https://doi.org/10.15407/frg2022.06.516
UDC 004:576.316.3:57.063.7:582.282.232

MOLECULAR GENETIC IDENTIFICATION OF YEAST ISOLATE MF22_1

M.O. FOMINA, O.Ya. FILIPISHENA, L.V. POLISHCHUK

D.K. Zabolotny Institute of Microbiology and Virology, National Academy of Sciences of Ukraine
154 Akademika Zabolotnogo St., Kyiv, 03143, Ukraine
e-mail: M.Fomina@ukr.net

Microscopic fungi including yeasts are important part of human microbiota. Among them, yeasts of genus *Candida* are very common and often the most predominant part of mycobiota. *Candida* spp., including *Candida albicans* and non-*Candida albicans* species, can be opportunistic pathogens affecting people with depressed immune system. The problem with some non-*Candida albicans* species, e.g. *C. glabrata* and *C. krusei*, is that they are highly resistant to antifungal therapy and, at the same time, difficult to identify accurately at the species level by widely clinically used conventional biochemical methods. The aim of this work was precise molecular genetic identification of human sputum yeast isolate MF22_1, allegedly belonging to *Candida* spp., by using multiple genetic markers. We used four fragments of its chromosomal DNA, which were sequenced and deposited by us in GenBank NCBI database: 1) ITS (GenBank OM479428, 548 bp), 2) 28S rRNA (OM479513, 607 bp), 3) 18S rRNA (OM4794321, 737 bp), and 4) RNA polymerase II gene — RPB2 (OM524388, 1,217 bp). The part of Nucleotide collection database [http://www.ncbi.nlm.nih.gov/nuccore/] containing information on the nucleotide structure of fungal DNA (taxid: 4751) was studied using BLASTN [http://blast.ncbi.nlm.nih.gov/Blast.cgi]. The BLASTN analysis established that all four sequenced genetic markers: ITS, 28S rRNA, 18S rRNA, and RPB2 of the studied isolate MF22_1 had the maximum similarity to the corresponding sequences of the type strains of *Pichia kudriavzevii* species. Therefore, it was confirmed that the isolate belonged to species *Pichia kudriavzevii*, which anamorph (or non-ascosporic state) is *Candida krusei*. This ubiquitous in the environment species is a common clinical isolate responsible for about 2 % incidences of yeast infections caused by *Candida* species in humans. Comparative analysis of a primary structure of ITS region demonstrated the great similarity of the ITS sequence of *Pichia kudriavzevii* MF22_1 to the majority of other *Pichia kudriavzevii* clinical isolates from human sputum, stool, blood etc., which are preserved in different culture collection of the institutions specializing in medical studies of yeasts around the world. For these clinical isolates of *Pichia kudriavzevii*, no clear correlation was observed between the similarity of ITS sequences and the type of biomaterial sampled from humans.

Key words: *Candida* spp., yeast, identification, ITS region, 18S rRNA, 28S rRNA, RPB2.

Citation: Fomina M.O., Filipishena O.Ya., Polishchuk L.V. Molecular genetic identification of yeast isolate MF22_1. Fisiol. rast. genet., 2022, **54**, No. 6, pp. 516—527. https://doi.org/10.15407/frg2022.06.516

The fungal, particularly yeast microbiota of humans and mammalian animals has been largely understudied compared to the bacterial component of microbiome [1]. However, there is growing evidence that fungi can play a crucial role in modulation of host immunity and consequently in health or disease of a macro-organism [1, 2]. From the very moment of their birth macro-organisms are exposed to the colonization by mycobiota originated, firstly, from maternal microbiome (vertical transmission), and then, throughout life, from environment and available foods. The most abundant and diverse fungal microbiota is characteristic for the mammalian gut where the most predominant members of fungal communities usually belong to genus *Candida* [1, 2]. Many representatives of *Candida* spp., including the major fungal species in the human gut *Candida albicans*, are typically regarded as opportunistic pathogens meaning that they only affect patients with depressed immune system. At the same time *C. albicans* is the most frequently detected fungus in faeces of healthy humans, and therefore, is considered a normal component of the human gut microbiota [1]. The pathogenesis caused by yeasts is thought to be largely associated with the disbalance of fungal microbiota which is in its turn tightly connected to bacterial dysbiosis.

Apart of intestine, such yeasts as *C. albicans* can also colonize other body sites including mouth, skin, and vagina. *Candida* spp. yeasts often colonize the upper respiratory tract in healthy individuals, and this oropharyngeal colonization is reflected in finding such yeasts in sputum at microbiological examination. However, *Candida* spp. can cause thrush or even pneumonia in severely immunocompromised patients [3, 4]. It was also found that *Candida* spp. never caused pneumonia alone but contributed to the pathological process in association with bacterial pathogens or opportunistic members of normal respiratory bacterial microbiota [3]. That is why the microbe-microbe interactions within the oral microbiome including inter-kingdom fungal-bacterial signalling affecting microbial pathogenicity have attracted increasing attention [4].

