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SHORT COMMUNICATIONS

Isolation of a Divergent Strain of *Candida saitoana* from the Anyui Mummy of a Steppe Bison (*Bison priscus*)

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Yeasts are a traditional model for investigation of eukaryotic genetics. Yeasts are also a convenient model for developing the principles of phylogenetic classification and the species concept due to the comparatively small size of the group and the previously accumulated knowledge on both the phenotypic and molecular biological characteristics of yeast species.

The main criteria for classification of yeast species have initially been morphological, with subsequent investigation of their physiological characteristics. The present stage, while considering all accumulated knowledge, is based on the application of molecular biological techniques, primarily, the analysis of conservative rDNA regions. Until recently, the reference point for establishing the criterion of a phylogenetic species in yeasts has been the percentage of differences in the ribosomal genes, more specifically, in the D1/D2 domains of the 26S (LSU) rDNA [1]. Quite recently, another rDNA region, ITS, was chosen as such a universal barcode marker for the whole kingdom of Fungi [2]. However, a large amount of data indicates that in some cases, including yeasts, independent application of these two regions is not always applicable, so it is necessary to use them jointly [3]. Moreover, the level of differences in the ribosomal genes for differentiation between yeast species is being discussed [4]. It is impossible to solve this problem without taking into account all the possible deviations from the type strains, which are stored in collections as "standard" strains. The discovery of genetically divergent strains makes it possible to specify the phylogenetic boundaries of the species, and also contributes to development of the understanding that a phylogenetic species is not something discrete; slight deviations are always possible, and their level is to be studied in the future.

In 2012, in the course of the investigation of the yeasts associated with the Pleistocene bison (steppe bison) mummy found recently in permafrost in the lower reach of Anyui River (Chukotka, Russia), the strain *Candida saitoana* VKPM Y-3988 was isolated, which had substantially divergent rDNA nucleotide sequences, as well as certain specific phenotypic features. The strain was isolated from the wool sampled from the surface of the carcass. Its identification was carried out by analyzing the nucleotide sequences of rDNA regions: ITS1-5.8S-ITS2 and the D1/D2 domains of the 26S (LSU). The protocols for DNA isolation, amplification, and sequencing have been reported previously [5]. The MAFFT 6 [6] and MEGA 4 [7] software package were used for phylogenetic analysis. The strain sequences used for construction of the phylogenetic tree were obtained from the NCBI (ncbi.nlm.nih.gov) GenBank and the CBS (cbs.knaw.nl) databases. The nucleotide network was constructed using the TCS1.21 program [8]. The physiological tests were carried out according to *The Yeasts* [3]. Comparative information on the physiology of the strains was derived from the CBS database and from *The Yeasts* [3, 9].

Phylogenetic analysis by the nucleotide networks method based on the D1/D2 domains of the 26S (LSU) rDNA nucleotide sequences of the strains related to the isolated one according to the NCBI GenBank database showed that several genetically divergent strains differing in 1 bp from the type strain have been known for the species *Candida saitoana* (Fig. 1). The newly isolated strain is a previously unknown divergent strain of the species differing from the type strain by 2 bp, which is comparable to distinctions between *C. saitoana* and *C. pseudoglaebosa*, which have been confirmed as independent species by DNA-DNA reassociation [10].

The nucleotide networks method was previously used for successful demonstration of the presence of phylogeographical groupings of other ascomycetous species based on analyzing the data on the variability of D1/D2 domains of the 26S (LSU) rDNA nucleotide sequences [4]. *C. saitoana* strains with the nucleotide sequences deposited in the NCBI GenBank, originate from Italy, China, Russia (Moscow oblast and Chukotka), Finland, and Japan. The strains from China (one of the strains) and Finland differ from the *C. saitoana* type strain and from other strains conspecific to it by 1 bp; the strain from Chukotka differs by 2 bp. Although the sample available is insufficient to

