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ARTICLE

Pullusurfactans A–E, new biosurfactants produced by *Aureobasidium pullulans* A11211-4-57 from a fleabane, *Erigeron annus* (L.) pers.

Jong-Shik Kim[1] · In-Kyoung Lee[2] · Bong-Sik Yun[2]

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Abstract

Yeast biosurfactants have potent applications in medical, cosmeceutical, and food industries due to their specific modes of action, low toxicity, and applicability. In this study, biosurfactant-producing yeasts were screened for various industrial applications. Among them, *Aureobasidium pullulans* strain A11211-4-57 with potent surfactant activity from fleabane flower, *Erigeron annus* (L.) pers., was selected. From culture supernatant of strain A11211-4-57, five new low-surface-tension chemicals designated as pullusurfactans A–E were identified through consecutive chromatography steps, involving ODS, silica gel, Sephadex LH-20, and ODS Sep-pak cartridge columns. Based on mass and NMR measurements, structures of pullusurfactans A–E were determined as *myo*-inositol lipids with molecular formulae of $C_{20}H_{35}O_9$, $C_{18}H_{32}O_8$, $C_{20}H_{35}O_9$, $C_{24}H_{42}O_9$, and $C_{18}H_{32}O_8$, respectively. These compounds exhibited potent biosurfactant activities (22.90, 22.40, 32.28, 25.28, and 22.44 mN/m, respectively). These results suggest that these novel biosurfactants have potential use as biosurfactants in industrial aspect.

Introduction

The progression in biotechnology suggests that biosurfactants derived from yeasts have many potential applications. They are advantageous over chemically synthesized surfactants in that they are biodegradable and relatively nontoxic or nonpathogenic, thus allowing for use in food and pharmaceutical industries [1–5]. Most surfactants currently used in industries are synthetic products chemically made from petroleum. More than about ten million surfactants have been manufactured worldwide via chemical synthesis. However, due to growing public concerns over environmental pollution, there is an imperative need to develop biosurfactants that can replace chemically synthesized surfactants. Yeast-derived biosurfactants have eco-friendly characteristics. They can be produced in large scales via fermentation. Thus, they can be applied to various fields, including oil recovery, medicines, foods, cosmetics, and percutaneous drug delivery system (DDS) [6]. Although various yeast-derived surfactants have been used, they have shown advantages due to their relatively low surfactant activities. Thus, there is still a need to develop biosurfactants with much stronger activities.

We isolated a biosurfactant-producing yeast strain identified as *A. pullulans* from fleabane flowers (*Erigeron annus* (L.) pers.). *A. pullulans* is a ubiquitous yeast [7, 8]. Recently, it has been reported that *A. pullulans* produces pullulan [9], β-glucan [10], (poly)malic acid [11], lipase [12], laccase [13], lipid composition [14, 15], siderophores [16], and biosurfactants [17–21]. This *A. pullulans* A11211-4-57 strain that we isolated in this study exhibited potent biosurfactant activity. In this report, we describe phylogenetic identification of this biosurfactant-producing yeast. We also report the isolation and structure determination of its active compounds pullusurfactans A–E (**1–5**, Fig. 1) as well as their biosurfactant activities.

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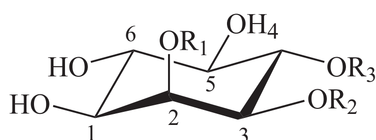
✉ Jong-Shik Kim
jskim@gimb.or.kr

✉ Bong-Sik Yun
bsyun@jbnu.ac.kr

[1] Gyeongbuk Institute for Marine Bio-industry, Uljin, Korea

[2] Division of Biotechnology and Advanced Institute of Environmental and Bioscience, College of Environmental and Bioresource Sciences, Chonbuk National University, Iksan-si, Korea



	R_1	R_2	R_3
1	Acetyl	Hexanoyl	Hexanoyl
2		Hexanoyl	Hexanoyl
3	Hexanoyl	Acetyl	Hexanoyl
4	Hexanoyl	Hexanoyl	
5	Hexanoyl	Hexanoyl	Hexanoyl

Fig. 1 Structures of pullusurfactans A–E (**1**–**5**)

Materials and methods

Reagents

Solvents including hexane, ethyl acetate, chloroform, and methanol used in each purifying step and column chromatography were purchased from SK Chemicals Co., Ltd. (Korea) and Daejung Chemical & Materials Co., Ltd. (Korea). HPLC solvents were purchased from Merck (Germany) and Baxter (Burdick & Jackson, USA). NMR solvents such as $CDCl_3$ were purchased from Sigma-Aldrich (USA). For the isolation and purification of surfactants, silica gel TLC (Merck, Kieselgel 60 F, 70–230 mesh, USA), ODS TLC (Merck, RP-18, F_{254}, USA), Sephadex LH-20 (Pharmacia, bead size 25–100 µm, Sweden), and ODS Sep-pak cartridge (Alltech, RP-18, USA) were employed.

