Review article

Life cycle and inheritance of the red yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*)

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Historical aspects

Phaffia rhodozyma was isolated in Japan in 1976 by Miller and co-workers (1). The authors isolated ten similar strains from exudates of broad-leafed trees. These yeasts produced carotenoid pigments, reproduced vegetatively by budding and fermented several sugars. All the isolates belonged to one species (2), and represented a new genus, that was named after the yeast taxonomist and ecologist Herman Phaff. The yeast lacked, as far as they could determine, a sexual life cycle.

Morphology

Vegetative cells reproduce by budding, are ellipsoidal and occur singly, in pairs and occasionally in short chains. The manner of bud formation is characteristic for heterobasidiomycetes yeasts (3, 4). The ultrastructure also refers to a basidiomycete origin: the vegetative cell wall is multilayered and ruptured forming a "collar" where the new bud emerges (1). The cell wall polysaccharides contain mainly β-(1→3) and β-(1→6) glucan and also a α-(1→3) glucan and none or a small amount of chitin (5, 6). The cells are surrounded by a capsule of acidic polysaccharides containing D-xylose.

Organisation of the cytoskeleton during the cell cycle

Budding associated changes in microtubules and actin studied by fluorescence microscopy revealed that the dynamics of microtubule arrangement is more similar to that of fission yeasts or animal cells than to *S. cerevisiae* (7). Interphase cells had a centrally positioned nucleus and bundles of cytoplasmic microtubules, which were not connected to the nucleus. During mitosis the whole nucleus moved into the bud, and divided there. Subsequently cytoplasmic microtubules disappeared and were replaced by a spindle. The nucleus elongated and the spindle poles moved apart separating the daughter nuclei while still in the bud. One of them moved back to the mother cell. At the end of telo phase and during cytokinesis, the spindle dissociated and cytoplasmic microtubules reappeared in the mother and daughter cells.

In non-budding cells only actin patches and no actin microfilaments could be visualized. They were regularly scattered all over the cytoplasm except for the nucleus area. In budding cells actin patches were present in both the bud and the mother cell.

Organization of the genome

Pulse-field gel electrophoresis is a valuable tool for studying the organization of yeast genomes. Electrophoretic karyotypes determined by OFAGE and CHEF techniques for *P. rhodozyma* isolates revealed considerable chromosomal length polymorphism. The numbers and sizes of the chromosomal bands varied between 7 to 12 (8) or 9-17 (9), ranging in size from 0.83 Mb to 3.5 Mb or 0.48 - to 3.1 Mb respectively in the strains studied.

Currently little is known about the structural and functional organization of *P. rhodozyma* genome in general. The first two genes (coding for actin and glyceraldehyde-3-phosphate-dehydrogenase) were isolated recently (10, 11) and two papers related to transformation have been published (12, 13).

Extrachromosomal genetic elements

Genetic elements of *P. rhodozyma* other than the nuclear chromosomes are mitochondria, DNA plasmids and dsRNA viruses. Although mitochon-

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drial DNA is essential for respiratory metabolism, it is not required for viability in this species (14). Of the other extrachromosomal elements DNA plasmids seem to be vital (15, 16), but dsRNA viruses are dispensable (17).

Mitochondria visualised in a *P. rhodozyma* strain by means of $DIOC_6$ (3) appeared as vesicles of various sizes and in large numbers, often lying adjacent to microtubules, in the cytoplasm of vegetative cells (7). *Phaffia rhodozyma* is a petite-positive basidiomycetous yeast: small colonies occur during cultivation which do not have functioning mitochondria. This was proved by measuring the difference between the O_2 consumption of the wild-type and the petite cells and isolated mitochondria and by the inability of to use nonfermentable carbon sources by the petites. The widely used petite inducer, ethidium bromide was effective (14). Petite mutants of *P. rhodozyma* unlike those of *Saccharomyces cerevisiae* arose by point mutation or small deletions even after short- or long-time ethidium bromide treatment. The grand strain had about 13 kb mtDNA in size estimated by restriction mapping (18). This is the smallest mitochondrial genome known in yeasts (19).

Linear DNA plasmids are known to exist widely in microorganisms. Studies on *P. rhodozyma* demonstrated the localisation of such plasmids in the crude mitochondrial fraction (16). Though yeast linear DNA plasmids are generally insensitive to ethidium bromide, *P. rhodozyma* strains could be efficiently cured by ethidium bromide treatment (20). A DNA hybridization study revealed strong sequence homology among some plasmids belonging to the same and different strains. No homology was found between the *P. rhodozyma* and other linear DNA plasmids. Some of the fragments of the plasmids were sequenced and data were compared by databases. Significant homology was not detected to any of the sequences (21). The only known function of yeast linear DNA plasmids is that they confer killer character on the host cells (*Kluyveromyces lactis*, *Pichia acaciae*). No such activity could be detected in any of the *P. rhodozyma* strains (16, 20, 21).

