***Fellomyces ogasawarensis* sp. nov. and *Fellomyces distylii* sp. nov., yeasts isolated from a plant in Japan**

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***Fellomyces ogasawarensis* and *Fellomyces distylii*, new yeast species isolated from a dead leaf of a plant (*Distylium lepidotum* Nakai, family Hamamelidaceae) collected in the Ogasawara Islands, isolated islands in the Pacific Ocean about 1000 km south of Japan, are described. The phylogenetic relationship of *F. ogasawarensis* and *F. distylii* with other members of the genus *Fellomyces* was estimated from 18S rRNA gene sequence analysis. The type strain of *F. ogasawarensis* is strain OK-81 (= JCM 9861) and that of *F. distylii* is strain OK-83 (= JCM 9862).**

Keywords: new *Fellomyces* species, 18S rRNA gene sequencing

INTRODUCTION

The genus *Fellomyces* was established by Yamada & Banno based on a single species, *Fellomyces polyborus* (16), which was originally described as *Sterigmatomyces polyborus* (8). This genus can be distinguished from *Sterigmatomyces* by the production of conidia, which are freed by an end-break in the sterigmata, and the presence of Q₁₀ as the major ubiquinone isoprenologue (16). Four species, *Fellomyces fuzhouensis*, *Fellomyces horovitziae*, *Fellomyces penicillatus* and *Fellomyces polyborus*, are currently recognized in the genus *Fellomyces* (1).

In the course of a survey of basidiomycetous yeasts living on the leaves of plants in the Ogasawara Islands, isolated islands in the Pacific Ocean south of Japan, two strains of stalked-conidium-forming yeasts were isolated. Comparison of the phenotypic properties of the new isolates and their levels of DNA hybridization with *Fellomyces* species revealed that these strains represented two new species of the genus *Fellomyces*. The names *Fellomyces ogasawarensis* and *Fellomyces distylii* are proposed for these yeasts.

METHODS

Yeast isolation and cultivation. A sample of a dead leaf of *Distylium lepidotum* Nakai (family Hamamelidaceae) was collected on Ani-jima Island of the Ogasawara Islands, Japan. The procedure used for yeast isolation and purification has been described previously (5, 7). Strains were

grown on YM agar (Difco) at 25 °C. *F. fuzhouensis* JCM 7367^T (T = type strain), *F. horovitziae* JCM 8358^T, *F. penicillatus* JCM 6907^T and *F. polyborus* JCM 6908^T were used for taxonomic comparisons. All strains were grown at 25 °C in YM broth (Difco), harvested by centrifugation, and washed with saline/EDTA buffer (0.15 M NaCl, 1 M EDTA; pH 8.0) for extraction of ubiquinone and nuclear DNA.

Physiological and chemotaxonomic characteristics. The isolates were characterized phenotypically by the methods currently used in yeast taxonomy (15) with some modifications. Assimilation of nitrogen compounds was examined on solid media with starved inoculum as described by Nakase & Suzuki (6). The vitamin requirements were investigated according to Komagata & Nakase (4). The maximum growth temperature was determined in YM broth (Difco) by using metal block baths.

Sugars in the whole cell were analysed by HPLC after hydrolysis as described previously (12). Ubiquinones were extracted and purified by the method of Yamada & Kondo (17) with slight modifications and determined by HPLC as described previously (3).

Nucleic acid analyses. DNA extraction, purification and reassociation were performed by the procedure of Hamamoto & Nakase (3). Nuclear DNA base compositions were determined by HPLC as described by Tamaoka & Komagata (13), with a minor modification (3).

The following oligodeoxynucleotides were synthesized on the basis of conserved regions at the 5' and 3' termini of eukaryotic 18S rRNAs with 12- and 13-nucleotide-long tails (enclosed in brackets), respectively, for use by the PCR-Direct Cloning System (Clontech): 5'-[CTCGCTCGCC-CA]ATCTGGTTGATCCTGCCAGT-3' and 5'-[CTGGTTCCGGCCCA]GATCCTTCCGCAGGTTACCC-3'. The DNA of the 18S rRNA coding gene was amplified by using PCR (9) with a *Taq* DNA polymerase (Takara) and the

The DDBJ accession numbers for the 18S rRNA gene sequences determined in this study are listed in Table 1.

