

ORIGINAL ARTICLE

# Screening and characterization of microorganisms with FR901379 acylase activity

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A simple and rapid screen was performed for microorganisms producing cyclic lipopeptide acylase, an enzyme that deacylates the acyl side chain of FR901379 to yield a cyclic peptide, FR179642, which is an important intermediate in producing micafungin. Among ~3800 newly isolated strains from soil samples, 5 microorganisms expressing high acylase activity were selected and classified, 3 as *Streptomyces* spp. and 2 as filamentous fungi. This is the first time that these strains have been identified as cyclic lipopeptide acylase producers. Both fungi and streptomycetes showed differing optimum pH and temperature profiles for acylase activity.

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**Keywords:** acylase; echinocandin; FR901379; screening

## INTRODUCTION

FR901379,<sup>1</sup> a product of the fungus *Coleophoma empetri* F-11899, is an echinocandin-like compound that inhibits the synthesis of 1,3- $\beta$ -D-glucan in the fungal cell wall. This compound has good water solubility due to sulfate ester moiety on the homophenylalanine group, whereas other echinocandin-like compounds, such as echinocandin B, aculeacin, mulundocandin and pneumocandin are barely water soluble. This insolubility is one of the reasons why they have not been developed for clinical use. In contrast, FR901379 shows significant hemolytic activity as a result of its long acyl chain and is therefore also inappropriate for clinical use. Therefore, we attempted to produce improved derivatives by substituting the acyl chain; to achieve this, production of a key intermediate, FR179642 or deacylated FR901379, was critical. Acyl peptides are generally unstable to chemical deacylation and therefore enzymatic deacylation is widely applied. Therefore, we used bioconversion to obtain FR179642 (Figure 1). In the past, deacylated products were produced by enzymatic deacylation using penicillin G acylase,<sup>2</sup> cephalosporin C acylase,<sup>3</sup> echinocandin B acylase<sup>4</sup> or aculeacin A acylase.<sup>5</sup> At first, we used acylase produced by *Actinoplanes utahensis* that was well known for removing acyl side chains from echinocandins. However, we had to change the procedure to use a more specific acylase with higher potency toward FR901379 because a large quantity of the deacylated FR901379 was required to synthesize the derivatives for optimizing the side chain. Therefore, we undertook a screening process to identify a novel lipopeptide acylase to remove the acyl side chain (palmitoyl) from FR901379 and, as a result, discovered several strains that showed this acylase activity.

In this study, we describe the screening process for FR901379 acylase-producing strains and their subsequent taxonomic characterization, as well as some comparisons of enzymatic activity with the known lipopeptide acylase-producing microorganism.

## MATERIALS AND METHODS

### Reagents and microorganisms

FR901379 and echinocandin B were prepared from the fermentation broth of *C. empetri* F-11899<sup>1</sup> and *Aspergillus nidulans* var. *echinulatus* A-32204, NRRL 3860,<sup>6</sup> respectively. Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan).

### Screening for FR901379 acylase-producing microorganisms

*Microorganisms.* Approximately 3300 actinomycetes and 500 fungi isolated from soil samples collected from various parts of Japan and stored at Fermentation Research Laboratories, Astellas Pharma (Aichi, Japan) were examined.

*Assay method.* As the first screening step, microorganisms were grown on agar supplemented with 1 mg ml<sup>-1</sup> of FR901379. Once colonies reached a diameter of 5–10 mm, the plate was overlaid with agar medium containing 1 × 10<sup>3</sup> per ml cells of *Candida albicans* Yu-1200 and incubated at 30 °C for 16 h. The candidate microorganism producing FR901379 acylase was identified as the colony allowing growth of *C. albicans* (Figure 2). This method is based on the observation that *C. albicans* is sensitive to FR901379, but is insensitive to its deacylated compound FR179642. The second step consisted of enzymatic hydrolysis of FR901379 by the cultured broth of the candidate microorganism, and determination of the production of FR179642 by HPLC.

