Alveolarides: Antifungal Peptides from *Microascus alveolaris* Active against Phytopathogenic Fungi.

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Supporting Information

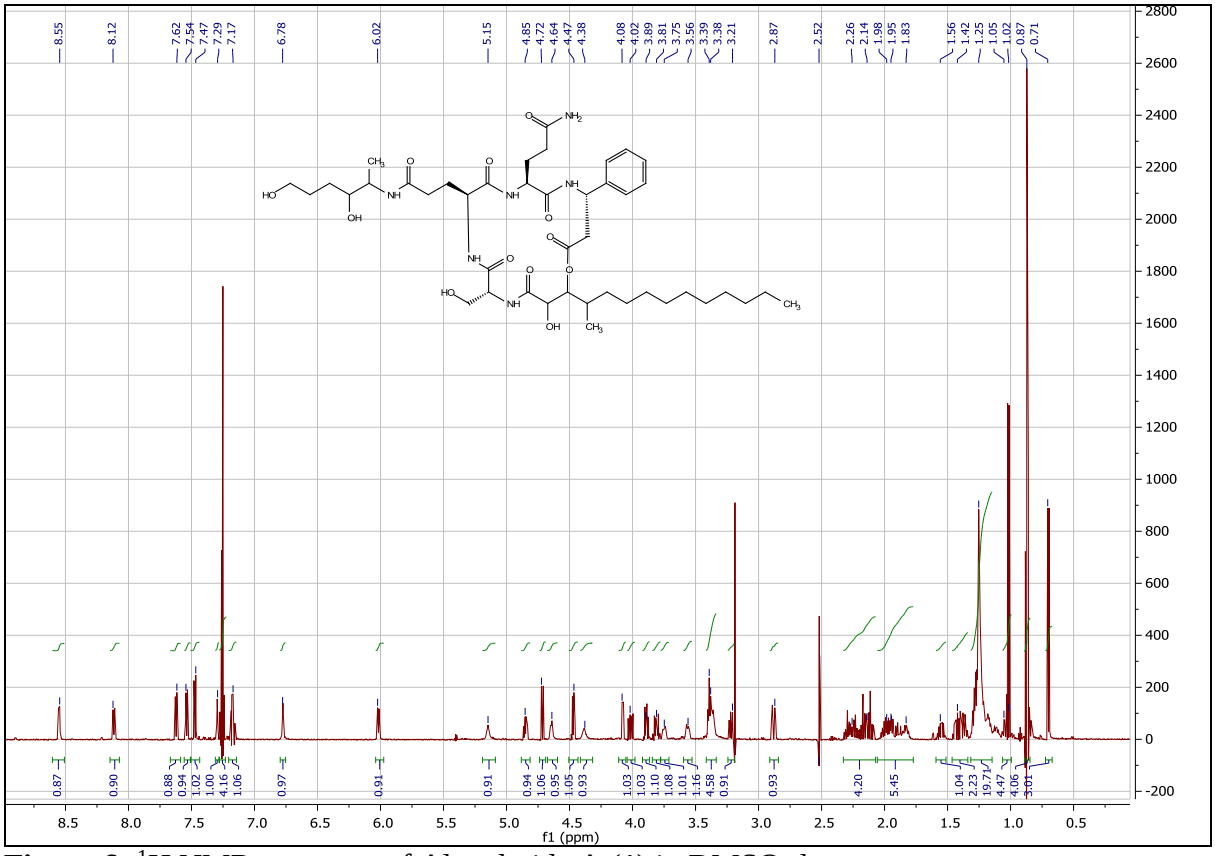

Figure 6: ^{1}H NMR spectrum of Alveolaride A (**1**) in DMSO-d_6

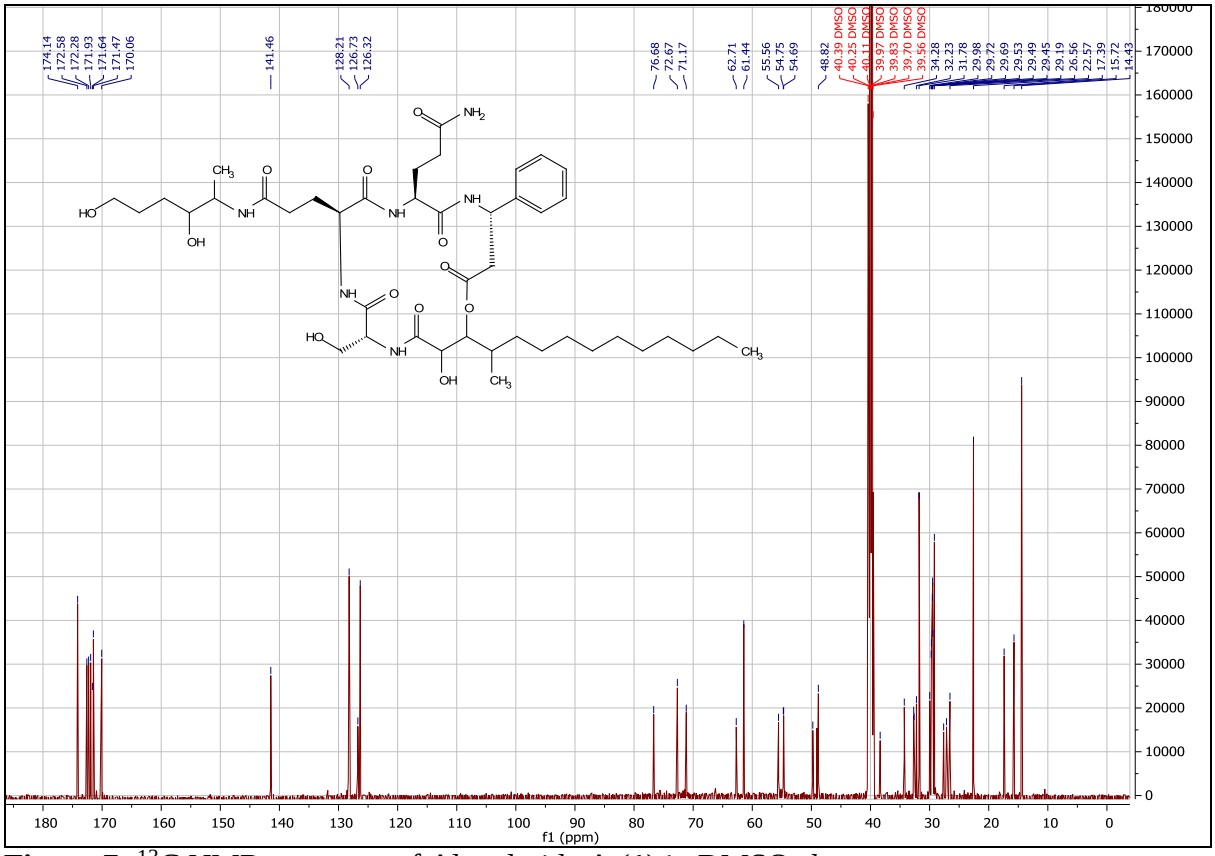

Figure 7: ^{13}C NMR spectrum of Alveolaride A (**1**) in DMSO-d_6

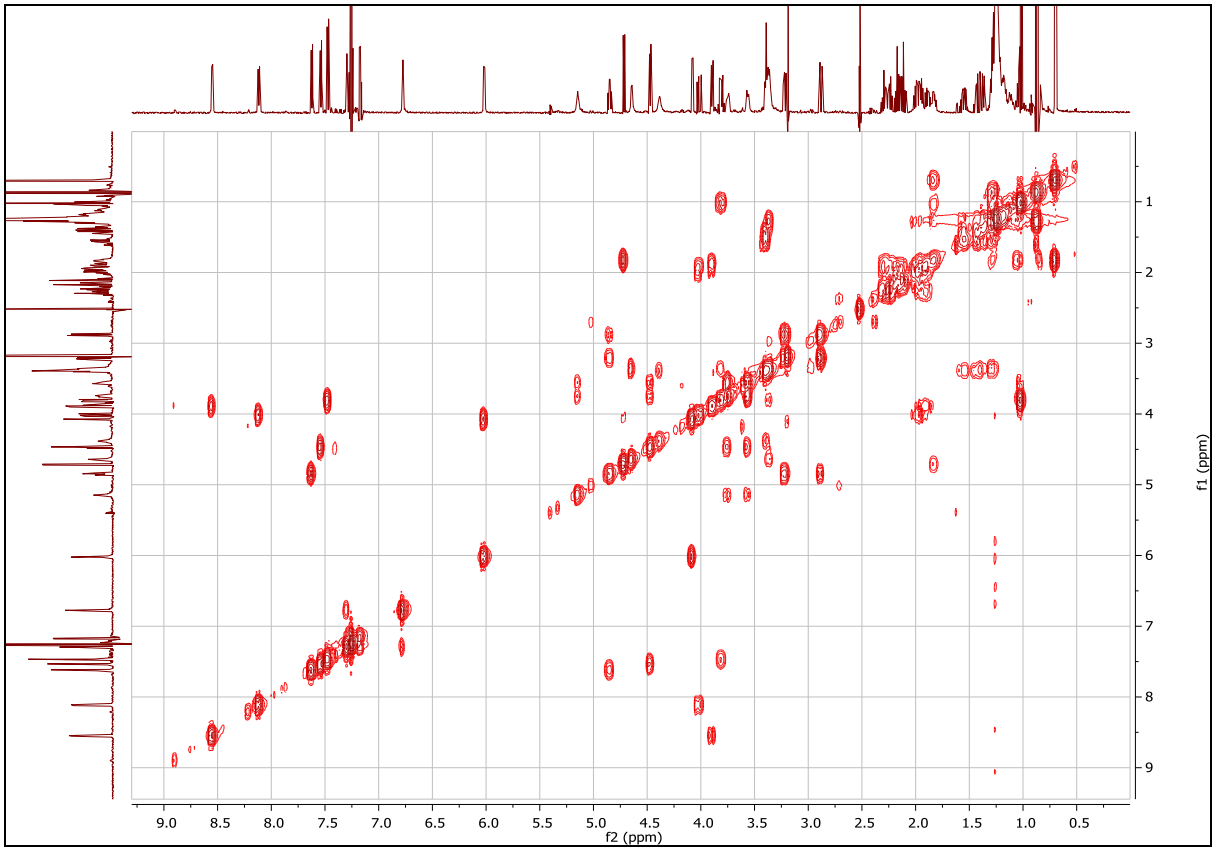

Figure 8: H-H COSY NMR spectrum of Alveolaride A (**1**) in DMSO-d_6

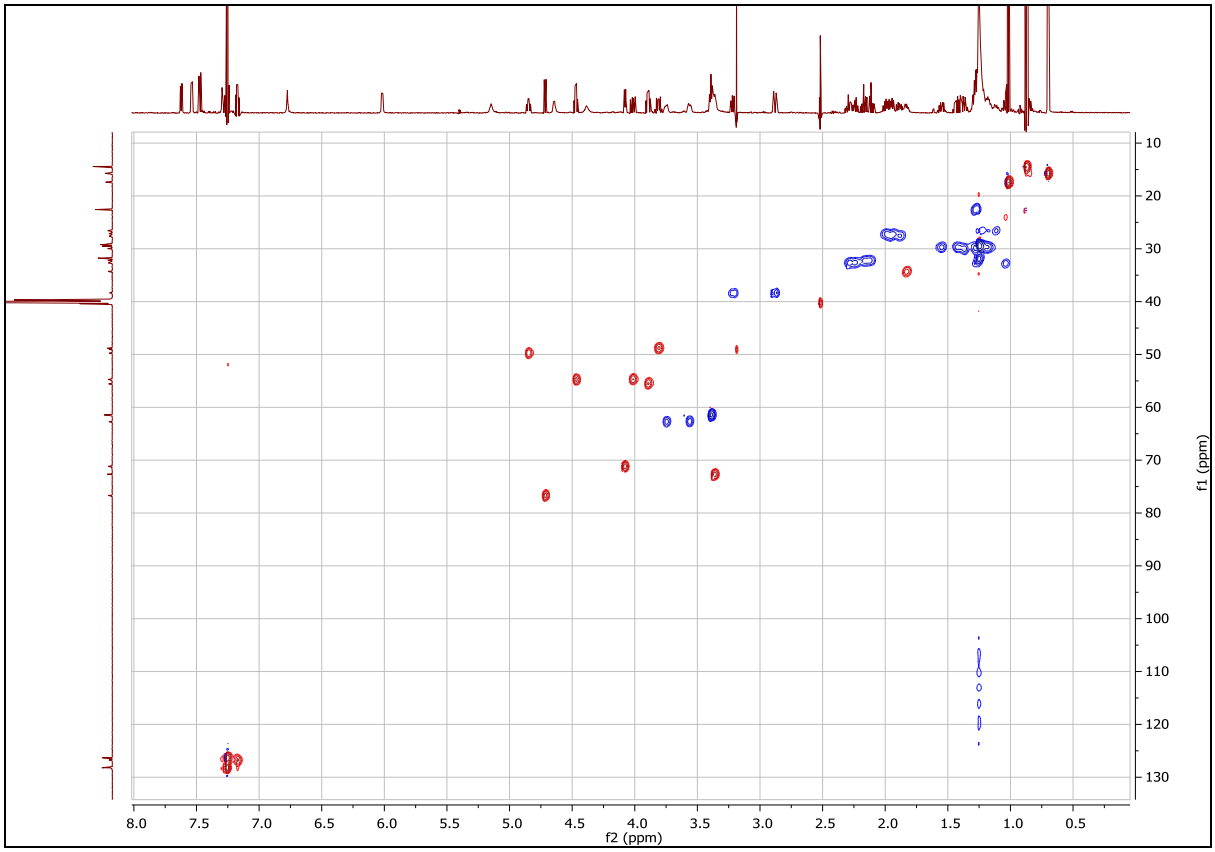

Figure 9: HSQC spectrum of Alveolaride A (**1**) in DMSO-d_6