Screening of biosurfactant-producing yeast

Fleabane is an annual or biennial herb of Compositae. It is a North America native plant that often colonizes disturbed areas such as pastures, vacant fields, waste areas, roadsides, and railways. Sampling area is located at N 36° 59′ 54.49″ and E 129° 24′ 17.28″. Sampling and treatment of fleabane flower (*Erigeron annus* (L.) pers.) and screening of biosurfactant-producing yeasts were performed as described previously [17, 18]. The selected yeasts were deposited at Korean Culture Center of Microorganisms (KCCM) on February 7, 2013 (Accession No. KCCM11373P).

Phylogenetic analysis of *A. pullulans* A11211-4-57

Sequencing of 18S rRNA and fatty acid elongase (*ELO*) genes from *A. pullulans* A11211-4-57 strain was performed as previously described [17, 18]. Nucleotide sequences reported in this paper were deposited at DDBJ/GenBank under the following accession numbers: AB746227 (18 S rRNA) and AB746303 (*ELO*). Alignment for sequences of 18S rRNA and *ELO* genes was performed using Clustal Omega (EMBL-EBI website). Phylogenetic trees were constructed by neighbor-joining (NJ) method using MEGA5 for Windows by repeating the analysis on 1000 bootstrap samples [22]. Kimura 2-parameter genetic distance was calculated [23].

Structure determination

FAB-mass and high-resolution FAB-mass spectra were measured on a JEOL JMS-700 MSTATION mass spectrometer (Japan) using glycerol or *m*-nitrobenzyl alcohol as a matrix. For high-resolution FAB-mass, polyethylene glycol was used as an internal standard. Nuclear magnetic resonance (NMR) spectra were obtained on a JEOLJNM-ECA600, 600 MHz FT-NMR Spectrometer at 600 MHz for ^{1}H NMR and at 150 MHz for ^{13}C NMR in $CDCl_3$. Chemical shifts are given in ppm (δ) with tetramethylsilane as the internal standard. For NMR spectra, two-dimensional NMR such as ^{1}H-^{1}H COSY, HMQC, and HMBC as well as one-dimensional NMR such as ^{1}H NMR and ^{13}C NMR were employed.

Surfactant activity

Surface tension variation of the purified biosurfactants was determined using a Du Noüy ring tensiometer (Sigma Model 700 instrument, KSV Instruments Ltd., Helsinki, Finland) which is submersed in a liquid. As the ring is pulled out of the liquid, the force required is measured to determine the surface tension of the liquid.

Result and discussion

Phylogenetic identification of a yeast strain A11211-4-57

We isolated a yeast strain A11211-4-57 with potent surface activity from a fleabane, *Erigeron annus* (L.) pers. through screening cultured yeast isolates on agar plates from homogenized flower samples using drop-collapse test as described previously [17, 18]. The phylogenetic analysis and NCBI BLAST search of 18S rRNA gene sequence of biosurfactant producer A11211-4-57 showed that this isolate was an ascomycetes yeast belonging to family Dothioraceae. The yeast was identified to belong to species *A. pullulans* on the basis of high similarity to *A. pullulans* DSM62074 (99%, 1671/1681), *A. pullulans* DSM3497 (99%, 1671/1681), and *A. pullulans* DSM3497 (99%, 1671/1681) (Fig. 2). To determine interspecies that this strain belonged to, additional analysis of *ELO* gene of A11211-4-