In recent years there has been a significant increase in the incidence of *Candida* infections related to high morbidity and mortality as well as the growing number of infections caused by non-*Candida albicans* yeasts belonging to genus *Candida* [5]. Some non-*Candida albicans* yeasts are known to possess higher intrinsic antifungal resistance compared to *C. albicans*. Such epidemiological shift of *Candida* pathogens, when the infection incidents, caused by both *Candida albicans* and non-*Candida albicans* yeasts, made up approximately 50 % each, together with the increased incidence of these infections required rapid and precise methods of identification of *Candida* species [5, 6]. The comparative assessment of biochemical and molecular genetic methods of taxonomic identification of *Candida* species demonstrated that the latter are more accurate and faster than biochemical approaches [5]. For example, the *Candida* species with the least level of correct identification by biochemical methods (Auxacolor and Vitek 2) were *C. glabrata* and *C. krusei,* which are usually associated with high levels of antifungal resistance.

Thus, the rapid and precise identification of yeast pathogens may become critical for the start of treatment and antifungal therapy strategy [5, 6]. And the molecular genetic approaches, particularly PCR-based

assays which often use rRNA genes and spacers as the genetic markers, may provide rapid, sensitive and accurate diagnostics of fungal pathogens.

The aim of our work was precise molecular genetic identification of human sputum yeast isolate MF22_1, allegedly belonging to *Candida* spp., by using multiple genetic markers including ribosomal operon regions.

Materials and methods

Yeast strain MF22_1 from the collection of D.K. Zabolotny Institute of Microbiology and Virology of National Academy of Sciences of Ukraine was originally isolated from the sputum sample during routine bacteriological examinations of a hospitalized cardiac patient in Kyiv, Ukraine in 2019. Initial biochemical and morphological examination of the isolate on site suggested that it belonged to *Candida* spp., however failed to provide its exact identification at the level of the species. In order to detail the taxonomic position of this yeast strain we performed its molecular genetic identification using four fragments of its chromosomal DNA, which were sequenced and deposited by us in GenBank NCBI database: 1) internal transcribed spacer — ITS (GenBank OM479428, 548 bp), 2) 28S rRNA or large subunit — LSU (OM479513, 607 bp), 3) 18S rRNA or small subunit — SSU (OM4794321, 737 bp), and 4) RNA polymerase II gene — RPB2 (OM524388, 1,217 bp). The primary structure of ITS fragment of the strain MF22_1 was used in our study as a reference sequence #1; combined ribosomal cluster sequences [ITS + LSU + 18S rRNA, 2814 bp] served as a reference sequence #2; and RPB2 sequence was a reference sequence #3.

The commonly available Internet database of the NCBI server: «Nucleotide collection» [http://www.ncbi.nlm.nih.gov/nuccore/] was used in this work. The part of the Nucleotide collection database containing information on the nucleotide structure of fungal DNA (taxid: 4751) was analyzed. Sequence alignment was performed using the software package of the same BLASTN server [http://blast.ncbi.nlm.nih.gov/Blast.cgi]. We used the basic settings of the BLASTN program without changes.

Results and discussion

Molecular genetic identification of yeast strain MF22_1 in this study was based on the analysis of the sequences of four genetic markers: ITS, LSU, 18S rRNA, and RPB2.

The ITS fragment of ribosomal operon is regarded as a genetic marker with the highest probability of successful identification for the broadest range of fungi, providing the most clearly defined barcode gap between inter- and intraspecific variation [7—9]. Thus, over decade, ITS region has been used as a universal DNA barcode marker for Fungi, the second largest kingdom of eukaryotic life [8]. In fungi, the existence of teleomorph and anamorph stages in their life cycles presents the fundamental problem of fungal taxonomy. Teleomorphic and anamorphic stages usually differ greatly in their phenotype, making it difficult to directly associate an asexual anamorph with a sexual teleomorph. Moreover, different strains of the same fungal species may manifest such differences in their phenotype, such

as micro- and macro-morphology, or nutrients utilization and other physiological characteristics, often leading to their description as different species, ultimately leading to long lists of synonyms, which is very common, for example, for yeasts [10—12].

In addition to ITS region, other genetic markers from ribosomal operon (large subunit — LSU or 28S rRNA, and small subunit — SSU or 18S rRNA) are also commonly used for fungal identification [8, 13, 14]. Thus, the nuclear ribosomal LSU is a popular phylogenetic marker in certain groups of microscopic fungi which had superior species resolution in some taxonomic groups (e.g. the early diverging lineages and the ascomycetous yeasts). However, this marker was otherwise slightly inferior to the ITS. Regarding the nuclear ribosomal SSU, it has poor resolution on the species and intra-species level in fungi.

ITS use as bar coding of fungal DNA helps to identify and link the anamorph and teleomorph stages of fungi, and thus reduce the multiple names of fungi. The ITS sequence includes 5′-end of 18S rRNA (or SSU) gene, ITS1, 5.8S rRNA gene, ITS2, and 3′-end of 28S rRNA (or LSU) gene, and in this work for strain MF22_1 is referred to reference sequence #1.

As a result of analysis of the GenBank Internet database (Nucleotide collection; Fungi) using the BLASTN (megablast) program we found over 5,000 available sequences of ITS region for genomic DNA fragments of representatives of fungi, which were similar to the reference sequence #1 for strain MF22_1. These sequences similarity ranged from 100 % to 77 %, and they were found in the fragments varying in molecular size from 136 bp up to 11848804 bp. It should be noted that some of the detected sequences similar in primary structure to reference sequence #1 for strain MF22_1 are not defined by species but only by higher taxa of the kingdom Fungi: classes, orders, families or genera (Fig. 1).