For the second screening step, actinomycetes were inoculated into the medium (glucose 4%, yeast extract 1%, KH<sub>2</sub>PO<sub>4</sub> 0.1%, pH adjusted to 6.5

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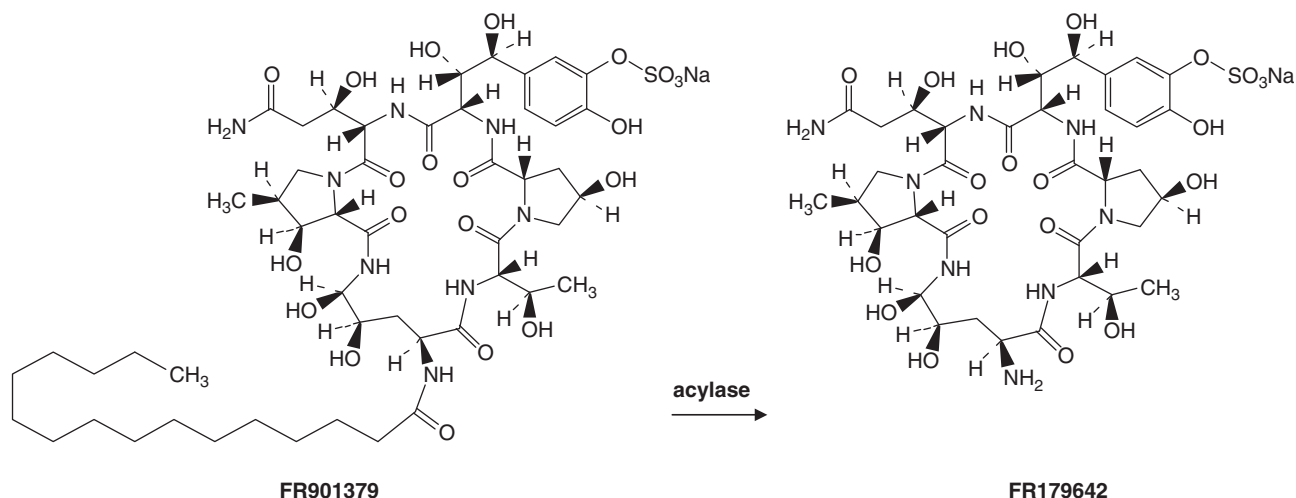


Figure 1 Enzymatic hydrolysis of FR901379 by acylase.

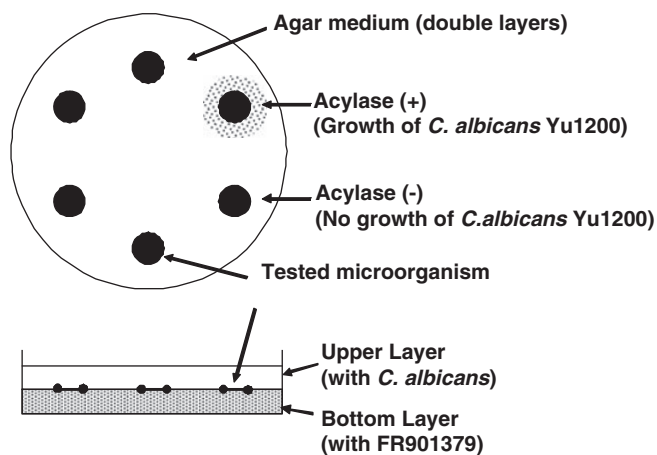


Figure 2 Schematic diagram of first screening step for FR901379 acylase-producing microorganisms.

before sterilization), whereas fungi were inoculated into the same medium supplemented with  $1 \text{ mg ml}^{-1}$  of sterilized FR901379, as some of the tested fungal strains showed deacylation activity only when cultured with a medium supplemented with FR901379. Inoculated media were cultured at  $25^\circ\text{C}$  for 2–5 days until microbes were well grown in the liquid culture.