Table 1. Yeast strains compared in this study and their small-subunit rRNA gene sequence accession numbers

Species	Strain*	DDBJ, EMBL and/or GenBank accession no.
<i>Athelia bombacina</i>	ATCC 20629	M55638
<i>Auricularia auricula-judae</i>	UC 1475109	L22254
<i>Bulleromyces albus</i>	MUCL 30301 ^T	X60179
<i>Calocera cornea</i>	UC 1475111	L22256
<i>Candida albicans</i>	Unknown	X53497
<i>Cryptococcus albidus</i>	JCM 2334 ^T	D31655
<i>Cystofilobasidium capitatum</i>	IAM 13521 ^T	D12801
<i>Dacryomyces chrysospermus</i>	UC 1475112	L22257
<i>Fellomyces distylii</i>	JCM 9862 ^T	AB001036†
<i>Fellomyces fuzhouensis</i>	JCM 7367 ^T	AB001032†
<i>Fellomyces horovitziae</i>	JCM 8358 ^T	AB001033†
<i>Fellomyces ogasawarensis</i>	JCM 9861 ^T	AB001035†
<i>Fellomyces penicillatus</i>	JCM 6907 ^T	AB001034†
<i>Fellomyces polyborus</i>	JCM 6908 ^T	D64117
<i>Fibulobasidium inconspicuum</i>	JCM 6898	D64123
<i>Filobasidium floriforme</i>	IAM 14212	D13460
<i>Filobasidiella neoformans</i>	IAM 14211	D12804
<i>Kockovaella thailandica</i>	JCM 7824 ^T	D64133
<i>Kurtzmanomyces nectairei</i>	JCM 6906 ^T	D64122
<i>Mixia osmundae</i>	IFO 32408	D14163
<i>Mrakia frigida</i>	IAM 14136 ^T	D12802
<i>Neurospora crassa</i>	Unknown	X04971
<i>Pseudohydnum gelatinosum</i>	UC 1475114	L22260
<i>Saccharomyces cerevisiae</i>	Unknown	M27607
<i>Spongipellis unicolor</i>	Unknown	M59760
<i>Sporidiobolus johnsonii</i>	Unknown	L22261
<i>Sporobolomyces roseus</i>	Unknown	X60181
<i>Sterigmatomyces halophilus</i>	JCM 6905 ^T	D64119
<i>Sterigmatosporidium polymorphum</i>	JCM 6902 ^T	D64120
<i>Thanatephorus cucumeris</i>	Unknown	M92990
<i>Tilletia caries</i>	Unknown	U00972
<i>Tilletiopsis washingtonensis</i>	JCM 8362 ^T	D83192
<i>Tremella foliacea</i>	Unknown	L22262
<i>Tremella globospora</i>	Unknown	U00976
<i>Tremella moriformis</i>	Unknown	U00977
<i>Trichosporon cutaneum</i>	MUCL 30308 ^T	X60182
<i>Tsuchiyaea wingfieldii</i>	JCM 7368 ^T	D64121
<i>Udeniomyces piricola</i>	JCM 2958 ^T	D31659
<i>Ustilago hordei</i>	Unknown	U00973
<i>Ustilago maydis</i>	Unknown	X62396
<i>Xanthophyllomyces dendrorhous</i>	JCM 9042 ^T	D31656

*IAM, IAM Culture Collection, Tokyo, Japan; JCM, Japan Collection of Microorganisms, Saitama, Japan; MUCL, Mycothèque de l'Université Catholique, Louvain-la Neuve, Belgium; UC, University Herbarium, University of California, Berkeley, USA. T, Type strain.

† Sequence determined in this study.

oligonucleotides described above for primers. The PCR products were cloned directly in the linearized pDIRECT vector (Clontech) and the double-stranded DNA templates for sequencing were prepared according to the directions of the manufacturer. Sequencing was performed by using the Pharmacia Autocycle Sequencing kit (*Taq* DNA polymerase) as described by Suh *et al.* (10, 11).

Analysis of sequence data. The yeast strains which were used for the phylogenetic analysis are listed in Table 1. The five 18S rDNA sequences which we determined were aligned with the sequences of 36 other basidiomycetes and ascomycetes retrieved from the GenBank and DDBJ libraries by using the multiple-alignment program CLUSTAL W (14). The alignments were manually adjusted. An unrooted phylo-

genetic tree was constructed by using the NEIGHBOR program contained in the PHYLIP package, version 3.5 (2). The confidence values of branches were determined by performing a bootstrap analysis in which 1000 replicates were used (2).

