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SHORT COMMUNICATION

# Biotechnological strains of *Komagataella* (*Pichia*) *pastoris* are *Komagataella phaffii* as determined from multigene sequence analysis

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**Abstract** *Pichia pastoris* was reassigned earlier to the genus *Komagataella* following phylogenetic analysis of gene sequences. Since that time, two additional species of *Komagataella* have been described, *K. pseudopastoris* and *K. phaffii*. Because these three species are unlikely to be resolved from the standard fermentation and growth tests used in yeast taxonomy, the identity of biotechnologically important strains of *K. pastoris* was determined from multigene sequence analyses. Results from this study show that the strain of '*Pichia pastoris*' commonly used in gene expression studies is actually *K. phaffii*.

**Keywords** *Pichia pastoris* · *Komagataella phaffii* · Methanol yeasts · Gene expression system

## Introduction

*Komagataella* (*Pichia*) *pastoris* has many biotechnological applications. Initially, *K. pastoris* was developed as a source of single-cell protein because the species can grow on either glucose or methanol, and high cell densities can be maintained under fermentation conditions [11]. Later, a gene expression system was developed to produce large quantities of medically and industrially important proteins [1, 2]. Consequently, two aspects of the species have contributed to its utility: (1) fermentation techniques were developed for maintaining extremely high cell densities in excess of 100 g/l dry weight, and (2) because *K. pastoris* assimilates methanol, the expression system is linked with alcohol oxidase, which is abundantly produced in the presence of methanol. The importance of this gene expression system is emphasized by its commercialization by the Invitrogen Corporation (Carlsbad, CA, USA) as the *Pichia pastoris* Expression Kit, which is widely used in molecular biology studies to express foreign genes [2].

Gene sequence analysis has shown that the genus *Pichia* is not monophyletic despite the phenotypic similarity of most species. The methanol-assimilating species *P. pastoris* and *Pichia angusta*, an obligate synonym of *Hansenula polymorpha*, were found to be distantly related to one another and to *Pichia membranifaciens*, the type species of the genus *Pichia*. Yamada et al. [12, 13] proposed the transfer of *P. pastoris* to the newly described genus *Komagataella*, and the transfer of *P. angusta* as *Ogataea polymorpha* to the newly described genus *Ogataea*. The reason for the change in species name was that prior usage of the taxonomic combination *P. polymorpha* required selection of a new species name when *H. polymorpha* was transferred to *Pichia*, and *P. angusta* was chosen because it was believed that *H. angusta* was an obligate synonym of *H. polymorpha* [4]. With the transfer of this species from *Pichia* to *Ogataea*, the new combination *O. polymorpha* could be validly used. The basis for the proposed new genera was from analysis of divergence in partial sequences of nuclear large subunit (LSU) ribosomal RNA (rRNA) and nuclear small subunit (SSU) rRNA. Because relatively few species were included in each of the analyses, it was unclear how closely related *P. pastoris* and *P. angusta* (*H. polymorpha*) might be to numerous unexamined species, and the proposals were not initially accepted [5]. However, analysis of domains D1/D2 of the LSU rRNA gene sequences for all currently known ascomycetous yeasts substantiated the separation of *Komagataella*, *Ogataea*, and *Pichia* [7], and

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the phylogenetic separation demonstrated from single-gene sequence analyses was supported from multigene sequence analyses [9]. Consequently, *Komagataella*, *Ogataea,* and *Pichia* represent separate, phylogenetically circumscribed genera.

Gene sequence analyses have also shown that *K. pastoris* is not the only member of the genus *Komagataella*. *Komagataella pseudopastoris*, initially described as *Pichia pseudopastoris* [3], was recognized from LSU and SSU rRNA gene-sequence divergence, and *K. phaffii* was recognized as distinct from *K. pastoris* and *K. pseudopastoris* from D1/D2 LSU rRNA gene-sequence divergence [6]. Although genetically separate, the three species of *Komagataella* are often not reliably resolved from one another by reactions on the standard fermentation and assimilation tests commonly used in yeast taxonomy.

