ZKBS
The Central Committee on Biological Safety

Az. 45270_e December 2021

Position Statement of the ZKBS on the suitability of haploid laboratory strains of *Saccharomyces cerevisiae* as part of biological safety measures according to § 8 para. 1 GenTSV

1. General Information

With the entry into force of the amendment to the Genetic Engineering Safety Regulation (GenTSV) in March 2021, it is necessary that, in accordance with § 7 para. 5 GenTSV, the continued existence of already recognised biological safety measures (here: vector and recipient systems) is confirmed by the Central Committee on Biological Safety. Section 8, paragraph 1 of the amended GenTSV specifies the conditions under which the use of a recipient organism can be recognised as part of a biological safety measure. 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 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 haploid laboratory strains of *Saccharomyces cerevisiae* fulfil the abovementioned conditions.

Haploid laboratory strains of *S. cerevisiae* were already recognised as suitable recipient organisms for biological safety measures in the "Guidelines for protection against hazards from *in vitro* recombinant nucleic acids" in force since 1978 (most recently in the 5th revised version of 1986). This was also continued in the Genetic Engineering Act of 1990. In the decades of widespread use of haploid *S. cerevisiae* laboratory strains as biological safety measures, they have proven to be safe without exception.

1.1. Scientific description

The species *S. cerevisiae* belongs to the *Saccharomycetaceae* family. The family includes yeasts that reproduce by budding and belongs to the Ascomycetes. *S. cerevisiae* is distributed worldwide and occurs in a wide range of habitats. *S. cerevisiae* cells are facultatively aerobic and have an ellipsoidal or spheroid shape depending on the number of chromosome sets. *S. cerevisiae* can reproduce both by sexual and asexual reproduction [1]. The majority of all

wild and domesticated *S. cerevisiae* strains reproduce vegetatively and have a diploid chromosome set [2, 3].

Under nutrient-rich conditions, *S. cerevisiae* reproduces asexually by budding. In this process, the mother cell initially produces a small outgrowth that continuously enlarges. The outgrowth is cut off as a bud after a nucleus has migrated into the outgrowth. In the absence of nitrogen and fermentable carbon sources, diploid *S. cerevisiae* cells that contain both MATa and MATα mating types sporulate. This results in meiotic division, producing four haploid ascospores. These spores are more resistant to chemical and physical environmental influences than vegetative cells [4, 5]. When the spores encounter sufficient nutrients, they germinate, whereupon two haploid cells of the opposite mating type can fuse to form a diploid cell. The mother cell itself can switch mating type after division and mate with the daughter cell. This reproductive mechanism is called homothally. The switching of the mating type is initiated by the homothallic switching endonuclease, which inserts a new a or α gene into the MAT gene locus via recombination [6]. If the cell is unable to switch mating types, haploid cells will continue to divide mitotically until they encounter a germinated spore of the opposite mating type. This reproductive mechanism is called heterothally. Most *S. cerevisiae* wild isolates are homothallic [7].

Since the 1930s, *S. cerevisiae* strains have been the subject of genetic studies. For this purpose, the strains were cultivated on agar plates and stab cultures and, since the 1950s, also cryopreserved [8]. Early advances in yeast genetics were made with haploid laboratory strains. Haploid strains are particularly suitable for this purpose, as genetic analyses are possible regardless of the dominance of an allele. A large proportion of the haploid laboratory strains of *S. cerevisiae* are derived from the heterothallic strain S288c [9, 10]. The strain was bred in the 1960s by Robert Mortimer for genetic and molecular biology studies [10, 11]. The spontaneous and introduced mutations of haploid *S. cerevisiae* laboratory strains are diverse and well characterised, making the strains versatile in basic research and biotechnology. Auxotrophs, for example, contribute to reducing the survivability of yeasts in the environment. The genomes of various haploid laboratory strains of *S. cerevisiae* have been completely sequenced [12–14].

Haploid laboratory strains of *S. cerevisiae* are scientifically very well characterised model organisms with a taxonomically clear classification.

1.2. Pathogenic potential of haploid laboratory strains of *S. cerevisiae*

Only a few *S. cerevisiae* strains have been described as human pathogens. They are responsible for 1 - 4 % of all severe fungal infections in humans [15]. These strains are associated with inflammation of the skin and mucous membranes in immunocompetent patients and systemic infections of the bloodstream in immunocompromised patients. Most clinical strains are characterised by four virulence factors. They grow at temperatures above 37 °C, are capable of forming pseudohyphae, can attach to epithelial cells and proliferate *in vivo* [16].