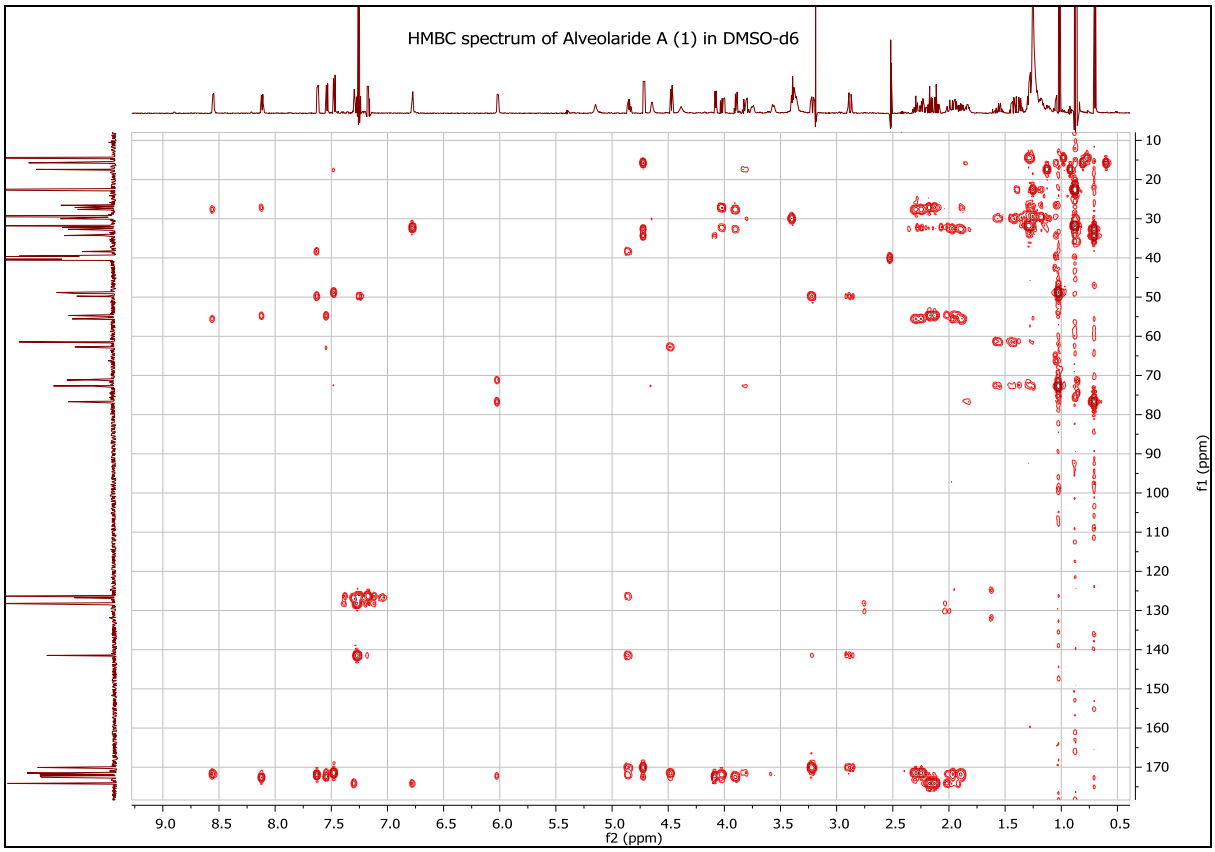

Figure 10: HMBC spectrum of Alveolaride A **(1)** in DMSO-d_6

Experimental Section

Alveolaride A tetra acetate (1a): $C_{51}H_{78}N_6O_{16}$, $[\alpha]_D^{25}$ -25.0 (*c* 0.04, MeOH); IR (neat) ν_{max} 3197.9, 2926.0, 2854.7, 1735.2, 1657.4, 1532.3, 1453.2, 1369.4, 1228.7, 1167.9, 1024.7, 952.3 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6): δ 8.72 (d, *J* = 5.5 Hz, 1H, 16-NH), 8.36 (d, *J* = 8.0 Hz, 1H, 11-NH), 7.77 (d, *J* = 8.9 Hz, 1H, 19-NH), 7.68 (d, *J* = 8.0 Hz, 1H, 3-NH), 7.42 (d, *J* = 8.0 Hz, 1H, 26-NH), 7.32 (s, *J* = 2.4 Hz, 1H, 1H, 