The reaction mixture for enzymatic hydrolysis contained  $1 \text{ mg ml}^{-1}$  FR901379,  $50 \text{ mM}$  phosphate buffer (pH 7.0) and cultured broth in a final volume of  $1 \text{ ml}$ . The mixture was incubated at  $30^\circ\text{C}$  for 1 day and centrifuged at  $2000 \text{ r.p.m.}$  for 20 min. The supernatant was analyzed by HPLC (Shimadzu Corporation, Kyoto, Japan) to detect deacylated FR901379 (FR179642). The HPLC system comprised a LiChrospher 100RP-18 column ( $250 \text{ mm} \times 4 \text{ mm}$  i.d.) (Merck KGaA, Darmstadt, Germany) and a mobile phase of 5% acetonitrile/0.5% ammonium dihydrogenphosphate, with a UV detector at  $210 \text{ nm}$  and a flow rate of  $1 \text{ ml per min}$ . The retention time of FR179642 was  $\sim 6.3 \text{ min}$ .

### Taxonomic studies

Cultural characteristics were observed on several media incubated at  $30^\circ\text{C}$  for 14 days and at  $25^\circ\text{C}$  for 14 days for actinomycetes and fungi, respectively. Color names used in this study were taken from the Methuen Handbook of Colour.<sup>7</sup>

## RESULTS

### Screening for FR901379 acylase-producing microorganisms

A total of 145 strains were selected in the first screening step. Among these, 22 actinomycetes and 2 fungi were confirmed to produce the deacylated compound FR174692 by HPLC. In all, three strains of actinomycetes, that is, no. 4811, no. 6907 and no. 8703, which showed relatively higher activity than others, and two fungi, no. 30 084 and no. 30 085, were chosen for further characterization.

### Characterization of FR901379 acylase-producing strains

Strain no. 4811, no. 6907 and no. 8703 were isolated from a soil sample collected in Fukushima Prefecture. The cultural characteristics on yeast extract–malt extract agar, oatmeal agar, inorganic salts–starch agar, glycerin–asparagine agar, peptone–yeast extract–iron agar and tyrosine agar, as well as the light and scanning electron microscopy observations of the respective growths are summarized in Table 1.

For both no. 4811 and no. 8703, the color of the aerial mycelium was yellowish gray to greenish gray, the reverse side color of growth was yellowish brown to brown, the soluble pigment was light brown and neither intracellular pigments nor soluble pigments were pH sensitive. No melanoid pigments were produced in strain no. 4811, whereas melanoid pigments were produced in no. 8703 in the tryptone–yeast extract broth, peptone–yeast extract–iron agar and tyrosine agar. The color of the aerial mycelium of strain no. 6907 was yellowish gray to bluish gray, the reverse side color of growth was light brown to brown and the intracellular pigments were not pH sensitive. Melanoid pigments were produced in tryptone–yeast extract broth, peptone–yeast extract–iron agar and tyrosine agar.

The physiological characteristics are summarized in Table 2. Strain no. 4811 and no. 8703 did not use inositol, sucrose and raffinose but peptonized milk; the temperature range for growth was  $4.0\text{--}35.0^\circ\text{C}$ . In contrast, strain no. 6907 used all carbon sources tested but did not peptonize milk; the temperature range for growth was  $9.0\text{--}40.0^\circ\text{C}$ .

The vegetative mycelium of strain no. 4811, no. 6907 and no. 8703 developed well with irregular, but not fragmented, branching. The aerial mycelium extending from the vegetative mycelium branched monopodially to form elongated spore chains. The spore chain morphology of the aerial mycelium of strain no. 4811 and no. 8703 was straight to flexuous, thus belonging to the RF type according to the classification described by Pridham *et al.*<sup>8</sup> The spore chain