RESULTS AND DISCUSSION

Two strains, OK-81 and OK-83, were characterized by the production of conidia, which were freed by an end-break in the sterigma, the lack of ballistoconidia, the presence of xylose in the whole cells, and the presence of Q₁₀ as the major ubiquinone isoprenologue. On the basis of these results, these two isolates were assigned to the genus *Fellomyces*.

Strains OK-81 and OK-83 differ from the four known species of *Fellomyces* in nuclear DNA base composition, and exhibit low levels of DNA relatedness to the type strains of the known species and also between each other (Table 2). Apparently, strains OK-81 and

OK-83 represent two distinct new species of the genus *Fellomyces*. Table 3 shows the phenotypic characteristics among six species of the genus *Fellomyces*.

The names *Fellomyces ogasawarensis* and *Fellomyces distylii* have been given to these new species and they are described below.

Latin diagnosis of *Fellomyces ogasawarensis* sp. nov.

In liquid YM post dies 3 (25 °C), cellulae sphaericae vel ovoideae (2.0–3.0 × 4.0–8.0 µm). Conidia singula in apicem cylindraceorum sterigmatum formata et ad apicem sterigmatis sine projectione liberata. Post 1 mensem sedimentum et pelliculum formantur. Cultura in agarō YM post 1 mensem (25 °C), albo-isabellinus, rugosa, non nitida, margine integer. Pseudohyphae nullae. Ballistospores non formantur. Fermentatio nulla. Glucosum, galactosum, L-sorboseum, saccharosum,

Table 2. Levels of DNA complementarity for *Fellomyces* species

Species	Strain*	G + C mol%†	Percentage relative binding of DNA from:					
			JCM 9861 (= OK-81)	JCM 9862 (= OK-83)	JCM 7367	JCM 8358	JCM 6907	JCM 6908
<i>F. ogasawarensis</i>	JCM 9861 ^T (= OK-81)	53.3	100	24	16	12	3	10
<i>F. distylii</i>	JCM 9862 ^T (= OK-83)	51.4	14	100	13	10	2	7
<i>F. fuzhouensis</i>	JCM 7367 ^T	54.6	3	6	100			
<i>F. horovitziae</i>	JCM 8358 ^T	52.1	3	5		100		
<i>F. penicillatus</i>	JCM 6907 ^T	47.9	2	5			100	
<i>F. polyborus</i>	JCM 6908 ^T	48.2	2	7				100

* T, Type strain.

† Determined by HPLC in this study.

Table 3. Characteristics differentiating *F. ogasawarensis* and *F. distylii* from known species

+, Positive; –, negative.

	<i>F. ogasawarensis</i>	<i>F. distylii</i>	<i>F. fuzhouensis</i>	<i>F. horovitziae</i>	<i>F. penicillatus</i>	<i>F. polyborus</i>
G + C mol%	53.3	51.4	54.6	52.1	47.9	48.2
Assimilation of:						
Soluble starch	+	+	+	–	+	+
L-Rhamnose	+	–	+	–	+	+
Ethanol	–	–	+	–	–	–
Erythritol	–	+	+	–	+	+
Galactitol	+	+	+	–	+	+
D-Mannitol	+	+	+	–	+	+
Glucono-δ-lactone	+	+	+	+	–	+
2-Ketogluconic acid	+	+	+	–	+	+
DL-Lactic acid	+	–	+ / –	–	+ / –	–
Inositol	–	–	+	–	+	+

Fig. 1. *F. ogasawarensis* JCM 9861. Conidia in the sterigmata produced on YM agar after 5 d at 25 °C. Bar, 10 µm.

maltosum, cellobiosum, trehalosum, lactosum, melibiosum, raffinose, melezitose, amylosum solubile, D-xylosum, L-arabinosum, D-arabinosum, D-ribosum, L-rhamnosum, glycerolum, ribitolum, galactitolum, D-mannitolum, D-glucitolum, salicin, glucono-δ-lactonum, acidum 2-ketogluconicum, acidum 5-ketogluconicum, acidum DL-lacticum, acidum succinicum, acidum citricum, acidum D-glucuronicum et acidum D-galacturonicum assimilantur, at non inulinum, ethanolum, erythritolum, methyl α-D-glucosidum nec inositolum. Lysinum assimilatur at non kalium nitricum, natrium nitrosum, ethylaminum nec cadaverinum. Maxima temperatura crescentiae: 28–29 °C. Ad crescentiam thiaminum necessarium est. Materia amyloidea iodophila non formatur. Ureum hydrolysat. Diazonium caeruleum B: positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 53.3 mol% (per HPLC). Ubiquinonum majus: Q₁₀. Xylosum in cellulis praesens.

