**Development of extracts and fermented officinal plants for food use endowed with sensory, antimicrobial and nutraceutical properties**

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The first two activities of the PhD thesis project are described. Firstly, growth curves of fermenting bacteria were constructed obtaining the number of colonies per millilitre (CFU/ml). Secondly, *Calendula officinalis* aqueous extracts were fermented with strains of lactic acid bacteria for 24 hours, correlating colonies per millilitre (CFU/ml) and pH values.

**Sviluppo di estratti e fermentati di piante officinali per uso alimentare con funzione sensoriale, antimicrobica e nutraceutica**

Le prime due attività del progetto di tesi di dottorato sono descritte. In primo luogo, sono state costruite le curve di crescita dei batteri fermentanti, ottenendo il numero di colonie per millilitro (UFC/ml). In secondo luogo, gli estratti acquosi di *Calendula officinalis* sono stati fermentati con ceppi di batteri lattici per 24 ore, correlando il numero di colonie per millilitro (UFC/ml) e i valori di pH.

**Key words**: fermentation, lactic acid bacteria, plant extract, calendula.

# **1. Introduction**

This poster reports the main results of the first two activities concerning:

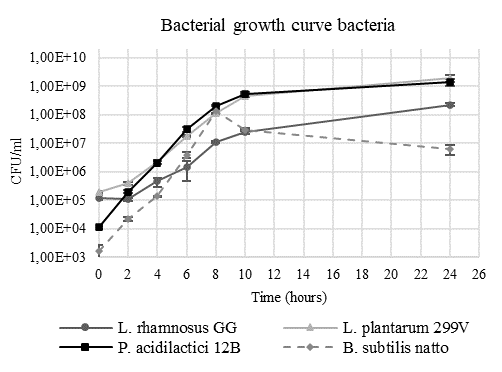
1) construction of growth curves of the following fermenting bacteria: *Lactobacillus rhamnosus* GG, *Lactobacillus plantarum* 299V, *Pediococcus acidilactici* 12B and *Bacillus subtilis natto*;

2) *Calendula officinalis* aqueous extracts fermentation with *Lactobacillus plantarum* 299V and *Pediococcus acidilactici* 12B.

# **2. Materials and Methods**

1) Fresh De Man, Rogosa and Sharpe (MRS) broth (peptone from casein 10 g/L; meat extract 8 g/L; yeast extract 4 g/L; D(+)-glucose 20 g/L; dipotassium hydrogen phosphate 2 g/L; Tween® 80 1 mL/L; di-ammonium hydrogen citrate 2 g/L; sodium acetate 5 g/L; magnesium sulfate 0.2 g/L; manganese sulfate 0.04 g/L.) was inoculated with 1% from -20 °C frozen stock bacterial cultures and incubated overnight at 37 °C; afterwards, new fresh MRS broth was inoculated with 0,01% from the overnight culture. This culture was plated by spread plate technique on MRS agar medium, every 2 hours from time zero to 10 hours, and then at 24 hours; plates were incubated overnight at 37 °C.

2) *Calendula officinalis* aqueous extract was inoculated with 105 CFU/ml of lactic acid bacteria (*Lactobacillus plantarum* 299V and *Pediococcus acidilactici* 12B, separately) and incubated overnight at 37 °C; the inoculated extract was plated at time zero and after 24 hours and its pH value was measured with pHmeter HI5521 (Hanna Instruments Inc., Woonsocket, United States).



**Figure 1.** Growth curves of L. rhamnosus GG, L. plantarum 299V, P. acidilactici 12B and B. natto plotted as CFU/ml on time.

**3. Results and Discussion**

**3.1 Fermenting bacteria growth curves**

Table 1 and Figure 1 show the kinetics of bacterial growth: for all four bacteria, the exponential phase starts approximately after 2 hours, but *L. rhamnosus* GG, *L. plantarum* 299V and *P. acidilactici* 12B reach the stationary phase approximately after 10-12 hours, while at that time *B. natto* begin to decrease the number of viable cells, probably due to sporification process of this bacteria, that occurs once the stationary phase is achieved.