More than half (53 %) of all detected sequences, 2,642 sequences were found to be similar to ITS sequences belonging to fungi from 28 genera of the Pichiaceae family. Of these, 2,114 sequences (43 %) belonged to the strains of species *Pichia kudriavzevii* (synonym *Issatchenkia orientalis*) and its anamorphs, including the most famous anamorph *Candida krusei* (see Fig. 1). Thus, according ITS data, it can be suggested that the studied strain MF22_1 belongs to species *Pichia kudriavzevii.*

Pichia kudriavzevii and its anamorph *Candida krusei* are ubiquitous yeasts widely distributed in the environment [12, 15, 16]. They are often found in soil, fruits and various agricultural products, natural fermentations

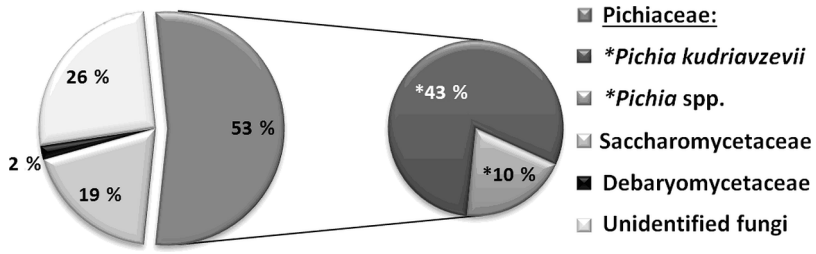

Fig. 1. Diagram of the taxonomic organization of 5,000 fungal ITS-sequences similar to the ITS sequence of yeast strain MF22_1 (reference sequence #1)

and foods. However it is not regarded as a food spoilage species, in contrary it is used as a cheese flavouring and ripening agent in the handcrafted fermented food production [15, 16]. Moreover, the strains of this species under the names *Pichia kudriavzevii* and its former synonyms *Issatchenkia orientalis* and *Candida glycerinogenes* are used in the biotechnology for industrial production of glycerol and succinate [12]. At the same time this species is of clinical importance being often isolated from humans and animals [12, 16].

In our study over two thousands yeasts in GenBank database, identified by ITS fragments as species *P. kudriavzevii* (see Fig. 1), were isolated from various sources: plants, soil, food, beverages, animal feed, industrial equipment, human and animal tissues, blood and secretions, from around the world, different regions and countries (e.g. Poland, Hungary, Finland, France, USA, Mexico, Brazil, Argentina, Nigeria, Tunisia, Ghana, Egypt, China, Japan, South Korea, Vietnam, Indonesia, Kazakhstan, and Australia).

Among the 50 strains with the most similar ITS primary structure (Qc = 100 %, I > 90 %), there were 20 type (permanent reference) strains of *Pichia kudriavzevii* from the collection of CBS (The Convention of Biological Diversity, Montreal, Canada). The localization of substitutions in ITS sequence of *P. kudriavzevii* MF22_1 was detected using the program BLASTN (MSA viewer). It was shown that most of substitutions are located outside the 5.8S rRNA gene locus.

If compared to other *P. kudriavzevii* strains, the changes in ITS sequence of the studied strain *P. kudriavzevii* MF22_1 were found in a number of nucleotide bases (17—18, 79, 110, 443 and 538). As mentioned above, many *P. kudriavzevii* strains with DNA and RNA sequences added to GenBank were clinical isolates e.g., from human secretions and blood.

For clinical specimens of human excretions, it was reported that *Candida* species in general were more frequently isolated from: 1) urine, 2) sputum/bronchial wash/bronchoalveolar lavage, and 3) vaginal swabs, comprising 39 %, 25 % and 11 %, respectively [5]. The greatest variety of *Candida* species that also included *C. krusei* was observed for the second kind of specimens.

The studied yeast culture MF22_1 was also isolated from human sputum. It was of interest to establish strain MF22_1 genetic relatedness with other isolated from humans *P. kudriavzevii* strains based on the similarity of a primary structure of ITS region. Among analysed GenBank ITS sequences of *P. kudriavzevii*, we found that 11.5 % sequences belonged to clinical isolates from the collections of the research departments specializing in medical studies of yeasts. That is *P. kudriavzevii* strains isolated from human stool samples were from the collection of Pasteur Institute of Tunis, Tunisia; isolates from human sputum samples were from the collection of Al-Neelain University, Sudan; human blood isolates were from the collection of Federal University of Sao Paulo, Brazil; and isolates from human bio-specimens were from the collection of Laboratorium Parasitologi, Universitas Indonesia, Jacarta, Indonesia.

ITS-fragment BLASTN alignment for studied isolate MF22_1 and other human-isolated *P. kudriavzevii* strains revealed that their structures can vary ranging from complete sequence identity to dozens of differences

such as mismatches, gaps, unalignment regions. The typical results of this alignment are illustrated using some selected strains in Fig. 2. Such differences may depend on the collection where certain *P. kudriavzevii* clinical isolates are preserved. For example many *P. kudriavzevii* strains from the collections of Pasteur Institute of Tunis, Al-Neelain University and Federal University of Sao Paulo demonstrated complete identity (I = 100 %) of their ITS sequences to those of strain MF22_1 (reference sequence #1) and the genetically well-studied strain *P. kudriavzevii* CY902, isolated from grape skin in China (see Fig. 2).

