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## Introduction

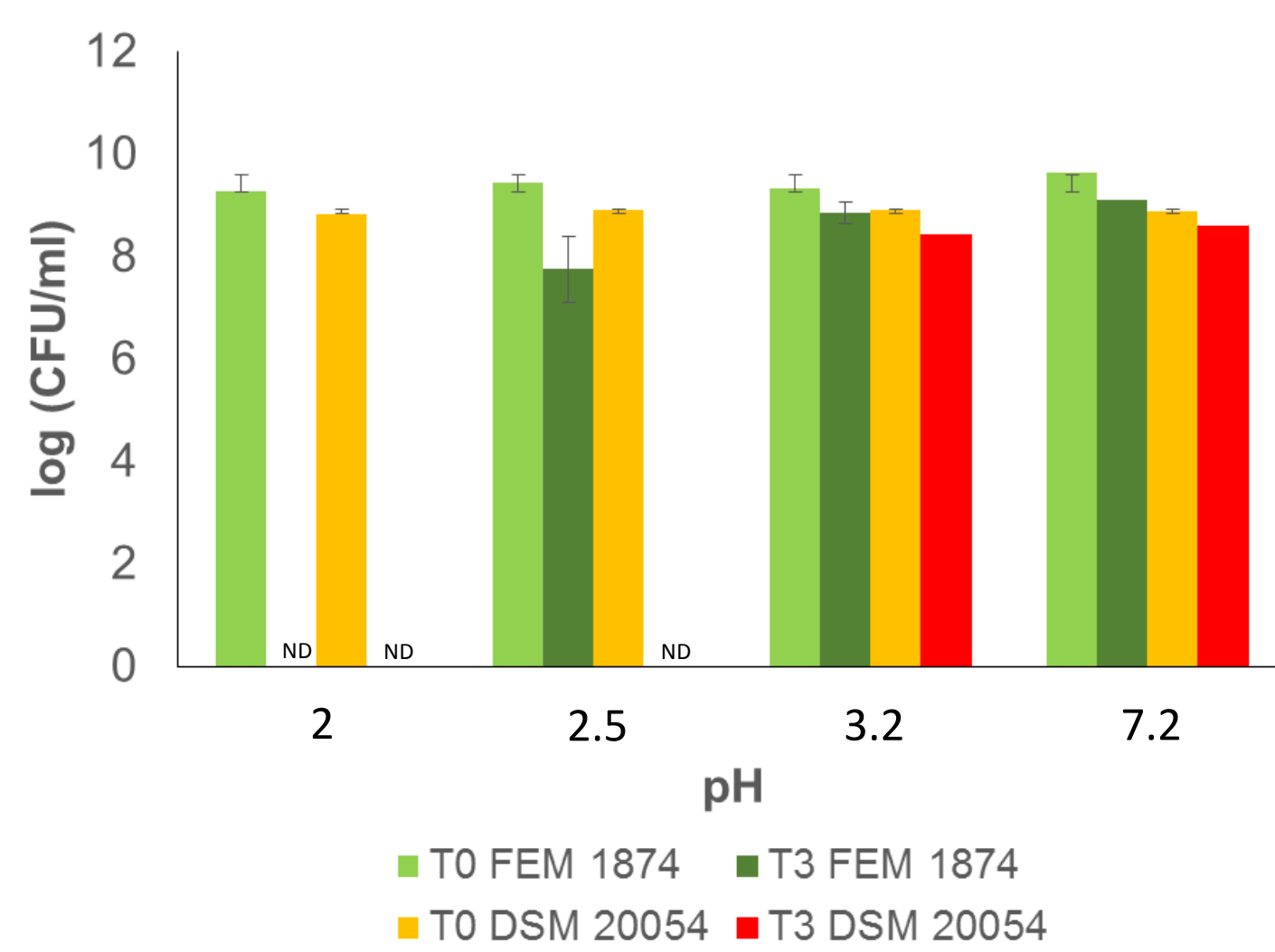
Probiotics and their metabolites can act as brain modulators through the gut:brain axis system. In this respect  $\gamma$ -aminobutyric acid (GABA) gut bacteria produced, has been identified as a possible factor improving brain function, inhibiting inflammation and regulating energy metabolism.