In haploid laboratory strains of *S. cerevisiae*, these characteristics are not manifested. Most laboratory strains have little or no ability to grow at temperatures above 37 °C [17, 18]. Due to mutations in the FL08 gene, strain S288c and its derived strains are unable to form pseudohyphae [19]. In contrast to clinical isolates, haploid laboratory strains are unable to

attach to epithelial cells [20]. In the mouse model, S288c-derived diploid laboratory strains are unable to colonise tissues and organs upon intravenous injection of 2 x 10^7 colony-forming units [21]. Whereas clinical isolates are able to colonise the brain, pancreas, liver, kidney and lungs of animals. The haploid laboratory strain S288c as well as commercial *S. cerevisiae* strains are used as probiotics in piglets without any harmful effects on the animals [22, 23].

Some *S. cerevisiae* strains are associated with phytopathogenicity. Thus, a damaging effect of pseudohyphae-forming isolates on grapevines has been described [24]. Haploid laboratory strains are apathogenic for plants, as they are mostly unable to form pseudohyphae [25].

Haploid laboratory strains of *S. cerevisiae* are apathogenic and thus pose no risk to humans, animals or plants.

1.3. Multiplication ability of haploid laboratory strains of *S. cerevisiae* outside genetic engineering facilities

Multiple studies with soil and water samples show that haploid laboratory strains of *S. cerevisiae* cannot survive in the environment. For example, haploid laboratory strains and commercial baker's yeast strains do not survive longer than 20 days in non-sterile suspended soil samples [26, 27]. When culture broth is added to suspended soil samples, haploid laboratory strains are overgrown by the competing microflora and are no longer detectable after less than 20 days [26, 28]. In the case of release in wastewater, haploid laboratory strains are no longer detectable after 20 days [26].

These data show that haploid laboratory strains of *S. cerevisiae* are only able to survive in soil and water for a short time. Permanent establishment in the environment does not occur.

1.4. Horizontal gene transfer from haploid laboratory strains of *S. cerevisiae* to other organisms

Horizontal gene transfer in fungi can occur through processes such as transformation and sexual inheritance of genetic material, the molecular processes of which have been extensively studied [29–31].

In contrast to various bacterial species, most fungi are not able to actively take up DNA from the environment and thus do not exhibit any natural competence for transformation with free DNA. For *S. cerevisiae*, however, evidence is available that points to a natural competence. For instance, yeasts become competent to take up DNA during the stationary growth phase in the presence of sugars and the absence of other nutrients [32]. Furthermore, *S. cerevisiae* can take up DNA after switching from an isotonic to a hypotonic culture media [33].

In genetic engineering operations, DNA enters *S. cerevisiae* cells under conditions that make the cell wall and cell membrane permeable to it, e.g. by using physical (electroporation) or chemical (polyethylene glycol/Ca^{2+} shock) methods.

In addition to the uptake of DNA by means of transformation, yeasts are able to pass on genetic material via sexual reproduction. Thus, heterothallic haploid *S. cerevisiae* strains can produce viable hybrids with related species of the *Saccharomyces* genus. Hybrid cells are less likely to be produced during mating the less closely related the species are to each other. The offspring of closely related parental species usually carry the genetic material of both parents, whereas

in distantly related parental species the offspring carry the genetic material of only one parental species and fragments of the genetic material of the other parent [34].

In summary, horizontal gene transfer by haploid laboratory strains of *S. cerevisiae* is only possible to a small extent and is limited to closely related species.

2. Recommendation

According to Section 8 para. 1 GenTSV, haploid laboratory strains of *S. cerevisiae* are recognised as part of a biological safety measure.

3. Reasoning

Haploid laboratory strains of *S. cerevisiae* fulfil the requirements of § 8 para. 1 GenTSV for recognition as recipient organisms for biological safety measures. They are scientifically very well described and apathogenic for humans, animals and plants. The survival of haploid laboratory strains outside genetic engineering facilities has been well studied and it has been shown that the yeasts are only able to survive for short periods in soil and water. These yeasts therefore do not pose a risk to the legal interests as defined in § 1 Para. 1 GenTG. Horizontal gene transfer from *S. cerevisiae* to other microorganisms is generally very low and limited to close relatives of the same genus.

Haploid strains that are not derived from established laboratory strains are usually not sufficiently characterised scientifically and with regard to their pathogenic potential for humans, animals and plants to be suitable as recipient organisms for biological safety measures.

Information on whether individual haploid laboratory strains of *S. cerevisiae* are suitable as recipient strains for biological safety measures according to the criteria laid down in this Position Statement is collected and made available in the database of recipient strains for biological safety measures maintained by the ZKBS administrative office.