At the same time, the collection of the University of Jakarta included the strains, whose primary ITS structures had the greatest number of differences compared to reference sequence. According to GenBank information, the strains of this Indonesian collection were identified as *P. kudriavzevii* using both ITS and LSU as genetic markers. For some strains (e.g. CK8, CK9, CK10, CK12) localization of the differences in ITS fragment structures were observed within probable 5.8S rRNA gene region which is known to contain a highly conserved DNA sequence (see Fig. 2). In general, most of the differences in the ITS structure of *P. kudriavzevii* strains of Indonesian collection were localized within sequences of ITS1 and ITS2 regions. The structural differences of the analyzed ITS of *P. kudriavzevii* strains from University of Jakarta collection significantly differed from those for not only studied strain MF22_1 (reference sequence #1), but also for *P. kudriavzevii* type strains and *P. kudriavzevii* strains isolated from various sources.

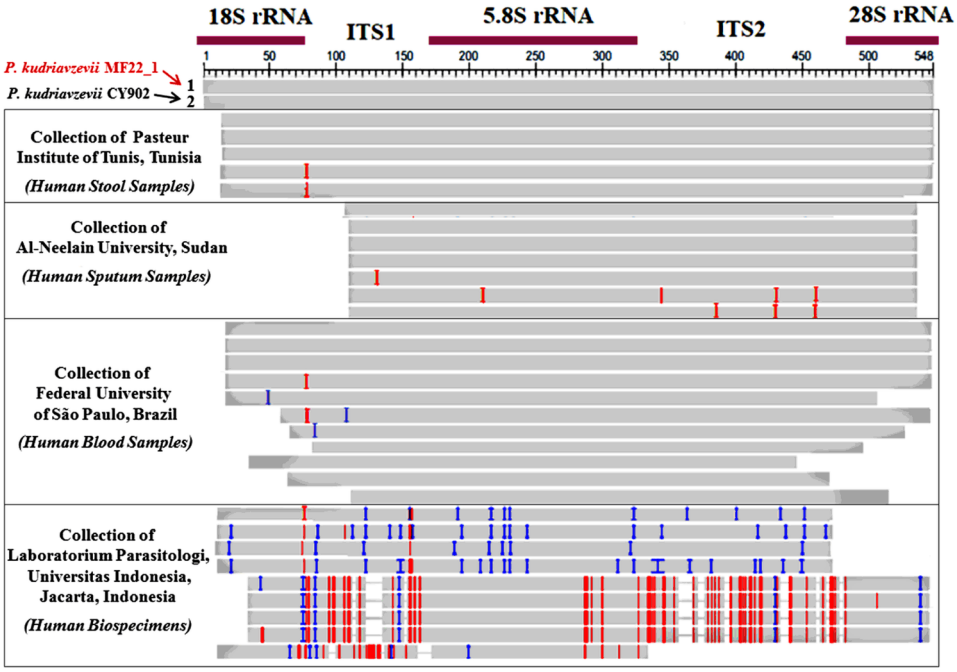

Fig. 2. Differences in ITS nucleotide sequences of *Pichia kudriavzevii* clinical isolates from different collections compared to (1) the studied strain *P. kudriavzevii* MF22_1 (reference sequence #1) and (2) isolated from grape skin strain *P. kudriavzevii* CY902

Based on the available data on *P. kudriavzevii* clinical isolates from analysed collections, it was not possible to draw the clear conclusion whether an actual source of isolation (blood, stool, sputum etc.) had an impact on the differences in the ITS region of these strains.

It should be noted that one of the reasons that the regions of ribosomal operon are particularly attractive targets in phylogenetic studies on microorganisms is because multiple copies are present in each genome, and the genes have both conserved regions for designing broad-range primers and more variable regions for identification of fungi and bacteria [6, 17]. The presence of multiple ITS regions has been also revealed in genome of some *P. kudriavzevii* strains. For example, for the mentioned above strain *P. kudriavzevii* CY902, it was found the existence of 14 copies of ITS regions located on three chromosomes (Table). Chromosome III contains 10 copies of rRNA cluster. One of these copies (84432—84961 bp) differs from all other in tandem with 2 mismatches and 20 gaps (Qc = 96 %, I = 94 %).