## Study design

"Wild" Alpine cheese isolate *Lactobacillus brevis* FEM 1874 was characterized for potential probiotics traits and GABA production. Acid, bile and pancreatic fluid resistance was assessed and the genetic loci for GABA production and export characterized for the subsequent knock-out vectors construction.

### 1) FEM 1874 survives in gastrointestinal physicochemical condition

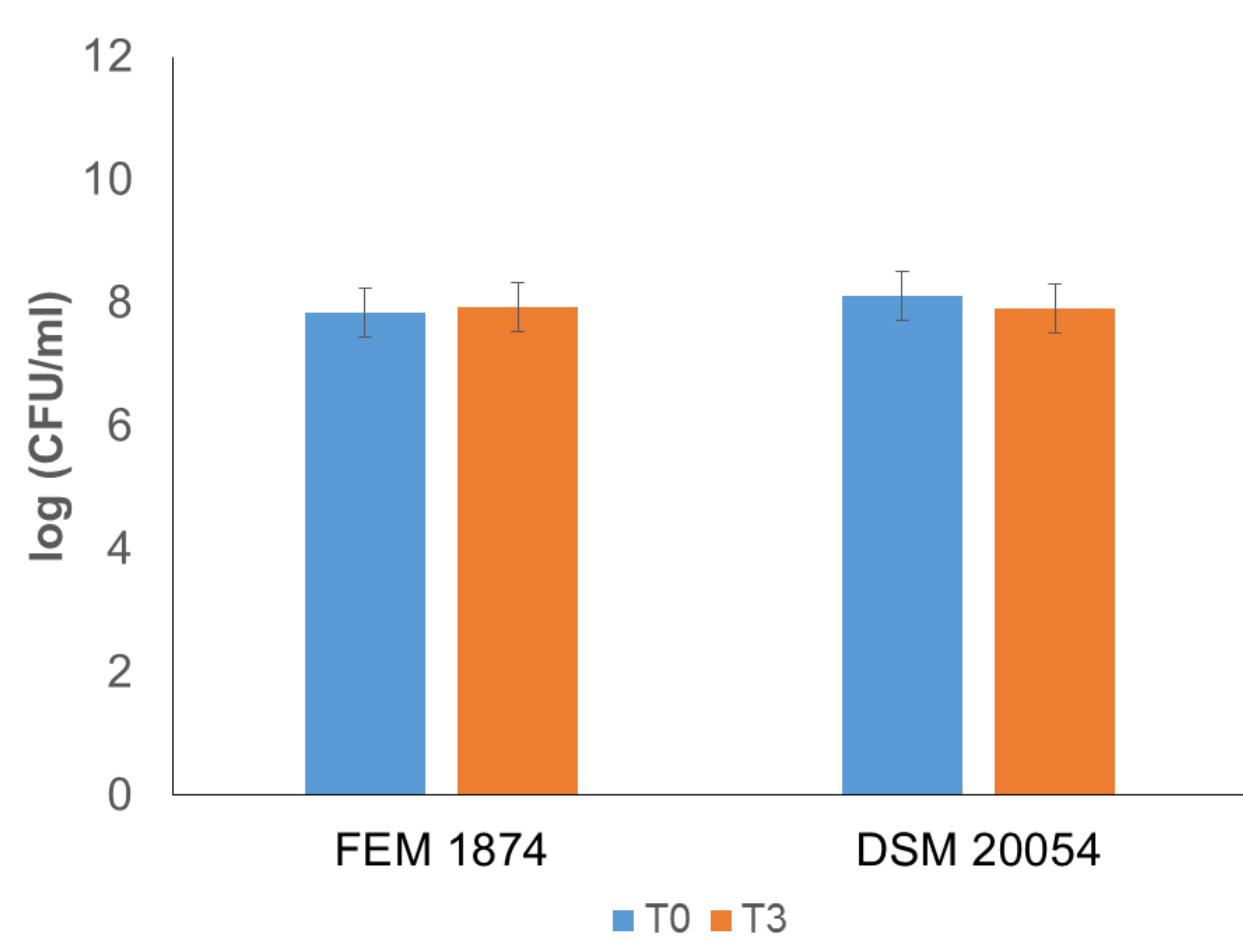
**Acid tolerance (pH 2, 2.5, 3.2).** Acid treatment performed in PBS at 0 and 3h (T0 and T3 resp). Cell counts compared to type strain grown in the same condition (mean  $\pm$  sd, N = 3).



