Antonie van Leeuwenhoek **51** (1985) 167–177

Improvement of sporulation in the yeast *Yarrowia lipolytica*

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BARTH, G. and WEBER, H. 1985. Improvement of sporulation in the yeast *Yarrowia lipolytica.* Antonie van Leeuwenhoek **51**: 167–177.

Strains of *Yarrowia lipolytica* forming exclusively spherical ascospores were developed through inbreeding. These strains are more suitable for micromanipulation than other inbred strains forming helm-shaped ascospores. External factors affecting sporulation frequency and tetrad formation in this yeast were investigated. Optimal formation of complete tetrads occurred at a narrow range of pH values around 6.0. Citrate was found to stimulate sporulation strongly. A synthetic medium containing citrate was developed to obtain standard conditions for maximum sporulation.

INTRODUCTION

After the discovery of sexual reproduction in the yeast *Candida lipolytica* (Wickerham et al., 1970), all strains of this yeast exhibiting sporulation were assigned to the perfect genus *Saccharomycopsis* (Yarrow, 1972; Bassel and Mortimer, 1973; Barnett et al., 1979). In 1980 Van der Walt and Von Arx reclassified this ascigerous teleomorph to the new genus *Yarrowia. Yarrowia lipolytica* (Wickerham, Kurtzman et Herman) van der Walt et von Arx comb. nov. was defined as the type species for this genus. In wild-type strains of *Y. lipolytica* and mutants isolated for genetic investigations, the frequency of sporulation is low and does not allow tetrad analysis, because asci containing three or four viable spores are rare (Wickerham et al., 1970; Bassel et al., 1971; Esser and Stahl, 1976). Formation of complete tetrads and spore viability could be improved by several inbreeding programs (Bassel et al., 1971; Gaillardin et al., 1973; Ogrydziak et al., 1978; Kurischko et al., 1983). Both wild-type and inbred

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strains of this yeast form helm- or hat-shaped ascospores (Lodder, 1970; Ogrydziak et al., 1978; Kreger-Van Rij, 1980; Van der Walt and Von Arx, 1980). Isolation of single ascospores from such strains by micromanipulation is possible but laborious and complicated because these spores are closely attached to each other (Ogrydziak et al., 1978, 1982). In contrast to the situation in certain other yeasts used in genetic studies, sporulation in *Y. lipolytica* is known so far to occur only on complex sporulation media where asci are formed almost exclusively on hyphi (Ogrydziak et al., 1978; Weber, 1979). Hitherto conditions for optimal sporulation of this yeast were not intensively investigated. To facilitate tetrad analysis in *Y. lipolytica*, we bred strains with higher fertility parameters and spherical ascospores which are more suitable for micromanipulation. Furthermore, we improved the conditions for sporulation and developed a defined sporulation medium which guarantees standard conditions for sporulation in this yeast.

Materials and Methods

Yeast strains

Strains of *Y. lipolytica* used in the experiments are listed in Table 1. Nutritional mutants produced by UV mutagenesis were used in crosses between haploid strains (Barth and Weber, 1983). For mating types the original designations A and B of Wickerham et al. (1970) are used, which correspond with the designation by the symbols + and − (Esser and Stahl, 1976; Stahl, 1978).

Media and culture conditions

YEPG (yeast extract – peptone – glucose medium) contained 1% Bacto yeast extract (Difco), 2% Bacto peptone and 2% glucose (Leupold, 1955, modified). MMT (minimal medium with thiamin) was used as described by Barth and Künkel (1979). YM (yeast extract – malt medium) contained 0.5% Bacto peptone, 0.3% Bacto yeast extract, and 0.3% malt (Wickerham, 1951). ASM (acetate sporulation medium) contained 0.14% sodium acetate and 0.04% glucose. VSM (vegetable juice sporulation medium) was used as described by Weber (1979). For CSM (citrate sporulation medium), minimal medium according to Barth and Künkel (1979) was supplemented with 20 $g \cdot l^{-1}$ sodium citrate $\cdot 5\,H_2O$ as carbon source; in addition 2 $mg \cdot l^{-1}$ biotin, 20 $mg \cdot l^{-1}$ of other vitamins (*p*-aminobenzoic acid, inositol, niacin, calcium pantothenate, pyridoxin, riboflavin and thiamin hydrochloride) and 50 $mg \cdot l^{-1}$ nucleotide bases (adenine, guanine, cytosine hydrochloride, thymine, uracil) were added after autoclaving.

Media were solidified with 2% Difco agar.

Mating

The method for conjugation was described by Barth and Weber (1983).