

## *Supplementary Material*

### **A multi-method approach for selecting riboflavin-overproducing *Weissella cibaria* strains after treatment with roseoflavin: from DNA sequencing of FMN riboswitch to phenotypical selection of mutants strains.**

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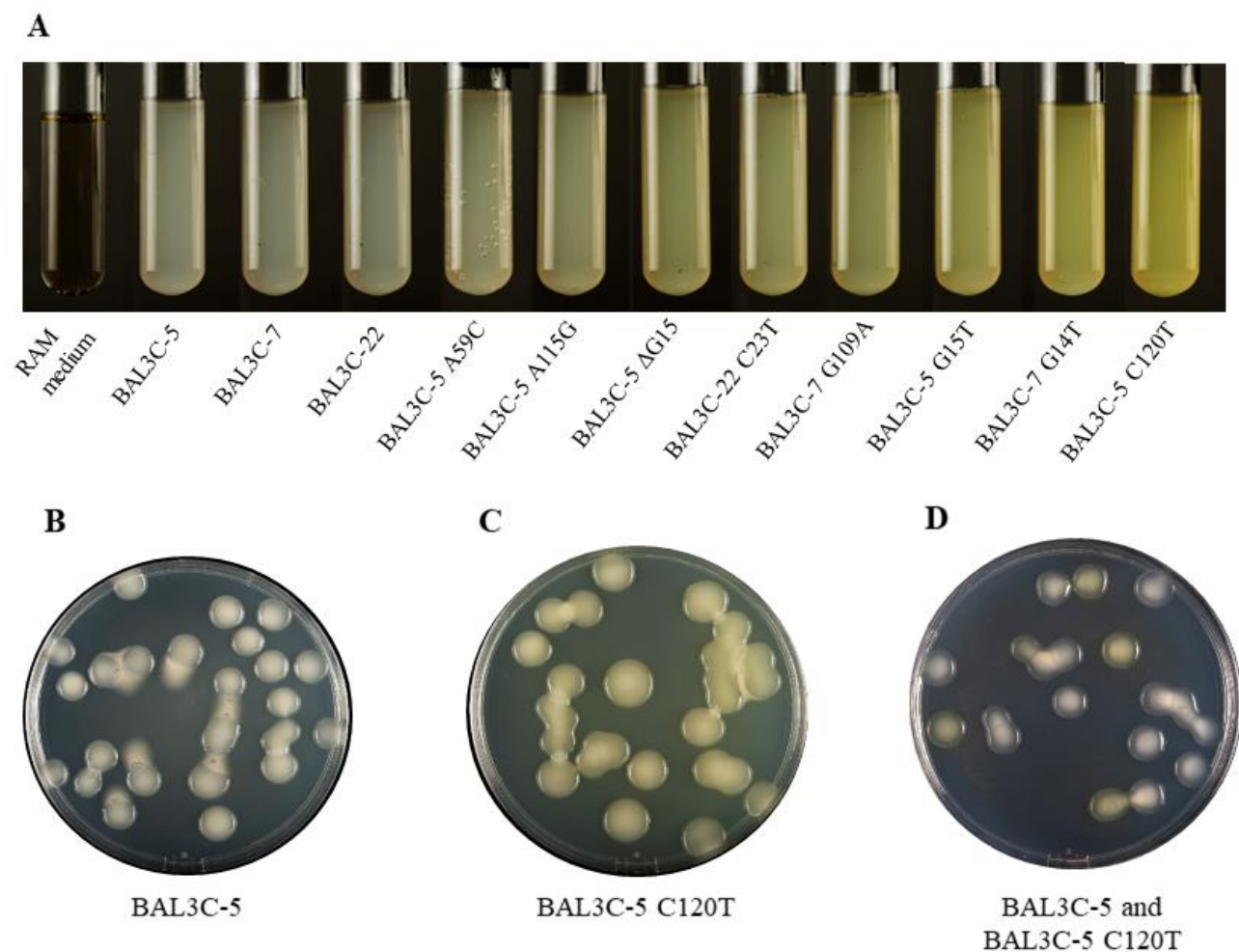
