Review

Biology of the red yeast *Xanthophyllomyces dendrorhous* (*Phaffia rhodozyma*)

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Phaffia rhodozyma is a red-pigmented basidiomycetous yeast that produces astaxanthin. Because of this property, it is fermented commercially on a large scale. Astaxanthin is used as a food colorant for fish, and as an antioxidant, it is potentially useful in the human pharmaceutical industry. This review summarizes the published biology of this yeast: its morphology and ultrastructure, organization of the cytoskeleton, and the nuclear and extrachromosomal genomes. Alteration of sexual and vegetative phases in the life cycle and its biotechnological importance are also described.

Key Words——astaxanthin; cytoskeleton; dsRNA virus; genome organization; *Phaffia rhodozyma*.

Historical aspects *Phaffia rhodozyma* M. W. Miller, Yoneyama & Soneda was isolated in Japan in 1976 by Miller and co-workers (1976). 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 and represented a new genus, which 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 of *P. rhodozyma* reproduce by budding, are ellipsoidal, and occur singly, in pairs, or occasionally in short chains. The manner of bud formation is characteristic of heterobasidiomycetous yeasts (Kreger-van Rij, 1998; Johnson and An, 1991). The ultrastructure of cells also refers to a basidiomycete origin: the vegetative cell wall is multilayered and ruptured forming a "collar" where the new bud emerges (Miller et al., 1976). The cell wall polysaccharides contain mainly β- (1→3) and β- (1→6) glucan and also α- (1→3) glucan and none or a small amount of chitin (Johnson et al., 1979; Kucsera and Takeo, 1998). The cells are surrounded by a capsule of acidic polysaccharides containing D-xylose.

Organization of the cytoskeleton during the cell cycle A study by fluorescence microscopy of budding-associated changes in microtubules and actin revealed that the dynamics of microtubule arrangement is more similar to that of fission yeasts or animal cells than to *Saccharomyces cerevisiae* (Slaninova et al., 1999). 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 telophase 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 visualised. 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 orthogonal-field-alternation gel electrophoresis (OF-AGE) and contour-clamped homogeneous electric field techniques (CHEF) for *P. rhodozyma* isolates revealed considerable chromosomal length polymorphism. The numbers and sizes of the chromosomal bands varied from 7 to 12 (Nagy et al., 1994) or 9 to 17 (Adrio et al., 1995), ranging in size from 0.83 Mb to 3.5 Mb or 0.48 Mb to 3.1 Mb respectively in the strains studied.

Little is currently known about the structural and functional organization of *P. rhodozyma* genome in general. Several genes, coding for actin (Wery et al., 1996), glyceraldehyde-3-phosphate-dehydrogenase (Verdoes et al., 1997), endo-β-1,3(4) glucanase and aspartic protease (Bang et al., 1999), and a carotenoid biosynthetic gene (Verdoes et al., 1999) were recently

isolated and several papers related to transformation have been published (Adrio and Veiga, 1995; Wery et al., 1997; Kim et al., 1998; Martinez et al., 1998).

The ploidy level of the nucleus of vegetative cells in different *P. rhodozyma* strains is uncertain. The extensive chromosome length polymorphism compared with the results of genetic analysis from crosses of auxotrophic and color strains may indicate that some of the strains studied are diploid or aneuploid (Kucsera et al., 1998). A similar conclusion was drawn from flow cytometric analysis of propidium iodide-stained cells (Medwid, 1998).

Extrachromosomal genetic elements Extrachromosomal genetic elements of *P. rhodozyma* include mitochondrial DNA (mtDNA), DNA plasmids (Pfeiffer et al., 1995), and dsRNA viruses (Castillo and Cifuentes, 1994). Although mitochondrial DNA is essential for respiratory metabolism, it is not required for viability in this species (Kucsera et al., 1995a). Of the other extrachromosomal elements, DNA plasmids seem to be vital (Pfeiffer et al., 1994b), but dsRNA viruses are dispensable (Pfeiffer et al., 1994a).