The discovery of *K. pseudopastoris* and *K. phaffii* raised the question of which yeast is being used in studies of gene expression. It is believed that the strain used for this purpose is one of those cited in the US Patent of Wegner [11], and those strains were received by Eugene Wegner (C.P. Kurtzman, unpublished communication) from the US Agricultural Research Service (ARS) Culture Collection before it was recognized that the cultures maintained as *P. pastoris* also included *K. phaffii*. The two strains of *P. pastoris* cited in the patent were deposited in the ARS Patent Culture Collection as NRRL Y-11430 (strain 21-1) and NRRL Y-11431 (strain 21-2). Strain histories were not provided, which is typical for patent collection deposits. Consequently, the purpose of this work was to determine the identity of the *P. pastoris* strains commonly used in gene-expression research.

## Materials and methods

### Yeast strains compared

The species and strains compared are listed in Table 1 with their sources and GenBank accession numbers of the genes sequenced. The yeast from the *P. pastoris* Expression Kit was isolated on a streak plate of YM agar (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g glucose, 20 g agar, 1 l distilled water) and accessioned as NRRL Y-48124. All strains examined are available from the ARS Culture Collection (NRRL), National Center for Agricultural Utilization Research, Peoria, IL, USA.

### DNA preparation, gene sequencing and phylogenetic analysis

The methods used for DNA isolation and purification were reported earlier [7, 8]. DNA characterization was initiated by polymerase chain reaction (PCR) amplification of the gene regions of interest followed by sequencing reactions using the ABI Big Dye Terminator v3.0 Cycle Sequencing Kit. Sequences of both DNA strands were determined by capillary electrophoresis using an ABI 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA). Oligonucleotide primers for amplification and sequencing of

**Table 1** Strains of *Komagataella* species compared

| Species | Strain designation[a] | | Source of strain | GenBank accession numbers[b,c] | | | |
|---|---|---|---|---|---|---|---|
| | NRRL | CBS | | D1/D2 LSU | Mito SSU | EF-1α | RPB1 |
| *Komagataella pastoris* | Y-1603 | 704 | Type strain of *Pichia pastoris* | U75963 | EF547704 | EF552478 | GQ327955 |
| | Y-11431 | | US patent 4,414,329 | | | | |
| *Komagataella phaffii* | Y-7556 | 2612 | Type strain of *K. phaffii* | AF017407 | EF547706 | EF552480 | GQ327957 |
| | Y-11430 | | US patent 4,414,329 | | | | |
| | Y-48124 | | Invitrogen *Pichia pastoris* expression system | | | | |
| *Komagataella pseudopastoris* | Y-27603 | 9187 | Type strain of *Pichia pseudopastoris* | AF403149 | EF547705 | EF552479 | GQ327956 |
| *Ogataea glucozyma* | YB-2185 | 5766 | Type strain of *Pichia* (*Hansenula*) *glucozyma* | U75520 | EU018527 | EU014736 | GQ327959 |
| *Pichia membranifaciens* | Y-2026 | 107 | Type strain of *Pichia membranifaciens* | U75725 | EF547677 | EF552451 | GQ327958 |

[a] NRRL: Agriculture Research Service (ARS) culture collection, National Center for Agricultural Utilization Research, Peoria, IL, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands

[b] *D1/D2 LSU rRNA* gene-sequence accession numbers (GenBank) for domains 1 and 2 nuclear large subunit ribosomal RNA; *Mito SSU* mitochondrial small subunit rRNA; *EF-1α* translation elongation factor-1α; *RPB1* RNA polymerase 1

