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Growth optimization of *Malassezia furfur* on Sabouraud Dextrose Agar (SDA) supplemented with extracted egg yolk oil: Determination of minimum volume requirements

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Background: *Malassezia furfur* is a lipophilic yeast, causes various skin diseases. In-vitro research on this clinically significant pathogen has been hampered in Sri Lanka by lack of accessible, cost-effective culture media for isolation and subculture.

Objective: To evaluate the growth characteristics of *M. furfur* on SDA supplemented with varying concentrations of egg yolk oil and to determine the minimum volume required for optimal growth.

Methods: *M. furfur* was isolated from Pityriasis versicolor (03) patients attending Teaching Hospital Jaffna, on SDA with ghee media, and was confirmed microscopically, macroscopically, and biochemically. Egg yolk oil was extracted by solvent extraction method (2-propanol and hexane). SDA culture plates with different volumes of egg yolk oil (2x, x, x/2, x/4, x/8, and x/16; x refers to oil extracted from one egg yolk) were streaked with colonies of *M. furfur* and incubated at 32 °C. Growth level (5-point scale), isolation, and colony size (mm) were taken on the 3rd and 5th days of incubation. Each experiment was triplicated.

Results: *M. furfur* was confirmed microscopically and biochemically by ‘spaghetti-meatballs’ appearance and catalase positivity. The oil volume extracted from one egg yolk and yield in percentage were 2 mL and 33.33 % respectively. On day 3, all plates showed growth level 4, isolating colonies on SDA at 2x, x, x/2, x/4, and x/8 oil volumes, with a colony size of 1.5±0.1 mm except in the x/16 plate. On day 5, isolated colonies were observed only in x/2, x/4, and x/8 plates with a colony size of 2.0 ±0.1 mm, which aligns with the ideal colony size of *M. furfur* (2-3 mm). The oil volumes: x/2, x/4, and x/8 exhibited similar growth levels, isolation, and colony size.

Conclusions: This study indicates that SDA with egg yolk oil promotes *M. furfur* growth, with x/8 (0.25 mL) as the minimum volume for optimal growth.