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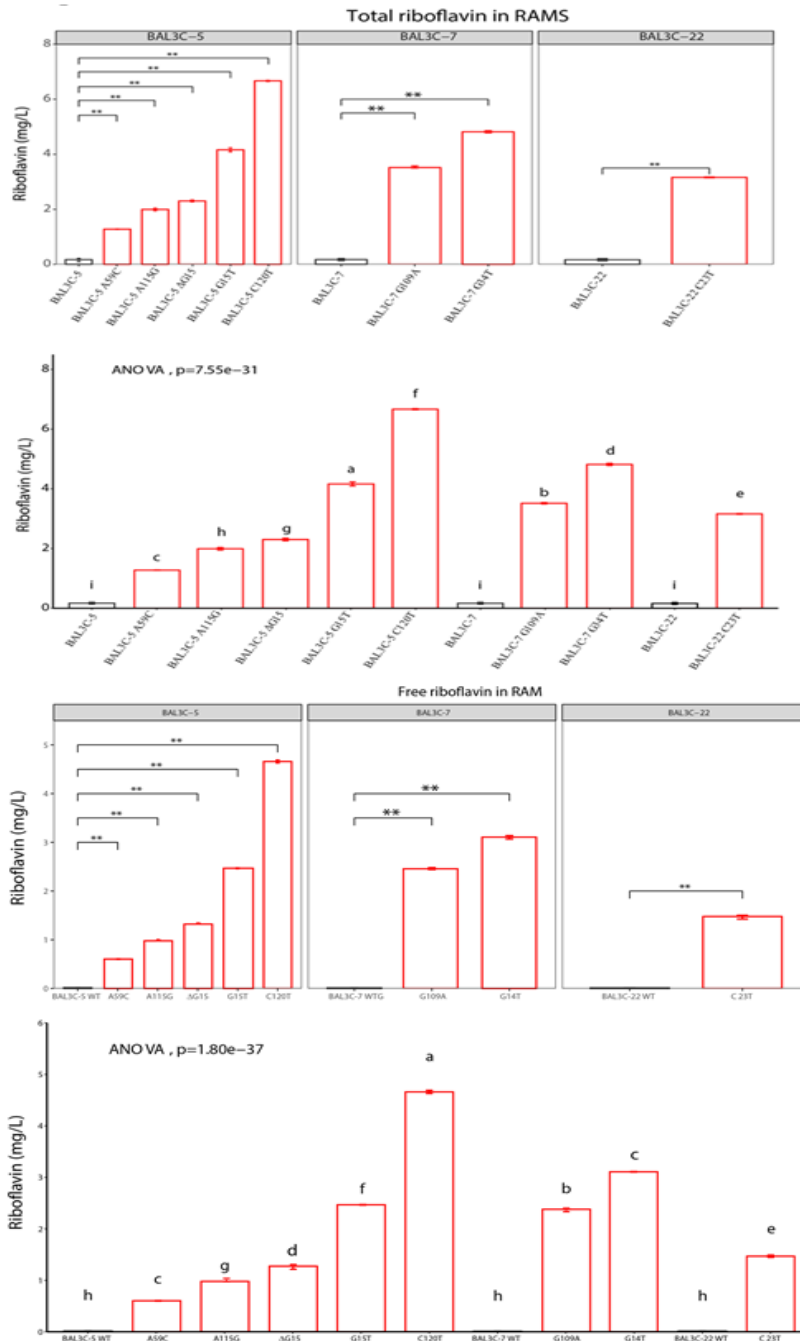
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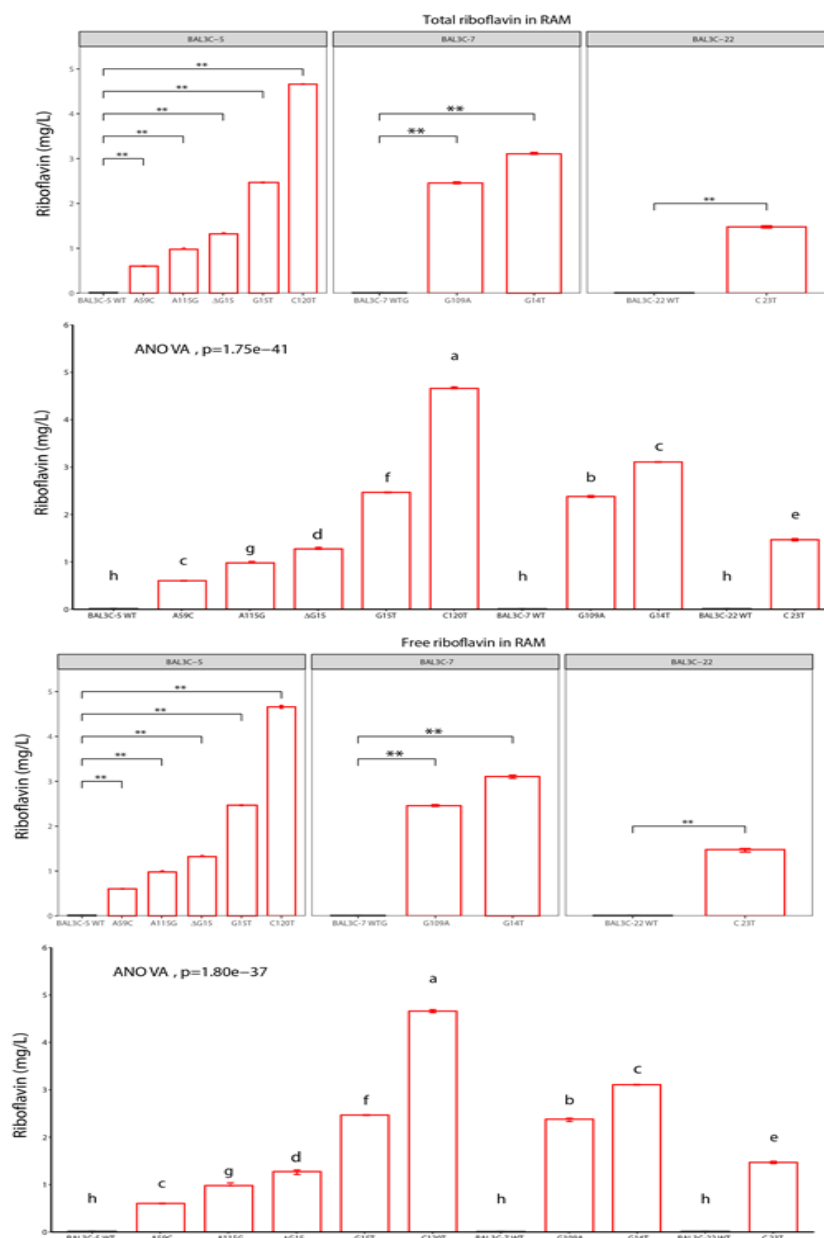
**Keywords:** lactic acid bacteria, *Weissella cibaria*, riboflavin, dextran, FMN-riboswitch



