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Short Communication

PCR-amplified ITS length variation within the yeast genus *Metschnikowia*

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Variations in the ITS region have been applied in studies of the yeast genus *Zygosaccharomyces* and in some other eukaryotes (Baldwin et al., 1995; James et al., 1996). The ITS product length variability has been studied in the genus *Saccharomyces* showing that species belonging to the *Saccharomyces sensu stricto* group have amplified ITS region products of over 800 bp, whereas those belonging to the *sensu lato* group and *Saccharomyces kluyveri* have smaller ITS lengths (Huffman et al., 1992; Molina et al., 1992; Valente et al., 1996; Vaughan–Martini and Martini, 1993).

The ascomycetous yeast genus *Metschnikowia* is a well-studied group forming needle-shaped ascospores without any appendages (Kreger van-Rij, 1984). There are 10 species currently assigned to the genus, mostly associated with flowers or fruits, but some are associated with marine invertebrates and aquatic habitats (Fell and Pitt, 1969; Giménez–Jurado, 1992; Lachance, 1993). Molecular probes for identification of species of *Metschnikowia* are currently being developed (Giménez–Jurado et al., 1995; Henriques et al., 1991). Mendonça–Hagler et al. (1993) showed by the partial sequencing of rRNA that all species except *Metschnikowia gruessii* and *Metschnikowia agaveae*, which were not yet described, are of the same monophyletic line. We analysed the variability in size of the ITS region in all species included in the genus *Metschnikowia* to test its application as a characteristic for taxonomy.

The cultures listed in Table 1 were grown in 2 ml of modified GYP broth (5% glucose, 0.5% yeast extract, 0.5% peptone pH 5.5–6.5), and DNA extraction was done as described by Valente et al. (1996). The amplification reaction was done in a 30 µl volume topped with 10 µl of mineral oil using a Gibco BRL PCR kit (Sào Paulo, Brazil; Gaithersburg, MA, U.S.A.). The mixture for 1 reaction was 23.9 µl of milli-Q water, 3 µl of 10× amplification buffer plus $MgCl_2$ (1.5 mM final concentration), 0.6 µl of 10 mM dNTP mixture, 0.3 µl of Taq DNA polymerase (1.5 U), 0.6 µl of each primer (0.1 µM final concentration), and 1 µl of target genomic DNA (20–30 ng). The primers used were ITS1 (5′TCCGTAGGTGAACCTGCGG 3′) and ITS4 (5′TCCTCCGCTTATTGATATGC 3′) as described by White et al. (1990). The mixture was subjected to an initial denaturing cycle of 3 min at 95°C, followed by 33 cycles of 45 s at 95°C, 30 s at 55°C, 45 s at 72°C, and a final extension step of 7 min at 72°C. The PCR products were revealed by electrophoresis in 2% agarose gels using a 100 bp DNA ladder (Gibco) as the size standard. Fluorescent ethidium bromide-stained bands were photographed under UV light. Band sizes were estimated using the method of Schaffer and Sederoff (1981).

The PCR products corresponding to the ITS region of *Metschnikowia* species were restricted in size to between 360 and 450 bp (Fig. 1), making them among the smallest of the yeasts tested which ranged from

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Table 1. List of the cultures studied, their reference, origin, and approximate size in base pairs (bp) of the ITS product amplified by primers ITS1 and ITS4.

