Development of Artificial Sebum-containing Leeming and Notman Agar Medium to Enhance the Growth of *Malassezia*

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Short Report

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Abstract

Modified Leeming and Notman agar medium (mLNA) has been widely utilized to grow lipophilic fungi belonging to the genus *Malassezia*. We developed a new artificial sebum-containing mLNA to obtain higher yields of *Malassezia* spp. The olive oil in mLNA was replaced with artificial sebum composed of triglyceride (triolein), diglyceride (glyceryl distearate), fatty acids (palmitic acid, myristic acid, pentadecanoic acid, and oleic acid), and squalene, and the Tween 60 was replaced with glyceryl stearate. Nine human-associated *Malassezia* species grew well on the artificial sebum-containing mLNA, and the yield of the most predominant fungus on human skin, *Malassezia restricta*, in artificial sebum-containing mLNA was double that in standard mLNA.

Full Text

Lipophilic fungi belonging to the genus *Malassezia* are the most predominant fungi in the human skin microbiome on all sites of the body except the soles of the feet (1). As sebum is the nutrient source for *Malassezia*, the scalp and face, where the sebaceous glands are well developed, are particularly highly colonized. Although *Malassezia* are commensal microorganisms on the human skin, these fungi can cause seborrheic dermatitis, pityriasis versicolor, and folliculitis, and may also exacerbate atopic dermatitis (2). Medium for cultivating *Malassezia* should include fatty acids, as they are required by these fungi. Two media were developed previously for cultivation of *Malassezia* spp.: Leeming and Notman agar (LNA) and Dixon medium (3-5). The whole-fat cow's milk initially included in the LNA was later replaced by olive oil and this modified LNA (mLNA) is now the most commonly used medium for cultivation of these fungi (Table 1). Although all *Malassezia* species can grow well on mLNA, we developed a new medium by adding artificial sebum to mLNA, to more closely mimic the human skin environment and thus improve the growth rate and obtain a high microorganism yield.

Thirty-three strains of nine human-associated *Malassezia* species were examined in this study: *Malassezia dermatis* (3 strains), *Malassezia furfur* (4 strains), *Malassezia globosa* (5 strains), *Malassezia japonica* (3 strains), *Malassezia obtusa* (3 strains), *Malassezia restricta* (5 strains), *Malassezia slooffiae* (4 strains), *Malassezia sympodialis* (3 strains), and *Malassezia yamatoensis* (3 strains). The strain numbers and sources are listed in Table S1. The olive oil in mLNA was replaced by artificial sebum composed of triglyceride (triolein), diglyceride (glyceryl distearate), fatty acids (palmitic acid, myristic acid, pentadecanoic acid, and oleic acid), and squalene, and the Tween 60 was replaced by self-emulsifying glyceryl stearate (Table 1). The medium was prepared by adding all components to an Erlenmeyer flask, heating at 60°C to dissolve solid components with stirring, and autoclaving at 121°C for 15 minutes. The autoclaved medium was allowed to cool to about 50°C, and 20 mL was then poured into each 9-cm-diameter plate and allowed to solidify at room temperature. The fungi grown in each medium were collected and washed with phosphate-buffered saline (PBS, pH 7.2), and 100 µL of a suspension adjusted to A_{630} = 5 was added to each 9-cm-diameter agar plate and spread well. The agar plates were incubated at 32°C. *Malassezia* cells were harvested from the plates every day for 10 days and washed with PBS, and the weight of the fungi was measured.

As *Malassezia* species colonize oily sites of the skin, such as the scalp, face, and neck, the growth ability of *Malassezia* should be increased in medium with components approximating such oily sites. Therefore, the olive oil in mLNA was replaced with artificial sebum containing triglyceride, triolein, diglyceride, glyceryl distearate, palmitic acid, myristic acid, pentadecanoic acid, and oleic acid, with a high proportion of free fatty acids and squalene, as natural human sebum is composed of triglycerides, diglycerides, free fatty acids, squalene, cholesterol esters, free cholesterol, and wax esters (6). Cholesterol esters, free cholesterol, and wax esters are present in low proportions in sebum and/or have a high melting point. The addition of a large amount of a component with a high melting point to the medium would result in its immediate solidification during preparation, which would make preparation difficult. Therefore, we did not add these components to this medium. In order to change the olive oil in the mLNA to artificial sebum and produce a stable emulsified medium, the surfactant was changed from Tween 60 to the self-emulsifying glyceryl stearate, its amount was doubled because the physical properties and hydrophilic-lipophilic balance (HLB) of the artificial sebum components and olive oil were different, and Tween 60 could not emulsify them.

We compared the growth of nine species of *Malassezia* in mLNA medium and artificial sebum-containing mLNA medium. The fungal yield in the stationary phase on the two media was higher in the order of high yield group [*M. slooffiae*, *M. japonica*, *M. yamatoensis*, *M. furfur*], intermediate yield group [*M. dermatis*, *M. sympodialis*, *M. obtusa*], and low yield group [*M. globosa*, *M. restricta*]. The yields of *M. slooffiae*, *M. japonica*, *M. yamatoensis*, and *M. obtusa* in the stationary phase were almost the same between artificial sebum-containing mLNA and mLNA. However, the yields of the five other *Malassezia* species were 1.3–2.2-fold higher in artificial sebum-containing mLNA than mLNA. In particular, the yield of *M. restricta* in artificial sebum-containing mLNA was double that in mLNA. *M. restricta* is the predominant species in the human skin fungal microbiome in all sites of the body, followed by *M. globosa* and *M. sympodialis*, while the six other *Malassezia* species show little colonization of the skin. Considering the characteristics of the skin microbiome, it is noteworthy that the yield of *M. restricta* in artificial sebum-containing mLNA was double that in mLNA. As there was no significant difference in the time to reach the stationary phase between the two media, the artificial sebum-containing mLNA was characterized by a particularly high yield of predominant fungal species in the skin microbiome. As the lipases secreted by each *Malassezia* species have different substrate specificities, it may be possible to increase the yields of *M. slooffiae*, *M. japonica*, *M. yamatoensis*, and *M. obtusa* by changing the composition of the artificial sebum included in the medium.

