Komagataella phaffii Biotechnology

Subjects: Biotechnology & Applied Microbiology

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The need for a more sustainable society has prompted the development of bio-based processes to produce fuels, chemicals, and materials in substitution for fossil-based ones. In this context, microorganisms have been employed to convert renewable carbon sources into various products. The methylotrophic yeast *Komagataella phaffii* has been extensively used in the production of heterologous proteins. The obligate aerobic yeast *Komagataella phaffii* is a non-pathogenic certified and generally recognized as a safe (GRAS) microorganism. It is classified in the Saccharomycetales order and Saccharomycetaceae family.

Keywords: Komagataella phaffii ; Pichia pastoris ; strain engineering ; metabolic engineering

1. Introduction

The urgency for effective strategies to mitigate climate change is evident. Environmental issues caused by deliberate petroleum exploitation to produce chemicals, energy, and materials are a major concern regarding global warming. In this context, the global economy needs to be transformed faster, and the circular bioeconomy seems to be the right way to achieve it. This new form of economy aims to reuse, recycle, and remanufacture biomass (from agroecological systems, forestry, and urban wastes) to generate valuable products, such as fuels, biomaterials, and fine chemicals [1]. The circular bioeconomy benefits from integrated frameworks to utilize biomass and address its uses to human needs. One of the major areas that compose this framework is biotechnology, which has the potential to lead bioeconomy global advances even further [2]. The extensive range of roles that biotechnology plays alongside bioengineering provides new techniques to modify the DNA of several microorganisms and plants. Such modifications can improve the utilization of lignocellulosic biomass as raw material through the heterologous protein expression and the production of renewable chemicals, biofuels, and materials [3].

Lignocellulosic biomass polymeric carbohydrates, cellulose, and hemicellulose are mainly composed of glucose and xylose. Glucose is a C6 sugar easily consumed by most microorganisms and can be converted into an extensive range of different chemicals. Xylose, a C5 sugar, can be naturally consumed by filamentous fungi, yeasts, and bacteria. However, this pentose is more challenging to metabolize with the same efficiency as glucose. Nonetheless, xylose can also be converted into various chemicals, such as xylitol [4] and xylonic acid [5]. Recently, advances in genetic manipulation techniques have opened up the range of microorganisms that can produce fine chemicals from glucose and xylose with higher yields and productivity. The yeast *Komagataella phaffii* is one example of it.

Komagataella phaffii (previously *Pichia pastoris*) is a well-known methylotrophic yeast. It has many valuable features such as low nutritional requirement, the ability to reach high cell densities (even in acidic culture media), and is widely employed in biotechnological processes to produce heterologous proteins of commercial interest [6]. Therefore, its ability to use methanol as a carbon and energy source, its non-fermentative utilization of glucose under aerobic conditions, and its efficiency to grow on glycerol are some of the main reasons for the preferential choice of this yeast for bioprocess development [7]. These characteristics transformed *K. phaffii* into a suitable host for many industrial applications. More recently, this yeast has been called the “biotech yeast” for its broad utilization as a cell factory for synthetic biology and metabolic engineering aiming for valuable chemical compound productions (**Figure 1**) [7][8].

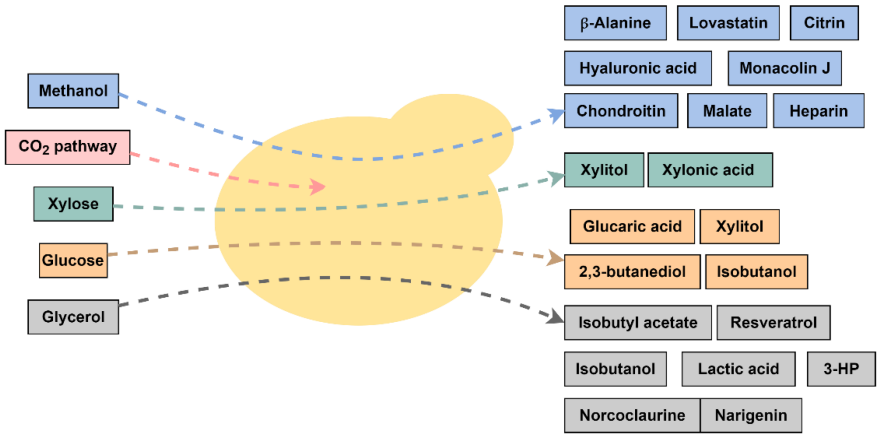

Figure 1. Representative scheme of the different carbon sources and chemicals produced by engineered *K. phaffii* strains. 3-HP = 3-hydroxy propionic acid.

K. phaffii has already been used as a successful host for heterologous protein production. However, optimizing *K. phaffii* to produce chemicals is still necessary, mainly aiming at methanol metabolism, fermentation parameters, and the construction of new genetic tools, such as promoters and plasmids. New methods, platforms, and strategies have been developed to enable more feasible ways to engineer this yeast to address these necessities [9]. Strategies to increase the efficiency rate of target integration genes in the *K. phaffii* genome through the knockout of homologous genes (*ku70* [10] and *Dnl4p* [11]), and also the utilization of CRISPR/Cas9 to facilitate the genome editing of *K. phaffii* [12], have already been used. Several examples of the available tools to enhance *K. phaffii* utilization as a good biotechnology host are summarized in [13].