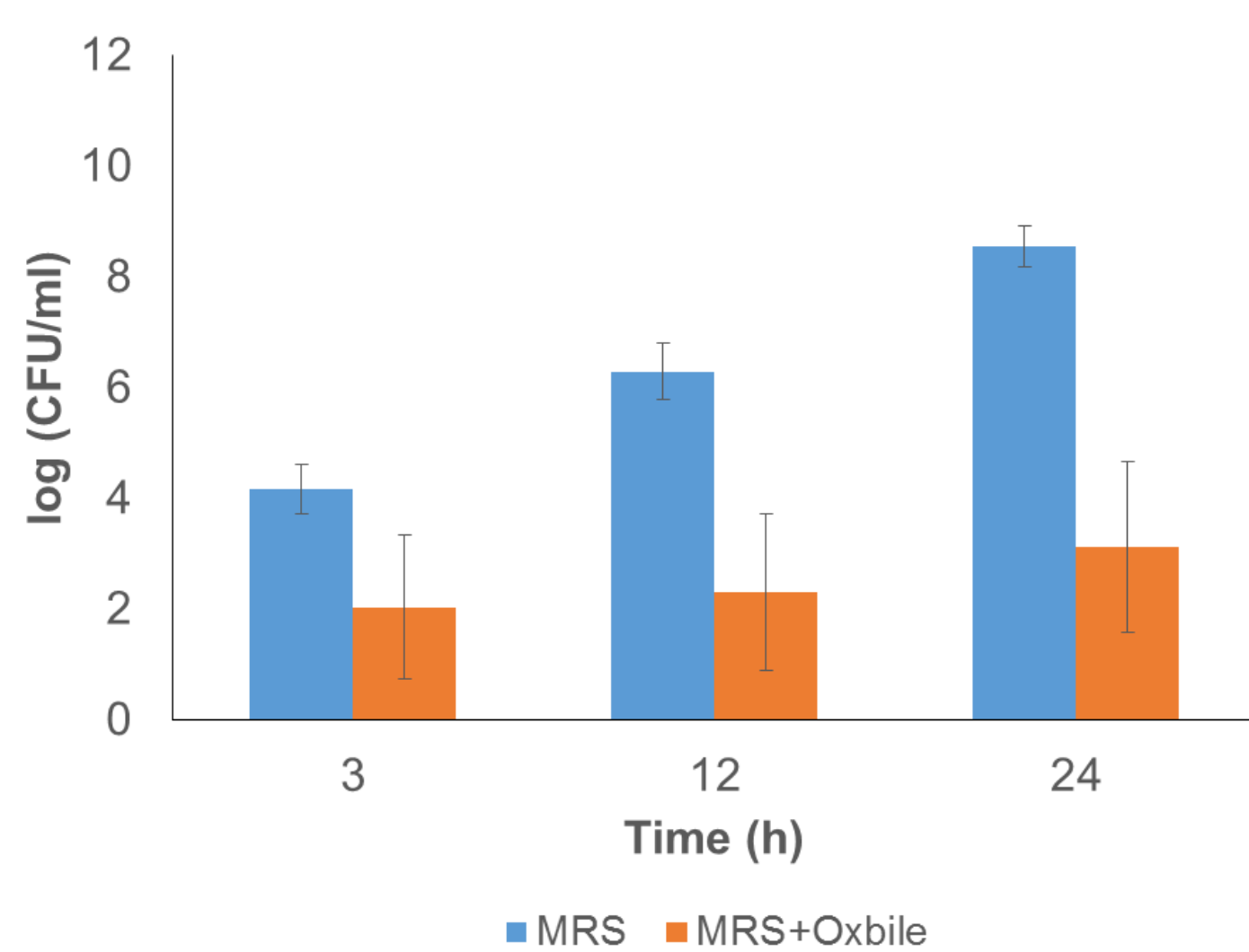
**Bile Salt Hydrolysis (BSH) assay.** BSH activity performed on MRS agar plates supplemented with 0.5% of bile acids. (mean  $\pm$  sd, N = 3).