Mitochondria visualised in a *P. rhodozyma* strain by means of 3,3′-dihexyloxacarbocyanine iodide ($DIOC_6(3)$) appeared as vesicles of various sizes and in large numbers, often lying adjacent to microtubules in the cytoplasm of vegetative cells (Slaninova et al., 1999). *P. 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 petite cells and their isolated mitochondria and by the inability of the petites to use nonfermentable carbon sources. The widely used petite inducer, ethidium bromide, was as effective in this yeast as in *S. cerevisiae* (Kucsera et al., 1996).

All the examined *P. rhodozyma* strains except strain CBS 5905 contain numerous linear DNA plasmids (Fig. 1). Studies demonstrated their localization in the crude mitochondrial fraction (Pfeiffer et al., 1994a). Though yeast linear DNA plasmids are generally insensitive to ethidium bromide, *P. rhodozyma* strains could be efficiently cured by ethidium bromide treatment (Pfeiffer et al., 1995). 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 with databases, but no significant homology was detected with any of the deposited sequences (Pfeiffer et al., 1996b). The only known function of yeast linear DNA plasmids is that they confer killer character on the host cells (*Kluyveromyces lactis* (Dombrowski) van der Walt, *Pichia acaciae* van der Walt). No such activity could be detected in any of the *P. rhodozyma* strains (Pfeiffer et al., 1994b; 1995; 1996b).

Several *P. rhodozyma* strains harbour cytoplasmic dsRNA viruses (Castillo and Cifuentes, 1994; Pfeiffer et al., 1994a), while others are virus-free. Studies revealed three different dsRNA patterns. Strains with one, three and four types of dsRNA molecules were found (Fig. 2). Elongated icosahedral virus-like particles (VLPs) 34×26 nm in size were detected in strains carry-
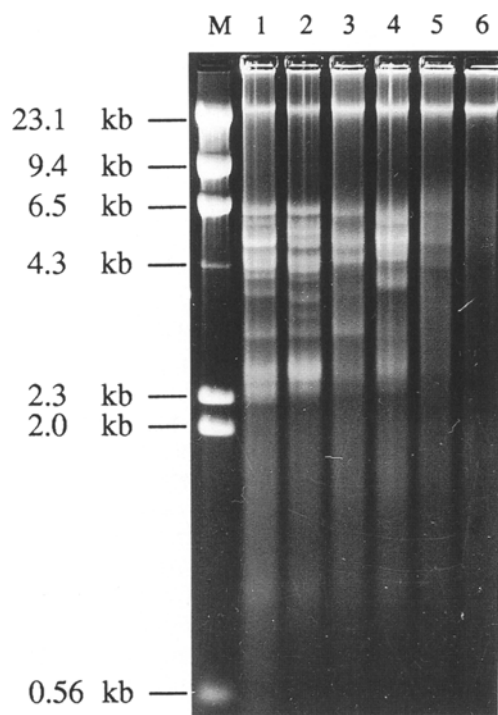

Fig. 1. DNA plasmid pattern of *Phaffia rhodozyma*. M, *Hind* III-digested λ DNA; lane 1, ATCC 24261; lane 2, ATCC 24229; lane 3, ATCC 24203; lane 4, CBS 6938; lane 5, CBS 5908; lane 6, CBS 5905.
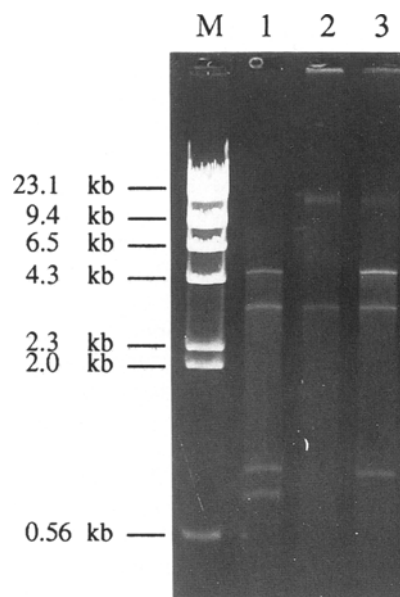