Species	Strain (IM-UFRJ)	Reference	Origin	Size of ITS product
Metschnikowia bicuspidata	51.625^T	CBS 5575	*Diplostomum flexicaudum*	380
	50.627	CBS 6010	Pacific Ocean, California, U.S.A.	380
	50.493	UCD 67-002	Freshwater pond, New Zealand	380
Metschnikowia australis	51.177^T	CBS 5847	Antarctic Ocean	380
Metschnikowia krissii	51.167^T	CBS 4823	Seawater	400
Metschnikowia zobellii	51.173^T	CBS 4821	Seawater	410
Metschnikowia reukaufii	50.498^T	UCD 62-311	*Epilobium angustifolium* flower	380
Metschnikowia pulcherrima	50.497^T	UCD C-214	Grapes	360
Metschnikowia gruessii	51.175^T	CBS 7657	*Hebe salicifolia* nectaries	360
Metschnikowia agaveae	51.499^T	UWO 92207.1	Rots of leaves of agave plant	450
	51.500	UWO 92210.1	Rots of leaves of agave plant	450
Metschnikowia lunata	50.496^T	UCD 77-62	*Vicia craca* flower	360
Metschnikowia hawaiiensis	51.169^T	CBS 7432	*Ipomoea acuminata* flower	360
Candida pulcherrima?	50.329	Pagnocca et al., 1989	Sepetiba Bay, Rio de Janeiro, Brazil	610
Candida pulcherrima-like	50.213	Pagnocca et al., 1989	Mangrove sediment, Rio de Janeiro, Brazil	580
Candida reukaufii-like	50.218	Hagler et al., in press	River in Tijuca Forest, Rio de Janeiro, Brazil	380
Candida reukaufii-like A	51.616	Rosa et al., 1995	*Ipomoea litoralis* flower	380
	51.617	Rosa et al., 1995	*Ipomoea litoralis* flower	380
	51.618	Rosa et al., 1995	*Ipomoea litoralis* flower	380
	51.615	Rosa et al., 1995	*Ipomoea litoralis* flower	380
Candida reukaufii A	51.634	Rosa et al., 1995	*Ipomoea litoralis* flower	370
	51.632	Rosa et al., 1995	*Ipomoea litoralis* flower	370
	51.633	Rosa et al., 1995	*Ipomoea litoralis* flower	380
	51.635	Rosa et al., 1995	*Ipomoea litoralis* flower	380
	51.636	Rosa et al., 1995	*Ipomoea litoralis* flower	380
Saccharomyces cerevisiae	51.174^T	CBS 1171	Brewers yeast	850
Torulaspora delbrueckii	50.663^T	UCD 69-034	Unknown	720
Kluyveromyces aestuarii	51.172^T	CBS 4438	Biscayne Bay	670
Yarrowia lipolytica	50.678	Hagler and Mendonça–Hagler, 1981	Guanabara Bay	370
Issatchenkia terricola	50.453^T	UCD 66-022	South African soil	430

^{T}, type culture. IM-UFRJ, Coleção de Culturas de Leveduras do Instituto de Microbiologia Prof. Paulo de Góes, UFRJ, Rio de Janeiro, Brazil; CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; UCD, Dept. of Food Science and Technology, University of California Davis, CA, U.S.A.; UWO, University of Western Ontario, Canada.

360 to 850 bp (Table 1, Fig. 1). The ITS length does not vary within the species *Saccharomyces cerevisiae* (Valente et al., 1996), and we found that it did not vary among *Metschnikowia bicuspidata* strains either. This suggests that although ITS size alone can not identify yeasts, it could be used as an exclusionary characteristic to detect some cultures misidentified on the basis of phenotypic similarity, such as *Metschnikowia* species or their anamorphs. Several Brazilian strains putatively identified as *Metschnikowia* anamorphs had ITS products mostly of less than 400 bp, supporting their identification (Table 1). However, strains IM-UFRJ (Coleçáo de Culturas de Leveduras do Instituto de Microbiologia Prof. Paulo de Góes, UFRJ) 50.213 and 50.329, previously identified as *C. pulcherrima*-like and *C. pulcherrima*, had products of about 580 and 610 bp, respectively, showing that they were misidentified.

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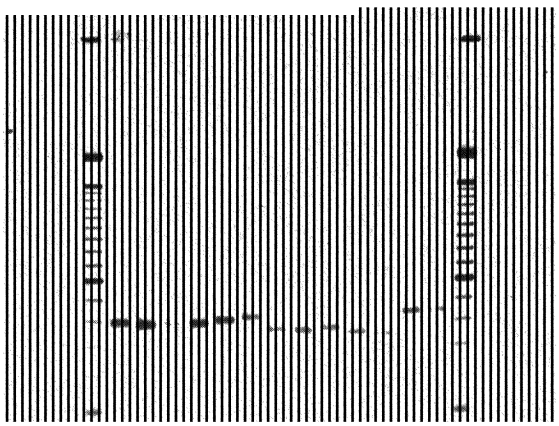

Fig. 1. A 2.0% agarose gel showing size in base pairs of PCR-amplified ITS using primers ITS1 and ITS4.

Slots 1 and 15 (from left to right), 100 bp DNA ladder, slot 2, *Metschnikowia bicuspidata* (51.625^T); slot 3, *M. bicuspidata* (50.493); slot 4, *M. bicuspidata* (51.627); slot 5, *M. australis* (51.177^T); slot 6, *M. krissii* (51.167^T); slot 7, *M. zobellii* (51.173^T); slot 8, *M. gruessii* (51.175^T); slot 9, *M. pulcherrima* (50.497^T); slot 10, *M. reukaufii* (50.498^T); slot 11, *M. hawaiiensis* (51.169^T); slot 12, *M. lunata* (50.496^T); slot 13, *M. agaveae* (51.499^T); slot 14, *M. agaveae* (51.500). The numbers in parentheses are IM-UFRJ strains.

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