Information on GenBank database Pichia kudriavzevii strains with highly similar SSU, ITS and LSU primary structure to the P. kudriavzevii MF22_1 reference sequence #2

P. kudriavzevii strain code	Accession numbers, Query Coverage (Qc, %) and Sequence Identity (I, %) of the GenBank database *P. kudriavzevii* strains			Strain source
	SSU	ITS	LSU	
CBS 2052 *	MW281657.1 Qc = 96, I = 99.94	KY104588.1 Qc = 93, I = 97.61	KY108844.1 Qc = 100, I = 99.84	Italy, *Homo sapiens*
CBS 573	MW281689.1 Qc = 96, I = 99.10	MW284507.1 Qc = 87, I = 99.53	AJ508568.1 Qc = 92, I = 99.65	N/A
CBS 2046 *	MW281656.1 Qc = 97, I = 99.76	KY104568.1 Qc = 92, I = 99.41	KY108835.1 Qc = 100, I = 99.84	Argentina, *Homo sapiens*
CBS 5146 *	MW281678.1 Qc = 97, I = 99.58	KY104591.1 Qc = 86, I = 98.32	KY108837.1 Qc = 96, I = 98.81	Brasil, *Homo sapiens*
ATCC 6258*	M60305.1 Qc = 91, I = 97.99	OL351344.1 Qc = 93, I = 99.61	KC601854.1 Qc = 96, I = 100	US, culture collection
DIC	JF715173.1 Qc = 57, I = 99.70	JF715197.1 Qc = 92, I = 99.01	JF715184.1 Qc = 100, I = 99.51	India, mango
Y4	JF715171.1 Qc = 57, I = 99.10	JF715195.1 Qc = 93, I = 98.83	JF715182.1 Qc = 97, I = 100	India, mango
Yeast chromosomes with sequences similar to SSU, ITS and LSU sequences of *P. kudriavzevii* MF22_1 (reference sequence #2)				
CY902	chromosome III — CP039612.1 Qc = 100, I = 99.89 (2)** chromosome II — CP039617.1 Qc = 100, I = 99.89 (10) chromosome I — CP039615.1 Qc = 100, I = 99.86 (2)			China, grape skin
SJP	chromosome I — CP021088.1 Qc = 100, I = 99.89 (5) chromosome III — CP021090.1 Qc = 100, I = 99.86 (1) chromosome V — CP021092.1 Qc = 100, I = 99.82 (1)			South Korea
CBS573 *	chromosome I — CP028774.1 Qc = 100, I = 99.82 (2) chromosome II — CP028773.1 Qc = 100, I = 99.82 (1)			N/A
CBS5147	chromosome I — CP028531.1 Qc = 100, I = 99.82 (1) chromosome II — CP028532.1 Qc = 100, I = 99.79 (1)			Russia, fruit juice

Notes: * — type material of *P. kudriavzevii*, ** — number of rRNA clusters, N/A — not available.

The complete sequence of 18S rRNA gene (SSU, 1737 bp) and the partial sequence of 28S rRNA gene (LSU, 607 bp) were also obtained for the studied strain *P. kudriavzevii* MF22_1. Hence, along with *P. kudriavzevii* MF22_1 ITS region sequence, they were compared to the sequences of entire ribosome cluster or its large fragment of other *P. kudriavzevii* strains in the analysis of their phylogenetic relationships. In total, the integrated *P. kudriavzevii* MF22_1 sequence of the ribosomal cluster contained 2814 bp (reference sequence #2). It should be considered that the data of databases may vary, and contain sequences of different molecular size, such as the fragments of individual genes (e.g. LSU, RPB2), the whole genes (e.g. 18S rRNA), the fragments containing sequences of several genes (e.g. ITS region) or whole chromosomes (e.g. *P. kudriavzevii* CBS573 chromosome IV: CP028776.1). A BLASTN analysis of the GenBank database (Nucleotide collection, Fungi) was carried out to search for the yeast strains with genomes containing the most similar sequences to the structure of the reference sequence #2 of *P. kudriavzevii* MF22_1. This database contains tens of thousands of *P. kudriavzevii* sequences of the fragments of different rRNA gene clusters. On the one hand, there are deposited small fragments of genes, such as 18S rRNA gene sequences of *P. kudriavzevii* strains DIC (JF715173.1, 559 bp) and Y4 (JF715171.1, 556 bp). On the other hand, only for a small number of strains in the database, there are the sequences of both analyzed genes SSU and LSU, as well as ITS region, which are presented as the sets of three separate fragments and also as a part of a complete chromosome sequence for some *P. kudriavzevii* strains (see Table).

Of the thousands of identified DNA sequences of the representatives of *P. kudriavzevii* species (or its synonyms *Issatchenkia orientalis* or *Candida krusei*) in the database, only 25 fragments of the rRNA clusters of 10 chromosomes had the Query coverage 100 % (see Table). The similarity of their primary structures to the reference sequence #2 ranged from 99.89 % to 99.79 %. In eukaryotes, rRNA genes clusters are known to exist as tandem multicopy repeats. The database sequences contain mostly 1—2 copies, but chromosome I of *P. kudriavzevii* CY902 contains a tandem of 10 copies (see Table).

A number of *P. kudriavzevii* strains, that demonstrated high similarity to the reference sequence #2, are the type strains of this species from the largest in the world culture collections such as CBS (Westerdijk Fungal Biodiversity Institute, Netherlands) and ATCC (American Type Culture Collection, USA).

It should also be noted that the sequences of individual copies on one chromosome differ in the number of discrepancies with the reference sequence #2 of *P. kudriavzevii* MF22_1. For example, one copy (83845—86640 bp) out of ten copies on chromosome II of *P. kudriavzevii* strain CY902 has a lower similarity with reference sequence #2 (99.3 %) compared to other copies (99.9 %).

