

Position Statement of the ZKBS
on the suitability of established laboratory strains
of *Komagataella phaffii* as part of biological safety
measures according to § 8 para. 1 GenTSV

1 General Information

According to § 7 paragraph 5 GenTSV, new biological safety measures (here: vector and recipient systems) must be approved by the Central Committee on Biological Safety. The conditions for the use of a recipient organism as part of a biological safety measure are set out in § 8 Paragraph 1 of GenTSV. These are fulfilled if 1. a scientific description and a taxonomic classification of the recipient organism are available, 2. the propagation of the recipient organism is only possible under conditions that are rarely or not at all encountered outside genetic engineering facilities, 3. the recipient organism is not pathogenic for humans, animals or plants and does not have any environmentally hazardous properties and 4. the recipient organism only engages in minor horizontal gene exchange with other species.

This Position Statement examines and evaluates whether established laboratory strains of *Komagataella phaffii* fulfil the abovementioned conditions.

1.1 Scientific description

The species *Komagataella phaffii* (formerly *Pichia pastoris*) belongs to the *Phaffomycetaceae* family [1, 2]. This family includes budding yeasts that are classified as ascomycetes [3]. *K. phaffii* was first isolated from the exudate of a California black oak tree in Yosemite National Park in 1954. The species colonises the exudates of trees [4]. *K. phaffii* cells are methylotrophic, aerobic, and have an ellipsoidal or spheroidal shape [1]. Strains of the species are primarily haploid and reproduce vegetatively, but can also reproduce sexually under nitrogen-limited culture conditions [5].

Under nutrient-rich conditions, *K. phaffii* reproduces asexually by budding. *K. phaffii* cells are able to mate when nitrogen sources are limited and suitable carbon sources are present [5]. Sexual reproduction is homothallic, i. e. independent of the mating type (MAT). After induction of mating, cells can invert a 138 kb region on chromosome 4 and thus change their mating type (so-called mating-type switching) [6]. During mating, two cells of different mating types fuse to form a *MATa/MATα* diploid cell. This is followed by meiotic division, which results in four haploid ascospores [4]. The recombination rate during meiosis is about 3.5 times reduced

in *K. phaffii* than in *Saccharomyces cerevisiae*, with an average of 25 crossover events per meiosis [4].

The first *K. phaffii* isolates were stored in the culture collection of the University of California from the 1960s onwards. All *K. phaffii* strains used in basic research and biotechnology can be traced back to one strain (NRRL Y-11430; synonym CBS 7435), which is derived from one of the strains isolated from this strain collection in 1954 [7]. Due to the methylotrophic property of the yeast, the Philips Petroleum Company patented this strain in 1979. The structure of the strain's mannan cell wall is altered compared to wild-type strains because of a *HOC1* open reading frame truncation. This improves transformability and protein secretion [7]. Original plans involved the use of this yeast as a high-protein animal feed. However, rising methanol prices in the wake of the 1970s oil crisis made production uncompetitive compared to other high-protein animal feeds such as soy. In the following years, the strain was used as a platform for protein expression and the patents were transferred to other licensees in 1993 [8]. Since 2023, a licence-free *K. phaffii* strain (NCYC 2543) has been made available as an expression system. This strain is also derived from the strain collection of the University of California and its genome sequence is practically identical to that of NRRL Y-11430 [7]. *K. phaffii* is primarily used as a protein expression platform in basic research and biotechnology [9–11]. The spontaneous and introduced mutations of *K. phaffii* strains are diverse and well characterised [7, 8]. The genomes of various laboratory strains of *K. phaffii* have been fully sequenced [7, 12]. The two aforementioned strains (NRRL Y-11430 and NCYC 2543) and their derivatives are hereinafter referred to as established laboratory strains of *K. phaffii*.

The established laboratory strains of *K. phaffii* are scientifically very well characterised model organisms with a taxonomically defined classification.

1.2 Pathogenic potential of established laboratory strains of *Komagataella phaffii*

There are no reports of *K. phaffii* infections in humans, animals or plants. The *K. phaffii* genome does not contain any virulence factors found in other pathogenic yeasts [12–14]. Pathogenic yeasts often possess the ability to undergo pseudohyphal growth. *K. phaffii* possesses the FLO gene clusters that enable this phenotype, but the activation of these genes and the expression of the phenotype only occurs at low growth rates, high pH and nitrogen and carbon limitation [15, 16].

