

# Comparative phylogenetics of $\beta$ -galactosidases LAC of ascomycetous yeasts

Elena S. Naumova<sup>1</sup>, Lyudmila V. Lyutova<sup>1,2</sup>, Alla V. Shnyreva<sup>2</sup>, Gennadi I. Naumov<sup>1</sup>

<sup>1</sup>State Research Institute of Genetics and Selection of Industrial Microorganisms, NRC "Kurchatov Institute",  
1-Dorozhnyi proezd, 1, Moscow 117545, Russia; +7-(495)3148173, [lena\\_naumova@yahoo.com](mailto:lena_naumova@yahoo.com);

<sup>2</sup>Lomonosov Moscow State University, Leninskiye gory 1-12, Moscow, 119234, Russia

## Introduction

The enzyme  $\beta$ -galactosidase is widely distributed in plant and animal tissues, however is extremely rare in yeasts. Due to the presence of the  $\beta$ -galactosidase enzyme, dairy yeasts *Kluyveromyces lactis* and *K. marxianus* are able to ferment lactose. Polymeric LAC loci have a complex structure and consist of two closely linked structural genes *LAC4* ( $\beta$ -galactosidase) and *LAC12* (lactose permease), and a regulatory sequence (Dickson & Riley 1989; Fairhead & Dujon 2006; Naumov 2008).

## Results

Using yeast whole genome databases, we have conducted a comparative analysis of amino acid sequences of the  $\beta$ -galactosidase *LAC4* genes of *Kluyveromyces lactis*, *K. marxianus*, *Debaryomyces hansenii*, *Scheffersomyces stipitis* and *Sugiyamaella lignohabitans*. Search for homologous  $\beta$ -galactosidases in the yeast genera studied was performed in GenBank using the BLAST software. The  $\beta$ -galactosidase *LAC4* of *K. lactis* NRRL Y-1140 was used as a query (Fig. 1).

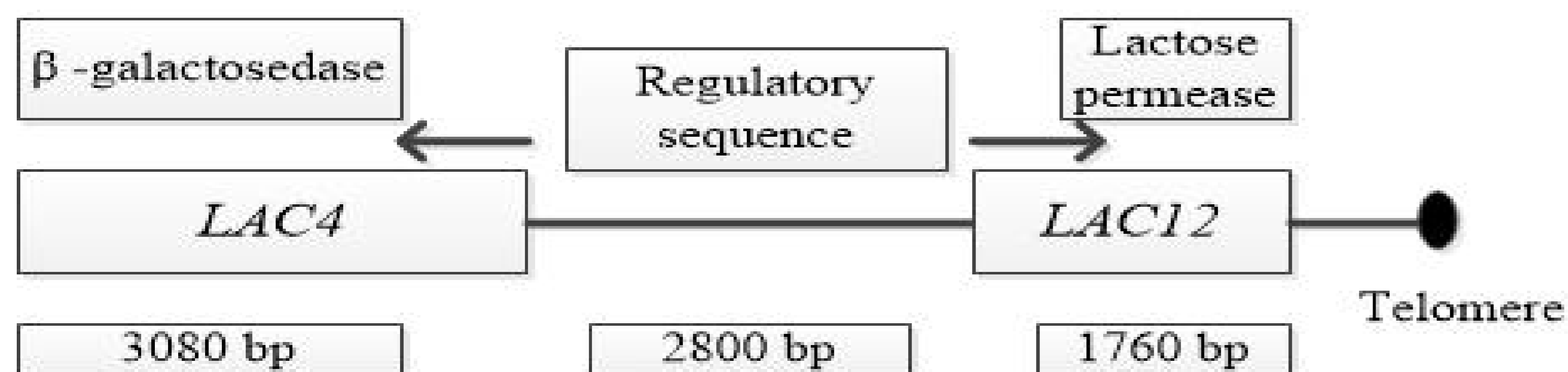


Fig. 1. Complex structure of *LAC4-LAC12* locus located in telomeric region of chromosome II of *Kluyveromyces lactis* NRRL Y-1140

The phylogenetic analysis revealed significant differences between *LAC4* proteins of *Kluyveromyces* and other three studied genera of *Scheffersomyces*, *Sugiyamaella* and *Debaryomyces* (Fig. 2). On the other hand, the *LAC4* proteins of *Kluyveromyces* yeasts divided into two distinct subgroups corresponding to ecological origin of strains: dairy products and natural sources. The group of dairy strains is heterogeneous and includes both *K. lactis* and *K. marxianus* yeasts (98% identity). Note that the phylogenetic tree based on the D1/D2 domain sequences of the yeasts analyzed is in good agreement with the divergence of the  $\beta$ -galactosidases *LAC4* in the strains studied (Fig. 2 and 3).

