Lacticaseibacillus parahuelsenbergensis and *L. styriensis*, isolated from grass and corn silage

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Introduction

In a screening project at 45°C, two rod-shaped, non-motile, non-spore-forming, facultative anaerobic, Gram-stain positive lactic acid bacteria, designated as LD0937^T and SCR0063^T, were isolated from grass and corn silage. Polyphasic approach characterisation identified these as novel species of the *Lacticaseibacillus* genus [1].

Material & Methods

- A core gene phylogenetic tree was constructed using the UBCG ver3.0 tool [2] and default options (Figure 1). The tree was visualized using the MEGA 11 software (version 11.0.3.) [3].
- For phenotypic characterization, the novel strains and all reference bacteria were cultivated on MRS agar plates at 37°C for 24 h under semi-anaerobic conditions in an anaerobic box with anaerogen bags. Growth tests were conducted in MRS broth with a range of parameters, including temperature (5 to 55°C), pH (3.0 to 10.5) and NaCl salt concentrations (4% w/v to 10% w/v). Using the commercially available test kit API 50 CH system (bioMèrieux), the capacity for carbohydrate metabolism was measured (Table 1).

Results & Discussion

According to the analysis of the core genome, the isolates LD0937^T and SCR0063^T can be identified as novel species, *L. parahuelsenbergensis* LD0937^T and *L. styriensis* SCR0063^T (Figure 1).

Strain LD0937^T showed weak growth at pH 3.5 and good growth at pH 10.0 and in medium containing 8% w/v NaCl, whereas SCR0063^T showed weak growth in medium containing 8% w/v NaCl and good growth at pH 3.5 to pH 10.0, in contrast to the closest related strains. Both novel isolates could produce acid from L-Arabinose, LD0937^T from L-Sorbose and SCR0063^T from α-methyl-D-Mannoside, which was confirmed with functional genomics.

Figure 1. Phylogenetic analysis of the novel isolates LD0937^T and SCR0063^T core genome using UBCG tool. Reference strains are the phylogenetically closest related *Lacticaseibacillus* type strains. *Lactobacillus delbrueckii* subsp. *delbrueckii* DSM 20074^T served as outgroup.

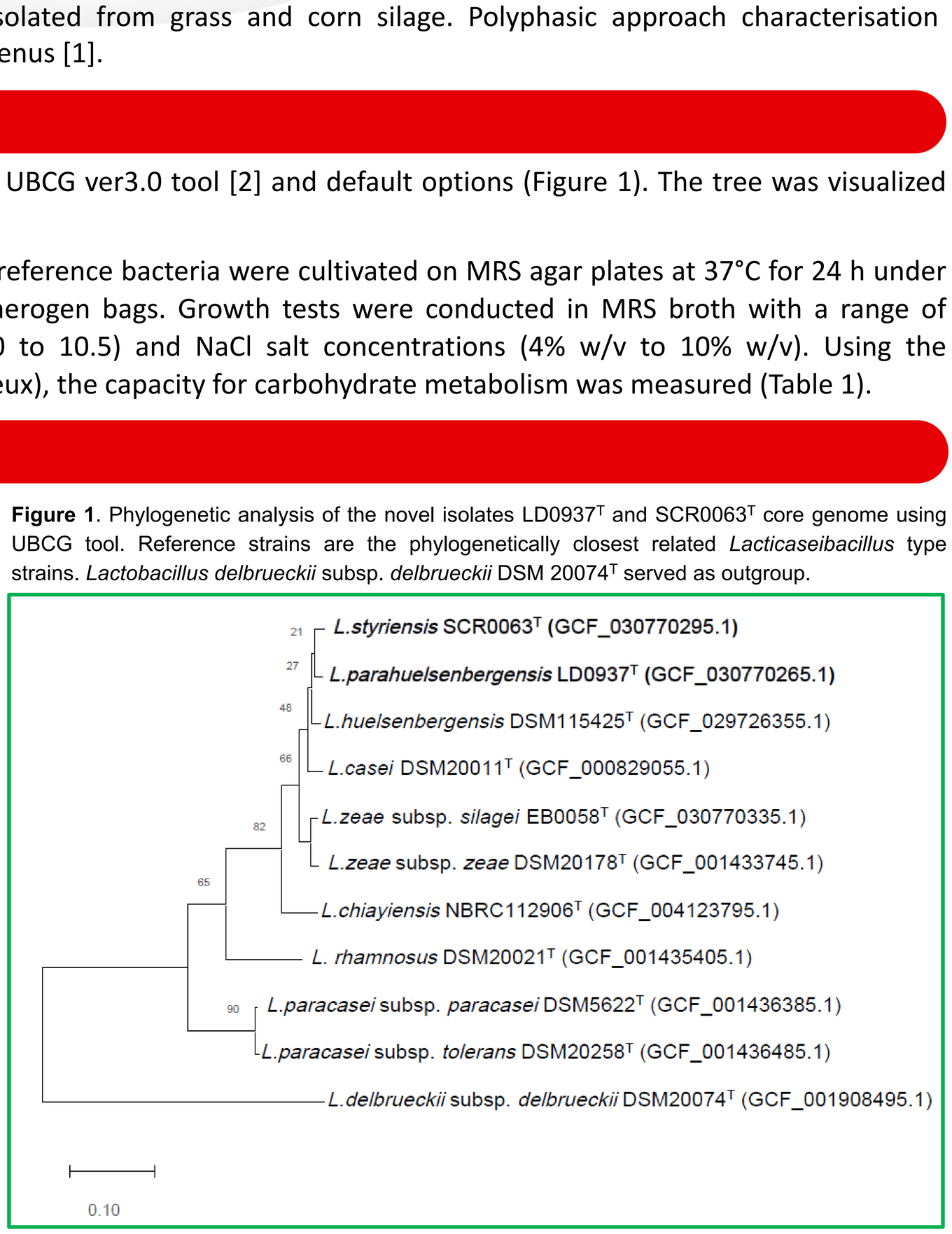

Table 1. Differential phenotypic and growth characteristics of the two isolates and their phylogenetically closest related type strains *L. huelsenbergensis* DSM 115425^T and *L. casei* DSM20011^T.

	Growth at:			Acid production from:			
Strain	pH 3.5	pH 10.0	8% NaCl	L-Arabinose	α-methyl-D-Mannoside	L-Sorbose	Dulcitol
LD0937^T	w	+	+	+	-	+	-
SCR0063^T	+	+	w	+	+	-	-
***L. huelsenbergensis* DSM115425^T**	+	-	w	-	-	-	+
***L. casei* DSM20011^T**	-	-	-	-	-	-	-

+ ... good growth, w … weak growth, - … no growth

Conclusion

Based on this polyphasic approach, using genomic, phylogenetic, and phenotypic analysis, LD0937^T and SCR0063^T represent two novel species within the *Lacticaseibacillus* group: ***Lacticaseibacillus parahuelsenbergensis*** with the type strain LD0937^T (=DSM 116105 =NCIMB 15471) and ***Lacticaseibacillus styriensis*** with the type strain SCR0063^T (=DSM 116297 =NCIMB 15473).

References:

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