Lipids of *Pediococcus cerevisiae* and some methicillin-resistant substrains

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The phospholipids of *Pediococcus cerevisiae* were identified as phosphatidyl glycerol, lysylphosphatidyl glycerol, cardiolipin, phosphatidic acid and an unknown. Evidence was obtained for the presence of mono- and diglucosyl diglycerides. The major fatty acids were C18:1, C16:0, and C16:1, with smaller amounts of C14:0, C14:1, and C18:0. The methicillin-resistant strains did not contain more lipid or lipid phosphate than the parent strain when they were grown in the presence of methicillin. The percentages of fatty acids in the organisms were not markedly different. Some variation in the proportions of the phospholipids was noted.

INTRODUCTION

It has been reported that in some instances the lipid composition of bacteria resistant to antibiotics is quantitatively and/or qualitatively different from that of the sensitive strains (Hugo and Stretton, 1966; Dunnick and O'Leary, 1970; Anderes, Sandine and Elliker, 1971; Vaczi, 1966, and Vaczi and Farkas, 1961).

Several methicillin-resistant and -dependent strains of *Pediococcus cerevisiae* were available (Widdowson and White, 1971), the resistance of which was unexplained (Wilkinson and White, 1973). The lipids of these organisms were examined to see if there was any correlation between resistance and lipid composition.

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The lipid composition of *P. cerevisiae* has been examined to some extent (Ikawa, 1963) and there has been a recent report of the fatty acid content of this organism (Uchida and Mogi, 1972). In the present study the phospholipids and glycolipids of the parent and resistant organisms have been examined in more detail and the fatty acid content has been determined. In addition, total lipid, lipid phosphate, and the percentages of neutral, "glyco" and phospholipids have been measured.

**MATERIALS AND METHODS**

**Organisms.** *Pediococcus cerevisiae* ATCC 8081 (sensitive to 10 µg of methicillin/ml) and *P. cerevisiae* CRD (a methicillin-dependent substrain resistant to up to 300 µg of methicillin/ml) were maintained as described by P. J. White (1968). *P. cerevisiae* 8081 R (resistant to up to 150 µg of methicillin/ml) and *P. cerevisiae* MRD2 (methicillin-dependent and -resistant to up to 300 µg of methicillin/ml) were described by Widdowson and White (1971) and were maintained like *P. cerevisiae* CRD.

**Medium.** The partly defined medium of P. J. White (1968) was used. Methicillin was sterilized by filtration and was included (100 µg/ml) in the medium for growth of the methicillin-resistant organisms.

**Culture conditions.** The organisms were grown in 1 litre of medium in a 2-litre conical flask and were incubated without shaking at 37 °C. When the lipids were to be labelled, 500 µCi of H32P04 were included in the medium. Organisms were always harvested in the exponential phase of growth (after about 8 hr with parent organisms, and about 24 hr with the resistant organisms).

**Harvesting and washing of organisms.** Organisms were harvested at 6000 g for 10 min at 2 °C and were washed once by resuspension in cold distilled water.

**Determination of dry weight.** The washed organisms from 1 litre of culture were resuspended in distilled water (20 ml) and duplicate 1 ml samples were placed in preweighed vials. The vials were heated at 105 °C overnight and weighed.

**Extraction of lipid.** Lipids were extracted by a modification of the Bligh and Dyer method (1959). A suspension of organisms from 1 litre of culture (200–400 mg dry wt) in water was made up to 100 ml with 0.05 M phosphate buffer pH 7.5. Methanol (250 ml) and 125 ml of chloroform were added to the suspension in a 1-litre separatory funnel, shaken vigorously and allowed to stand for two hours. Chloroform (125 ml) and 125 ml of water were added, shaken and allowed to stand overnight. The lower phase (containing the lipid) was passed through anhydrous sodium sulphate and rotary evaporated to dryness. The lipid residue