[c] Strains of the same species had identical sequences for all four genes

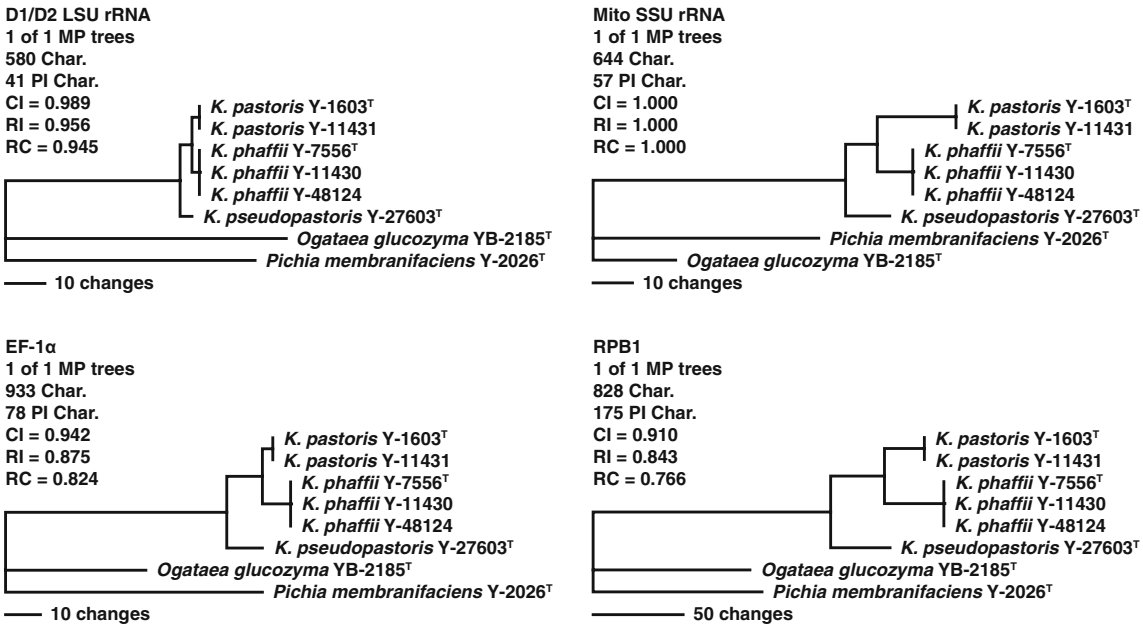


**Fig. 1** Phylogenetic analyses of species of *Komagataella* using gene sequences from domains 1 and 2 nuclear large subunit ribosomal RNA (D1/D2 LSU rRNA), mitochondrial small subunit (SSU) rRNA, translation elongation factor-1α (EF-1α), and RNA polymerase 1 (RPB1). Each gene analysis represents the single most parsimonious tree from maximum parsimony analysis. The number of parsimony informative (PI) characters for each gene sequence ranges from 7.1 to 21.1%. *Ogataea glucozyma* was designated as the outgroup species in the analyses. *CI* consistency index, *RI* retention index, *RC* rescaled consistency index, *T* type strain

genes for D1/D2 LSU rRNA, mitochondrial SSU rRNA, and translation elongation factor-1α (EF-1α) were reported earlier [7, 8]. Primers for amplification and sequencing of RNA polymerase 1 (RPB1) were the following: Forward, YRP1-6F, 5′-GCAYAARATGTCYATGATGG; YRP1-9F, 5′-TGGRTTCCWRAYTGGGAYGG; YRP1-11F, 5′-TCCAYGCYATGGSTGGTMGWGAAGG. Reverse, YRP1-10R, 5′-GCAATRGTATCACCAATACC; YRP1-12R, 5′-CGACGTTGAATWTAACCTGTTTC; YRP1-14R, 5′-CCMACCATTTCACCWGGRTGAAC. Phylogenetic analyses of the four gene sequences were determined with the maximum parsimony program included in PAUP*4.063a [10]. Sequences were visually aligned for analysis. *Ogataea glucozyma* was the designated outgroup species.

## Results and discussion

Phylogenetic analyses of sequences for the nuclear genes D1/D2 LSU rRNA, EF-1α, and RPB1 and for the mitochondrial SSU rRNA gene gave congruent species trees (Fig. 1). Differences in branch lengths for each of the gene trees suggest differences in nucleotide substitution rates, but species relationships are the same for each gene tree. *O. glucozyma* represented the genus *Ogataea* and *P. membranifaciens* represented the genus *Pichia* in the analysis.

There were no nucleotide differences between strains of the same species for each of the genes sequenced. Because histories were unavailable for the biotechnological strains, it is possible that they may be the type strains. Nonetheless, the analyses showed that NRRL Y-11431 is *K. pastoris*, whereas NRRL Y-11430 and NRRL Y-48124 are *K. phaffii*. From this analysis, culture deposits for US Patent 4,414,329 [11] included strains of *K. pastoris* (NRRL Y-11431) and *K. phaffii* (NRRL Y-11430), and the Invitrogen *P. pastoris* Expression Kit uses *K. phaffii* NRRL Y-48124. Assuming that NRRL Y-48124 was one of the strains listed in US Patent 4,414,329, then *K. phaffii* was apparently selected over *K. pastoris* by chance and used for developing the cloning system.

The preceding results do not diminish the importance and application of the *P. pastoris* gene expression system, but they do clarify which of the phenotypically cryptic *Komagataella* species has been used. These results reflect on a small scale the dramatic impact that gene sequence analyses are having on the recognition of yeast species and their assignment to phylogenetically circumscribed genera.

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