Strain	BSH activity
<i>L. brevis</i> DSM 20054	-
<i>L. brevis</i> FEM 1874	+

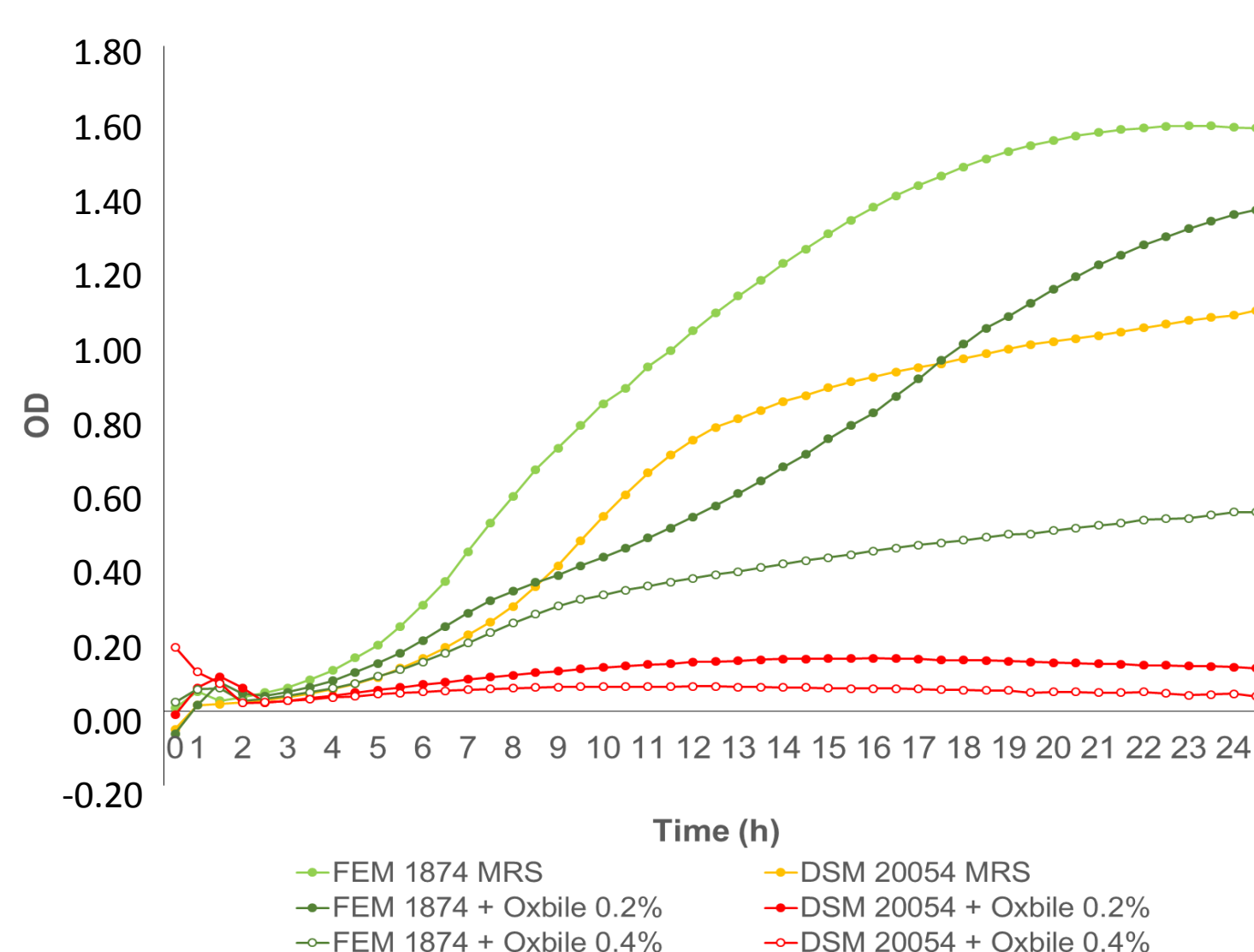
**Pancreatic fluid tolerance.** Tolerance test performed in NaHCO<sub>3</sub> with 1.9 mg/ml pancreatin for 3h. Cell counts compared to type strain grown in the same condition (mean  $\pm$  sd, N = 3).