**Table 1 Cultural characteristics of strain no. 4811, no. 6907 and no. 8703**

Medium	No. 4811	No. 6907	No. 8703
Yeast extract–malt extract agar (ISP-2)	G: good A: abundant, yellowish gray (2B2) R: brown (7F4) S: scanty, brown	G: good A: abundant, yellowish gray (white 4A2) R: grayish orange (5B6) S: none	G: good A: abundant, yellowish gray (2B2) R: gray brown (5F4) S: none
Oatmeal agar (ISP-3)	G: good–moderate A: abundant, yellowish gray (2C3) R: brown (7F4) S: trace, brown	G: moderate A: abundant, bluish gray (22C2) R: light brown (4D4) S: none	G: moderate A: abundant, yellowish gray (2C3) R: gray brown (4C4) S: none
Inorganic salts–starch agar (ISP-4)	G: good A: abundant, yellowish gray (2C3) R: yellowish brown (5E4) S: none	G: good A: abundant, bluish gray (19C2) R: brown (6F4) S: none	G: good A: abundant, yellowish gray (2C3) R: dark gray (1F6) S: none
Glycerin–asparagine agar (ISP-5)	G: good A: abundant, yellowish gray (2C3) R: brown (6E4) S: none	G: good A: abundant, bluish gray (22B2) R: reddish brown (8E4) S: none	G: good A: abundant, yellowish gray (2C3) R: olive brown (4E5) S: none
Peptone–yeast extract–iron agar (ISP-6)	G: moderate A: poor, white R: light brown (6D5) S: none	G: moderate A: none R: grayish brown (9F3) S: dark brown	G: moderate A: poor, white R: yellowish brown (5D5) S: none
Tyrosine agar (ISP-7)	G: moderate A: moderate, yellowish gray (2B2) R: brown (7F4) S: none	G: good A: moderate, yellowish white (4A2) R: dark magenta (13F3) S: dark brown	G: moderate A: moderate, yellowish gray (2B2) R: brown (7F4) S: none

Abbreviations: A, aerial mycelium; G, growth; R, reverse side color; S, soluble pigment.

**Table 2 Physiological studies of strain no. 4811, no. 6907 and no. 8703**

	No. 4811	No. 6907	No. 8703
Temperature range for growth	4.0–35.0 °C	9.0–40.0 °C	4.0–35.0 °C
Liquefaction of gelatin	+	+	+
Coagulation of milk	±	+	±
Peptonization of milk	+	–	+
Hydrolysis of starch	+	+	+
Production of melanoid pigments	–	+	–
<i>Carbon utilization</i>			
D-glucose	+	+	+
L-arabinose	+	+	+
D-xylose	+	+	+
Inositol	–	+	–
Mannitol	+	+	+
D-fructose	+	+	+
L-rhamnose	±	+	+
Sucrose	–	+	–
Raffinose	–	+	–

+, positive; ±, weakly positive; –, negative.

morphology of the aerial mycelium of no. 6907 was straight to flexuous or incomplete loops, thus belonging to the RF or RA types. Each spore chain consisted of  $\geq 20$  spores. The spores were smooth-surfaced (glabrous) and cylindrical. The spore size was  $0.5\text{--}0.7 \times 0.7\text{--}1.1 \mu\text{m}^2$  for no. 4811,  $0.5\text{--}0.7 \times 0.7\text{--}1.3 \mu\text{m}^2$  for no. 6907 and  $0.5\text{--}0.3 \times 0.6\text{--}1.1 \mu\text{m}^2$  for no. 8703. No sclerotium, sporangium or zoospore were observed.

With regard to the amino-acid composition of the cell wall, the whole-cell lysate was analyzed by methods described by Becker *et al.*<sup>9</sup> and Yamaguchi<sup>10</sup> with results demonstrating LL-diaminopimelic acid in all strains. Therefore, the cell wall of these three strains was considered to belong to type 1.

On the basis of the above-mentioned morphological observations and chemical analysis, strain no. 4811, no. 6907 and no. 8703 were considered to belong to the genus *Streptomyces* according to the taxonomic classification described by Pridham *et al.*<sup>8</sup> Accordingly, the characteristics of these strains were compared with those of other *Streptomyces* species as described in the literature, namely by Shirling *et al.*,<sup>11–13</sup> Skerman *et al.*<sup>14</sup> and Moore *et al.*<sup>15</sup> These comparisons indicated that the described characteristics of *Streptomyces anulatus* were substantially identical to those of strain no. 4811 and no. 8703, and therefore they were identified and named *S. anulatus* no. 4811 and *S. anulatus* no. 8703, respectively. Comparisons with no. 6907 were unable to identify a specific species for the strain, and it was therefore named *Streptomyces* sp. no. 6907.