In addition to the traditional analysis of ribosomal cluster sequences, a structure of nuclear gene RPB2, which encodes the second largest subunit of RNA polymerase II, is also used in phylogenetic studies of fungi. The reasons are that this is a single-copy gene of large molecular size, and

is characterized by a low rate of evolutionary changes, which is a prerequisite for providing a better understanding of the evolution of fungi, and a more precise definition of these microorganisms taxonomic relationships [18—20].

In our study, the obtained sequence of DNA-dependent RNA polymerase II for isolate *P. kudriavzevii* MF22_1 served as reference sequence #3 for RPB2 analysis. BLASTN analysis of the Internet database (Fungi) revealed 191 fungal sequences with only 21 sequences of strains that belong to family Pichiaceae. Of them, only six sequences of representatives of the species *P. kudriavzevii* from different collections were found. These six RPB2 sequences manifested the highest similarity to the reference sequence #3 (Qc = 100 %, I = 99.59—98.77 %), including one sequence belonging to the type culture of the species *P. kudriavzevii* CBS573 (Qc = 100 %, I = 99.59 %).

It was established that there are just a few differences in the primary structure of the RPB2 gene of *P. kudriavzevii* MF22_1 from the sequences of such gene of the representatives of *P. kudriavzevii* species (Fig. 3). At the same time, the number of differences was significantly greater when RPB2 sequence for *P. kudriavzevii* MF22_1 was aligned with RPB2 sequences of strains of other *Pichia* species (see Fig. 3).

Thus, the BLASTN analysis established that all four sequenced genetic markers: ITS, SSU, LSU, and RPB2 of the studied isolate MF22_1 had the maximum similarity to the corresponding sequences of type strains of *P. kudriavzevii* species and consequently the minimum number of nucleotide bases which are distinct from them.

Currently both names of this species: teleomorph *P. kudriavzevii* and anamorph *C. krusei* are still used in modern scientific literature mainly because name *Candida krusei* is still widely used in clinical routine and medical research. Presented by polyphyletic group of species, the genus *Candida* has served as a «dumping ground» for ascomycetous budding yeasts not forming ascospores [21]. After introduction of molecular genetic methods of yeasts identification, almost all clades of teleomorphic ascomycetous yeasts include representatives of *Candida* spp. As soon as an

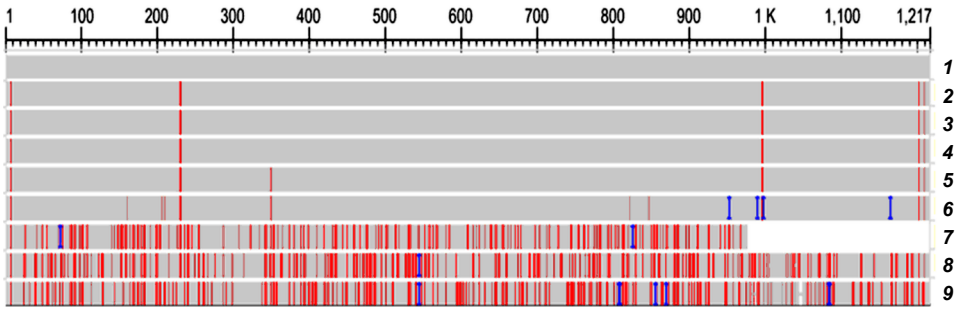

Fig. 3. Localization of differences in a nucleotide sequence of RPB2 gene of a number of selected strains of family Pichiaceae, if compared to the reference sequence #3 for (*1*) RPB2 of *P. kudriavzevii* MF22_1; (*2*) *P. kudriavzevii* CBS573; (*3*) *P. kudriavzevii* CY902; (*4*) *P. kudriavzevii* CW-CM; (*5*) *P. kudriavzevii* CBS5147; (*6*) *P. kudriavzevii* SJP; (*7*) *P. norvegensis* CBS 8403; (*8*) *Candida sorboxylosa* NRRL Y-17669; (*9*) *Kregervanrija fluxuum* NRRL YB-4273

ascosporic state of *Candida* species is discovered, this species should be recognized as a member of a teleomorphic genus and named appropriately; and in our case *C. krusei* is recommended to be named *P. kudriavzevii* [12, 21].

Candida krusei is a common clinical isolate responsible for about 2 % of yeast infections caused by *Candida* species in humans. However, this species is characterised by a combination of, on one side, resistance of this species to azoles with, on the other side, difficulties with its precise taxonomic identification by conventional biochemical and physiological approaches (e.g. Vitek) [5, 12]. Phylogenetic analysis did not distinguish clinical and environmental isolates into separate clades, meaning that *P. kudriavzevii*/*C. krusei* infections can be easily acquired from the environment [12]. Therefore, the special caution may be needed in the wide use of drug-resistant *P. kudriavzevii* strains in biotechnology and food applications.

Thus, the use of multiple genetic markers including ITS (OM479428), 28S rRNA (OM479513) and 18S rRNA (OM4794321) of ribosomal operon, as well as RNA polymerase II gene — RPB2 (OM524388) allowed to perform precise taxonomic identification of yeast isolate MF22_1 from human sputum at the species level. It was established that the isolate MF22_1 belonged to *Pichia kudriavzevii* (anamorph *Candida krusei*) which is considered as clinically important species. Comparative analysis of a primary structure of ITS region demonstrated the great similarity of the ITS sequence of *Pichia kudriavzevii* MF22_1 to the majority of other *Pichia kudriavzevii* clinical isolates from human sputum, stool, blood etc. in different culture collection around the world. There was no clear correlation between the ITS sequences of *Pichia kudriavzevii* clinical isolates and the type of material sampled from humans.

