CHAPTER 10

Yarrowia lipolytica

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1 History of *Yarrowia lipolytica* Research

Interest in *Candida lipolytica* (Harrison) Diddens et Lodder 1942 initially arose from its rather uncommon physiological characteristics. Strains of this species were more often isolated from lipid- or protein-containing substrates like cheese or sausage than from sugar-containing substrates. Indeed, strains of *Candida lipolytica* used few sugars (mainly glucose) as carbon source, but did readily assimilate various polyalcohols, organic acids, or normal paraffins. They were noted in the late 1940s by dairy technologists (Peters and Nelson 1948a,b) for their high extracellular protease and lipase activities, although these purified enzymes were never put to work industrially.

With the emergence of single-cell protein projects in the mid 1960s, a strong industrial interest stemmed from the fact that strains of this species were able to use n-paraffins which were cheap and abundant at that time, as sole carbon source. It was also observed that *C. lipolytica* was able to produce high amounts of organic acids (2-ketoglutaric acid and citric acid) when grown on these substrates (Tsugawa et al. 1969). Large-scale industrial production of citric acid or SCP using *Y. lipolytica* thus permitted the accumulation of extensive data on its behavior in very large fermentors.

The species was classified as a *Candida* at that time, since no sexual state had been described. The perfect form of *C. lipolytica* was identified in the late 1960s by Wickerham at the Northern Regional Research Laboratory of the USDA at Peoria. A culture isolated in 1945 from a jar of fiber tailings in a corn processing plant was found to form asci attached to hyphal elements when put on suitable media. One to four spores of various size and shape could be isolated from these asci, but spore viability was found to be very low. Two mating types, called A and B, were identified among the progeny. Nearly all other wild-type isolates from the species would mate to one of these two types, albeit at very low frequency, suggesting that most

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K. Wolf, *Nonconventional Yeasts in Biotechnology*

natural isolates are haploid (or near haploid). The perfect form was reclassified first as *Endomycopsis lipolytica* (Wickerham et al. 1970), then as *Saccharomycopsis lipolytica* (Yarrow 1972), and finally as *Yarrowia lipolytica* (van der Walt and von Arx 1980). Further details on its taxonomic position will be discussed below.

Wickerham's strains were sent both to Mortimer's laboratory in Berkeley and to Heslot's group in Paris, which independently started genetic studies on this species. Initial studies were plagued by low mating frequencies, poor sporulation ability, and low ascospore viability. These defects could be partially alleviated by inbreeding programs, but no perfect set of strains could be obtained: mating frequencies remained low, and spore viability plateaued at around 80% (Gaillardin et al. 1973; Ogrydziak et al. 1978). Protoplast fusion was attempted to overcome some of these difficulties (Esser and Stahl 1976; Weber et al. 1980). Later groups joining the *Y. lipolytica* club initiated new and independent inbreeding programs, starting with different strains, in Poland (Bojnanska 1977), East Germany (Kurischko et al. 1983; Barth and Weber 1985), and in private companies (British Petroleum or Pfizer USA). As a result, several inbred lines of *Y. lipolytica* exist nowadays, and linkage groups were defined by tetrad dissection or chromosome loss in several of these lines (Ogrydziak et al. 1978, 1982; Kurischko 1984, 1986; De Zeeuw, pers. comm.). It is still unclear today how conserved these linkage groups are across the different lines, which probably differ grossly at the level of chromosome structure (see below); clearly, the different lines are poorly interfertile; genetic exchange between them is difficult and commonly requires several rounds of backcrosses before acceptable behavior is restored.

Happily, however, these various groups entered *Y. lipolytica* genetics for different reasons and focused on specific aspects of its biology: Krebs cycle (Akiyama et al. 1973a,b; Finogenova et al. 1982; Barth 1985), lysine metabolism (Gaillardin et al. 1975), secretion of extracellular enzymes (Ogrydziak and Mortimer 1977), alkane degradation (Bassel and Mortimer 1982), mating and parasexual processes (Weber 1979; Barth and Weber 1984; Kurischko 1986), mitochondrial genetics (Matsuoka et al. 1982), virus-like particle (Groves et al. 1983; Tréton et al. 1985), ribosomal RNA genes (van Heerikhuizen et al. 1985). An exhaustive review on this early work has been published (Heslot 1990).

A fair amount of data both on genetic and physiological aspects of this species was thus accumulated in the mid 1980s, when finally a transformation system became available (Davidow et al. 1985; Gaillardin et al. 1985). Gene cloning and successful expression of heterologous proteins, which appeared to be efficiently secreted to the growth medium, initiated a second wave of interest in this yeast (for an early review, see Gaillardin and Heslot 1988). The availability of an amazingly efficient and precise system for integrative transformation, the fine dissection of the processing pathway of an abundantly secreted extracellular protease (Matoba et al. 1988), the discovery of a signal recognition particle closely resembling that of higher eukaryotes (Poritz et al. 1988), all seemed to pave the way for the rapid development of *Y. lipolytica* as a model organism for studies on protein secretion. Some tools were, however, still missing, and forcefully resisted availability: for instance, and for reasons very unclear at that time, no replicative vector could be