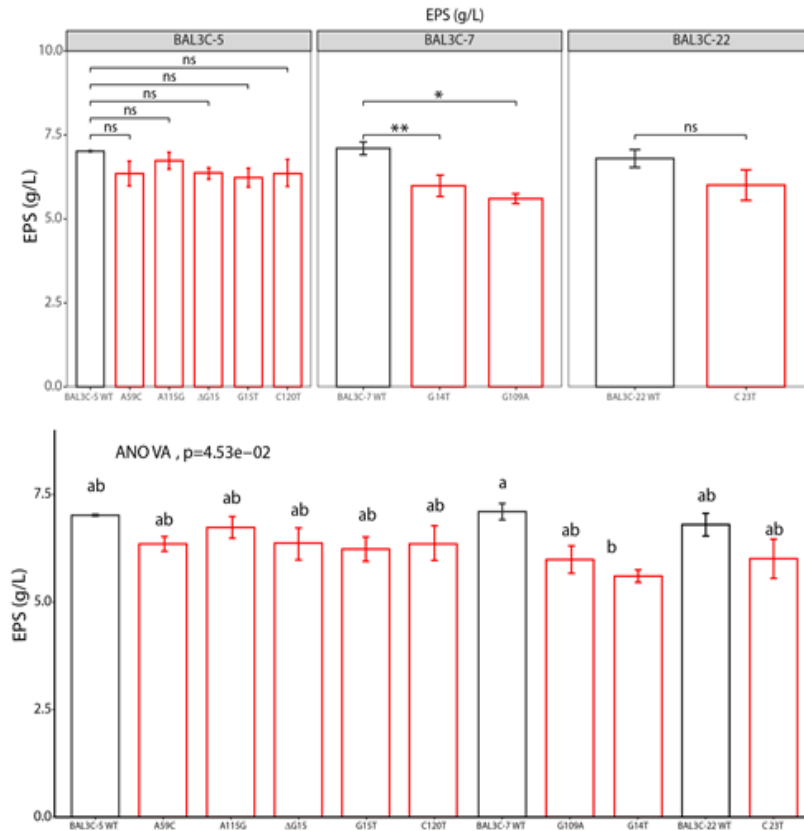
**Supplementary Figure S1. Analysis of *W. cibaria* strains in liquid (A) and solid (B, C, D) media.**  
**A.** The *Weissella cibaria* wt and mutant strains grown in RAMS. **B.** Mucous white colonies of parental strain in MRSS. **C.** Mucous yellow colonies of the BAL3C-5 C140T RF-overproducing strain. **D.** Mix culture of BAL3C-5 (with colonies) and BAL3C-5 C140T mutant (yellow colonies) strains.