14-NH_2,), 7.25 (m, 2H, H-5, H-9), 7.23 (m, 2H, H-6, H-8), 7.16 (m, 1H, 7-H), 6.79 (d, *J* = 2.4 Hz, 1H, 14-NH_2), 5.09 (d, *J* = 1.0 Hz, 1H, 29-H), 4.93 (m, 1H, 3-H), 4.84 (m, 1H, 26-H), 4.81 (m, 2H, 21-H, 30-H), 4.26 (dd, *J* = 11.1, 6.7 Hz, 1H, 27-H_a), 4.16 (dd, *J* = 11.1, 4.6 Hz, 1H, 1H, 27-H_b), 4.06 (m, 1H, 20-H), 4.03 (m, 1H, 11-H), 3.96 (q, *J* = 6.5 Hz, 1H, 24-H), 3.85 (dt, *J* = 9.2, 5.5 Hz, 1H, 16-H), 3.29 (dd, *J* = 17.0, 6.1 Hz, 1H, 2-H_a), 2.97 (dd, *J* = 17.0, 3.3 Hz, 1H, 2-H_b), 2.30 – 1.90 (m, 8H, 12-H, 13-H, 17-

H, 18-H), 1.67 (m, 1H, H-31), 1.53 (m, 4H, H-22, H-23), 1.24 (s, 16H, 33-H, 34-H, H-35, H-36, H-37, H-38, H-39, 40-H), 1.14/0.96 (m, 2H, H-32), 0.85 (t, *J* = 7.2 Hz, 3H, 41-H), 0.99 (d, *J* = 6.9 Hz, 3H, 20-CH_3), 0.62 (d, *J* = 6.7 Hz, 3H, 30-CH_3). ^{13}C NMR (151 MHz, DMSO): δ 174.0 (CO, C-14), 172.3 (CO, C-15), 172.1 (CO, C-10), 171.5 (CO, C-19), 170.8 (C, 24-*CO*CH_3), 170.7 (C, 21-*CO*CH_3), 170. 6 (C, 27-*CO*CH_3), 170.3 (C, 29-*CO*CH_3), 169.8 (CO, C-1), 169.7 (CO, C-25), 167.5 (CO, C-28), 141.4 (C, C-4), 128.2 (C, C6, C-8), 126.7 (CH, C-7), 126.1 (C, C-5, C-9), 75.3 (CH, C-30), 74.7 (CH_2, C-21), 73.0 (CH, C-29), 64.1 (CH_2, C-27), 64.0 (CH_2, C-24), 56.0 (CH, C-16), 55.3 (CH, C-11), 51.6 (CH, C-26), 48.9 (CH, C-3), 46.3 (CH, C-20), 38.2 (CH_2, C-2), 34.0 (CH, C-31), 32.4 (CH_2, C-18), 32.3 (CH_2, C-13), 32.2 (CH_2, C-32), 31.7 (CH_2, C-39), 29.45 (CH_2), 29.45 (CH_2), 29.40 (CH_2), 29.27(CH_2), 29.2 (CH_2), 27.6 (CH_2, C-17), 27.4 (CH_2, C-22), 27.1 (CH_2, C-12), 26.14, 24.7 (CH_2, C-23), 22.6 (CH_2, C-40), 21.54, 21.20, 21.16, 20.88, 17.5 (CH_3, C-20), 15.4 (CH_3, 31-CH_3), 14.42 (CH_3, C-41). (+)-ESI-HRMS *m/z*: 1031.5622 $[M+H]^+$ (1031.5547, calcd. for $C_{51}H_{79}N_6O_{16}^+$) , 1053.5381 $[M+Na]^+$ (1053.5367, calcd. for $C_{51}H_{78}N_6O_{16}Na^+$).