Fig. 2. dsRNA patterns of *Phaffia rhodozyma* strains. M, *Hind* III-digested λ DNA; lane 1, ATCC 24261; lane 2, ATCC 24229; lane 3, ATCC 24203.
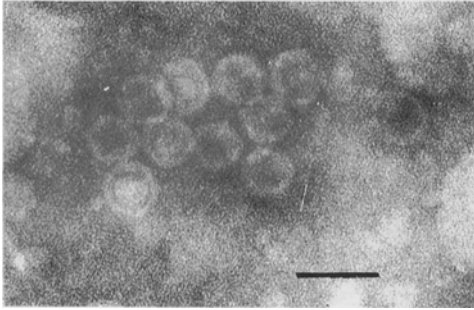
Fig. 3. Electron micrograph of VLPs stained with uranyl acetate. The bar represents 50 nm.

ing four or three types of dsRNAs (Fig. 3). One dsRNA molecule of 3.7 kb was found not to form part of the VLP genome (Pfeiffer et al., 1996c). The presence of the VLPs had little detectable effect on the reproduction and fitness of the host (Pfeiffer et al., 1996a; 1997).

Life cycle As attempts to mate various strains in the hope of observing subsequent dikaryotic mycelium and teliospore formation (which are characteristic of the basidiomycetous sexual life cycle) were unsuccessful, the new genus was previously placed in the group of Deuteromycotina (Miller et al., 1976).

Recently, however, sexual activity was induced by supplementation with exogenous polyols, especially ribitol (Golubev, 1995) or by depletion of nitrogen from the culture medium (Kucsera et al., 1995b). The teleomorphic state was described as *Xanthophyllomyces dendrorhous* Golubev (1995). 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 (Kucsera et al., 1995b). 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 cells 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 (Fig. 4).

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. These formed tetraploid zygotes, which underwent immediate meiosis, giving rise to diploid progenies again. However, the presence of aneuploids in the population could not be excluded (Kucsera et al., 1998).

Extrachromosomal inheritance was also demonstrated in *P. rhodozyma* (Pfeiffer et al., 1996c): a dsRNA virus-containing strain and a virus-free strain were crossed, and RNA was isolated from the tetrads. All the progenies contained viruses, providing reliable evidence for the efficient transmission of the VLPs via basidio-
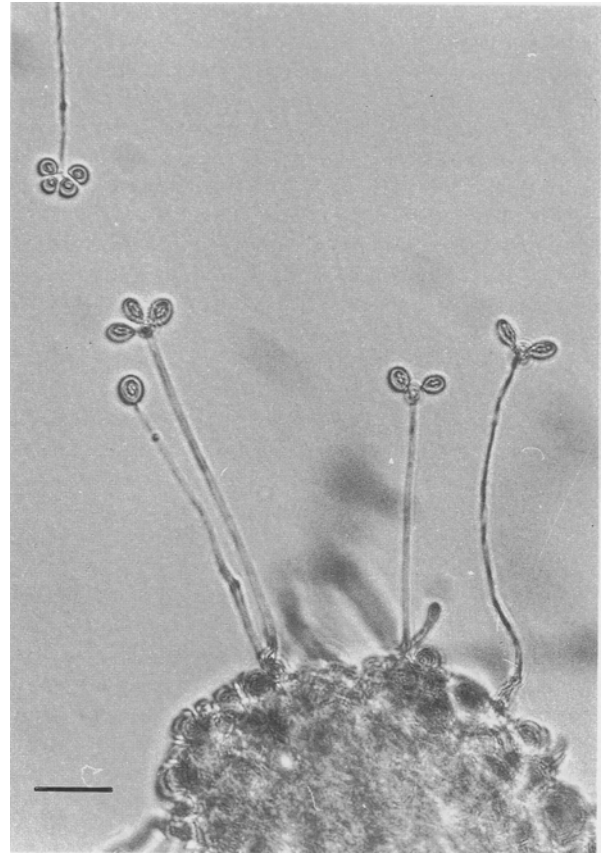

