Numerical Analysis and Computerized Identification of the Yeast Genera *Candida* and *Torulopsis*

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SUMMARY

Numerical analysis of the published standard descriptions of 104 species of *Candida* and 48 species of *Torulopsis* suggested that the number of species should be reduced to 78 and 33 respectively. Four examples are noted of closely matched species differing in ability to assimilate nitrate; these species may be combined as nitrate-variable species. Although the ability to form pseudomycelium is the only important difference between the genera, only three examples were noted of *Candida* and *Torulopsis* species sufficiently closely matched to be regarded as synonymous. Close relationships with species of the perfect genera were more common. A system, derived from the results of the analysis, is described for computerized identification of species of both genera.

INTRODUCTION

The genera *Candida* and *Torulopsis* were described by van Uden & Buckley (1970) as a heterogeneous collection of asporogenous yeasts which could not be classified conveniently elsewhere. Both genera therefore include yeasts of widely different properties with respect to morphology, fermentation, and production of a true mycelium or pseudomycelium. Production of a pseudomycelium is an unsatisfactory criterion for distinguishing between the two genera, and van Uden & Buckley noted a number of transitional species with poorly developed pseudomycelia.

Previously published numerical analyses of the genera *Candida* and *Torulopsis* have concerned only a restricted range of species, e.g. *Candida albicans* and related species (Kocková-Kratochvílová *et al.* 1967; Kocková-Kratochvílová & Ondrušová, 1972; Shechter, Landau & Dabrowa, 1972), and a selection of 22 *Torulopsis* species (Kocková-Kratochvílová, 1973). Therefore an analysis of the published properties of all valid species of *Candida* and *Torulopsis* was attempted, by the system used previously for the genera *Saccharomyces*, *Hansenula* and *Pichia* (Campbell, 1972, 1973a).

METHODS

Characters for numerical analysis. The analyses were performed on the published descriptions of species by van Uden & Buckley (1970), van Uden & Vidal-Leiria (1970) and by authors who subsequently described new species. The 58 characters described in the study of *Hansenula* and *Pichia* (Campbell, 1973a) were reduced to 53 by elimination of spore characters for analyses limited to *Candida* and *Torulopsis*. However, the allocation of five taxonomic units to ascospores was later restored for comparison of *Candida* and *Torulopsis* with ascosporogenous genera of similar morphological and physiological properties.

The standard properties of each species were described on 80-column punched cards (Campbell, 1972). Normally two cards were required to describe the limits of variability of a species, i.e. in one description all variable properties were scored positive and in the other description all variable properties were scored negative. Rarely-isolated species with no variable properties in the standard descriptions required only one card per species.

Calculation of the matching coefficient S_{sm} (Sneath & Sokal, 1973) was by the computer programs 1 and 2 described by Campbell (1973b), and the results were drawn as dendrograms in steps of 5 % at the highest matching coefficient of the group.

Species for analysis. The 71 species of *Candida* described by van Uden & Buckley (1970) and the 36 species of *Torulopsis* described by van Uden & Vidal-Leiria (1970) were analysed. In addition, the following recently described species were included: *Candida amylo lenta* (van der Walt, Scott & van der Klift, 1972), *C. australis* (Goto, Sugiyama & Iizuka, 1969), *C. boleticola* (Nakase, 1971a), *C. bombi* (Montrocher, 1967), *C. butyri* (Nakase, 1971a), *C. chilensis* (Grinbergs & Yarrow, 1970), *C. chiropterorum* (Grose & Marinkelle, 1968), *C. citrea* (Nakase, 1971b), *C. dendronema* (van der Walt, Scott & van der Klift, 1971a), *C. edax* (van der Walt & Nel, 1968), *C. entomaea* (van der Walt *et al.* 1971a), *C. fibrae* (Nakase, 1971c), *C. fragicola* (Nakase, 1971c), *C. homilentoma* (van der Walt & Nakase, 1973), *C. hylophila* (van der Walt *et al.* 1971a), *C. incommunis* (Ohara, Nonomura & Yamazaki, 1965), *C. insectamans* (van der Walt *et al.* 1972), *C. insectorum* (van der Walt *et al.* 1972), *C. lactosa* (Dwidjoseputro & Wolf, 1970), *C. naeodendra* (van der Walt, Johannsen & Nakase, 1973), *C. nitrativorans* (van der Walt *et al.* 1972), *C. oleophila* (Montrocher, 1967), *C. pseudolipolytica* (Blagodatskaya & Kocková-Kratochvílová, 1973), *C. quercuum* (Nakase, 1971a), *C. rugopelliculosa* (Nakase, 1971b), *C. silvanorum* (van der Walt *et al.* 1971a), *C. silvicultrix* (van der Walt *et al.* 1972), *C. sorboxylosa* (Nakase, 1971b), *C. steatolytica* (Yarrow, 1969a), *C. suecica* (Rodrigues de Miranda & Norkrans, 1968), *C. tsukubaensis* (Onishi, 1972), *C. valdiviana* (Grinbergs & Yarrow, 1970), *Torulopsis auriculariae* (Nakase, 1971c), *T. bombicola* (Spencer, Gorin & Tulloch, 1970), *T. dendrica* (van der Walt *et al.* 1971b), *T. fragaria* (Barnett & Buhagiar, 1971), *T. fructus* (Nakase, 1971c), *T. humilis* (Nel & van der Walt, 1968), *T. insectalens* (van der Walt *et al.* 1971b), *T. kestoni* (Scarr & Rose, 1966), *T. mannito-faciens* (Onishi & Suzuki, 1969), *T. musae* (Nakase, 1971c), *T. nemodendra* (van der Walt *et al.* 1971b), *T. peltata* (Yarrow, 1968), *T. philyla* (van der Walt *et al.* 1971b), *T. psychrophila* (Goto *et al.* 1969) and *T. silvatica* (van der Walt *et al.* 1971b). The list of species is incomplete since recently described species could not be included. Some earlier descriptions of species were not in the form used by Lodder (1970) and could not be applied to our system. *Torulopsis peltata*, although now transferred to the genus *Selenotila* (Yarrow, 1969b), was retained in the analysis since no later examination was planned for that genus.