Hydrolysis Product of 1 : $C_{43}H_{72}N_6O_{13}$, $[\alpha]_D^{25}$ -12.3 (*c* 0.073, CH_3OH); ^{1}H NMR (600 MHz, DMSO-d_6): δ 12.27 (s, 1H, COOH), 8.39 (d, *J* = 8.3 Hz, 1H, NH-3), 7.95 (d, *J* = 8.0 Hz, 1H, 11-NH), 7.88 (d, *J* = 7.7 Hz, 1H, 16-NH), 7.85 (d, *J* = 7.7 Hz, 1H, 26-NH), 7.41 (d, *J* = 8.6 Hz, 1H, 20-NH), 7.36 – 7.28 (m, 4H, 5-H, 6-H, 9-H, 8-H), 7.24 (d, *J* = 2.8 Hz, 1H, 14-NH_2), 7.23 (m, 1H, =7-H), 6.79 (s, 1H, 14-NH_2), 5.59 (d, *J* = 6.6 Hz, 1H, 29-OH), 5.18 (m, 1H, 3-H), 5.13 (t, *J* = 5.5 Hz, 1H, 27-OH), 4.74 (d, *J* = 7.2 Hz, 1H, 30-OH), 4.62 (d, *J* = 5.7 Hz, 1H, 21-OH), 4.36 (brs, 1H, 24-OH), 4.23 (m, 1H, 26-H), 4.21 (m, 1H, 11-H), 4.17 (m, 1H, 16-H), 3.99 (dd, *J* = 6.4, 2.2 Hz, 1H, 29-H), 3.78 (m, 1H, 20-H) , 3.74 (m, 1H, 27-H_a), 3.60 (dt, *J* = 10.7, 4.8 Hz, 1H, 27-H_b), 3,43 (m, 1H, 30-H), 3.37 (m, 2H, 24-H), 3.33 (m, 1H, 21-H), 2.71 (dd, *J* = 7.3, 3.4 Hz, 2H, 2-H), 2.08 (m, 4H, 13-H, 18-H), 1.87 (m, 2H, 12-H), 1.73 (m, 2H, 17-H), 1.66