**Effect of bile salts after low pH treatment.** Bile acid (Oxibile) treatment performed in MRS over time after pH 2.5 exposure (3h) (mean  $\pm$  sd, N = 3).

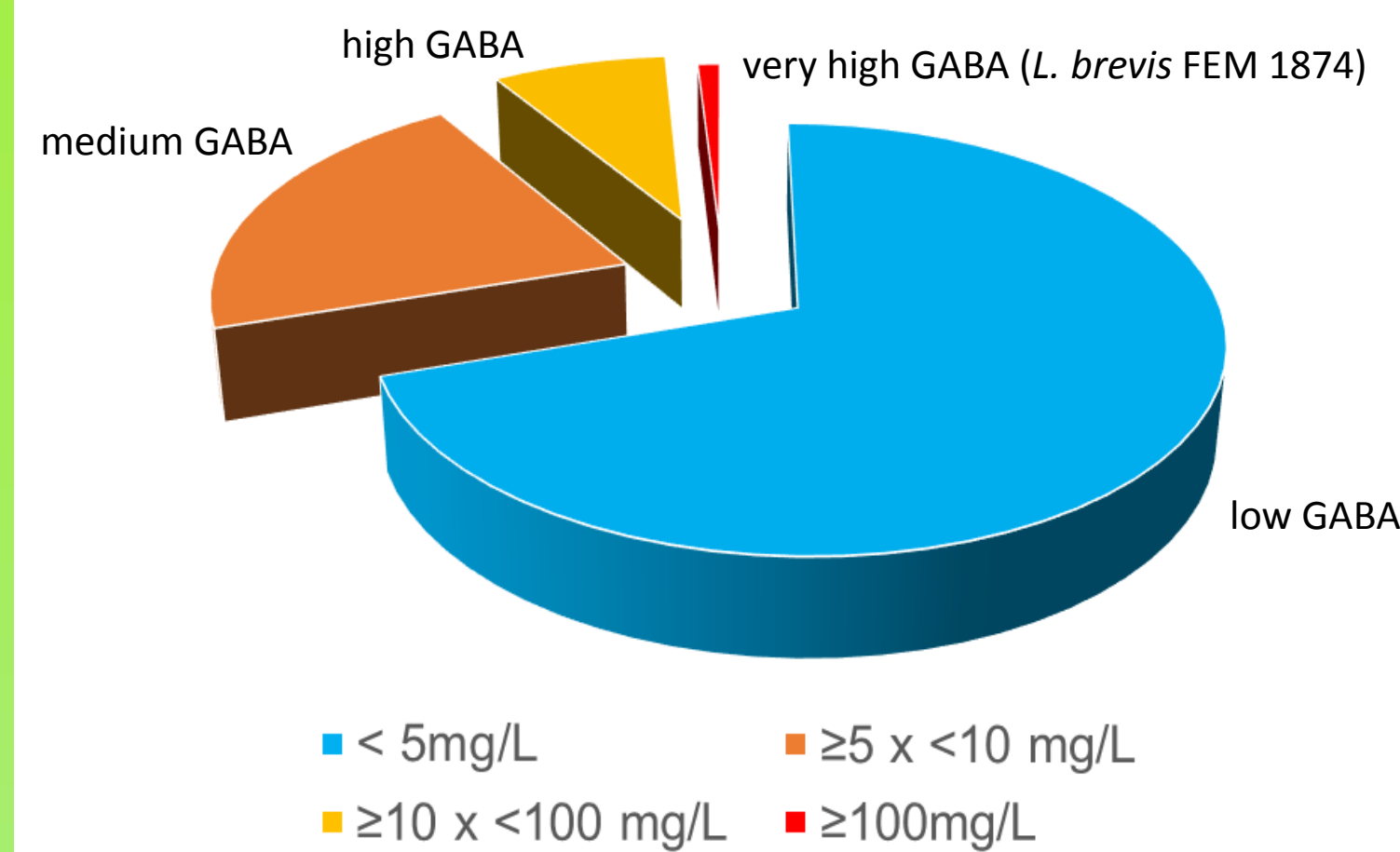