*S. anulatus* no. 4811, *S. anulatus* no. 8703 and *Streptomyces* sp. no. 6907 were originally deposited with the National Institute of Bioscience and Human Technology (NIBH) under the accession numbers FERM P-15377, FERM P-15507 and FERM P-15506, respectively, and subsequently converted to the accession numbers FERM BP-5808, FERM BP-5810 and FERM BP-5809, respectively, according to the Budapest Treaty.

Fungal strain no. 30084 and no. 30085 were isolated from a sample collected in Jouhoku-machi, Higashi Ibaraki-gun (Ibaraki, Japan). These strains showed restricted growth on various media and formed colonies varying in color according to the different type of culture media used: for example, greenish gray, brownish orange, yellowish white for no. 30084, greenish gray, yellowish gray, brown, etc. and

brown soluble pigments diffusing into the medium for no. 30 085. On several media, strain no. 30 084 formed anamorphs showing a conidial structure consisting of a dendritic conidiophore rising up from the surface of the medium with arthroconidia at its branches. Although no sporogenic organ was observed normally in no. 30 085, this strain formed a minimal quantity of asexual spores after prolonged incubation on corn meal agar.

The cultural characteristics of these fungi on various agar media are summarized in Table 3. Colonies of no. 30 084 on malt extract agar grew regressively and spread to attain diameters of 1.5–2.0 cm after 2 weeks of incubation at 25 °C. The colony was circular and was either raised as a whole or elevated peripherally and depressed in the center. The strain formed anamorphs in abundance, presenting a powdery surface. The colony was greenish gray with a yellowish-gray peripheral zone and the reverse side was light yellow with a yellowish-white peripheral zone. On potato dextrose agar, the colony also showed restricted growth and spread to attain diameters of 1.5–2.0 cm under the same cultural conditions as described above. The surface of the

colony was centrally elevated or convex, wrinkled and somewhat felt-like, forming a small amount of anamorphs. The colony was brownish orange to light brown with an orange-white peripheral zone, and the reverse side was brown with an orange-white peripheral zone.

The morphological characteristics of strain no. 30 084 were recorded by observing its growth in Miura's medium.<sup>16</sup> The conidiophore of strain no. 30 084 stood erect from the surface of the medium and consisted of a tan-colored linear trunk with colorless intricate branches. This dendritic structure made the conidiophore clearly distinguishable from the vegetative hypha. The conidiophore was 90–220 (or 240) µm in height, with its trunk being 2–3 (or 3.5) µm wide. The branches were bifurcated or trifurcated in succession and spread to occupy the space 30–50 µm in height and 40–60 µm in width over the top of the conidiophore. Each branch was fragmented along multiple septae, forming conidia in either form of a rod or an ellipsoid truncated at one or both ends. The conidium was colorless, smooth-surfaced, unicellular and varied in size from 2–4 × 1.5–2 µm<sup>2</sup>. Individual conidia were linked through vestiges of cell walls of their