Literature

1. **Sherman F** (2002). Getting started with yeast. *Methods Enzymol* **350**:3–41.
2. **Cubillos FA, Vásquez C, Faugeron S, Ganga A, Martínez C** (2009). Self-fertilization is the main sexual reproduction mechanism in native wine yeast populations. *FEMS Microbiol Ecol* **67**(1):162–70. doi:10.1111/j.1574-6941.2008.00600.x.
3. **Tsai IJ, Bensasson D, Burt A, Koufopanou V** (2008). Population genomics of the wild yeast *Saccharomyces paradoxus*: Quantifying the life cycle. *Proc Natl Acad Sci U S A* **105**(12):4957–62. doi:10.1073/pnas.0707314105.
4. **Knight SJ, Goddard MR** (2016). Sporulation in soil as an overwinter survival strategy in *Saccharomyces cerevisiae*. *FEMS Yeast Res* **16**(1):1–8. doi:10.1093/femsyr/fov102.
5. **Neiman AM** (2011). Sporulation in the Budding Yeast *Saccharomyces cerevisiae*. *Genetics* **189**(3):737–65. doi:10.1534/genetics.111.127126.
6. **Kostriken R, Strathern JN, Klar AJ, Hicks JB, Heffron F** (1983). A site-specific endonuclease essential for mating-type switching in *Saccharomyces cerevisiae*. *Cell* **35**(1):167–74. doi:10.1016/0092-8674(83)90219-2.
7. **Katz Ezov TAL, Chang S-L, Frenkel Z'E, Segrè AV, Bahalul M, Murray AW, Leu J-Y, Korol A, Kashi Y** (2010). Heterothallism in *Saccharomyces cerevisiae* isolates from nature: effect of HO locus on the mode of reproduction. *Mol Ecol* **19**(1):121–31. doi:10.1111/j.1365-294X.2009.04436.x.

