

# Evaluation of different culture media for enumeration and differentiation of *Lactobacillus* species



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## Introduction

Design and development of new selective media for enumeration and differentiation of a wider range of *Lactobacillus* strains of industrial interest still remains a big challenge. Different species even strains require different groups of nutrients in different concentrations and forms. In addition, depending on the nature of the samples, the efficiency of cultivation the active cells by standard techniques may be greatly reduced. No cultivation-based methodology is applicable to all members of the genus *Lactobacillus*, as there is significant variability between species and strains. Due to the limitations of using traditional methods of cultivation in the laboratory, optimization process of the media composition is crucial to achieve the most reliable enumeration of microorganisms in different metabolic states of living probiotic cells. That's way we choose four commonly used culture media as MRS, LBD, RCA, mRCA to test.

Test <i>Lactobacillus</i> strain	Culture media
<i>Lactobacillus delbrueckii</i> subsp. <i>Bulgaricus</i> NBIMCC1132	MRS, LBD, RCA, mRCA
<i>Lactobacillus acidophilus</i> NBIMCC504	
<i>Lactobacillus rhamnosus</i> NBIMCC507	
<i>Lactobacillus plantarum</i>	

## Results

The main reference method used for the enumeration and control of lactic acid products in the food and pharmaceutical industries is ISO 15214 and for this reason we also used it to establish the effectiveness of the tested culture media.

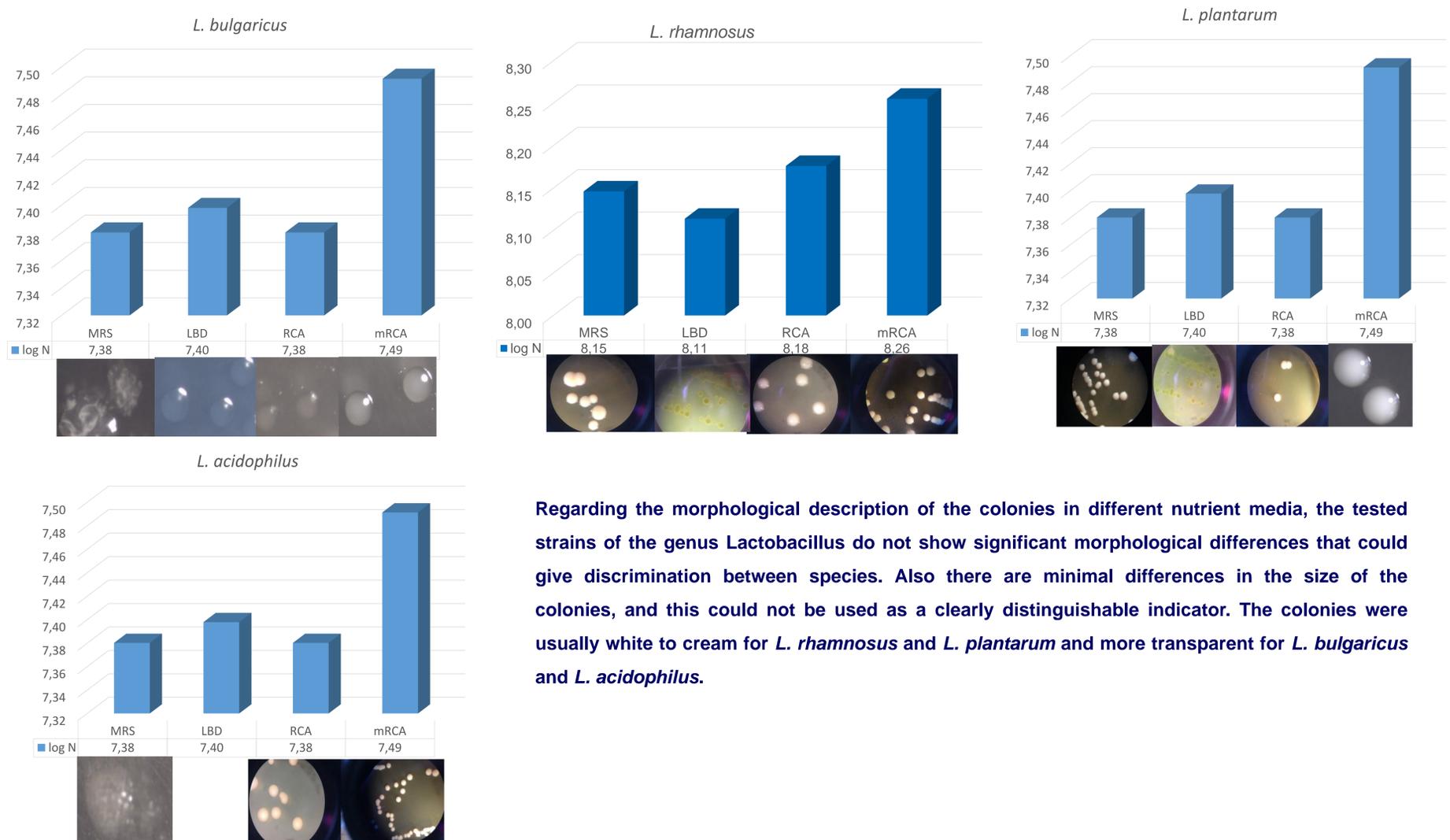
Table 1. Total count of tested *Lactobacillus* strains, cfu/ml.

Strain	Media, cfu/ml			
	MRS	LBD	RCA	mRCA
<i>L. bulgaricus</i>	$2,8 \times 10^6$	$1,1 \times 10^6$	$1,7 \times 10^6$	$2,0 \times 10^7$
<i>L. acidophilus</i>	$1,9 \times 10^6$	$1,0 \times 10^6$	$1,2 \times 10^6$	$1,4 \times 10^7$
<i>L. rhamnosus</i>	$1,4 \times 10^8$	$1,3 \times 10^8$	$1,5 \times 10^8$	$1,8 \times 10^8$
<i>L. plantarum</i>	$2,4 \times 10^7$	$2,5 \times 10^7$	$2,4 \times 10^7$	$3,1 \times 10^7$

The initial inoculum of each strain was standardized on the McFarland scale to a density of 0.5, which for bacterial species is determined within  $1.5 \times 10^8$  cfu/ml.

The analyzes clearly demonstrate that the mRCA medium has the best reproducibility of the total number of each strain tested. MRS medium possessed relatively good reproducibility, but a little bit lower than mRCA. The next medium is RCA and the lowest efficacy is observed in LBD medium, with one exception for *L. plantarum* strain, where the lowest number is reported in RCA medium. It is impressive that the representative of the species *L. rhamnosus* has the best growth indicators, followed by *L. plantarum*, the next one was *L. bulgaricus* and the weakest growth is reported for *L. acidophilus*.

Fig. 1 Comparison of the growth range of tested *Lactobacillus* strains on different culture media.



Regarding the morphological description of the colonies in different nutrient media, the tested strains of the genus *Lactobacillus* do not show significant morphological differences that could give discrimination between species. Also there are minimal differences in the size of the colonies, and this could not be used as a clearly distinguishable indicator. The colonies were usually white to cream for *L. rhamnosus* and *L. plantarum* and more transparent for *L. bulgaricus* and *L. acidophilus*.

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