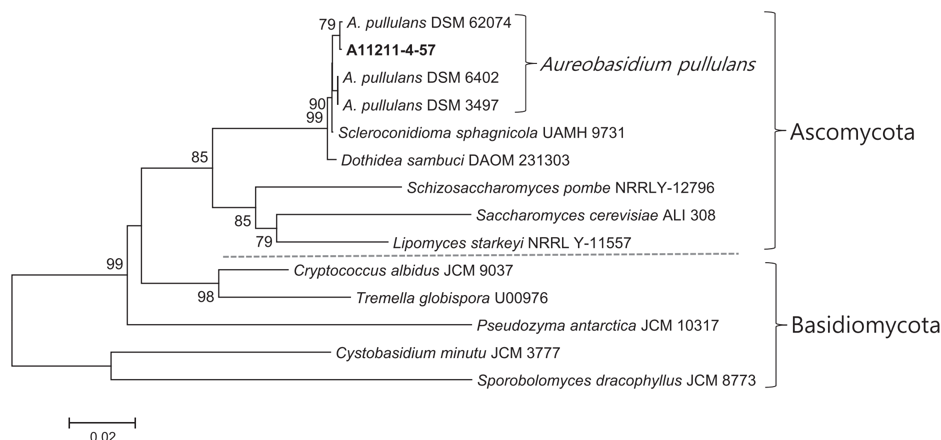


Fig. 2 Phylogenetic trees of 18S *rRNA* gene from *A. pullulans* A11211-4-57 with reference sequences. Bootstrap cutoff values of 75% are noted at branch junctions

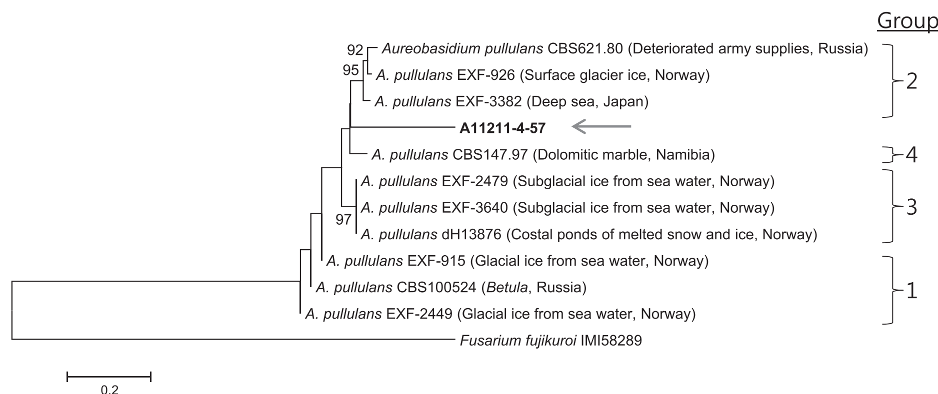


Fig. 3 Phylogenetic trees of fatty acid elongase gene (*ELO*) from *A. pullulans* A11211-4-57 with reference sequences. Bootstrap cutoff values of 75% are noted at branch junctions

57 was performed. Results revealed that this yeast strain did not belong to any known Group of *A. pullulans* interspecies (Fig. 3). Phylogenetic analyses of both genes suggested that *A. pullulans* A11211-4-57 should be classified as an unknown group based on Zalar et al. [24]. Recently, studies on biosurfactants from *A. pullulans* have been extended [17–21]. The present report reveals that *A. pullulans* also produces five novel biosurfactants.

Isolation and purification

Culture broth (about 25 liters) of *A. pullulans* A11211-4-57 was lyophilized and dissolved in water. The resulting solution was partitioned with hexane (18 L) and ethyl acetate (18 L) consecutively. Surfactant activity was detected in the ethyl acetate-soluble portion. This ethyl acetate-soluble portion was dried over magnesium sulfate anhydrous, concentrated under reduced pressure, and separated on a column of silica gel (Sigma-Aldrich, St. Louis, MO, USA), and eluted with an increasing amount of methanol in chloroform ($CHCl_3$:MeOH, 50:1 to 2:1, v/v, stepwise). Three fractions of $CHCl_3$:MeOH (50:1, v/v), $CHCl_3$:MeOH (20:1, v/v), and $CHCl_3$:MeOH (10:1, v/v) showed significant surfactant activities. Fraction $CHCl_3$:MeOH (20:1, v/v) was divided into two active fractions by reversed-phase octadecyl-silica (ODS) column chromatography eluted with 60–100% aqueous methanol. One fraction was subjected to Sephadex LH-20 column chromatography using 70% aqueous methanol as an eluting solvent followed by silica gel column chromatography using chloroform: methanol (40:1 to 10:1, v/v, stepwise) to provide compounds **1** (30 mg), **2** (15 mg), and **3** (5 mg). The other fraction was subjected to Sephadex LH-20 column chromatography using 70% aqueous methanol followed by preparative silica gel TLC using chloroform: methanol (10:1, v/v) to afford compound **4** (8 mg). An active fraction $CHCl_3$:MeOH (50:1, v/v) was separated by reversed-phase ODS column chromatography eluted with 60–100% aqueous methanol. Active fractions were combined and subjected to Sephadex LH-20 column chromatography eluted with 70% aqueous methanol followed by silica gel column chromatography eluted with $CHCl_3$:MeOH (40:1 to 20:1, v/v, stepwise) to provide compound **5** (18 mg). Active fraction $CHCl_3$:MeOH (10:1, v/v) was separated by reversed-phase ODS column chromatography eluted with 50–100% aqueous methanol. An active fraction was subjected to Sephadex LH-20 column chromatography eluted with 70% aqueous methanol followed by silica gel column chromatography eluted with $CHCl_3$:MeOH (40:1 to 20:1, v/v, stepwise) to provide compound **2** (64 mg). Isolation and purification procedures

