

Comparison of M5 agar and MRS agar for differentiation of *L. plantarum* and *L. buchneri* in silage additives



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Introduction

Lentilactobacillus buchneri (*L. buchneri*) is commonly used in silage additives to improve aerobic stability of ensiled material (Kung and Ranjit, 2001). In silage additives containing *L. buchneri* and *Lactiplantibacillus plantarum* (*L. plantarum*), a differential determination of cell counts in the product is desirable. According to ISO 15214:1998 standard (Technical Committee ISO/TC 34, 1998), MRS agar should be used for the determination of cell counts of lactic acid bacteria. To differentiate between homo- and heterofermentative lactic acid bacteria, the M5 agar has been developed (Zúñiga et al., 1993). The aim of this study was to compare the differential determination of cell counts in a silage additive containing *L. buchneri* and *L. plantarum* using M5 or MRS agar.

Methods

- M5 agar was prepared according to Zúñiga et al. (1993).
- MRS agar plates were ready-to-use plates (BIOSOLUTE®, Th. Geyer GmbH & So KG, Renningen, Germany).
- Samples were prepared in duplicate by spread plate technique.
- M5 agar plates (dilutions 10⁻⁹ and 10⁻¹⁰) and MRS agar plates (dilution 10⁻¹⁰) were incubated at 37°C for 72h. The remaining MRS agar plates (dilution 10⁻⁹) were incubated at 30°C for 72h.
- Plates were read at 48h and 72h.