Acknowledgements. The authors would like to thank Dr. Ji Won Hong from Kyungpook National University, Daegu, South Korea for his invaluable help with sequencing fragments of chromosomal DNA of yeast isolate.

REFERENCES

1. Perez, J.C. (2021). Fungi of the human gut microbiota: roles and significance. International Journal of Medical Microbiology, 311, No. 3, 151490. https://doi.org/10.1016/j.ijmm.2021.151490
2. Fiers, W.D., Leonardi, I. & Iliev, I.D. (2020). From birth and throughout life: fungal microbiota in nutrition and metabolic health. The Annual Review of Nutrition, 40, pp. 323-343. https://doi.org/10.1146/annurev-nutr-013120-043659
3. Moss, B.J. & Musher, D.M. (2021). Candida species in community-acquired pneumonia in patients with chronic aspiration. Pneumonia, 13, No. 12. https://doi.org/10.1186/s41479-021-00090-x
4. Cannon, R.D. (2022). Oral fungal infections: past, present, and future. Frontiers in Oral Health, 3, 838639. https://doi.org/10.3389/froh.2022.838639
5. Costa, A.R., Silva, F., Henriques, M., Azeredo, J., Oliveira, R. & Faustino, A. (2010). Candida clinical species identification: molecular and biochemical methods. Annals of Microbiology, 60, pp. 105-112. https://doi.org/10.1007/s13213-009-0007-6
6. Khot, P.D., Ko, D.L. & Fredricks, D.N. (2009). Sequencing and analysis of fungal rRNA operons for development of broad-range fungal PCR assays. Appl. Environ. Microbiol., 75, No. 6, pp. 1559-1565. https://doi.org/10.1128/AEM.02383-08

7. Peay, K.G., Kennedy, P.G. & Bruns, T.D. (2008) Fungal community ecology: a hybrid beast with a molecular master. BioScience Journal., 58, No. 9, pp. 799-810. https://doi.org/10.1641/b580907
8. Schoch, C.L., Seifert, K.A., Huhndorf, S., Robert, V., Spouge, J.L., Levesque, C.A. & Chen, W. (2012). Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for Fungi. Proceedings of the National Academy of Sciences of the United States of America, 109, No. 16, pp. 6241-6246. https://doi.org/10.1073/pnas.1117018109
9. Bruns, T.D., White, T.J. & Taylor, J.W. (1991). Fungal molecular systematics. Annual Review of Ecology and Systematics, 22, No. 1, pp. 525-564. https://doi.org/10.1146/annurev.es.22.110191.002521
10. Couble, A., Domingo, J., Miroy, K. & Villard, J. (1996). Species-specific identification of Candida krusei by hybridization with the CkF1,2 DNA probe. Journal of Clinical Microbiology, 34, No. 7, pp. 1726-1731. https://doi.org/10.1128/JCM.34.1726-1731.1996
11. Kurtzman, C.P., Smiley, M.J. & Johnson, C.J. (1980). Emendation of the genus Issatchenkia kudriavzevii and comparison of species by deoxyribonucleic acid reassociation, mating reaction, and ascospore ultrastructure. International Journal of Systematic Bacteriology, 30, No. 2, pp. 503-513. https://doi.org/10.1099/00207713-30-2-503
12. Douglass, A.P., Offei, B., Braun-Galleani, S., Coughlan, A.Y., Martos, A.A.R., Ortiz-Merino, R.A. & Wolfe, K.H. (2018). Population genomics shows no distinction between pathogenic Candida krusei and environmental Pichia kudriavzevii: one species, four names. PLOS Pathogens, 14, No. 7, e1007138. https://doi.org/10.1371/journal.ppat.1007138
13. Yamada, Y., Maeda, K. & Mikata, K. (1994). The phylogenetic relationships of the hat-shaped ascospore-forming, nitrate-assimilating Pichia species, formerly classified in the genus Hansenula Sydow et Sydow, based on the partial sequences of 18S and 26S ribosomal RNAs (Saccharomycetaceae): the proposals of three new genera, Ogataea, Kuraishia, and Nakazawaea. Bioscience, Biotechnology, and Biochemistry, 58, No. 7, pp. 1245-1257. https://doi.org/10.1271/bbb.58.1245
14. Ueda-Nishimura, K. & Mikata, K. (2001). Reclassification of Pichia scaptomyzae and Pichia galeiformis. Antonie Van Leeuwenhoek (Journal), 79, No. 3-4, pp. 371-375. https://doi.org/10.1023/a:1012045906098
15. Zheng, X., Li, K., Shi, X., Ni, Y., Li, B. & Zhuge, B. (2018). Potential characterization of yeasts isolated from Kazak artisanal cheese to produce flavoring compounds. Microbiology open, 7, No. 1, e00533. https://doi.org/10.1002/mbo3.533.1
16. Kurtzman, C.P. (2011). Pichia E.C. Hansen (1904), (Chap. 57). In (Eds. C.P. Kurtzman, J.W. Fell, T. Boekhout) The Yeasts (pp. 685-707), Elsevier. https://doi.org/10.1016/B978-0-444-52149-1.00057-4
17. Espejo, R.T. & Plaza, N. (2018). Multiple ribosomal RNA operons in bacteria; their concerted evolution and potential consequences on the rate of evolution of their 16S rRNA. Front. Microbiol., 9, 1232. https://doi.org/10.3389/fmicb.2018.01232
18. Liu, Y.J., Whelen, S. & Hall, B.D. (1999). Phylogenetic relationships among ascomycetes: evidence from an RNA polymerase II subunit. Molecular Biology and Evolution, 16, No. 12, pp. 1799-1808. https://doi.org/10.1093/oxfordjournals.molbe
19. Liu, Y.J., Hodson, M.C. & Hall, B.D. (2006). Loss of the flagellum happened only once in the fungal lineage: phylogenetic structure of Kingdom Fungi inferred from RNA polymerase II subunit genes. BMC Evolutionary Biology, 6, No. 1, p. 74. https://doi.org/10.1186/1471-2148-6-74
20. Hansen, K., LoBuglio, K.F. & Pfister, D.H. (2005). Evolutionary relationships of the cup-fungus genus Peziza and Pezizaceae inferred from multiple nuclear genes: RPB2, β-tubulin, and LSU rDNA. Molecular Phylogenetics and Evolution, 36, No. 1, pp. 1-23. https://doi.org/10.1016/j.ympev.2005.03.010
21. Kurtzman, C.P. (2011). Discussion of Teleomorphic and Anamorphic Ascomycetous Yeasts and Yeast-like Taxa (Chap. 13). In (Eds. C.P. Kurtzman, J.W. Fell, T. Boekhout) The Yeasts (pp. 293-207), Elsevier. https://doi.org/10.1016/B978-0-444-52149-1.00013-6

