Biotransformation of Progesterone to 11-α-Hydroxyprogesterone
by Different Morphological Forms of Rhizopus arrhizus

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Summary
Medium supplementation with carboxymethyl cellulose resulted in production of Rhizopus arrhizus mycelium with an increased specific capacity to biotransform progesterone to 11-α-hydroxyprogesterone. This increase may be attributed to the observed differences in morphology. Morphologies of the control and CMC-grown mycelia were clumped, pelleted and dispersed respectively. Carbopol-grown mycelium, which manifested a clumped, dispersed morphology, intermediate between that of the control and CMC-grown mycelia, had a lower progesterone transforming capacity.

Introduction
Rhizopus arrhizus morphology in submerged culture can be varied with environmental conditions (Byrne & Ward, 1987). Morphologies range from discrete pellets to dispersed filamentous growth and mycelium may also form tissue-like masses in shake-flasks or fermenters (Byrne & Ward, 1989 a, b). Anionic polymers have been shown to result in production of dispersed filamentous growth in the case of R. arrhizus and other filamentous fungi (Byrne & Ward, 1987; Elmayergi et al., 1983; Morrin & Ward, 1989; Trinci, 1983). With Aspergillus and Penicillium species, culture conditions affect fungal morphology and formation of products, such as enzymes (Calam, 1976), organic acids (Kisser et al., 1980; Milsom & Meers, 1985) and antibiotics (Konig et al., 1982; Metz & Kossen, 1977).

R. arrhizus may be used in industrial fermentations for production of lipase, fumaric acid and 11-α-hydroxyprogesterone. We have investigated the
interrelationships between medium constituents, hyphal morphology and chemistry, and product formation using Carbopol-934 and carboxymethyl cellulose (CMC) to modify morphology (Byrne, 1985; Morrin & Ward, 1989). The different morphological forms have been characterised by phase contrast and electron microscopy and by examination of branching patterns. Rhodes et al. (1959, 1962) noted that efficient fumaric acid production by R. arrhizus required a finely dispersed growth consisting of minute particles of filamentous mold. We observed that biomass, produced in media containing CMC, which results in production of a dispersed morphology, manifested a higher fumaric acid production capacity, compared to biomass from the unsupplemented control which was pelleted. In this communication, we have investigated the effect of biomass, grown in control media and in media supplemented with CMC and Carbopol, on biotransformation of progesterone to 11-α-hydroxyprogesterone.

**Materials and Methods**

*Inoculum Preparation and Culture Conditions:*
Spores of *R. arrhizus* NRRL 2582, cultured on yeast/malt agar for 4 days at 30°C, were washed from the culture with water containing 0.1 ml/l Triton X-100 and counted (Byrne & Ward, 1987). Spores were inoculated into 250 ml Erlenmeyer flasks, containing 50 ml medium, and incubated at 30°C on an orbital incubator shaking at 150 rpm. The control medium contained 0.5% (w/v) soya peptone and 2.0% (w/v) glucose, pH 5.5. CMC and Carbopol-934 were added as supplements to the control at concentrations of 2.0% (w/v) and 0.3% (w/v) respectively.

*Biotransformation Procedure:*
After a 24h or 48h incubation time, mycelia were harvested aseptically by filtration, washed with water and re-inoculated into 50 ml of 10 mM sodium hydrogen phosphate buffer, pH 6.5, containing 50 mg progesterone (Aldrich, Milwaukee, WI), slurried in 2 ml ethanol. The biotransformation was carried out on the orbital shaker set at 30°C and 150 rpm for 24h.

*Steroid Analysis:*
The whole broth was extracted using 2 x 50 ml dichloromethane. The residual extracted mycelium was then broken using a bead beater (Biospec Products, Bartlesville, OK) for 3 x 1 min cycles, with temperature maintained at 4°C, and then extracted using 2 x 40 ml dichloromethane. Combined whole broth extracts and the combined broken mycelial extracts were each concentrated using a rotary evaporator and allowed to dry over P₂O₅. Dried extracts were resuspended in 10 ml acetonitrile and assayed using a Waters