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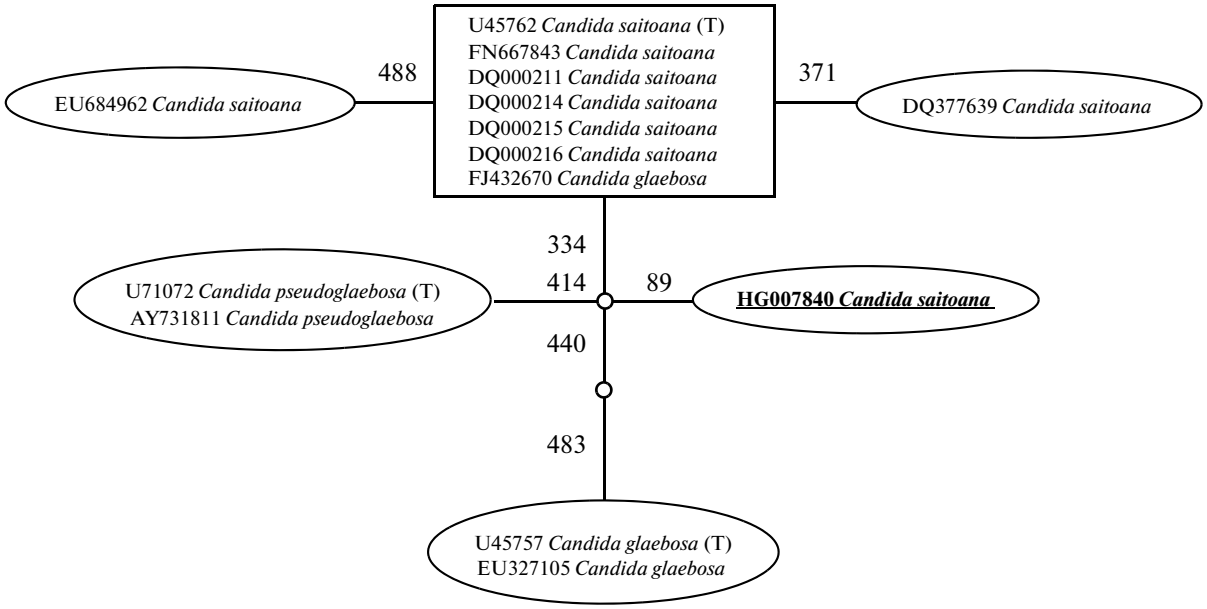

Fig. 1. Parsimony network analysis for the strains related to *C. saitoana* VKPM Y-3988 according to the data of the D1/D2 domains of the 26S (LSU) rDNA nucleotide sequences. Each connecting line denotes one substitution in the nucleotide sequence; the numerals indicate the substitution position. The type strains are designated with the character (T).

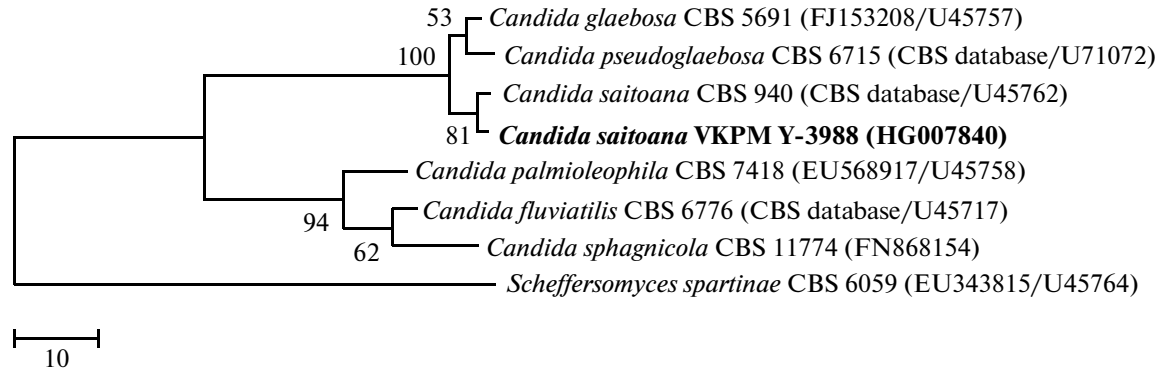

Fig. 2. Maximum parsimony analysis of the strain *C. saitoana* VKPM Y-3988 according to the nucleotide sequences of the rDNA regions: ITS1-5.8S-ITS2 and the D1/D2 domains of the 26S (LSU). The numerals above the branching points indicate the rate (>50%) of taxon groupings for 1000 alternative trees. The scale bar shows the number of substitution.

determine a certain phylogeographical trend reflecting the historical and geographical evolution of the species, but such genetic variation of *C. saitoana* straits could be a promising subject for such analysis.