Table 1 ^{1}H and ^{13}C NMR spectral data for compounds **1–5** in $CDCl_3$

No.	1		2		3		4		5	
	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)
1	69.7	3.73 (1H, br. d, $J=10.3$)	71.3	3.61 (1H, br. d, $J=8.9$)	69.8	3.73 (1H, br. d, $J=8.9$)	69.7	3.80 (1H, br. d, $J=8.9$)	69.7	3.71 (1H, dd, $J=10.0$, 2.4)
2	70.7	5.52 (1H, dd, $J=2.7$, 2.7)	70.2	4.20 (1H, br. s)	70.2	5.56 (1H, dd, $J=2.8$, 2.8)	70.8	5.49 (1H, br. s)	70.4	5.53 (1H, br. s)
3	69.2	4.94 (1H, dd, $J=10.3$, 2.7)	71.2	4.85 (1H, dd, $J=10.3$, 2.1)	69.5	4.94 (1H, dd, $J=10.3$, 2.8)	71.4	4.85 (1H, br d, $J=8.3$)	69.3	4.93 (1H, dd, $J=10.3$, 2.7)
4	71.4	5.26 (1H, dd, $J=10.3$, 9.6)	71.8	5.34 (1H, dd, $J=10.3$, 9.6)	71.5	5.28 (1H, dd, $J=10.3$, 9.6)	71.2	3.79 (1H, dd, $J=9.6$, 8.3)	71.4	5.26 (1H, dd, $J=10.3$, 9.6)
5	72.8	3.53 (1H, dd, $J=9.6$, 9.6)	72.9	3.50 (1H, dd, $J=9.6$, 9.6)	73.1	3.54 (1H, dd, $J=9.6$, 9.6)	74.4	3.50 (1H, dd, $J=9.6$, 9.6)	72.9	3.52 (1H, dd, $J=9.6$, 9.6)
6	73.3	3.80 (1H, dd, $J=10.3$, 9.6)	72.8	3.86 (1H, dd, $J=9.6$, 8.9)	73.5	3.81 (1H, dd, $J=9.6$, 8.9)	73.1	3.70 (1H, dd, $J=9.6$, 8.9)	73.4	3.78 (1H, dd, $J=10.0$, 9.6)
1′	170.7				173.5		173.5		173.5	
2′	20.8	2.14 (3 H, s)			34.1	2.41 (2 H, t, $J=7.3$)	34.1	2.27 (1H, m), 2.24 (1H, m)	34.0	2.39 (2 H, t, $J=7.6$)
3′					24.7	1.64 (2 H, m)	24.3	1.57 (2 H, m)	24.6	1.62 (2 H, m)
4′					31.1	1.32 (2 H, m)	31.2	1.25 (2 H, m)	31.2	1.31 (2 H, m)
5′					22.3	1.32 (2 H, m)	22.3	1.20–1.34 (2 H, m)	22.3	1.20–1.34 (2 H, m)
6′					13.9	0.88 (3 H, t, $J=7.2$)	14.1	0.88 (3 H, t, $J=7.2$)	13.9	0.84–0.90 (3 H, overlapped)
1″	172.9		173.1		170.0		173.8		172.8	
2″	33.9	2.20 (1H, m), 2.18 (1H, m)	34.3	2.27 (1H, m), 2.25 (1H, m)	20.6	1.96 (3 H, s)	34.0	2.38 (1H, m), 2.35 (1H, m)	33.9	2.18 (1H, m), 2.16 (1H, m)
3″	24.3	1.52 (2 H, m)	24.6	1.56 (2 H, m)			24.6	1.59 (2 H, m)	24.3	1.52 (2 H, m)
4″	31.1	1.24 (2 H, m)	31.3	1.26 (2 H, m)			31.2	1.30 (2 H, m)	31.1	1.25 (2 H, m)
5″	22.2	1.28 (2 H, m)	22.3	1.28 (2 H, m)			22.3	1.20–1.34 (2 H, m)	22.2	1.20–1.34 (2 H, m)
6″	13.8	0.87 (3 H, t, $J=7.2$)	13.9	0.87 (3 H, t, $J=6.9$)			13.9	0.87 (3 H, t, $J=7.2$)	13.8	0.84–0.90 (3 H, overlapped)
1‴	173.7		174.1		173.8				173.6	
2‴	34.2	2.33 (1H, m), 2.28 (1H, m)	34.0	2.34 (1H, m), 2.32 (1H, m)	34.2	2.33 (1H, m), 2.30 (1H, m)			34.2	2.32 (1H, m), 2.27 (1 H, m)
3‴	24.6	1.58 (2 H, m)	24.5	1.56 (2 H, m)	24.7	1.59 (2 H, m)			24.6	1.57 (2 H, m)
4‴	31.2	1.25 (2 H, m)	31.3	1.26 (2 H, m)	31.1	1.29 (2 H, m)			31.2	1.27 (2 H, m)
5‴	22.3	1.28 (2 H, m)	22.3	1.28 (2 H, m)	22.3	1.29 (2 H, m)			22.3	1.20–1.34 (2 H, m)
6‴	13.9	0.87 (3 H, t, $J=7.2$)	13.9	0.87 (3 H, t, $J=6.9$)	13.9	0.87 (3 H, t, $J=7.2$)			13.9	0.84–0.90 (3 H, overlapped)