**Growth rate with bile salts.** Bile acid (Oxibile) treatment performed in MRS over time (mean  $\pm$  sd, N = 3).



## Results

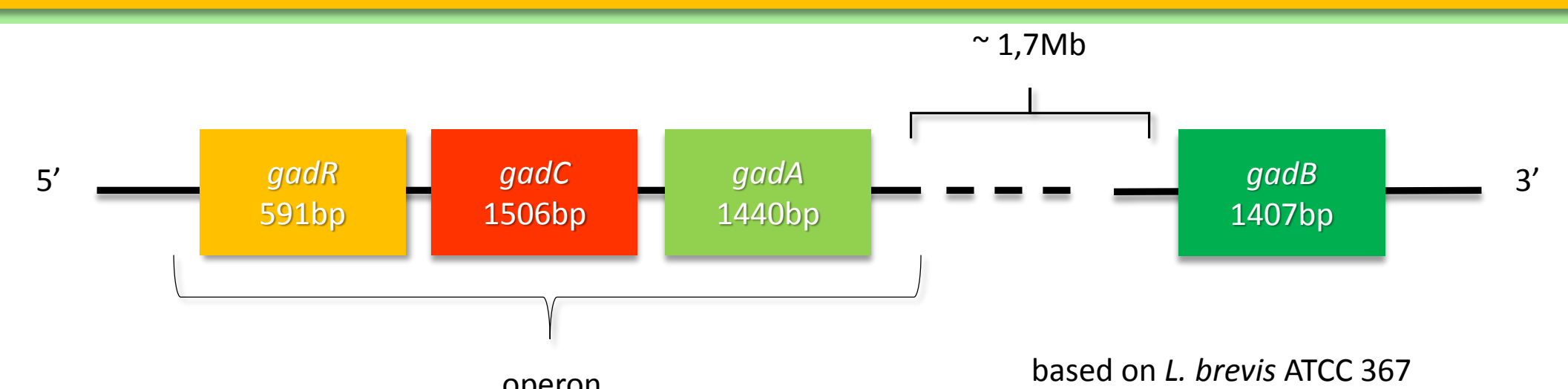
### 2) FEM 1874 produced high GABA level