**Table 1.** Number of CFU/ml of L. rhamnosus GG, L. plantarum 299V, P. acidilactici 12B and B. natto plated at different time points.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Time (hours) | *L. rhamnosus* GG | *L. plantarum* 299V | *P. acidilactici* 12B | *B. natto* | |
| 0 | 1,24\*105 ± 2,43\*104 | 1,97\*105 ± 2,32\*104 | 1,13\*104 ± 1,53\*103 | 1,67\*103 ± 1,15\*103 | |
| 2 | 1,09\*105 ± 1,21\*104 | 3,97\*105 ± 4,51\*104 | 2,01\*105 ± 2,57\*104 | 2,23\*104 ± 4,04\*103 | |
| 4 | 4,60\*105 ± 1,55\*105 | 2,28\*106 ± 4,20\*105 | 2,11\*106 ± 2,17\*105 | 1,41\*105 ± 5,57\*103 | |
| 6 | 1,43\*106 ± 9,61\*105 | 1,78\*107 ± 1,70\*106 | 3,10\*107 ± 5,61\*106 | 3,93\*106 ± 8,96\*105 | |
| 8 | 1,13\*107 ± 1,22\*106 | 1,14\*108 ± 1,76\*106 | 2,09\*108 ± 8,33\*106 | 1,33\*108 ± 1,40\*107 | |
| 10 | 2,53\*107 ± 4,73\*106 | 4,70\*108 ± 6,08\*107 | 5,50\*108 ± 1,05\*108 | 3,03\*107 ± 3,06\*106 | |
| 24 | 2,27\*108 ± 2,65\*107 | 1,94\*109 ± 5,17\*108 | 1,39\*109 ± 3,83\*108 | 6,33\*106 ± 2,52\*106 | |
| All data are expressed as mean ± standard deviation. | | | | |



**Figure 2.** Calendula extract non-fermented (right) and fermented (left) by P. acidilactici 12B.

**3.2 *Calendula officinalis* aqueous extract fermentation**

Table 2 and Table 3 report the number of CFU/ml in the inoculated calendula extracts plated at the moment of inoculum and after 24 hours of fermentation: *L. plantarum* 299V and *P. acidilactici* 12B were able to grow from about 105 to 108 CFU/ml.

The starting pH of calendula extracts was between 5,44 and 5,50 and both bacteria were able to lower it after 24 hours of fermentation, reaching values ranging from 3,74 to 4,03 (Table 2 and Table 3).

The fermented extracts appear more limpid, compared to the non-fermented ones, which present a higher number of particles in suspension (Figure 2), probably linked to consumption and/or transformation of plant material by bacterial metabolism.

**Table 2.** Number of CFU/ml of L. plantarum 299V in calendula extract, plated at time zero and after 24 hours of fermentation, with correspondent pH values.

|  |  |  |  |
| --- | --- | --- | --- |
| Time (hours) | CFU/ml | pH | |
| 0 | 2,60\*105 ± 2,97\*105 | 5,50 ± 0,10 | |
| 24 | 2,90\*108± 1,07\*108 | 3,74 ± 0,058 | |
| All data are expressed as mean ± standard deviation. | | |

|  |  |  |  |
| --- | --- | --- | --- |
| Time (hours) | CFU/ml | pH | |
| 0 | 1,26\*105 ± 8,44\*105 | 5,44 ± 0,025 | |
| 24 | 5,87\*108 ± 7,29\*108 | 4,03 ± 0,04 | |
| All data are expressed as mean ± standard deviation. | | |

**Table 3.** Number of CFU/ml of P. acidilactici 12B in calendula extract, plated at time zero and after 24 hours of fermentation, with correspondent pH values.

# **4. References**

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