Sexual co-flocculation by heterothallic cells of the fission yeast
Schizosaccharomyces pombe modulated by medium constituents

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Abstract

Novel simple synthetic media for inducing sexual co-flocculation in a short time after mixing heterothallic fission-yeast (Schizosaccharomyces pombe) cells of h− and h+ were devised; The most effective of these, mannose synthetic medium (MSM), contains 0.4% mannose as a carbon source in addition to galactose, KH₂PO₄ (pH4.0) and 4 vitamins. The addition of galactose to the medium suppressed the asexual self-flocculation but rather promoted the sexual co-flocculation. By transferring and mixing h− and h+ cells grown in malt-extract broth plus galactose into MSM, these heterothallic strains were revealed to be sexually ready through a long period of the log to stationary phases. Furthermore, a variety of C sources and NH₄Cl at various concentrations in various media were examined for their effects upon sexual co-flocculation, conjugation and sporulation; it was found that the sugar concentration strictly affected the progress of the sequence of sexual reproduction at 26 °C but not 30 °C and that sexual co-flocculation of the heterothallic strains was induced only under lower concentrations of C and N source than that for the homothallic one.

Abbreviations: ASF – asexual co-flocculation; SF – sexual co-flocculation; EMM2 – Edinburgh minimum medium; MSM – mannose synthetic medium; MEB – malt-extract broth; MEBG – MEB plus galactose; SSL – sporulation synthetic liquid; SPL – sporulation liquid; OD – optical density.

Introduction

Sex-directed flocculation has been observed for both budding and fission yeasts (Yanagishima & Yoshida 1981; Calleja 1987); it has an important role for attaining a high percentage of conjugation between cells of opposite mating types in liquid conditions. Sexual agglutinins in budding yeasts are glycoproteins and have been physiologically, chemically and genetically investigated in considerable detail (Lipke & Kurjan 1992). On the other hands, there are few investigations of sexual agglutination in the fission yeast Schizosaccharomyces pombe except for studies of heterothallic strains by Egel (1971) and of a homothallic strain examined by Calleja et al. (1981 & 1982). Accordingly, sexual agglutinating substances of the fission yeast have not yet been isolated.

Sexual co-flocculation, or agglutination, was indicated to precede conjugation in both homothallic (Calleja & Johnson 1971) and heterothallic (Egel 1971) strains of S. pombe; agglutinabilities in both strains were reported to be inducible.

We reported previously that cells of the heterothallic fission-yeast S. pombe, both 972 h− and 975 h+, exhibited asexual self-flocculation when separate cultures entered late log phase and exhibited sexual co-flocculation after an extended period when the cultures were mixed even though the sources of carbon and nitrogen had not been entirely consumed (Johnson et al. 1987). It suggests that an energy source at low concentration and mutual induction are needed for the
formation of agglutinins. According to observations of homothallic strains (Calleja 1987), culture conditions and nutrients affect the sequence of events in sexual reproduction to a considerable extent, obviously by effecting the differential expression of relevant genes.

In the present study we explore the components of synthetic media and conditions for controlling the expression of events in the sexual reproduction of heterothallic cultures and to stimulate sexual co-flocculation at high frequency without a lag period after mixing.

Materials and methods

Organisms

Leupold’s original (1955) heterothallic haploid strains, 972 h⁻ and 975 h⁺ (hereafter designated h⁻ and h⁺), of the fission yeast, Schizosaccharomyces pombe, and a homothallic haploid strain (NCYC 132) were used.

Media and culture conditions

Media used for pre- and main cultures were MEB: 2% malt extract broth (Oxoid) or MEBG: MEB plus 1% galactose; those for mixed culture were conditioned medium: cell-free MEBG in which cells were cultured for 24 h at 26 °C; SPL (Leupold 1970): sporulation agar without agar: 1% glucose, 0.1% KH₂PO₄ and 4 vitamins (10⁻⁶ g/ml inositol, 10⁻⁶ g/ml nicotinic acid, 1 µg/ml Ca²⁺-pantothenate and 10 ng/ml biotin) as in EMM2 (Edinbugh minimum medium 2, Mitchison 1970); SSL (synthetic sporulation liquid, Egel 1971): 1% glucose, 0.02% aspartic acid, 0.2% KH₂PO₄, 0.05% MgSO₄, 0.01% CaCl₂, 4 vitamins as in EMM2, trace metal ions (H₃BO₄, CuSO₄, KI, FeCl₃, MnSO₄, H₂MoO₄, ZnSO₄).

Cultures were maintained at the same temperature before (separate-cultures) and after (mixed-cultures) mixing. One loopful cells taken from a slant was inoculated into 10 ml of medium in a tightly capped 30-ml bottle and was precultured without shaking. After 24 h, the precultured cells were inoculated into the fresh medium for the main culture, whose volume was less than one third of Erlenmeyer flask’s capacity. Cells of h⁻ and h⁺ separately grown for a given time were mixed, transferred into a 100-ml or 50-ml flask equipped with a side-arm for measuring the turbidity and cultured by rotary-shaking. When the medium for the mixed-culture was to be different from the main culture, cells were washed once with 0.1% glucose solution and transferred. The volume of mixed culture was one fifth or one tenth of the flask’s capacity.

Slide culture was performed on a block of MSM-agar (2%), less than 1.0 mm in thickness and (10 × 5) mm² in size: About 1 µl of cell-suspension was put on the block on a slide and was covered with a coverslip (24 × 36 mm²). The edges of coverslip were sealed with silicone oil. An Olympus BH–2 phase-contrast microscope in an incubator and Fuji Neopan Presto film were used for photography.

Estimation of extent of sexual co-flocculation (SF) and definitions of induction time and of SF activity

The extent of flocculation was estimated turbidimetrically by measuring with a photometer (Spectronic 20D; Milton Roy Company, U.S.A.) the change of turbidity (optical density at 600 nm, OD) of the cell suspension tipped from main compartment into the sidearm. A first measurement was made after standing for just 5 min from immediately after the transfer, time enough to allow the flocs to settle to the bottom in the sidearm (Calleja & Johnson 1977). A second measurement was made after shaking vigorously to break the flocs into free cells. The extent of flocculation is expressed as the difference, ΔOD, between the two readings: which is equivalent to ΔKU defined by Calleja and Johnson (1977). We multiplied the measured ΔOD by 10³ as a convention for the extent of flocculation. When the extent of flocculation is 100, by that convention, after mixing of h⁻ and h⁺ cells, the flocs are large enough to be readily visible. Therefore, the time(h) required to attain 100 was defined as the induction time of SF for convenience though the real time for induction, to be precise, was shorter. Thus the activity of SF is inversely proportional to the time required for induction: the shorter time for induction, the higher activity of SF. Every experiment for flocculation was carried out more than ten times; patterns and amounts similar to those in the figures and the tables shown in this paper were obtained.

Determinations of cell number, septum index, % conjugation and % sporulation

The cell number was counted microscopically in a Bürker Türk hemocytometer slide (Erma Optical Works, Tokyo, Japan) after suitable dilution of cul-