8. **Lindegren CC** (1949). The yeast cell. Its Genetics and Cytology., 1st ed.. Educational Publishers, Inc, St. Louis
9. **Louis EJ** (2016). Historical Evolution of Laboratory Strains of *Saccharomyces cerevisiae*. *Cold Spring Harb Protoc* **2016**(7):585 - 593. doi:10.1101/pdb.top077750.
10. **Mortimer RK, Johnston JR** (1986). Genealogy of principal strains of the yeast genetic stock center. *Genetics* **113**(1):35–43. doi:10.1093/genetics/113.1.35.
11. **Mortimer RK, Lerner RS, Barr JK** (1957). Ultraviolet-induced biochemical mutants of *Saccharomyces cerevisiae*. *UCRL US At Energy Comm* **1957**(3746):2–10.
12. **Goffeau A, Barrell BG, Bussey H, Davis RW, Dujon B, Feldmann H, Galibert F, Hoheisel JD, Jacq C, Johnston M, Louis EJ, Mewes HW, Murakami Y, Philippsen P, Tettelin H, Oliver SG** (1996). Life with 6000 Genes. *Science* **274**(5287):546–67. doi:10.1126/science.274.5287.546.
13. **Araya CL, Payen C, Dunham MJ, Fields S** (2010). Whole-genome sequencing of a laboratory-evolved yeast strain. *BMC Genomics* **11**(1):88. doi:10.1186/1471-2164-11-88.
14. **Matheson K, Parsons L, Gammie A** (2017). Whole-Genome Sequence and Variant Analysis of W303, a Widely-Used Strain of *Saccharomyces cerevisiae*. *G3 (Bethesda)* **7**(7):2219–26. doi:10.1534/g3.117.040022.
15. **Raghavan V, Aquadro CF, Alani E** (2019). Baker's Yeast Clinical Isolates Provide a Model for How Pathogenic Yeasts Adapt to Stress. *Trends Genet* **35**(11):804–17. doi:10.1016/j.tig.2019.08.002.
16. **McCusker JH** (2006). *Saccharomyces cerevisiae:* an Emerging and Model Pathogenic Fungus. *In* Heitman J (ed), Molecular principles of fungal pathogenesis. ASM Press, Washington, D.C.
17. **Steinmetz LM, Sinha H, Richards DR, Spiegelman JI, Oefner PJ, McCusker JH, Davis RW** (2002). Dissecting the architecture of a quantitative trait locus in yeast. *Nature* **416**(6878):326–30. doi:10.1038/416326a.
18. **McCusker JH, Clemons KV, Stevens DA, Davis RW** (1994). *Saccharomyces cerevisiae* virulence phenotype as determined with CD-1 mice is associated with the ability to grow at 42 degrees C and form pseudohyphae. *Infect Immun* **62**(12):5447–55. doi:10.1128/iai.62.12.5447-5455.1994.
19. **Liu H, Styles CA, Fink GR** (1996). *Saccharomyces cerevisiae* S288C Has a Mutation in FL08, a Gene Required for Filamentous Growth. *Genetics* **144**(3):967–78.
20. **Murphy AR, Kavanagh KA** (2001). Adherence of clinical isolates of *Saccharomyces cerevisiae* to buccal epithelial cells. *Med Mycol* **39**(1):123–7. doi:10.1080/mmy.39.1.123.127.
21. **Clemons KV, McCusker JH, Davis RW, Stevens DA** (1994). Comparative pathogenesis of clinical and nonclinical isolates of *Saccharomyces cerevisiae*. *J Infect Dis* **169**(4):859–67. doi:10.1093/infdis/169.4.859.
22. **Zhaxi Y, Meng X, Wang W, Wang L, He Z, Zhang X, Pu W** (2020). Duan-Nai-An, A Yeast Probiotic, Improves Intestinal Mucosa Integrity and Immune Function in Weaned Piglets. *Sci Rep* **10**(1):4556. doi:10.1038/s41598-020-61279-6.
23. **Trckova M, Faldyna M, Alexa P, Zajacova ZS, Gopfert E, Kumprechtova D, Auclair E, D'Inca R** (2014). The effects of live yeast *Saccharomyces cerevisiae* on postweaning diarrhea, immune response, and growth performance in weaned piglets. *J Anim Sci* **92**(2):767–74. doi:10.2527/jas.2013-6793.
24. **Gognies S, Belarbi A, Ait Barka E** (2001). *Saccharomyces cerevisiae*, a potential pathogen towards grapevine, Vitis vinifera. *FEMS Microbiol Ecol* **37**(2):143–50. doi:10.1111/j.1574-6941.2001.tb00862.x.
25. **Gognies S, Barka EA, Gainvors-Claisse A, Belarbi A** (2006). Interactions Between Yeasts and Grapevines: Filamentous Growth, Endopolygalacturonase and Phytopathogenicity of Colonizing Yeasts. *Microb Ecol* **51**(1):109–16. doi:10.1007/s00248-005-0098-y.
26. **Fujimura H, Sakuma Y, Amann E** (1994). Survival of genetically-engineered and wild-type strains of the yeast *Saccharomyces cerevisiae* under simulated environmental conditions: a contribution on risk assessment. *J Appl Bacteriol* **77**(6):689–93.
27. **Ando A, Suzuki C, Shima J** (2005). Survival of Genetically Modified and Self-Cloned Strains of Commercial Baker's Yeast in Simulated Natural Environments: Environmental Risk Assessment. *Appl Environ Microbiol* **71**(11):7075–82. doi:10.1128/AEM.71.11.7075-7082.2005.
28. **Bröker M** (1990). A study on the survival of wild-type, laboratory and recombinant strains of the baker yeast *Saccharomyces cerevisiae* under sterile and nonsterile conditions. *Zentralbl Hyg Umweltmed* **190**(5-6):547–57.

29. **Fitzpatrick DA** (2012). Horizontal gene transfer in fungi. *FEMS Microbiol Lett* **329**(1):1–8. doi:10.1111/j.1574-6968.2011.02465.x.
30. **Mitrikeski PT** (2015). Pathways and Mechanisms of Yeast Competence: A New Frontier of Yeast Genetics . pp. 223–37. *In* Van den Berg, Marco A, Maruthachalam K (eds), Genetic Transformation Systems in Fungi, Volume 2. Springer
31. **Mitrikeski PT** (2013). Yeast competence for exogenous DNA uptake: towards understanding its genetic component. *Antonie Van Leeuwenhoek* **103**(6):1181–207. doi:10.1007/s10482-013-9905-5.
32. **Nevoigt E, Fassbender A, Stahl U** (2000). Cells of the yeast *Saccharomyces cerevisiae* are transformable by DNA under non-artificial conditions. *Yeast* **16**(12):1107–10. doi:10.1002/1097-0061(20000915)16:12<1107:AID-YEA608>3.0.CO;2-3.
33. **Neukamm B, Stahl U, Lang C** (2002). Endocytosis is involved in DNA uptake in yeast. *Biochim Biophys Acta* **1572**(1):67–76. doi:10.1016/s0304-4165(02)00279-9.
34. **Marinoni G, Manuel M, Sulo P, Petersen RF, Piskur J** (1999). Horizontal Transfer of Genetic Material among *Saccharomyces* Yeasts. *J Bacteriol* **181**(20):6488–96. doi:10.1128/JB.181.20.6488-6496.1999.