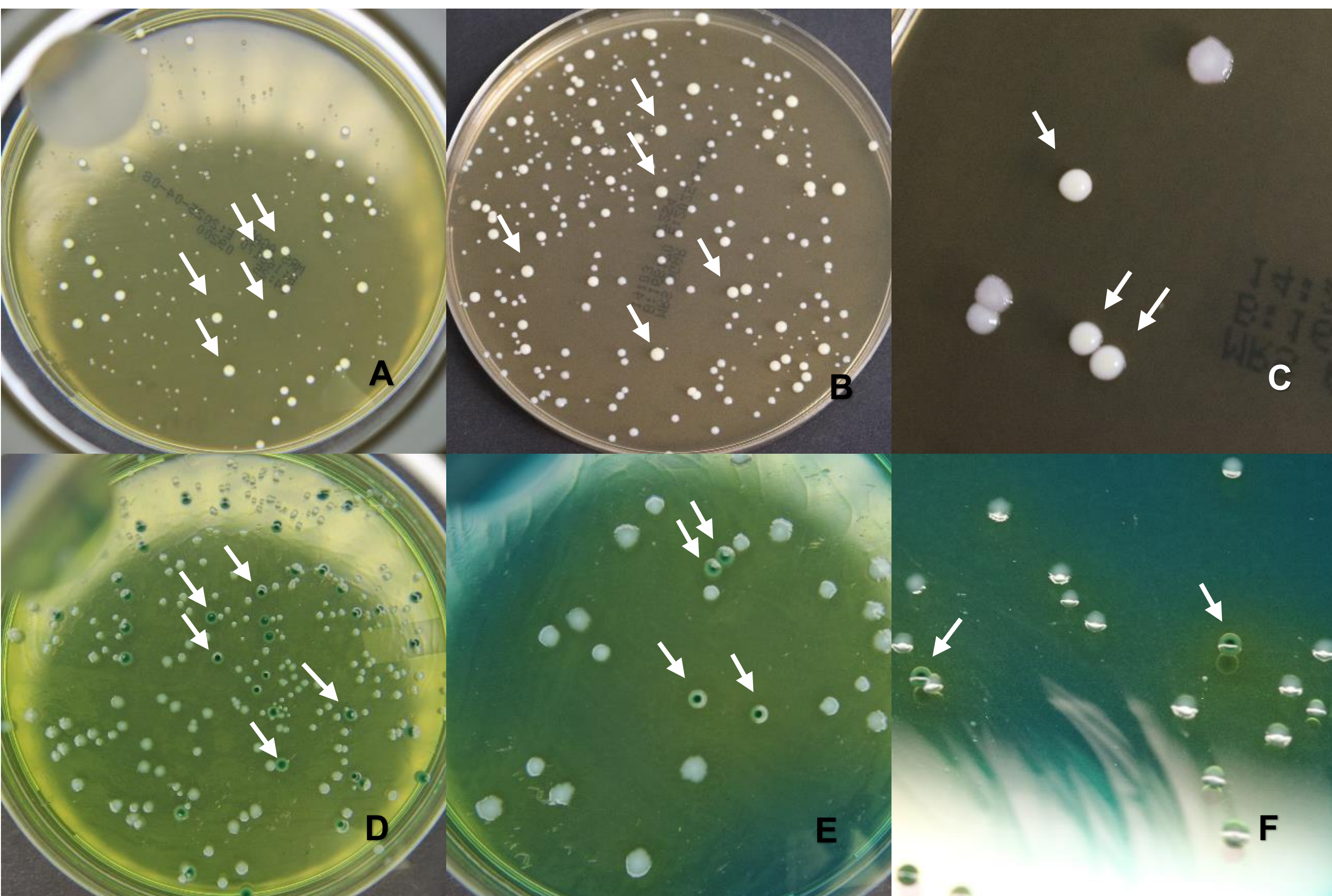


Figure 1. Pictures of *L. plantarum* and *L. buchneri*. Upper row: MRS agar incubated at 30°C for 48h (A), at 37°C for 48h (B), and at 37°C for 72h (C). A: large white beige colonies are *L. plantarum*. *L. buchneri* colonies grow slowly and are barely visible; B: at 37°C, *L. buchneri* colonies grow faster and the difference in size is smaller. C: after 72h at 37°C, *L. buchneri* colonies have an irregular margin, greyish color and are slightly larger than *L. plantarum*. Bottom row: M5 agar incubated at 37° for 48h (D) and at 37°C for 72h (E and F). D: *L. plantarum* can be differentiated by dark blue-green color of colonies; E: after 72h at 37°C, *L. plantarum* colonies are translucent and blue-green, flat and round, *L. buchneri* colonies are translucent, greyish, flat, with an irregular margin. F: on M5 agar, *L. buchneri* and *L. plantarum* colonies appear translucent if plate is held at an angle. *L. plantarum* are indicated by white arrows.

Results

Table 1. Colony morphology of *L. plantarum* and *L. buchneri* on MRS and M5 agar at 30°C or 37°C incubation temperature (dilution 10⁻⁹).

Agar	MRS		M5	
Incubation temperature	30°C		37°C	
Species	<i>L. plantarum</i>	<i>L. buchneri</i>	<i>L. plantarum</i>	<i>L. buchneri</i>
Incubation time	48h			
Diameter	1-1.5 mm	0.2 mm	1-1.5 mm	1 mm
Color	beige	greyish	blue-green	greyish
Morphology	round with entire margin, convex, opaque	round with entire margin, flat, translucent	round with entire margin, flat, translucent	round with irregular margin, flat translucent
Incubation time	72h			
Diameter	1.5-2 mm	0.5-1 mm	1.5-2 mm	1.5-2 mm
Color	beige	greyish	blue-green	greyish
Morphology	round with entire margin, convex, opaque	round with irregular margin, flat, translucent	round with entire margin, flat, translucent	round with irregular margin, flat translucent

Table 2. Cell count of *L. buchneri* and *L. plantarum* on MRS and M5 agar at 30°C or 37°C incubation temperature.

Colony forming units (CFU)	MRS		M5		P-value
	Mean	SD	Mean	SD	
Incubation time	48 h				
Total CFU ¹	3.1•10 ¹¹ (37°C)	4.2•10 ¹⁰	3.1•10 ¹¹ (37°C)	4.2•10 ¹⁰	0.95
<i>L. plantarum</i> ²	4.4•10 ¹⁰ (30°C)	5.0•10 ⁹	4.4•10 ¹⁰ (37°C)	3.6•10 ⁹	0.90
<i>L. buchneri</i> ¹	2.7•10 ¹¹ (37°C)	4.3•10 ¹⁰	2.7•10 ¹¹ (37°C)	4.5•10 ¹⁰	0.96
Incubation time	72 h				
Total CFU ¹	3.2•10 ¹¹ (37°C)	4.1•10 ¹⁰	3.2•10 ¹¹ (37°C)	4.2•10 ¹⁰	0.69
<i>L. plantarum</i> ²	4.3•10 ¹⁰ (30°C)	4.8•10 ⁹	4.4•10 ¹⁰ (37°C)	3.3•10 ⁹	0.67
<i>L. buchneri</i> ¹	2.8•10 ¹¹ (37°C)	4.2•10 ¹⁰	2.7•10 ¹¹ (37°C)	4.2•10 ¹⁰	0.67

¹ determined at dilution 10⁻¹⁰, ² determined at dilution 10⁻⁹

Conclusions

- Both agar types are suitable for determination of differential cell counts of *L. buchneri* and *L. plantarum*.
- On M5 agar plates (not commercially available), colonies can be differentiated by color.
- Differentiation of *L. buchneri* and *L. plantarum* colonies on standardized, commercially available MRS agar incubated at 30°C for 48h is easily possible due to different growth characteristics and colony morphology.
- The use of MRS agar (counted at 48h and 72h and incubated at 30°C and 37°C) is suitable for differential cell count of *L. buchneri* and *L. plantarum* by differentiation of colony morphology and growth characteristics.