Identification and characterization of lipid droplets in *Malassezia furfur*

Celis AM^{1,2,3}, Triana S^{2,3}, Ibarra H, Cardona J⁴, Restrepo S², Gonzalez A⁴, Wösten HAB¹, de Cock H¹

¹Microbiology, Department of Biology, Utrecht University, Utrecht, The Netherlands, ²Laboratorio de Micología y Fitopatología, Departamento de Ciencias Biológicas Universidad de Los Andes, Bogotá, Colombia, ³Grupo Investigación Celular y Molecular de Microorganismos Patógenos (CeMoP), ⁴Grupo de Diseño de Productos y Procesos (GDPP), Departamento de Ingeniería Química, Universidad de los Andes

Background: *Malassezia furfur* is a lipid-dependent yeast that is part of the human skin microbiota. Mechanisms underlying growth on skin including its lipid metabolism are largely unknown.

Methods: We followed physiological characterization of *M. furfur* in complex medium (mDixon) and minimal medium supplemented with sources of palmitic acid and/or oleic acid to evaluate the presence of the lipid droplets (LD) through confocal microscopy using Nile red staining. Lipidomic and genomic analysis were conducted in order to characterize the lipid composition and to identify the presence of the genes involved in neutral lipid synthesis in this yeast.

Results: Lipid droplets (LDs) are shown in *Malassezia*. Lipidomic analysis revealed the presence of phospholipids as well as TGs in LDs of *M. furfur* but SEs were not detected. TG species 52:4, 54:3, 54:4, and 54:5 were enriched in LDs after growth in the presence of Tween 80 or oleic acid (both donors of C18:1), while TG species 50:1 and 52:2 were enriched after growth in the presence of Tween 40 (donor of C16:0).

Figure 2. Triglycerides (TG), phosphatidylcholine (PC), and lysophosphatidylcholine (LPC) profiles of LDs isolated from *M. furfur* CBS1878 cultures grown in mDixon broth containing oleic acid (red bars), Tween 40 (green bars) or Tween 80 (blue bars). Error bars indicate standard deviation.

Figure 1. Growth curves (A, B) and localization of LDs (C, D) of *M. furfur* CBS1878 in mDixon and / or MM with palmitic acid and / or oleic acid (A, C) or after transfer to MM without fatty acids (B, D). LDs were localized in cells by Nile Red staining 0, 72, and 168 h after culturing in mDixon (a-c) or in MM supplemented with PA (d-f) OA (g-i), or PA+OA (j-l) (C). Alternatively, LDs were localized in cells that had been grown for 168 h in MM with PA and / or OA and that were subsequently depleted for PA (a-b), OA (b-c) or PA+OA (d-e) (D). Bar represents 5 μ m.

Conclusions: Results suggest that LDs of *M. furfur* relieve lipotoxicity caused by oleic acid. This would compensate for the inability of this yeast to degrade unsaturated fatty acids via β -oxidation, which is likely explained by the absence of a gene encoding $\Delta 3,2$ -enoyl-CoA isomerase.

General significance: We are presenting here new insights in the lipid metabolism of this yeast with specific attention to lipid droplets which is important to understand aspects in the adaptation to the host.