(m, 1H, 31-H), 1.53 (m, 2H, 23-H), 1.41/1.35 (m, 2H, 22-H), 1.38/1.05 (CH_2, 32-H), 1.27 (m, 40-H), 1.28 – 1.15 (m, 14H), 0.99 (d, *J* = 6.8 Hz, 3H, 20-CH_3), 0.91 (d, *J* = 6.7 Hz, 3H, 31-CH_3), 0.86 (t, *J* = 7.0 Hz, 3H, 41-H). ^{13}C NMR (151 MHz, DMSO) δ 174.3 (CO, C-28), 174.2 (CO, C-14), 172.2 (CO, C-1), 171.9 (CO, C-19), 171.4 (CO, C-15), 170.8 (CO, C-10), 170.6 (CO, C-25), 142.5 (C, C-4), 128.7 (2xCH, C-6, C-8), 127.4 (CH, C-7), 126.9 (2xCH, C-5, C-9), 76.1 (CH, C-30), 72.72 (CH, C-29), 72.68 (CH, C-21), 61.0 (CH2, C-27), 61.4 (CH2, C-24), 55.5 (CH, C-26), 52.92 (CH, C-16), 52.85 (CH, C-11), 49.8 (CH, C-3), 48.9 (CH, C-20), 41.2 (CH2, C-2), 35.3 (CH, C-31), 33.0 (CH_2, C-32), 32.4 (CH_2), 31.9 (CH_2), 31.8 (CH_2), 30.0 (CH_2) 29.7 (CH_2), 29.58 (CH_2, C-23), 29.54 (CH_2), 29.51 (CH_2), 29.2 (CH_2), 28.6 (CH_2, C-17), 28.3 (CH_2, C-12), 26.7 (CH_2), 22.6 (CH_2, C-40), 17.4 (CH_3, 20-CH_3), 15.7 (CH_3, 31-CH_3), 14.4 (CH_3, C-41). (+)-ESI-HRMS: *m/z*: 881.5239 $[M+H]^+$ (881.5230 calcd. for $C_{43}H_{73}N_6O_{13}^+$) , 903.5066 $[M+Na]^+$ (903.5050 calcd. for $C_{43}H_{72}N_6O_{13}Na^+$).

2,3-dihydroxy-4-methyltetradecanoic acid (DHMTDA): $C_{15}H_{30}O_4$, ^{1}H NMR (600 MHz, MeOH-d_4) δ 4.24 (d, *J* = 2.8 Hz, 1H, H-2), 3.59 (dd, *J* = 7.8, 2.8 Hz, 1H, H-3), 1.77 (m, 1H, H-4), 1.50 (m, 1H, H-5a), 1.25-1.45 (m, 19H), 1.19 (m, 1H, H-5b), 1.02 (d, *J* = 6.7 Hz, 3H, 4-C*H*$_3$), 0.93 (t, *J* = 7.0 Hz, 3H, H-14). ^{13}C NMR (151 MHz, MeOD) δ 175.6 (CO, C-1), 76.1 (CH, C-3), 71.5 (CH, C-2), 35.1 (CH, C-4), 32.8 (CH2, C-5), 31.7 (CH_2), 29.6 (CH_2), 29.4 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.1 (CH_2), 26.4 (CH_2), 22.3 (CH_2), 14.3 (4-*C*H_3), 13.0 (CH_3, C-14).

Identification of the Fungus PF1466

Fermentation

The strain PF1466 was inoculated in 500-ml Erlenmeyer flasks, each containing 100 ml of a seed medium (2.0% soluble starch, 1.0% glucose, 0.5% polypeptone, 0.6% wheat germ, 0.3% yeast extract, 0.2% soybean meal, pH 7.0 with NaOH). The flasks were incubated at 25°C for 3 days while shaking at 220 rpm. Aliquots of 3 ml of this seed cultured broth were transferred to 500-ml Erlenmeyer flasks containing 2 g of oatmeal and 80 g of water-absorbed brown rice as a solid production medium. The flasks were incubated statically at 25°C for 14 days.

Table 1. Cultural characteristics of *Microascus alveolaris* PF1466

	Growth in 2weeks at 25℃	Color [1)]
Potato dextrose agar	14～15 mm Velvety, with wrinkled and raised center	Surface: Olive (3F-8)～Olive yellow (3D-8), Grey (3C-1) Reverse: Yellowish grey (3C-2) Soluble pigment: -
2% Malt extract agar	6～12 mm Velvety, with wrinkled and raised center	Surface: Olive (3F-8)～Olive yellow (3D-8), Grey (3C-1) Reverse: Yellowish grey (3C-2) Soluble pigment: -
Oatmeal agar	37～40 mm velvety	Greenish grey (28F-2), White (28A-1)
LcA agar [2)]	25～27 mm velvety	Greenish grey (28F-2), White (28A-1)

1) Color notaions of the colonies were from Kornerup & Wanscher (1978).
2) MIURA's LcA medium

(Reference)
KORNERUP, A & J. H. WANSHER: Methuen Handbook of Colour, 1978
MIURA, K. & M. Y. KUDO: An agar-medium for aquiatic Hyphomycetes. Trans. Mycolo. Soc. Japan, 11: 116～118, 1970