Holotypus ex folio Distylium lepidotum, Japan, X, 1994, T. Nakase, JCM 9861 (originaliter ut OK-81) conservatur in collectionibus culturarum quas 'Japan Collection of Microorganisms', Wako, Saitama sustentat.

Description of *Fellomyces ogasawarensis* sp. nov.

Fellomyces ogasawarensis (o.ga.sa.war.en'sis. M.L. adj. *ogasawarensis* referring to Ogasawara, the remote islands in the Pacific Ocean of Japan where the type strain was isolated).

After growth in YM broth (Difco) for 3 d at 25 °C, the cells are spherical to ovoid (2.0–3.0 × 4.0–8.0 µm) and produce one or more sterigmata, each giving rise to a single conidium. The conidium is freed without forceful ejection by an end-break in the sterigma (Fig. 1). A sediment and a hydrophobic pellicle are formed after 1 month. After growth on YM agar (Difco) at 25 °C for 3 d, the streak culture is yellowish white. After 1

Fig. 2. *F. distylii* JCM 9862. Conidia in the sterigmata produced on YM agar after 5 d at 25 °C. Bar, 10 µm.

month, the streak culture is yellow-sienna, wrinkled, dull, and has an entire margin. On Dalmau plate cultures on corn meal agar after 10 d at 25 °C no true mycelia and pseudomycelia develop. No ballistoconidia are formed. Does not ferment D-glucose. Assimilates D-glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, L-rhamnose, glycerol, ribitol, galactitol, D-mannitol, D-glucitol, salicin, glucono-δ-lactone, 2-ketogluconic acid, 5-ketogluconic acid, DL-lactic acid, succinic acid, citric acid, D-glucuronic acid and D-galacturonic acid. Does not assimilate the carbon in inulin, ethanol, erythritol, methyl α-D-glucoside and inositol. Does not assimilate the following nitrogen sources: nitrate, nitrite, ethylamine and cadaverine. Assimilation of L-lysine is positive. Maximum growth temperature is 28–29 °C. Thiamin is required for growth. Growth does not occur on 50 % (w/w) glucose-yeast extract agar. No starch-like substance is produced. Does not liquefy gelatin. Growth occurs in the presence of 100 p.p.m. cycloheximide. Urease is positive. The diazonium blue B reaction is positive.

The G + C content of the nuclear DNA is 53.3 mol%, as determined by HPLC (Table 2). The major ubiquinone is Q₁₀. Xylose is present in the whole cells.

The type strain of *F. ogasawarensis*, strain OK-81, was isolated from a leaf of *Distylium lepidotum* Nakai in the Ogasawara Islands, Japan. This strain has been deposited in the Japan Collection of Microorganisms, Saitama, Japan, as JCM 9861.

Latin diagnosis of *Fellomyces distylii* sp. nov.

In liquid YM post dies 3 (25 °C), cellulae sphaericae vel ovoideae (2.0–3.0 × 4.0–6.0 µm). Conidia singula in apicem cylindraceorum sterigmatum formata et ad apicem sterigmatum sine projectione liberata. Post 1 mensem sedimentum et pelliculum formantur. Cultura in agar

Fig. 3. Phylogenetic tree of the species of the genus *Fellomyces* and related taxa. Trees was constructed as described in the text. Bootstrap values greater than 50% are shown. The bar indicates the distance corresponding to 1 base change per 100 nucleotide positions.

YM post 1 mensem (25 °C), *isabellinus*, *butyrosa*, *glabra*, *non nitida*, *marginis integer*. *Pseudohyphae nullae*. *Ballistospores non formantur*. *Fermentatio nulla*. *Glucosum*, *galactosum*, *L-sorbosum*, *saccharosum*, *maltozum*, *cellobiosum*, *trehalosum*, *lactosum*, *melibiosum*, *raffiniosum*, *melezitosum*, *amylum solubile*, *D-xylosum*,

L-arabinosum, *D-arabinosum*, *D-ribosum*, *glycerolum*, *erythritolum*, *ribitolum*, *galactitolum*, *D-mannitolum*, *D-glucitolum*, *salicinum*, *glucono-δ-lactonum*, *acidum 2-ketogluconicum*, *acidum 5-ketogluconicum*, *acidum succinicum*, *acidum citricum*, *acidum D-glucuronicum* et *acidum D-galacturonicum* assimilantur, at non *inulinum*,