Additional phylogenetic analysis using the data on the nucleotide sequences of two rDNA regions—ITS1-5.8S-ITS2 and D1/D2 domains of the 26S (LSU)—showed that phylogenetically the strain isolated was indeed closely related to the species *C. saitoana* (Fig. 2). The results showed that the difference between the species *C. glaebosa* and *C. pseudoglaebosa* was nine substitutions in the nucleotide sequence, while the difference between the strains *C. saitoana* CBS 940^T and *C. saitoana* VKPM Y-3988 was five substitutions, which accounts for ~0.5% of the differences. Earlier, these rDNA regions were used to investigate the population variability of the yeast of the genus *Saccharomyces* [11]. Thus, based on the data obtained, it was shown that the existing geographical variability of *S. cerevisiae* strains was up to 0.35% in regions of rDNA, i.e. ITS1-5.8S-ITS2 and D1/D2 domains of the 26S (LSU).

Phenotypic differentiation by conventional physiological tests between the species *C. glaebosa, C. pseudoglaebosa*, and *C. saitoana* is difficult [3, 10]. Moreover, the physiological characterization of the species *C. saitoana* is continuously under revision: for example, certain data from *The Yeasts*, 1998 [9] differ somewhat from *The Yeasts*, 2011 [3], which do not agree with the data for the collection strains, either

Variable assimilation characteristics for the species *C. saitoana* according to *The Yeasts* [3, 9], the CBS data, and the results obtained in the course of the present work

	1	2	3	4	5	6
L-Sorbose	+	V	+	+	+	+
Cellobiose	–	+	+	+	+	+
Lactose	–	V	+	+	–	+
Melibiose	–	+	+	+	V	+
Raffinose	–	+	+	+	+	+
Melezitose	+	V	–	–	+	–
Inulin	–	+	–	+	–	+
Soluble starch	–	V	–	–	–	–
L-Arabinose	–	V	+	+	+	+
D-Arabinose	–	–	–	–	V	–
D-Ribose	–	V	+	–	+	–
Glucosamine	–	V	–	+	+	–
Glycerol	+	+	+	+	+	–
Erythritol	–	–	–	–	+	–
Ribitol	–	+	+	+	+	+
Dulcitol	–	–	–	–	+	+
Salicin	–	+	+	+	+	+
Lactic acid	+	+	–	+	+	+
Citric acid	–	+	–	+	+	+

1, *The Yeasts*, 2011 [3]; 2, *The Yeasts*, 1998 [9]; 3, the type strain CBS 940 (CBS data); 4, strain CBS 6729 (CBS data); 5, strain CBS 8046 (CBS data); 6, strain VKPM Y-3988.

(table). However, taking into account the known diversity of varying characteristics of this species, the newly isolated strain has the previously unknown specific feature of being incapable of utilizing glycerol as a carbon source, which was not known previously for the species *C. saitoana*. The physiological data obtained are also of interest for other *C. glaebosa* clade species, since they are all capable of glycerol assimilation [3, 12].

Thus, it was shown by the example of the strain *Candida saitoana* VKPM Y-3988 that the study of microorganisms from rather unusual habitats or unexplored territories makes it possible to reveal the previously unknown phylogenetic and phenotypic divergent strains of well-known species. In turn, such findings contribute to the solution of the fundamental problems pertinent to understanding the range of all variabilities, which may be included in the notions of the 'species' and the 'genospecies' of such a convenient model group as yeasts.

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