**Table 3 Cultural characteristics of strain no. 30 084 and no. 30 085**

Medium	No. 30084	No. 30085
Malt extract agar	G: repressible, diameters 1.5–2.0 cm S: circular, elevated to centrally concave, powdery, abundant anamorphs. Colonies are greenish gray (1B2–1C2) with a yellowish-gray (4B2) peripheral zone R: pale yellow (4A3) with a yellowish-white (4A2) peripheral zone	G: repressible, diameters 2.0–3.0 cm S: circular, flat, felt-like to cottony. A pale orange exudate is produced. The colony is pale gray (1B1) to light gray (1C1) greenish gray (27F2), with an orange-white (5A2) peripheral zone R: dark green (28F to 4) with a yellowish-white (4A2) peripheral zone
Potato dextrose agar (Difco 3013)	G: repressible, diameters 1.5–2.0 cm S: circular, elevated to centrally concave, wrinkled, slightly felt-like, scanty anamorphs. Brownish orange (6C3) to light brown (6D6) with an orange-white (6A2) peripheral zone R: brown (7F6) with an orange-white (6A2) peripheral zone	G: repressible, diameters 2.0–3.0 cm S: circular, flat-to-elevated, slightly wrinkled, felt-like; an orange-colored exudate produced. Greenish gray (1B2) to pale gray (1B1) with an orangish white (6A2)-orangish gray (6B2) peripheral zone R: grayish brown (6F3) to dark brown (6F4 to 5), with a grayish orange (5B3)-brown peripheral zone. Diffusion of brown soluble pigments was observed
Czapek's solution agar	G: very repressible, diameters 0.5–1.0 cm S: circular to amorphous, flat, powdery, abundant anamorphs. Greenish gray (29C2) with a light-gray (1B1) peripheral zone R: light gray (1B1) to greenish gray (25D2)	G: very repressible, diameters 1.0–2.0 cm S: circular, flat, thin, no rise up of aerial hyphae, white (1A1) or yellowish gray (2C2) R: white (1A1) or yellowish gray (2C2)
Sabouraud's dextrose agar (Difco 0190)	G: repressible, diameters 1.5–2.0 cm S: circular, elevated to centrally convex, slightly felt-like, wrinkled. No anamorph formed. Grayish orange (6B3) to brownish orange (6C7), with an orangish-white (6A2) peripheral zone R: light brown (6D6), with an orangish-white (5A2) peripheral zone	G: repressible, diameters 2.0–2.5 cm S: circular, elevated to centrally convex; the surface wetted by an exudate and glossy; somewhat wrinkled, and the aerial hyphae in the center were consolidated into a bundle. Yellowish gray (4B2) to grayish yellow (4B3) but light brown (6D4) in the center R: dark brown (7F6–7) with a brown (7E7) peripheral zone, with diffusion of brown soluble pigments
Emerson YpSs diameters agar (Difco 0739)	G: very repressible, diameters 0.5–1.0 cm S: repressible, diameters 1.5–2.0 cm no rise up of aerial hyphae. No anamorph formed. yellowish white (4A2) R: yellowish white (4A2)	G: repressible, diameters 1.5–2.5 cm S: circular, flat-to-elevated, wrinkled, felt-like, gray with an olive tinge (2E2). Pale gray (2B2) in the peripheral zone R: dark brown (7F5–6) with a brown (7E5) peripheral zone. Diffusion of brown soluble pigments is noted
Corn meal agar diameters (Difco 0386)	G: very repressible, diameters 0.5–1.0 cm S: circular, flat, no rise up of aerial hyphae. Scanty anamorphs. White (1A1) to yellowish white (4A2) R: yellowish white brown (4A2)	G: slightly repressible, diameters 2.5–3.0 cm S: circular, flat and thin, with no rise up of aerial hyphae. Greenish gray (1B2–1C2) but olive (1F3) in the center R: greenish gray (1C2) but olive (1F3) in the center
MY20 agar	G: very repressible, diameters 1.0–1.5 cm S: circular, elevated to convex, powdery, wrinkled. Abundant anamorphs. Greenish gray (27D2) in the center, with a light-gray (1B1) peripheral zone R: yellowish white (4A2) to pale yellow (4A3)	G: much repressible, diameters 0.5–1.0 cm S: circular, elevated to convex, wetted by an exudate and glossy. The aerial hyphae in the center were consolidated into a bundle. Brown (6E4) R: dark brown (6F5), with diffusion of brown soluble pigments

Abbreviations: G, growth; S, surface; R, reverse.

branches. Vegetative hyphae were smooth-surfaced, septated, colorless and branched. The hyphal cell was cylindrical and 1.0–2.5  $\mu\text{m}$  in width. No clamydospores were observed. Strain no. 30 084 could grow at 3–32 °C, and the optimum temperature for growth was 24–28 °C. The above-mentioned characteristics of strain no. 30 084 were observed after incubation at 25 °C and compared with descriptions in several books<sup>17–19</sup> on the taxonomy of fungi. As a result, these characteristics were found to agree with descriptions of the genus *Oidiodendron*, and the strain was therefore identified as belonging to this genus and named *Oidiodendron* sp. no. 30 084. Although this strain was originally deposited with NIBH and assigned the accession number FERM P-15550, it was subsequently converted to a deposit according to the Budapest Treaty and assigned the accession number FERM BF-5943.