2. *Komagataella* Taxonomy and Diversity

The obligate aerobic yeast *Komagataella phaffii* is a non-pathogenic certified and generally recognized as a safe (GRAS) microorganism. It is classified in the Saccharomycetales order and Saccharomycetaceae family. For being capable of using methanol as the only carbon source, *K. phaffii* is methylotrophic and was anteriorly known as *Pichia pastoris*. Phylogenetic studies placed *P. pastoris* in the genus *Komagataella* proposed by Yamada et al. (1995) after the analysis of partial sequences of rRNAs subunits (18S and 26S) of the 12 strains of methanol assimilating yeasts [14]. Supporting this previous study, Kurtzman and Robnett (1998) compared gene sequences (D1/D2 LSU rRNA) of about 500 species of Ascomycetous yeasts. The multigenic sequence analysis sustained the phylogenetic position of *Pichia pastoris* in the *Komagataella* genus [15].

The first species of *P. pastoris* (described initially as *Zygosaccharomyces pastoris*) isolated from a chestnut tree was described in 1919 by Guilliermond [16]. *Komagataella* genus is currently composed of seven species, with most of them (including *K. phaffii*) isolated from tree exudates in North America and Europe (**Table 1**) [17]. All species have spherical to ovoid shapes white/cream colonies, and during asexual reproduction, haploid cells multiply via multilateral budding and do not have pseudohyphae or true hyphae. In sexual reproduction (diploid cells), the ascospore is hat-shaped, ranging from 1 to 4 spores (that can be conjugated or not) [18]. The *Komagataella* species can grow at high cell densities using methanol, glucose, or glycerol as a carbon source with a doubling time of 2 to 3 h, and ammonium can be used as a nitrogen source during growth [17][18]. Recently, it was demonstrated that some species from *Komagataella*, including *K. phaffii,* possess the capacity to grow on xylose [19].

Table 1. *Komagataella* species diversity and its characteristics.

Type Species	Strain	Genome Size (Mb)	Isolation	Origin	Carbon Sources	Ref
K. pastoris	*CBS 704*	9.6	*Aesculus* species	France		ASM170810v1 [A]
K. phaffii	*CBS 7435*	9.4	*Quercus velutina*	California, USA		ASM170808v1 [A]
K. ulmi	*CBS 12361*	9.6	*Ulmus americana*	Illinois, USA	Glucose Glycerol Methanol Ethanol Xylose	[19]
K. kurtzmanii	CBS 12817	9.6	*Fir flux*	Arizona, USA		
K. mondaviorum	CBS 15017	9.5	*Populus deltoides*	California, USA		
K. pseudopastoris	CBS 9187	10.6	*Salix alba*	Hungary		
K. populi	CBS 12362	9.3	*Populus deltoides*	Illinois, USA		

[A]: NCBI identifier. Table based on [19].

Since all species have similar phenotypic and fermentative features, they cannot be distinguished using the conventional taxonomy, for example, morphological tests, that are usually used for yeasts. Therefore, gene sequence analysis (D1/D2 LSU rRNA, ITS, EF-1α) and other gene markers tracking are necessary for the correct species placement and identification [18][20]. Several genome sequencing and transcriptome studies have been conducted throughout the years [21][22][23]. A recent study sequenced the genome of all seven type species of *Komagataella* and its strains (a total of 25 isolates), identifying strains capable of growing using xylose as the sole carbon source, and which have a higher tolerance to stress conditions, such as alkaline pH [19].

The genomes of the two most studied species of the genus, *K. pastoris* and *K. phaffii* (previously both species were classified as *P. pastoris*), have close genome structures to *K. ulmi* and *K. kurtzmanii,* respectively. All species are capable of growing in glucose, glycerol, methanol, and ethanol. All 25 strains could grow at acidic pH (4.0) after 7 days, whereas at pH 9.0, all the species were strongly affected. Only the *K. pseudopastoris* (anteriorly known as *P. pseudopastoris*) and *K. kurtzmanii* CBS 12,817 strains were able to adapt and had better growth when compared to the other species [19]. Commonly, non-engineered yeasts in this genus do not present exponential growth on xylose; however, as indicated in the study of Heistinger et al. (2022), all seven species are capable of using this sugar and grow at slow rates. The species *K. pastoris* CBS 704 and *K. populi* CBS 12,362 showed the best growth on xylose [19].

Despite the initial studies on *Komagataella* diversity, the strains of *K. phaffii* X33 (prototrophic strain) and GS115 (HIS^- phenotype) are the ones most used in biotechnological applications, and are mainly used in studies of heterologous expression [20]. In recent years, there have been impressive methodological developments to model and analyze the metabolism of *K. phaffii* and engineer its genome and metabolic pathways, including improvements in genome modification with homologous recombination and target-guided gene cloning using CRISPR/Cas9 [24]. Indeed, the information about the genetic and physiological profile of this specie and the availability of genetic tools to manipulate it has been crucial for works on metabolic engineering and recombinant protein production studies.

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