Fig. 2. Phylogenetic relatedness of  $\beta$ -galactosidases *LAC4* of *Kluyveromyces lactis*, *K. marxianus*, *Debaryomyces hansenii*, *Scheffersomyces stipitis* and *Sugiyamaella lignohabitans*. The scale corresponds to 20 substitutions per 100 amino acid residues. Bootstrap >70% is given.

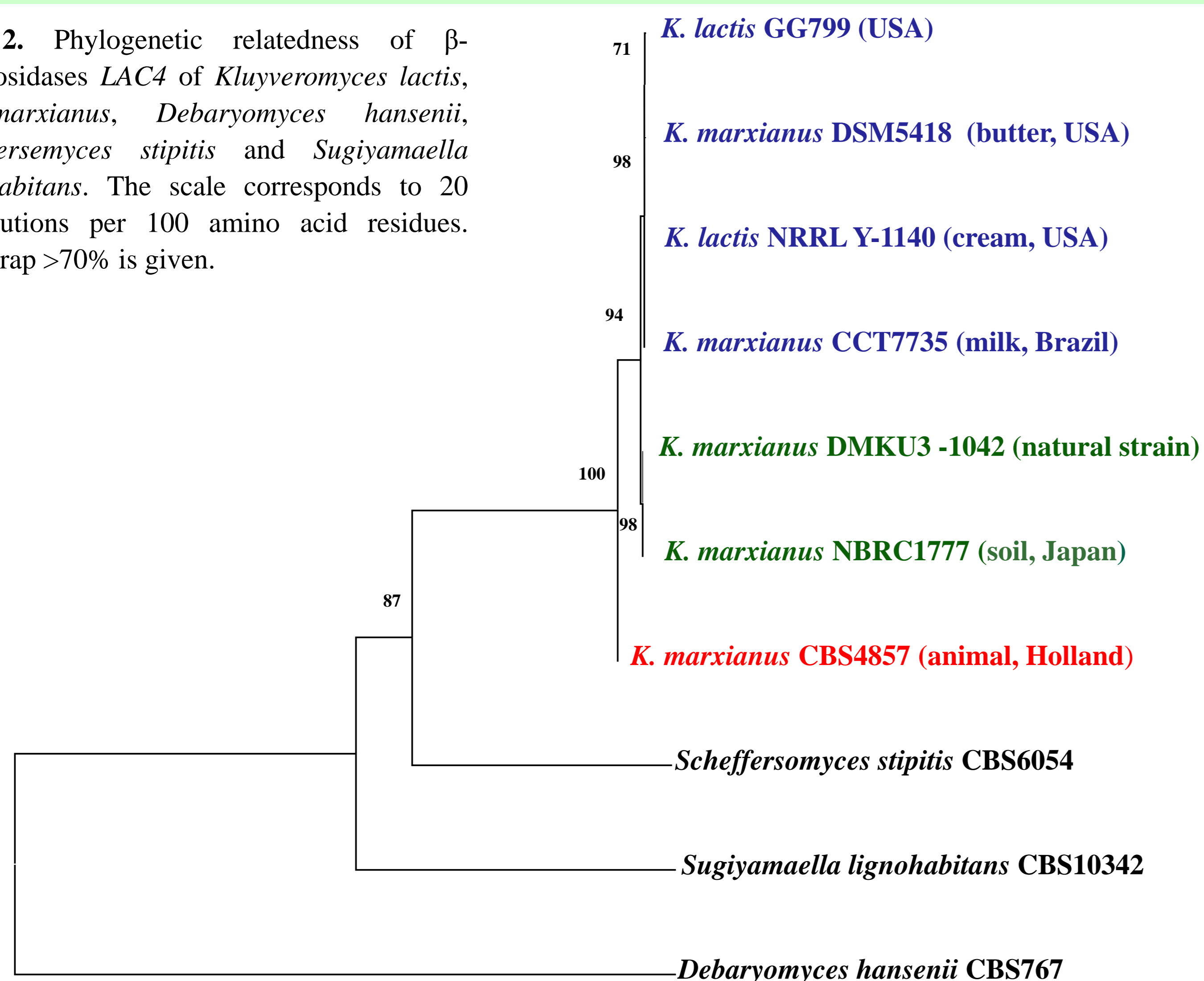
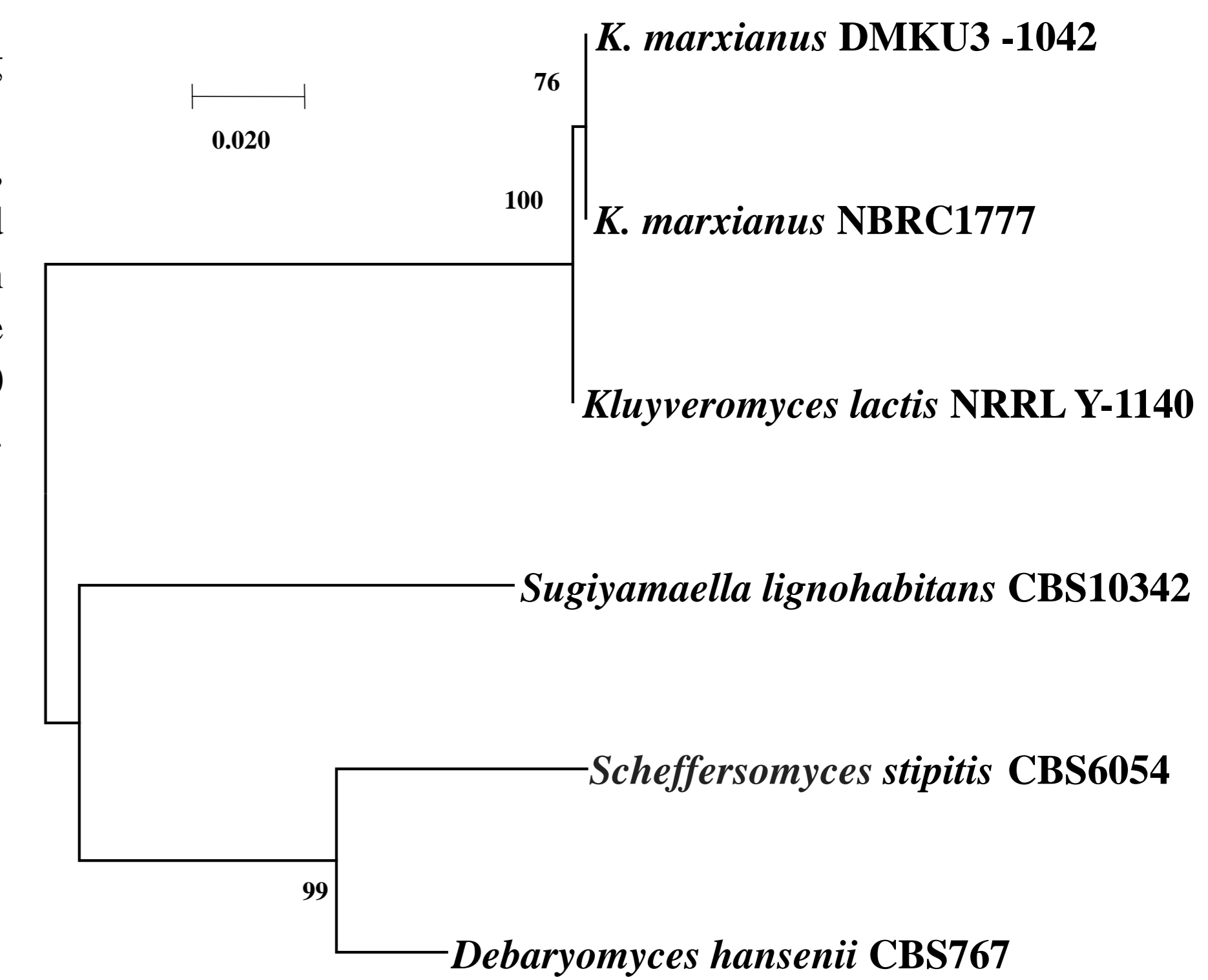