NMR spectra were recorded at 600 MHz for ^{1}H and 150 MHz for ^{13}C

Proton resonance integral, multiplicity, and coupling constant (J = Hz) in parentheses

for biosurfactants **1–5** are depicted in Supplementary Information.

Structure determination

Chemical structures of compounds **1–5** were determined by mass and NMR measurements. The molecular weight of compound **1** was determined to be 418 by FAB-MS measurement which provided quasi-molecular ion peaks at *m/z* 419.2 $[M + H]^+$ and 441.2 $[M + Na]^+$ in positive mode. Its molecular formula was established to be $C_{20}H_{34}O_9$ by high-resolution FAB-MS measurement (*m/z* 419.2252 $[M + H]^+$, Δ −2.9 mmu) in combination with ^{1}H and ^{13}C NMR data. This molecular formula dictates four degrees of unsaturation. The ^{1}H NMR spectrum of **1** measured in $CDCl_3$ showed signals at δ 5.52, 5.26, 4.94, 3.80, 3.73, and 3.53 due to six oxygenated methines. It also showed signals at δ 2.33/2.28, 2.20/2.18, 1.58, 1.52, and 1.2–1.4 belonging to eight methylenes and signals at δ 2.14, 0.87, and 0.87 corresponding to three methyls. Three hydroxyl protons at δ 4.59, 4.05, and 3.97 were also observed. In ^{13}C NMR spectrum, a total of 20 carbon peaks were observed, including three ester carbonyl carbons at δ 173.7, 172.9, and 170.7, six oxygenated methine carbons at δ 73.3, 72.8, 71.4, 70.7, 69.7, and 69.2, eight methylene carbons at δ 34.2, 33.9, 31.2, 31.1, 24.6, 24.3, 22.3, and 22.2, and three methyl carbons at δ 20.8, 13.9, and 13.8 (Table 1). Correlations between oxygenated methine protons in the ^{1}H–^{1}H COSY spectrum suggested the presence of an inositol moiety. It was found that, except the proton at δ 5.52 with coupling constant of 2.7 Hz, the rest of protons occupied an axial position based on their proton coupling constants. From these results, the inositol moiety was identified as a *myo*-inositol. Further, four partial structures in an acyl chain were identified. HMQC spectrum unambiguously assigned all proton-bearing carbons (Table 1). The structure of **1** was determined by HMBC spectrum which provided long-range correlations from oxygenated methine protons at δ 5.52, 4.94, and 5.26 to ester carbonyl carbons at δ 170.7, 172.9, and 173.7, respectively. These correlations revealed that C-2, C-3, and C-4 in inositol were acylated. The long-range correlation from a methyl proton at δ 2.14 to the carbonyl carbon at δ 170.7 connected an acetyl group to C-2. The long-range correlations from two methyl protons at δ 0.87 to methylene carbons at δ 31.1 and 31.2, respectively, from the methylene proton at δ 1.52 to an ester carbonyl carbon at δ 172.9 and a methylene carbon at δ 31.1, and from the methylene proton at δ 1.58 to an ester carbonyl carbon at δ 173.7 and a methylene carbon at δ 31.2 revealed the presence of two hexanoyl moiety as shown in Fig. 4. Consequently, the structure of compound **1** was determined to be a new *myo*-inositol derivative acylated by one acetyl and two hexanoyl groups.