The colony of strain no. 30 085 on potato dextrose agar showed restricted growth and spread to attain diameters of 2.0–3.0 cm after 2 weeks of incubation at 25 °C. The colony was circular, flat-to-elevated, slightly wrinkled and felt-like. An orange-colored exudate was observed on the surface of the colony, with the colony itself being greenish gray to pale gray with an orange white to orange-gray peripheral zone. The reverse side was grayish brown to dark brown with a grayish-orange peripheral zone and a diffusion of brown soluble pigments into the medium. The colony on Czapek's solution agar showed markedly restricted growth on Czapek's solution agar and spread circularly to attain diameters of 1.0–2.0 cm after 2 weeks of incubation at 25 °C. The surface of the colony was flat and thin, with no aerial hyphae. The surface color was white or yellowish gray with the reverse side being the same. The conidiophore of strain no. 30 085 was not clearly distinguishable from the vegetative and aerial hyphae, with 2–5 conidiogenous cells occurring in whirls, or at times singularly, on the side of the vegetative hyphae. Conidiogenous cells were colorless, smooth-surfaced, filamentous to elongated flask-shaped (lecythiform), measuring 18–37 (at times 45)  $\times$  1.5–2.0  $\mu\text{m}^2$  and formed a single conidium to several conidia in a continuous series at the tip. The mode of conidiogenesis appeared to be phialidic but no definite collarette was observed. The conidia were colorless, smooth-surfaced, prolate (ellipsoidal) to bacilliform (rod-shaped), unicellular and 3–5.5  $\times$  1.5–2.5  $\mu\text{m}^2$  in size. Vegetative hyphae were smooth-surfaced, septated, colorless and branched, and although they were usually linear, at times they could also be curved and remarkably crimped. The hyphal cell was cylindrical to filiform and 1.0  $\times$  5.0  $\mu\text{m}^2$  in width, containing a large number of intracellular vacuoles. These vacuoles were released extracellularly with cell aging to yield a highly viscous exudate. Although clamydospores, sclerotia and catenulate forms were not observed, in old cultures the pleurogenesis of a large number of globose cells was observed. Strain no. 30 085 was able to grow at 4–29 °C, and the optimum temperature for growth was 22–26 °C. on potato dextrose agar. The above-mentioned characteristics of strain no. 30 085 were observed after 14 days of incubation at 25 °C and compared with the descriptions in several books<sup>20–22</sup> on the taxonomy of fungi. As a result, strain no. 30 085 was found to resemble *Verticillium*. Although fungi of the genus *Verticillium* typically produce a large number of conidia as a mucoid mass at the tip of the phialide, we considered the inability of strain no. 30 085 to produce numerous conidia to be due to its poor sporogenous ability, and therefore identified this strain as a species of the genus *Verticillium* and named it *Verticillium* sp. no. 30 085. Although this strain was originally deposited with NIBH, assigned the accession number FERM F-15551 and designated as fungus no. 30 085, it was subsequently renamed *Verticillium* sp. no. 30 085 and converted to a deposit according to the Budapest Treaty with the accession number FERM BP-5944.

### Optimum pH and temperature for acylase activity and substrate specificity

The optimum pH and temperature for acylase activity against FR901379 were determined using the culture broth of each selected strain. Profiles of optimum pH and temperature for acylase activity that differed greatly between streptomycetes and fungi are shown in Figure 3. The acylase activity of fungi occurred at a lower pH and temperature than that of Streptomycetes. *Streptomyces* no. 6907 showed the most potent acylase activity and was consequently compared with *A. utahensis* NRRL12052 against FR901379 and echinocandin B. In this protocol, 20 ml of cultured broth of *Streptomyces* no. 6907 and *A. utahensis* NRRL12052 was prepared as described in the literature<sup>23</sup> and 1 ml of each substrate solution (200 mg ml<sup>-1</sup>) together with 2.9 g of disodium hydrogen phosphate and 60 ml of water were added. The reaction was carried out at 60 °C for 24 h. After completion, the reaction mixture was filtered through a membrane filter (0.45  $\mu\text{m}$ ) to remove high molecular proteins, and each deacylated product was quantified by HPLC. As shown in Table 4, the acylase activity of *Streptomyces* no. 6907 was more potent and specific to FR901379 than that of *A. utahensis* NRRL12052.