L-rhamnosum, ethanolum, methyl α -D-glucosidum, acidum DL-lacticum nec inositolum. Lysinum assimilatur at non kalium nitricum, natrium nitrosum, ethylaminum nec cadaverinum. Maxima temperatura crescentiae: 27–28 °C. Ad crescentiam thiaminum necessarium est. Materia amyloidea iodophila non formatur. Ureum hydrolysatur. Diazonium caeruleum B: positivum. Proportio molaris guanini + cytosini in acido deoxyribonucleinico: 51.4 mol% (per HPLC). Ubiquinonum majus: Q₁₀. Xylosum in cellulis praesens.

Holotypus ex folio Distylium lepidotum, Japan, X, 1994, T. Nakase, JCM 9862 (originaliter ut OK-83) conservatur in collectionibus culturarum quas 'Japan Collection of Microorganisms', Wako, Saitama sustentat.

Description of *Fellomyces distylii* sp. nov.

Fellomyces distylii (di'sty.li.i. M.L. gen. n. *distylii* of *Distylium* [*Distylium lepidotum*], the plant from which the type strain was isolated).

After growth in YM broth (Difco) for 3 d at 25 °C, the cells are spherical to ovoid (2.0–3.0 × 4.0–6.0 µm) and produce one or more sterigmata, each giving rise to a single conidium. The conidium is freed without forceful ejection by an end-break in the sterigma (Fig. 2). A sediment and a hydrophobic pellicle are formed after 1 month. After growth on YM agar (Difco) at 25 °C for 3 d, the streak culture is yellowish white. After 1 month, the streak culture is sienna, butyrous, smooth, dull, and has an entire margin. On Dalmau plate cultures on corn meal agar after 10 d at 25 °C no true mycelia and pseudomycelia develop. No ballisticonidia are formed. Does not ferment D-glucose. Assimilates D-glucose, galactose, L-sorbose, sucrose, maltose, cellobiose, trehalose, lactose, melibiose, raffinose, melezitose, soluble starch, D-xylose, L-arabinose, D-arabinose, D-ribose, glycerol, erythritol, ribitol, galactitol, D-mannitol, D-glucitol, salicin, glucono- δ -lactone, 2-ketogluconic acid, 5-ketogluconic acid, succinic acid, citric acid, D-glucuronic acid and D-galacturonic acid. Does not assimilate the carbon in inulin, L-rhamnose, ethanol, methyl α -D-glucoside, DL-lactic acid and inositol. Does not assimilate the following nitrogen sources: nitrate, nitrite, ethylamine and cadaverine. Assimilation of L-lysine is positive. Maximum growth temperature is 27–28 °C. Thiamin is required for growth. Growth does not occur on 50% (w/w) glucose-yeast extract agar. No starch-like substance is produced. Does not liquefy gelatin. Growth occurs in the presence of 100 p.p.m. cycloheximide. Urease is positive. The diazonium blue B reaction is positive. The G + C content of the nuclear DNA is 51.4 mol%, as determined by HPLC (Table 2). The major ubiquinone is Q₁₀. Xylose is present in the whole cells.

The type strain of *F. distylii*, strain OK-83, was isolated from a leaf of *Distylium lepidotum* Nakai in the Ogasawara Islands, Japan. The strain has been deposited in the Japan Collection of Microorganisms, Saitama, Japan, as JCM 9862.

Phylogenetic relatedness of *F. ogasawarensis* and *F. distylii*

We determined almost complete 18S rDNA sequences of the two new species and three known species of *Fellomyces*, *F. fuzhouensis*, *F. horovitziae* and *F. penicillatus*. The trees in Fig. 3 were constructed, using the NEIGHBOR program, from the data sets aligned by CLUSTAL W on 1501 18S rDNA sites (14). The percentage of bootstraps was derived from 1000 resamplings in the neighbour-joining tree. Values that were greater than 50% are shown. The branch comprising the species of the genus *Fellomyces* includes *Kockovaella thailandica* and *Sterigmatosporidium polymorphum*, which also known as stalked-conidium-forming yeasts. Our evolutionary tree indicates that the species of the genus *Fellomyces* belong to the Tremellomycetidae lineage.

