ESTABLISHING COLLECTION OF PETITE MUTANTS OF YEAST *SACCHAROMYCES CEREVISIAE* AND *S. PARADOXUS* FOR MODELING PLANT-FUNGI INTERACTIONS

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Introduction

Yield losses due to damage to agricultural plants by pathogenic fungi are a problem of modern agriculture, which makes the study of the biology of fungal infection process an important task of modern science. The ability of the yeast *S. cerevisiae* and some closely related species to survive with significant damage to mitochondrial DNA (petit mutation) provides us with the opportunity to study the state of fungal cells that have been treated with fungicides that inhibit mitochondrial respiration of pathogenic fungi.

Methods

To obtain rho^0 mutants, natural, laboratory and industrial strains of *S. cerevisiae* and strains of the closely related yeast species *S. paradoxus* were used, including those isolated from plant samples from the Chernobyl Exclusion Zone (fig.1).

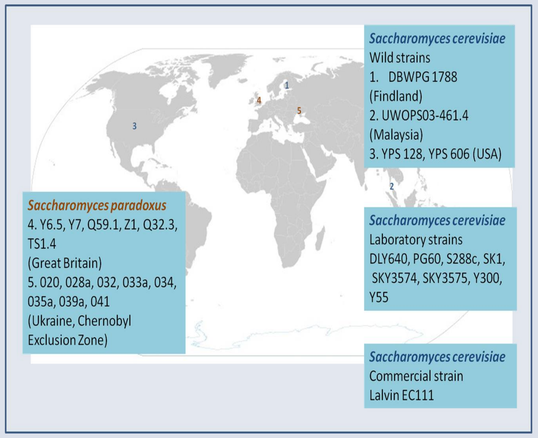

Fig. 1. Origins of yeast strains used for petite mutants isolations.

Petite mutants were isolated by double treatment of cells of the parent strain with ethidium bromide. Selection of petit mutants was carried out by loss of ability to grow on YPG medium containing a non-fermented carbon source glycerol. The loss of the mitochondrial genome was determined by the disappearance of mitochondrial nucleoids when analyzing DAPI-stained yeast cells using luminescent microscopy (Lumam-4 microscope).

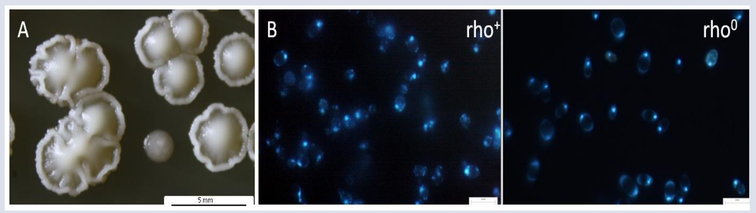

Fig. 2. A) *S.cerevisiae* strain SK1, petite mutant marked by arrow, 7day, YPD medium. B) Yeast cell with (*rho*$^+$) and without mitochondrial DNA (*rho*^{0}). Luminescence microscopy, DAPI staining.

Results

The impact of mitochondrial DNA loss on the formation of traits characteristic to infectious stages of fungal growth was assessed. Among them are the formation of pseudohyphal growth, the ability to form complex colonies and biofilms, cell flocculation and their invasion into the nutrient medium.

It was found that petite mutants lose the ability to form biofilms (mats) (fig.3). The formation of complex colonies and invasion into the nutrient medium was inhibited (fig.4).

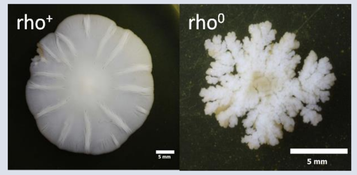

Fig. 3. Loss of ability to form biofilms in petite mutants, SK1 and SK1p, 14 days, YPD 0,3% a
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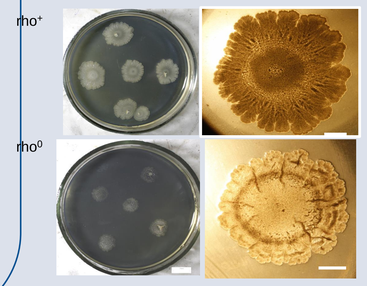

Fig. 4. Inhibition of media invasion in petite mutants, SK1 and SK1p, 14 days, YPD

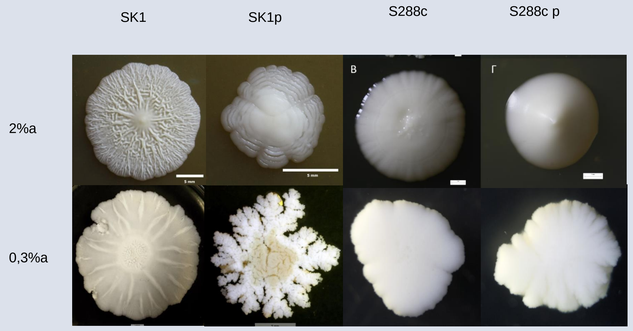

Fig. 4. Differences in manifestation of petite mutation between SK1 and S288c strains, 14 days, YPD

The results obtained allowed us to identify the SK1 strain of yeast *S. cerevisiae* as promising for modeling the dimorphic transition characteristic to infectious stages of some pathogenic fungi as well as the impact of mitochondrial DNA damage and the resulting respiratory disorder in mitochondria on this process.

Laboratory strains and their petits were used to study the influence of mitochondrial DNA status on the formation of adaptive regrowth (appearance of adapted cell subpopulations under stress conditions). After prolonged cultivation on YPD, YP0,1D and YPD+G media, adaptive regrowth on petit mutant colonies was hardly recorded, unlike on parent strains colonies (fig. 5). Our data indicate that the loss of mitochondrial DNA leads to a significant decrease in the adaptive response of yeast cells to changing living conditions by forming adaptive regrowth.

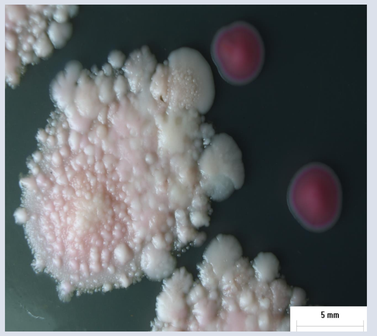

Fig. 5. Abundant adaptive regrowth on the surface of the *rho*$^+$ and its complete inhibition on *rho*^{0} yeast colony (*S.paradoxus* 032, YPD+G medium, 65days).

Conclusions

Thus, we maintain and expand the collection of petit mutants of yeasts *S.cerevisiae* and *S.paradoxus*, which includes natural, laboratory and commercial strains. The collection will be utilized to model the impact of mitochondrial inhibition in phytopathogenic fungi on their interaction with plant tissues.