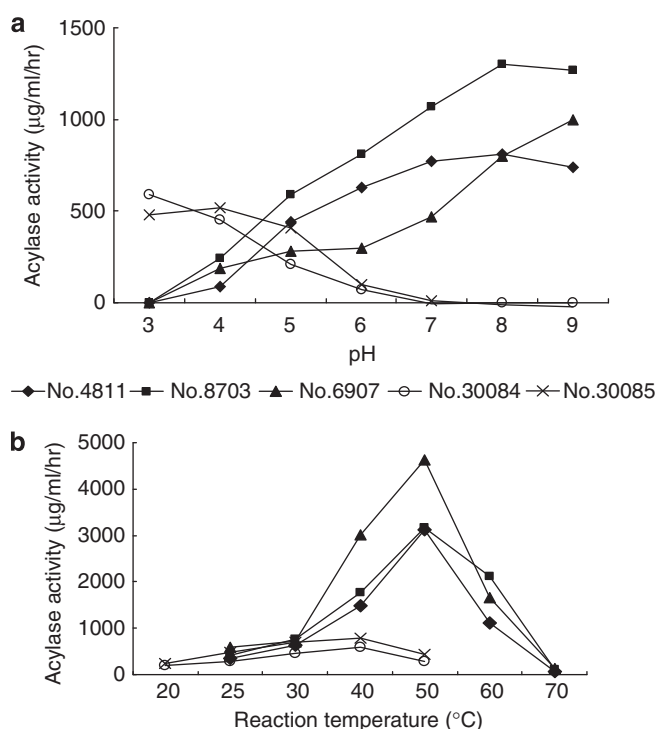


Figure 3 Optimum pH at 30 °C (a) and optimum temperature at pH 4 (b) for acylase activity produced by the selected strains.

Table 4 Relative activity of acylase produced by two actinomycetes

Microorganism	Substrate	
	FR901379	Echinocandin B
<i>A. utahensis</i> NRRL12052	420	100
<i>Streptomyces</i> sp. No. 6907	3900	438

Abbreviation: *A. utahensis*, Actinoplanes utahensis.

## DISCUSSION

Deacylation of microbial products is essential for the production of a number of important medicines. We used microbial deacylation to semi-synthesize a novel lipopeptide antifungal drug for deep-seated mycoses, through enzymatic deacylation of FR901379, followed by chemical reacylation with an optimized acyl group.<sup>24</sup> We required our original acylase to show potent activity against FR901379 for obtaining a large quantity of deacylated compound during the optimization study and for establishing industrial production. As *A. utahensis* produces an enzyme to deacylate several lipopeptide compounds, we searched for novel acylase-producing microorganisms among wild actinomycetes and fungal strains freshly isolated from soil samples. Consequently, three *Streptomyces* and two filamentous fungal strains were isolated. Taxonomic studies have shown that two of the three *Streptomyces* strains were species of *S. anulatus* and that each of the two fungal strains belonged to the genera *Verticillium* and *Oidiodendron*. Although it has been previously reported that *A. utahensis*,<sup>4,5</sup> *S. mobaraensis*<sup>25</sup> and *Streptomyces* sp.<sup>26</sup> produce acylases, our study is the first to find that *S. anulatus* also produces an acylase. Elucidation of the protein structure for acylases produced by these newly identified strains will be reported elsewhere, together with comparison of the amino-acid sequences with that of known acylases.

All acylases so far derived from actinomycetes are reported to belong to the family of penicillin acylases that have a heterodimer protein structure. Our preliminary data indicated that *Streptomyces* strains obtained in this study produce acylases with the same properties as those of penicillin acylase. In contrast, data regarding optimum pH and temperature for acylase activity suggested that fungal acylases have different properties from acylases produced by *Streptomyces*. Further, although penicillin acylases are constitutively produced, the addition of FR901379 to culture media was necessary to induce acylase activity in fungi. We are presently undertaking a detailed analysis on the structure and enzymology of these new acylases.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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