Received 09.11.2022

МОЛЕКУЛЯРНО-ГЕНЕТИЧНА ІДЕНТИФІКАЦІЯ ДРІЖДЖОВОГО ІЗОЛЯТУ MF22_1

М.О. Фоміна, О.Я. Філіпішена, Л.В. Поліщук

Інститут мікробіології і вірусології імені Д.К. Заболотного Національної академії наук України
03143 Київ, вул. Академіка Заболотного, 154
e-mail: M.Fomina@ukr.net

Мікроскопічні гриби, включно дріжджі, є важливою частиною мікробіоти людини. Серед них дріжджі роду *Candida* дуже поширені і часто становлять переважну частину мікобіоти. *Candida* spp., включно *Candida albicans* і види кандид non-albicans, можуть бути умовно-патогенними мікроорганізмами, що уражують людей із пригніченою імунною системою. Проблема з деякими видами кандид non-albicans, наприклад *C. glabrata* і *C. krusei*, полягає в тому, що вони є високорезистентними до протигрибкової терапії та, разом з тим, їх складно точно ідентифікувати на видовому рівні загальноприйнятими в клініці біохімічними методами. Метою цієї роботи була точна молекулярно-генетична ідентифікація за допомогою кількох генетичних маркерів дріжджового ізоляту MF22_1 з мокротиння людини, який за попередніми даними імовірно належав до *Candida* spp. Ми використали чотири фрагменти його хромосомної ДНК, секвеновані та депоновані нами в базі даних GenBank NCBI: 1) ITS (GenBank OM479428, 548 п.н.), 2) 28S рРНК (OM479513, 607 п.н.), 3) 18S рРНК (OM4794321, 737 п.н.) і 4) ген РНК-полімерази II — RPB2 (OM524388, 1217 п.н.). Частину бази даних колекції нуклеотидів [http://www.ncbi.nlm.nih.gov/nuccore/], що містить інформацію про нуклеотидну структуру ДНК грибів (таксид: 4751), було досліджено за допомогою BLASTN [http://blast.ncbi.nlm.nih.gov/Blast.cgi]. BLASTN аналіз показав, що всі чотири секвеновані генетичні маркери: ITS, 28S рРНК, 18S рРНК і RPB2 досліджуваного ізоляту MF22_1 мають максимальну схожість з відповідними послідовностями типових штамів виду *Pichia kudriavzevii*. Таким чином, було встановлено, що ізолят належить до виду *Pichia kudriavzevii*, анаморфою якого (або його неаскоспоровим станом) є *Candida krusei.* Цей всюдисущий у навколишньому середовищі вид є звичайним клінічним ізолятом, відповідальним за приблизно 2 % випадків дріжджових інфекцій, спричинених видами роду *Candida* у людей. Порівняльний аналіз первинної структури ділянки ITS продемонстрував велику подібність послідовності ITS *Pichia kudriavzevii* MF22_1 до більшості інших клінічних ізолятів *Pichia kudriavzevii* з мокротиння, калу, крові тощо, які зберігаються в різних колекціях культур установ, що спеціалізуються на медичних дослідженнях дріжджів, у всьому світі. Для цих клінічних ізолятів *Pichia kudriavzevii* не спостерігалося чіткої кореляції між послідовностями ITS і типом біоматеріалу, відібраного у людей.

Ключові слова: Candida spp., дріжджі, ідентифікація, ділянка ITS, 18S rRNA, 28S rRNA, RPB2.