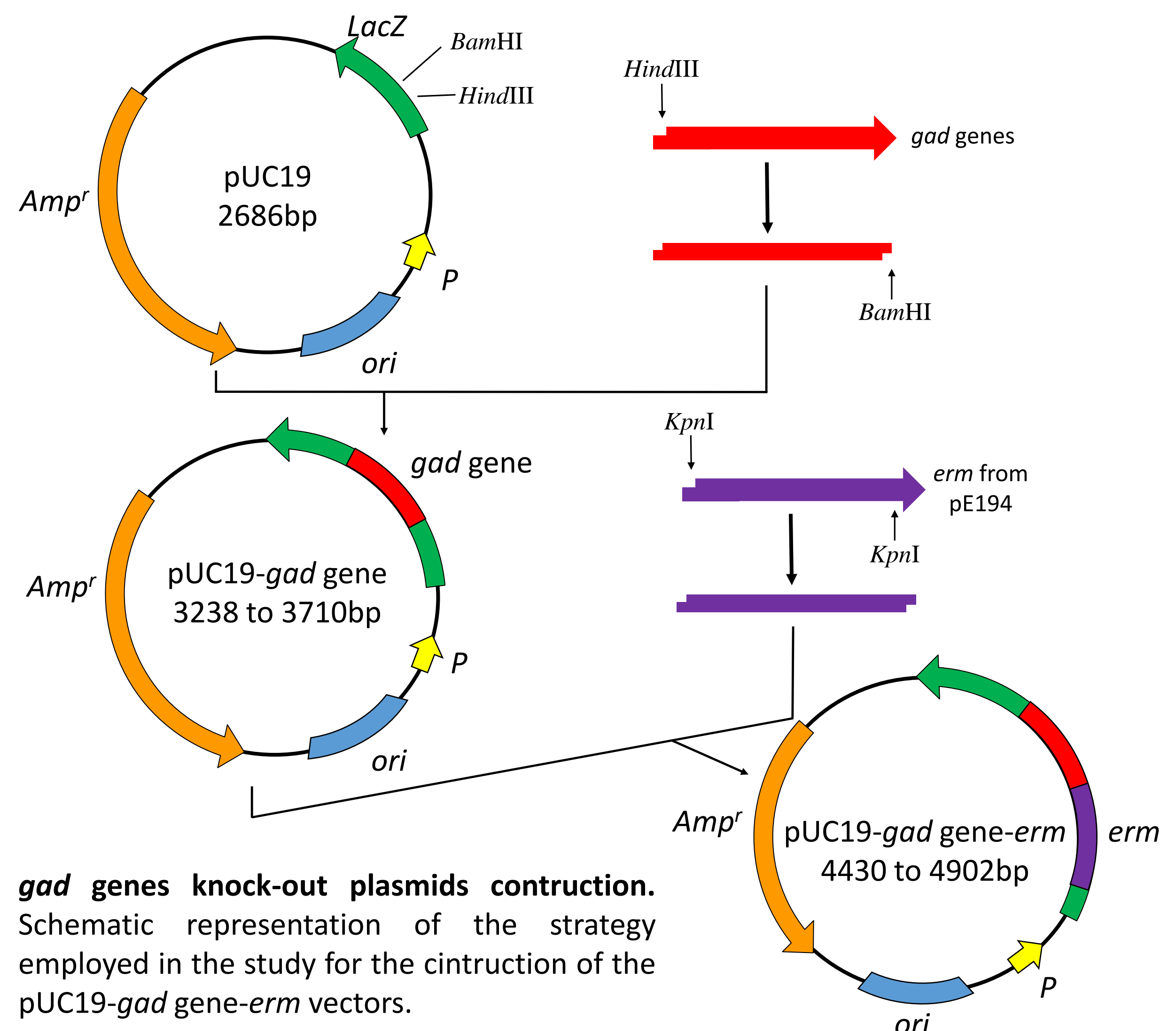
**«Wild» Alpine cheese isolates GABA production.** GABA production (mg/L) after 24h in experimental buffer + glutamate [1mg/ml]. Total isolate considered 110. Mean  $\pm$  sd, N = 3.

**GABA production.** GABA production after 24h in experimental buffer + glutamate [1mg/ml]. Mean  $\pm$  sd, N = 3

### 3) FEM 1874 GABA genes sequences analysis and knock-out generation strategy



**FEM 1874 GABA genes sequenced: 100% identity.** Based on *L. brevis* ATCC 367 gene sequencing, nine sets of primer were used to sequence all GABA genes. 100% of identity was found, only a SNP in antiporter (*gad C*) with synonymus mutation (V to V) were evidenced.



***gad* genes knock-out plasmids construction.** Schematic representation of the strategy employed in the study for the construction of the pUC19-*gad* gene-*erm* vectors.

## Conclusions

*Lactobacillus brevis* FEM 1874 was confirmed as a high GABA producing strain with potential as a probiotic for human use, given its ability to survive under simulated gastro-intestinal physicochemical conditions. However, its ability to produce GABA within the gut and consequently mediate health benefits via the gut:brain axis and immune system, must further be confirmed in vivo.

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