different (A), and by one-way Anova to establish differences in riboflavin production between groups (B) in both cases a *p* value ≤ 0.05 was considered significant.
Supplementary Figure S4. Real time analysis of the influence of FMN on riboflavin production by *W. cibaria* strains. The bacteria were grown in RAMS medium supplemented with 3 μM FMN in a Varioskan Flask System. The growth was estimated by measurement of the OD$_{600}$ nm (A) and flavin fluorescence (B) was measured upon excitation at a wavelength of 440 nm and detection of emission at a wavelength of 520 nm.
Supplementary Figure S5. GC-MS analysis of breads for detection of soluble dextran hydrolyzed to isomaltose. Chromatograms of breads produced by fermentation with BAL3C-22 B2 (A), BAL3C-22 B2 (B), with only the dough microbiota (without LAB) (C) and of wheat dough (D) are depicted. The samples were resuspended in H₂O and treated with the *Chaetomium erraticum* dextranase at 30 °C for 18 h, as described in Material and Methods, prior to the GC-MS analysis.
**Supplementary Figure S6.** Statistical analysis of free riboflavin (A) and flavins (B) levels in experimental breads produced with *W. cibaria* strains. Values are represented as mean ± standard deviation of three independent technical replicates. Statistical analyses were carried out by t-test to determine if levels of riboflavin and flavins synthesized by parental and mutant strains were significantly different ($p \leq 0.05$).
Supplementary Figure S7. Determination of soluble (A) and total (B) dextran levels in experimental breads produced with *W. cibaria* strains. Values are represented as mean ± standard deviation of three independent technical replicates. Statistical analyses were carried out by t-test to determine if levels of dextran produced by parental and mutant strains were significantly different (*p* ≤ 0.05).