Computerized identification of Candida and Torulopsis species. Identification of species of *Saccharomyces* and *Kluyveromyces* was conveniently accomplished by computer program 2 (Campbell, 1973b) but extension of the method to other genera increased to an inconvenient number the descriptions to be handled during each identification. A new program 3 compared the description of each unknown strain with all standard descriptions held in the data store; the names and matching coefficients of only the three highest matches were printed. Provided the highest match was $\geq 85\%$ this was normally accepted as identification.

RESULTS

Initially the genera *Candida* and *Torulopsis* were examined separately. In the analysis of the 48 species of the genus *Torulopsis* the entire genus was examined in a single test by

Fig. 2

Fig. 1

Fig. 3

Fig. 1. Taxonomic dendrogram of 104 species of the genus *Candida*, from analysis of standard descriptions of species.

Fig. 2. Taxonomic dendrogram of 48 species of the genus *Torulopsis*, from analysis of standard descriptions of species.

Fig. 3. Dendrogram of selected taxonomic groups to show relationships between species or groups of the genera *Candida*, *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Pichia*, *Saccharomyces* and *Torulopsis*.

program 2 (Campbell, 1973*b*); the groups of species thus detected were confirmed, or adjusted as necessary, by analysis of batches of 24 cards by program 1 (Campbell, 1973*b*). The large number of species in the genus *Candida* was beyond the capacity of a single examination by program 2. *Candida* species were examined in various combinations in groups of approximately 50 species; by four separate analyses the relationship of each species was shown to all other species of *Candida*. Again, the validity of the taxonomic groups so detected was confirmed by analysis of smaller groups. Dendrograms of these analyses are shown as Figs. 1 and 2.

In the genus *Candida*, clusters of six or more species were associated with *C. valida*, *C. albicans*, *C. maritima*, *C. boleticola* and *C. diddensii* (Fig. 1). Numerous smaller groups of 3 to 5 species were also noted. In the genus *Torulopsis*, 34 of the 48 species belonged to two main groups of species, related within each group at $S_{sm} > 80\%$ (Fig. 2). If species at $S_{sm} \geq 90\%$ are regarded as synonymous, in accordance with our system for other genera (Campbell, 1972, 1973*a*), the genus *Candida* should be reduced from 104 to 78 valid species and the genus *Torulopsis* from 48 to 33 species.

Each species of the genus *Candida* or *Torulopsis* was then compared with all species of the other genus and with the species already examined in the genera *Debaryomyces*, *Hansenula*, *Kluyveromyces*, *Lodderomyces*, *Pichia* and *Saccharomyces* (Campbell, 1972, 1973*a*). All species of these genera, as defined by Lodder (1970), were included in the comparison by programs 1 and 2 (Campbell, 1973*b*). Only groups of species are included in the resulting dendrogram (Fig. 3), except where more detail was occasionally required to show the close relationship between species of different genera. The main overlap between *Candida* and *Torulopsis* occurs in the groups containing *C. norvegensis*, *C. valida*, *T. glabrata* and *T. magnoliae*; some species of the genera *Kluyveromyces*, *Pichia* and *Saccharomyces* are also included in this cluster, and this complex group of species is illustrated in detail in Fig. 3. Otherwise, there is surprisingly little overlap between the genera, but some examples of the occurrence of similar species in different genera are discussed below.