Two strains from a dead leaf of a plant (*Distylium lepidotum* Nakai) fit the description of the genus *Fellomyces*, and they are described here as two new species, *F. ogasawarensis* and *F. distylii*. As a result of our description of two new species, a total of six species should be approved in the genus *Fellomyces*. Strains of this genus have been isolated from various sources, including animals, plants, and tunnels of some insects.

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REFERENCES

- Barnett, J. A., Payne, R. W. & Yarrow, D. (1990). *Yeasts: Characteristics and Identification*, 2nd edn. Cambridge: Cambridge University Press.
- Felsenstein, J. (1993). PHYLIP: phylogenetic inference package, version 3.5. University of Washington, Seattle, USA.
- Hamamoto, M. & Nakase, T. (1995). Ballistosporeous yeasts found on the surface of plant materials collected in New Zealand. 1. Six new species in the genus *Sporobolomyces*. *Antonie Leeuwenhoek* **67**, 151–171.
- Komagata, K. & Nakase, T. (1967). Reitoushokuhin no biseibutsu ni kansuru kenkyu. V. Shihan reitoushokuhin yori bunnri shita kobo no seijo (Microbiological studies on frozen foods. V. General properties of yeasts isolated from frozen foods) (in Japanese). *Shokuhin Eiseigaku Zasshi* **8**, 53–57.
- Nakase, T. & Suzuki, M. (1985). Ballistospore-forming yeasts found on the surface of the Japanese rice plant, *Oryza sativa* L. *J Gen Appl Microbiol* **31**, 457–474.
- Nakase, T. & Suzuki, M. (1986). *Bullera megalospora*, a new species of yeast forming large ballistospores isolated from dead leaves of *Oryza sativa*, *Miscanthus sinensis* and *Sasa* sp. in Japan. *J Gen Appl Microbiol* **32**, 225–240.
- Nakase, T. & Takashima, M. (1993). A simple procedure for the high frequency isolation of new taxa of ballistosporeous yeasts living on the surface of plants. *RIKEN Rev* **3**, 33–34.

8. Scott, D. B. & van der Walt, J. P. (1970). Three new yeasts from South Africa insect sources. *Antonie Leeuwenhoek* **36**, 389–396.
9. Suh, S.-O. & Nakase, T. (1995). Phylogenetic analysis of the ballistosporous anamorphic genera *Udeniomyces* and *Bullera*, and related basidiomycetous yeasts, based on 18S rDNA sequence. *Microbiology* **141**, 901–906.
10. Suh, S.-O., Takashima, M. & Nakase, T. (1996). Phylogenetic study of the anamorphic basidiomycetous yeasts *Rhodotorula lactosa* and *R. minuta*, and related taxa based on 18S ribosomal DNA sequences. *J Gen Appl Microbiol* **42**, 1–6.
11. Suh, S.-O., Takashima, M., Hamamoto, M. & Nakase, T. (1996). Molecular phylogeny of the ballistoconidium-forming anamorphic yeast genus *Bullera* and related taxa based on small subunit ribosomal DNA sequences. *J Gen Appl Microbiol* **42**, 501–509.
12. Suzuki, M. & Nakase, T. (1988). The distribution of xylose in the cells of ballistosporous yeasts – application of high performance liquid chromatography without derivatization to the analysis of xylose in whole cell hydrolysates. *J Gen Appl Microbiol* **34**, 95–103.
13. Tamaoka, J. & Komagata, K. (1984). Determination of DNA base composition by reversed-phase high-performance liquid chromatography. *FEMS Microbiol Lett* **25**, 125–128.
14. Thompson, J. D., Higgins, D. G. & Gibson, T. J. (1994). CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* **22**, 4673–4680.
15. van der Walt, J. P. & Yarrow, D. (1984). Methods for isolation, maintenance, classification and identification of yeasts. In *The Yeasts, a Taxonomic Study*, 3rd edn, pp. 45–104. Edited by N. J. W. Kreger-van Rij. Amsterdam: Elsevier.
16. Yamada, Y. & Banno, I. (1984). *Fellomyces*, a new anamorphic yeast genus for the Q10-equipped organisms whose conidium is freed by an end-break in the sterigma. *J Gen Appl Microbiol* **30**, 523–525.
17. Yamada, Y. & Kondo, K. (1973). Coenzyme Q system in the classification of the yeast genera *Rhodotorula* and *Cryptococcus* and the yeast-like genera *Sporobolomyces* and *Rhodospiridium*. *J Gen Appl Microbiol* **19**, 59–77.