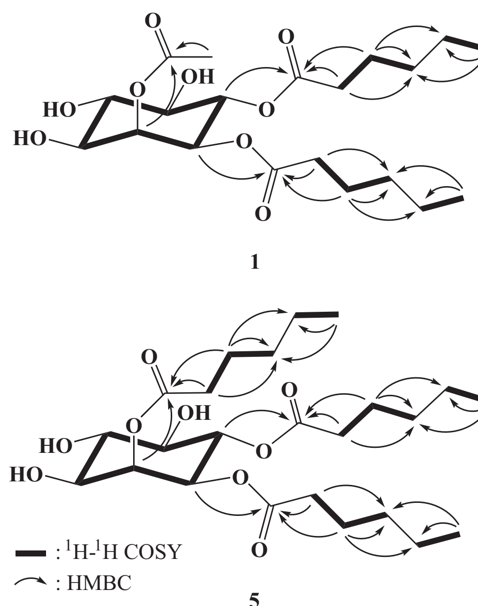


Fig. 4 Two-dimensional NMR correlations of pullusurfactans A and E

The molecular weight of compound **2** was determined to be 376 by FAB-MS measurement which provided a quasi-molecular ion peak at *m/z* 399.2 $[M + Na]^+$ in positive mode. Its molecular formula was established to be $C_{18}H_{32}O_8$ by high-resolution FAB-MS measurement (*m/z* 399.2012 $[M + Na]^+$, Δ + 1.7 mmu). ^{1}H and ^{13}C NMR spectra of compound **2** measured in $CDCl_3$ were very similar to those of **1** except that signals attributed to an acetyl group in compound **1** disappeared in compound **2** (Table 1). Two-dimensional NMR spectra including ^{1}H–^{1}H COSY, HMQC, and HMBC established the structure of compound **2**. HMBC spectrum provided long-range correlations from oxygenated methine protons at δ 4.85 (H-3) and 5.34 (H-4) to ester carbonyl carbons at δ 173.1 and 174.1, respectively. These correlations revealed that C-3 and C-4 positions in *myo*-inositol were acylated by hexanoyl moiety, respectively.

The molecular formula of compound **3** was established as $C_{20}H_{34}O_9$ by high-resolution FAB-MS measurement (*m/z* 419.2255 $[M + H]^+$, Δ −2.5 mmu), which was the same as that of compound **1**. ^{1}H and ^{13}C NMR spectra of compound **3** measured in $CDCl_3$ resembled that of compound **1** (Table 1). The structure of **3** was also assigned by two-dimensional NMR spectra including ^{1}H-^{1}H COSY, HMQC, and HMBC. Its partial structures, one inositol, one acetyl, and two hexanoyl, were elucidated by ^{1}H-^{1}H COSY spectrum which was the same as that of compound **1**. HMBC spectrum provided long-range correlations from oxygenated methine protons at δ 5.56, 4.94, and 5.28 to ester carbonyl carbons at δ 173.5, 170.0, and 173.8, respectively. They were assigned as carbonyl carbons of hexanoyl, acetyl, and hexanoyl, respectively, based on HMBC correlations. Thus,

the structure of compound **3** was determined to be a new myo-inositol derivative with 3-acetyl and 2,4-dihexanoyl groups.