In conclusion, we established artificial sebum-containing mLNA, taking into consideration the physical characteristics and HLB of natural human sebum. Using this new medium, we obtained higher yields of the predominant *Malassezia* species in the human fungal microbiome compared to those obtained using standard mLNA. The performance of the medium has not been examined using human skin specimens, but we expect to be able to obtain more colonies using artificial sebum-containing mLNA than with mLNA.

References

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Table

Table 1 xlsx is available in the Supplemental Files section.

Figures

Figure 1

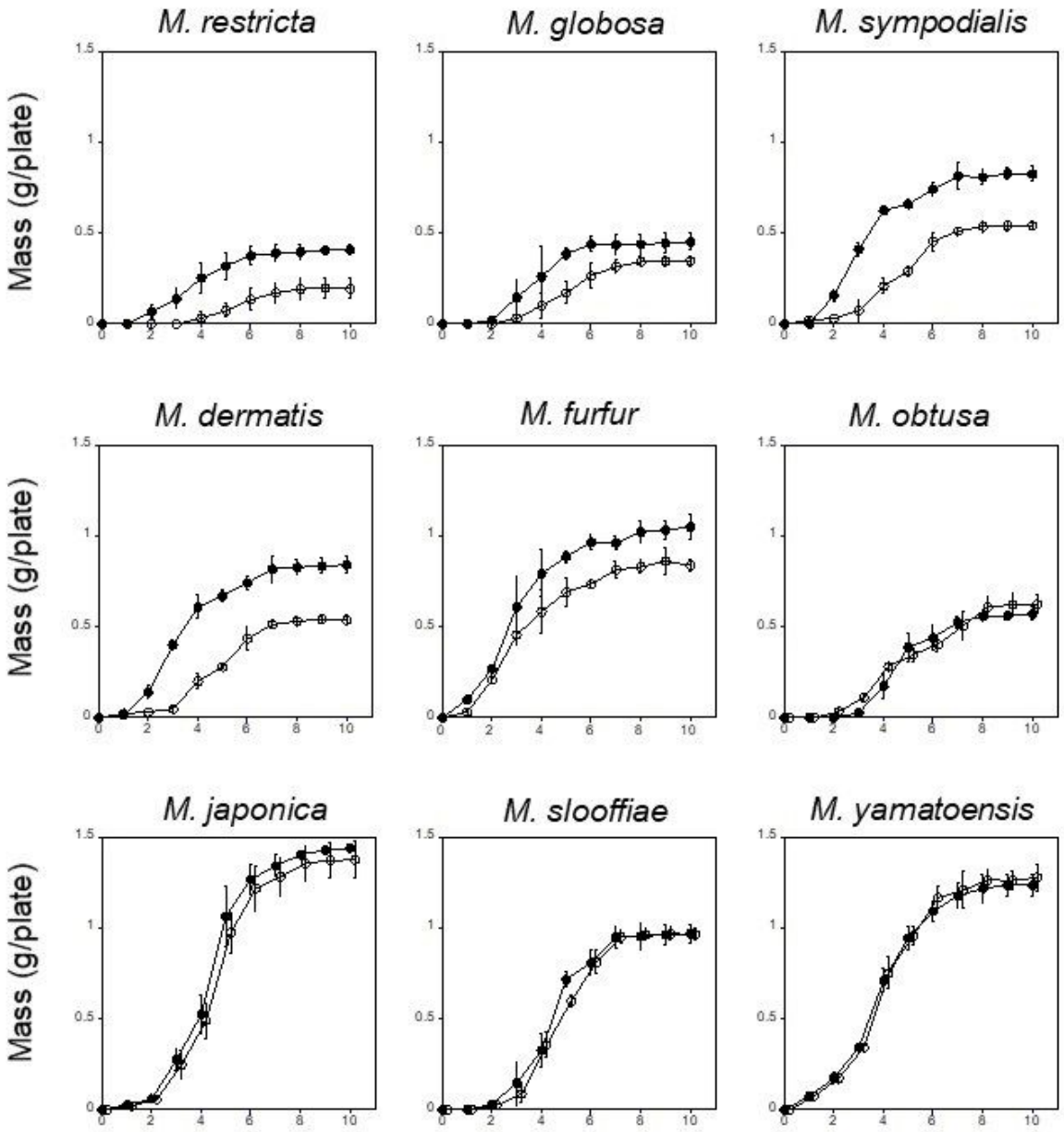

Figure 1

Growth curves of nine human-associated *Malassezia* species on artificial sebum-containing mLNA and mLNA.

Thirty-three strains of nine *Malassezia* species were grown at 32°C for 10 days, and the mass of each strain was measured daily. Closed circle, artificial sebum-containing mLNA; open circle, mLNA. The yields

are shown as the mean ± standard deviation.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.xlsx
- Tablev1.xlsx