The pathogenic potential of established laboratory strains of this yeast was investigated in several animal models. In a mouse model, intravenous injection of 1×10^6 colony forming units (CFU) of *K. phaffii* did not cause pathological changes in various types of tissue [17]. The yeast cells were cleared from the body within 144 hours post injection. The mice remained healthy and showed no abnormalities in food intake or body weight. As a control, mice were administered the same amount of a pathogenic *Candida albicans* strain. Mice infected with *C. albicans* suffered substantial weight loss and died within six days. In a separate study the oral administration of 1×10^7 CFU of *K. phaffii* was investigated in a mouse model [18]. The study showed that only a small number of *K. phaffii* cells could be detected in the gastrointestinal tract after 72 hours. No pathological effects or behavioural changes were observed in the test animals. Studies in broilers showed no adverse effects or behavioural changes when 1×10^6 to 10^7 CFU *K. phaffii* per g of dry feed was administered over a period of several weeks [19, 20]. Similarly, feeding 1×10^7 CFU *K. phaffii* per g of dry feed to quails for several weeks did not

affect the health of the animals [21]. Keeping brine shrimp in aquarium water with a concentration of 10^7 CFU *K. phaffii* per ml did not have any negative effects on the shrimp [22].

In conclusion, established laboratory strains of *K. phaffii* are non-pathogenic and pose no risk to humans, animals or plants.

1.3 Propagation ability of established laboratory strains of *Komagataella phaffii* outside of genetic engineering facilities

When non-sterile river water samples were inoculated with NRRL Y-11430-derived *K. phaffii* strains, the cell count of 5×10^6 CFU per ml was reduced by four logs within 20 days [23]. After 70 days in non-sterile river water samples, only a few *K. phaffii* cells (reduction by six logs) survived [23]. In suspensions of non-sterile soil samples (arable soil) in non-sterile river water, the cell count of the NRRL Y-11430-derived *K. phaffii* strains decreased by three logs to a maximum of 10^2 CFU per ml soil suspension within twelve days, and by six logs within 30 or 70 days (strain dependent) [23]. In both experiments, no stagnation of the cell number reduction could be observed. It can therefore be assumed that no single subpopulation of the yeast survived in the soil and water samples.

The *Saccharomyces cerevisiae* strain S288c, which is recognised as part of biological safety measures, was used as a control [23]. The cell count of the *S. cerevisiae* S288c strain in the river water samples was reduced from 1×10^6 CFU per ml by six logs after 70 days. In the soil samples, the cell count fell by three log units within twelve days. Due to the lack of a sufficiently selective medium over the natural germs in the environmental samples, no further data could be collected at later time points.

These data show that established laboratory strains of *K. phaffii* are only able to survive in soil and water for a relatively short time. Long-term establishment in the environment is not to be expected.

1.4 Gene transfer from *Komagataella phaffii* to other organisms

In contrast to various bacterial species, fungi are generally not able to actively transfer DNA via conjugation and transformation [24–26]. There is also no evidence for a natural DNA uptake capacity or other mechanisms of horizontal gene transfer in *K. phaffii*.

In genetic engineering operations, DNA only enters *K. phaffii* cells under conditions that make the cell wall and cell membrane permeable, e.g. by using physical (electroporation) or chemical methods (enzymatically mediated spheroplast generation, polyethylene glycol/ Ca^{2+} shock, LiCl) [27].

In addition to the uptake of DNA during the course of artificial transformation, yeasts are able to inherit genetic material through sexual reproduction. Crossbreeding within the *K. phaffii* species only occurs under specific culture conditions and is not detectable in natural environments, such as in river water samples [23]. Furthermore, *K. phaffii* strains can produce viable hybrids with related species of the genus *Komagataella* [28]. Species of the genus *Komagataella* are found in the exudate of European and American trees and in decaying wood [28]. The less closely related the species are, the less likely it is that hybrid cells will develop during crossing.

Overall, gene transfer in *K. phaffii* is only possible to a limited extent and is restricted to closely related species.

2 Recommendation

According to § 8 paragraph 1 GenTSV, established laboratory strains of *Komagataella phaffii* (NRRL Y-11430 and NCYC 2543, including derivatives) are recognised as part of a biological safety measure.

3 Reasoning

Established laboratory strains of the species *K. phaffii* meet the requirements of § 8 para. 1 GenTSV for recognition as recipient organisms for biological safety measures. They are both scientifically well-described and apathogenic for humans, animals and plants. The survival of established laboratory strains of *K. phaffii* outside of genetic engineering facilities has been well-studied and it has been shown that the yeasts are viable in soil and water for only short periods of time. These yeasts therefore do not pose a threat to the legal interests according to § 1 para. 1 GenTG. Horizontal gene transfer from *K. phaffii* to other microorganisms is not expected.

Information on whether individual laboratory strains of *K. phaffii* are suitable as recipients for biological safety measures in accordance with the criteria set out in this Position Statement will be collected and made available in the [database of recipient strains for biological safety measures](#), which is maintained by the ZKBS administrative office.

4 Literature

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