The molecular formula of compound **4** was established as $C_{18}H_{32}O_8$ by high-resolution FAB-MS measurement (*m/z* 399.1998 $[M+Na]^+$, $\Delta+0.3$ mmu), which was the same as that of compound **2**. ^1H and ^{13}C NMR spectra of compound **4** measured in $CDCl_3$ resembled that of compound **2** (Table 1). ^1H–^1H COSY spectrum suggested three partial structures: one *myo*-inositol and two hexanoyl groups. HMBC spectrum provided long-range correlations from oxygenated methine protons at δ5.49 (H-2) and 4.85 (H-3) to ester carbonyl carbons at δ173.5 and 173.8, respectively. These correlations revealed that compound **4** was a new *myo*-inositol derivative acylated at C-2 and C-3 positions by hexanoyl, respectively.

The molecular weight of compound **5** was determined to be 418 by FAB-MS measurement which provided quasi-molecular ion peaks at *m/z* 475.3 $[M+H]^+$ and 497.3 $[M+Na]^+$ in positive mode. Its molecular formula was established to be $C_{24}H_{42}O_9$ by high-resolution FAB-MS measurement (*m/z* 497.2705 $[M+Na]^+$, Δ −2.1 mmu) in combination with ^1H and ^{13}C NMR data. The ^1H NMR spectrum of compound **5** measured in $CDCl_3$ exhibited six oxygenated methine protons at δ 5.53, 5.26, 4.93, 3.78, 3.71, and 3.52 attributed to *myo*-inositol, 12 methylene protons at δ 2.39, 2.32/2.27, 2.18/2.16, 1.62, 1.57, 1.52, 1.31, 1.27, 1.25, and 1.20–1.34, and three methyl protons at δ 0.84–0.90. In ^{13}C NMR spectrum, 24 carbon peaks including three ester carbonyl carbons at δ 173.6, 173.5, and 172.8, six oxygenated methine carbons at δ 73.4, 72.9, 71.4, 70.4, 69.7, and 69.3, 12 methylene carbons at δ 22–35, and three methyl carbons at δ 13.9, 13.9, and 13.8 were evident (Table 1). Its partial structures due to one *myo*-inositol and three acyl chains were assigned by ^1H–^1H COSY spectrum as shown in Fig. 4. These partial structures were connected by a HMBC spectrum which provided long-range correlations from oxygenated methine protons at δ 5.53, 5.26, and 4.93 to ester carbonyl carbons at δ 173.5, 173.6, and 172.8, respectively. These results revealed that C-2, C-3, and C-4 in inositol were acylated. Three acyl groups were confirmed as all hexanoyl by long-range correlations from three methyl protons at δ 0.84–0.90 to methylene carbons at δ 31.2, 31.2, and 31.1, respectively, from the methylene proton at δ 1.62 to an ester carbonyl carbon at δ 173.5 and a methylene carbon at δ 31.2, from the methylene proton at δ 1.57 to an ester carbonyl carbon at δ 173.6 and a methylene carbon at δ 31.2, and from the methylene proton at δ 1.52 to an ester carbonyl carbon at δ 172.8 and a methylene carbon at δ 31.1 (Fig. 4). Consequently, the structure of compound **5** was determined to be a new *myo*-inositol derivative acylated at C-2, C-3, and C-4 positions by three hexanoyl groups.

Surface tension properties

Compounds **1–5** at 1 mg/L exhibited low surface tension (22.90, 22.40, 32.28, 25.28, and 22.44 mN/m) compared to water (72.8 mN/m). Results also revealed that compounds **1**, **2**, **4**, and **5** exhibited lower surface tension than other surfactants such as aureosurfactin (29.5 mN/m at 1.0 mg/L) and glycerol-liamocin (31.5 mN/m at 1.5 mg/L) [17, 18].

In conclusion, we isolated five new compounds (pullusurfactans A–E) with potent biosurfactant activities from culture broth of *A. pullulans* A11211-4-57, a yeast-like fungus isolated from a fleabane flower. Pullusurfactans A–E were purified from the culture filtrate and their chemical structures were determined to be myo-inositol lipids based on extensive mass and NMR measurements. Pullusurfactans exhibited potent surfactant activity. In preliminary test for toxicity, pullusurfactan complex showed no cytotoxicity up to 50 ppm against HeLa and SH-SY5Y cell lines. Their potent biosurfactant activities suggest that these novel biosurfactants have potential use in industrial aspect. Pullusurfactan complex is under development as a natural surfactant for cosmetics.

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Conflict of interest The authors declare that they have no conflict of interest.

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