Fig. 3. Neighbour-joining tree showing phylogenetic relatedness of *K. lactis*, *K. marxianus*, *Debaryomyces hansenii*, *Scheffersomyces stipitis* and *Sugiyamaella lignohabitans* based on D1/D2 26S rDNA nucleotide sequences. The scale corresponds to 20 substitutions per 1000 nucleotides. Bootstrap >70% is given.



We used molecular karyotyping and Southern blot hybridization to investigate chromosomal polymorphism of the *LAC4* genes in *K. lactis* strains isolated from various dairy products (Fig. 4, lanes 1-8). Strain VKPM Y-3737 is isolated from soil in Moscow region (Fig. 4, lane 9). With the general similarity of karyotype profiles, an important polymorphism of the sizes of chromosomes II and III is observed (Fig. 4A). Southern hybridization revealed different chromosomal location of *LAC4* genes in various strains (Fig. 4B). The data obtained suggest that fermentation of lactose in *K. lactis* is controlled by polymeric loci, at least *LAC1*, *LAC2* and *LAC3*. The study of new *LAC* genes is now in progress.

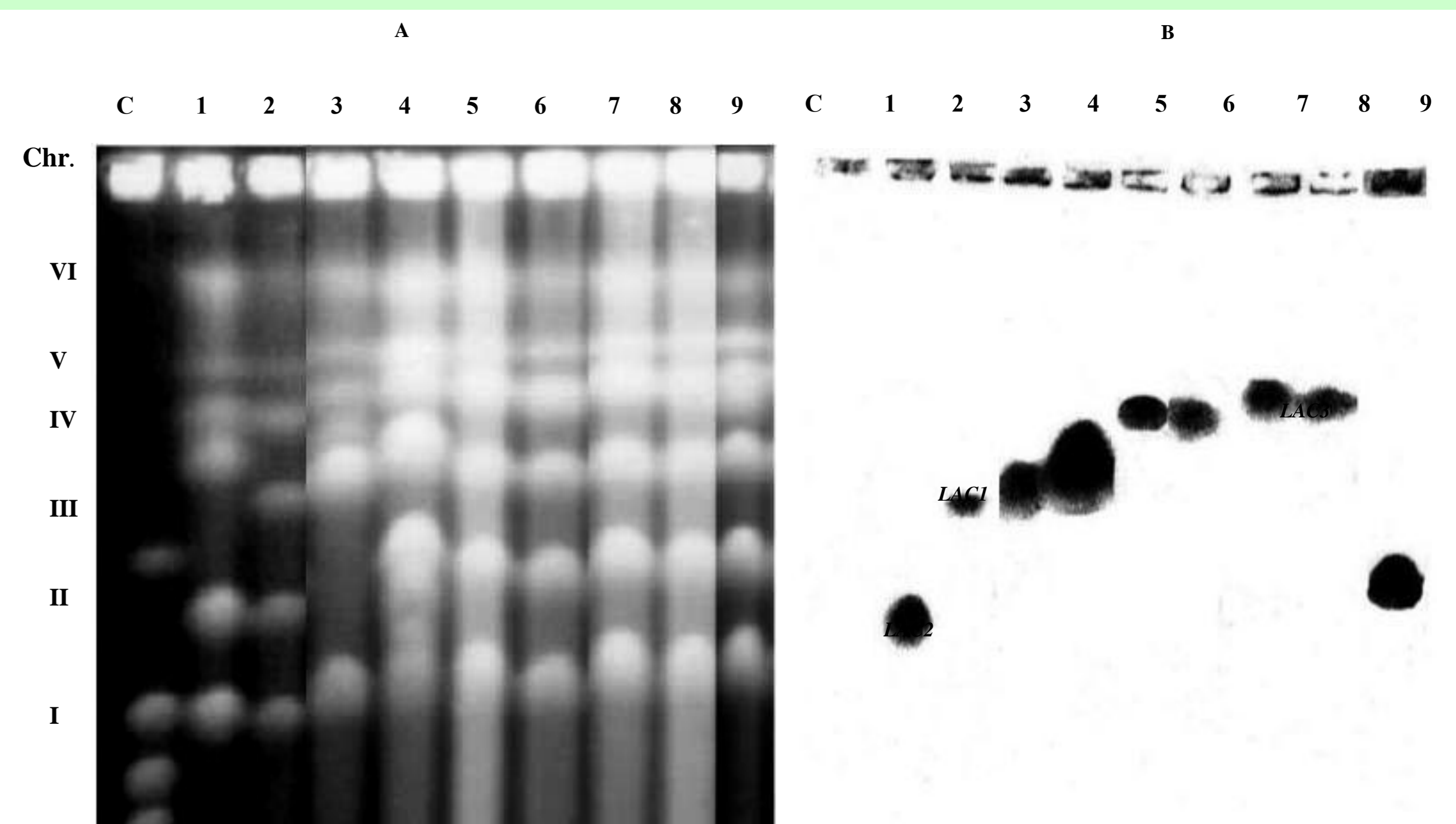


Fig. 4. Pulsed-field gel electrophoresis of *K. lactis* yeast chromosomal DNAs (A) and Southern hybridization with *LAC4* probe (B). Lanes: C, *S. cerevisiae* YNN 295 (chromosomal standard); *K. lactis*: 1 – NRRL Y-1140; 2 – NRRL Y-1118; 3 – VKM Y-869; 4 – VKM Y-870; 5 – VKM Y-1186; 6 – VKM Y-1333; 7 – VKM Y-1339; 8 – VKM Y-1343; 9 – VKPM Y-3737.

## Conclusion

The phylogenetic analysis of the  $\beta$ -galactosidases of dairy *K. lactis* and *K. marxianus* yeasts suggesting the common origin of their *LAC4* genes. With the discovery of polymeric *LAC* genes, new opportunities for studying their intraspecific and interspecific evolution have appeared.

## References

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