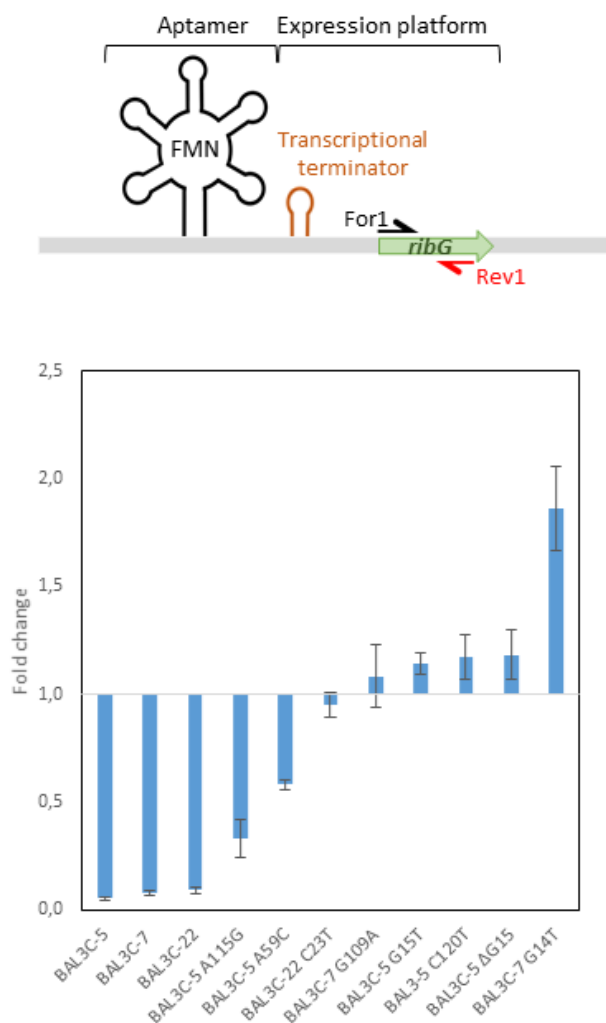
1 **Supplementary Figure S2. Statistical analysis of total and free RF production by the *W. cibaria* parental and RF-overproducing strains grown in RAMS medium.** Per each growth condition: Upper panel comparison of mutant strains with is corresponding parental strain. Lower panel, comparison of all the strains analysed. Values with different letters or different number of symbols (\*) indicate significant differences ( $p \leq 0.05$ ).



**Supplementary Figure S3. Statistical analysis of total and free RF production by the *W. cibaria* parental and RF-overproducing strains grown in RAM medium.** Per each growth condition: Upper panel comparison of mutant strains, which is corresponding parental strain. Lower panel, comparison of all the strains analysed. Values with different letters or different number of (\*) indicate significant differences ( $p \leq 0.05$ ).



**Supplementary Figure S4. Statistical analysis of dextran produced by the *W. cibaria* parental and RF-overproducing strains grown in RAMS medium.** Upper panel comparison of mutant strains, which is corresponding parental strain. Lower panel, comparison of all the strains analysed. Values with different letters or different number of (\*) indicate significant differences ( $p \leq 0.05$ ).



**Supplementary Figure S4. Analysis of influence of FMN on transcription of the *ribG* gene in the *W. cibaria* parental and RF-overproducing strains.** The bacteria were grown in RAMS and RAMS+3  $\mu$ m FMN media and using total RNA preparations, cDNA was synthesized and employed as substrate to perform RT-qPCR analysis. The fold change of mRNA levels in presence *versus* absence of FMN are represented.