Several *P. rhodozyma* strains harbour dsRNA viruses (17, 22). Studies revealed the existence of dsRNA molecules of different sizes in six strains. Polymorphism was demonstrated in both the length and the number of dsRNA. Strains with one-, three- and four-types of dsRNA molecules were found, while others proved to be dsRNA free. Elongated icosahedral virus-like particles (VLPs) 34x26 nm in size were detected in strains carrying four- or three-types of dsRNAs. One dsRNA molecule of 3.7 kb was found not to form part of the VLP genome (23). Little effect on the reproduction and fitness of the host due to the presence of the VLPs could be detected (24, 25).

Life cycle

As attempts to mate the various strains in the hope of observing subsequent dikaryotic mycelium and teliospore formation (which are characteristic for the basidiomycetous sexual life cycle) were unsuccessful the new genus was placed in the group of Deuteromycotina (1).

But recently sexual activity was induced by depletion of nitrogen from the culture medium (26) or by supplementation with exogenous polyols, especially ribitol (27). The teleomorphic state was described as *Xanthophyllomyces dendrorhous* (27). The most characteristic feature observed was pedogamy (conjugation between a mother cell and a bud) but mating between two different cells has also been observed. The sexual activity involved both mating and sporulation between two yeast cells under the same starvation conditions: the majority of the cells transformed into larger cells surrounded by refractile cell walls and accumulated numerous lipid granules. Cell clumping was observed, some of them formed short conjugation tubes and a few conjugated pairs were present. The septum disappeared in the conjugated cells. Later a slender holobasidium was formed, at the apex with 2-7 basidiospores (26).

Crosses between genetically marked strains, and pulse-field gel electrophoresis of the chromosomal DNA of cells derived from individual spores revealed evidence of karyogamy, meiosis and even recombination. The segregation ratio in tetrads pointed to diploid vegetative cells, which formed tetraploid zygotes and the immediate meiosis then gave rise to diploid progenies again. However, the presence of aneuploids in the population could not be excluded (28).

Extrachromosomal inheritance was also demonstrated in *Phaffia rhodozyma* (23): a dsRNA virus-

containing strain and a virus-free strain were crossed and from the tetrads RNA was isolated. All the progenies contained viruses providing reliable evidence on the efficient transmission of the VLPs via basidiospores during the sexual life cycle. Thus the mating process can also be effective in spreading yeast viruses.

Biotechnological importance - industrial and possible medical use of Phaffia rhodozyma

In recent years *P. rhodozyma* has become an important microorganism in biotechnology as it has potential use for both the food and pharmaceutical industry. This yeast is able to synthesize astaxanthin (3, 3'- dihydroxy - β, β'-caroten-4, 4'-dion) and other carotenoids, which are responsible for its orange to salmon-red colours (1, 4). Animals lack the ability to synthesize the carotenoids *de novo*, so carotenoids that are biosynthesized by microorganisms or by algae and plants must enter animals through their dietary intake. Aquacultured fishes removed from their natural food chain can not get algae or plankton, so they require pigment supplementation of their diet. As only a few species of microorganism produce astaxanthin in nature, *P. rhodozyma* has been exploited as an agent for pigmenting cultured fish and shellfish (29, 30). In addition recently astaxanthin has attracted considerable interest due to its beneficial effect on human health: potent antioxidant activity (31, 32) and possible role in delaying or preventing degenerative diseases (33, 34). Astaxanthin is extremely potent and its antioxidant activity has been reported to be stronger and to last longer than those carotenoids naturally present in vegetables such as β-caroten, lutein, etc.

So far only limited research has been done on the genetics and enzymology of astaxanthin biosynthesis in *P. rhodozyma*. The fermentation industry has focused on strain development for maximum production of astaxanthin, as the pigment content in the wild-type *P. rhodozyma* strains is low. For strain improvement several traditional methods have been successfully used, such as mutant isolation and selection (35, 36) or protoplast fusion (37, 38). Further progress in molecular biological studies of *P. rhodozyma* will certainly help on the isolation and characterization of genes coding for enzymes catalyzing specific steps in the biosynthesis of astaxanthin to increase the productivity.

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