Fig. 4. Micrograph of a colony with aerial basidiospores of *Phaffia rhodozyma* CBS 6938. The bar represents 500 μm.

spores during the sexual life cycle. Thus the mating process can also be effective in spreading yeast viruses.

Intraspecific strain homogeneity in *P. rhodozyma* Apart from the type strain, *P. rhodozyma* CBS 5905, all strains examined so far were able to sporulate. This raised the question of whether these different isolates and the type strain belong in one species. Isoenzyme analyses and DNA characterization by restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) pattern confirmed that the type strain and five other isolates do belong in one species (Varga et al., 1995). However, detailed genome analyses by electrophoretic karyotyping revealed extensive chromosome length polymorphism (Nagy et al., 1994; Adrio et al., 1995). The chromosome number of the different strains determined by different authors varied between 9 and 17, while number of chromosomes of CBS 5905 cells was 12 in all determinations. The asexual reproduction of the type strain may have stabilized the chromosomal pattern. Although there is no direct correlation between the electrophoretic karyotype and species specificity, several other results confirmed the species diversity: (i) analysis of extrachromosomal genetic elements in six wild-type *P. rhodozyma* strains demonstrated that all but the type strain contained double-stranded DNA plasmids

(Pfeiffer et al., 1995), (ii) only the type strain is able to produce a respiratory-deficient petite mutant spontaneously or by short-time ethidium bromide induction (Kucsera et al., 1996). Species diversity was further confirmed by ribosomal DNA internal transcribed spacer (ITS) and intergenic spacer (IGS) region nucleotide sequence analysis (Fell, 1999). All these results may suggest that these different isolates and the type strain do not belong in one species. Despite the data published earlier, *P. rhodozyma* CBS 5905 might be a different species from *X. dendrorhous* (CBS 5908, CBS 6938, ATCC 24203, ATCC 24229 and ATCC 24261).

Biotechnological importance – industrial and possible medical use of *P. rhodozyma* In recent years, *P. rhodozyma* has become an important microorganism as it has potential for use in both the food and pharmaceutical industries. This yeast is able to synthesize astaxanthin (3,3'-dihydroxy-β,β'-caroten-4,4'-dione) and other carotenoids, which are responsible for its orange to salmon-red colors (Miller et al., 1976; Johnson and An, 1991). Astaxanthin in nature is most commonly found in carapaces of crustaceans, muscles of salmonids, ovaries of fish and shellfish, and the skin of birds. 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 animals 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 (Lewis, 1990; Johnson and Schroeder, 1995). In addition, astaxanthin has recently attracted considerable interest due to its beneficial effect on human health: its potent antioxidant activity (Miki, 1991; Schroeder and Johnson, 1993) and its possible role in delaying or preventing degenerative diseases (Jyocouchi et al., 1991; Giovannucci et al., 1995). Astaxanthin is extremely potent, and its antioxidant activity has been reported to be stronger and to last longer than those of carotenoids naturally present in vegetables, such as β-carotene, lutein, etc.

So far, only limited research has been done on the genetics and enzymology of astaxanthin biosynthesis in *P. rhodozyma* (Cifuentes et al., 1997). 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 (An et al., 1991; An, 1997; Bon et al., 1997) or protoplast fusion (Chun et al., 1992, Retamales et al., 1998). Further progress in molecular biological studies of *P. rhodozyma* will certainly help in 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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