DISCUSSION

The dendrograms of the genera *Candida* and *Torulopsis* reveal close matching, $S_{sm} > 90\%$, between species which vary in their ability to assimilate nitrate as sole source of nitrogen. Current classification of the genera *Candida* (van Uden & Buckley, 1970) and *Torulopsis* (van Uden & Vidal-Leiria, 1970) does not recognize nitrate-variable species, but since variable species occur in other genera, e.g. *Brettanomyces* and *Cryptococcus* (Lodder, 1970), they are presumably acceptable also in *Candida* and *Torulopsis*. The only significant difference between *T. lactis-condensi* and *T. stellata* is the assimilation of nitrate by the former species. Guanine-cytosine (GC) proportions of DNA bases of both species are in the range 42.0 to 43.7% (Nakase & Komagata, 1971*a*) and the proton magnetic-resonance (p.m.r.) spectra are remarkably similar, although not identical (Gorin & Spencer, 1970). With this supporting evidence, the very close taxonomic relationship, $S_{sm} 90\%$, suggests that these two species should be regarded as a single nitrate-variable species. Other similar examples are also shown in Figs. 1 and 2. Thus, *C. freyschussii* and *C. quercuum* (nitrate-negative) are related at $S_{sm} 90\%$ to *C. melinii* (nitrate-positive), and may be considered as synonyms; *C. bogoriensis* (nitrate-positive) and *C. buffonii* (nitrate-negative) are also closely related.

Another complex group includes *T. halonitratophila*, which is related at $S_{sm} 90\%$ to, and therefore presumably synonymous with, a group of six nitrate-negative *Torulopsis* species. Of these species, *T. castellii* and *T. glabrata* differ only in fermentation of trehalose, which was not assessed in our tests, and therefore these species are identical in Fig. 2. *Torulopsis*

bovina, *T. inconspicua* and *T. pintolopesii* are mutually related at S_{sm} 95 %; their separation into three species by van Uden & Vidal-Leiria (1970) was based on their different habitats and optimum temperatures for growth, but morphologically and physiologically there is insufficient difference to justify more than one species. *Candida slooffii* and *Saccharomyces telluris* are closely related to this group and may represent a single species (van Uden & Buckley, 1970). In our results (Fig. 3) *Kluyveromyces delphensis* was also closely associated with this group.

In a taxonomic study of the genus (Kocková-Kratochvílová, 1973), strains of 22 species of *Torulopsis*, including five species previously transferred by van Uden & Vidal-Leiria (1970) to other genera, were arranged in nine main groups. Although the range of characters chosen by Kocková-Kratochvílová differed in some respects from that used here, the results are substantially the same, especially in the close similarity of *T. etchellsii* and *T. versatilis* (Kocková-Kratochvílová's phenon 6) and *T. apis*, *T. apicola*, *T. gropengiesseri* and *T. magnoliae* (phenon 7). In both Kocková-Kratochvílová's and our results the main groups of *Torulopsis* species are related at 75 to 80 %. *Torulopsis glabrata* and *T. pinus*, and *T. candida* and *T. wickerhamii* were placed in the same phenons by Kocková-Kratochvílová and in different groups in Fig. 2, but the percentage matching between the species is approximately the same in both analyses. Kocková-Kratochvílová analysed individual strains, whereas this report is based on standard descriptions of species, but the general agreement of the results is very satisfactory.

Similarities between species of different genera. Van Uden & Buckley (1970) noted a number of similarities between species of *Candida* and *Torulopsis* and perfect genera. They quoted some examples where *Candida* and *Torulopsis* were renamed when shown to form spores. Since these species are no longer regarded as *Candida* or *Torulopsis*, they are not included in the present analysis. Van Uden & Buckley also listed a number of similarities between species of ascosporogenous genera and species still classified as *Candida* or *Torulopsis*, and most of these similarities have been noted in the present analysis (Fig. 3). Thus, the *C. norvegensis* and *C. valida* groups are related to, and overlap considerably with, *Pichia fermentans*, *P. kudriavzevii* and *P. membranaefaciens*. Van Uden & Buckley (1970) suggested that *C. macedoniensis* is the imperfect stage of *Kluyveromyces marxianus*, and *C. pseudotropicalis* is the imperfect form of *K. fragilis*. The close similarity of these four species is confirmed, but in Fig. 3 both *Kluyveromyces* species are most closely related to, or are possibly the imperfect stage of *C. macedoniensis*. Although not shown in Fig. 3, there was a close match, S_{sm} 80 to 90 %, between *Lodderomyces elongisporus* and members of the *C. albicans* group, including *C. albicans* itself; van Uden & Buckley (1970) commented only on similarity to *C. parapsilosis*.

Other instances of closely matched species of different genera are apparent in Fig. 3, and others were shown in the more extensive results from which that dendrogram was prepared. Various groups of *Candida*, *Debaryomyces*, *Kluyveromyces*, *Pichia*, *Saccharomyces* and *Torulopsis* overlap, so that in one area there is a complex cluster of perfect and imperfect, weakly fermentative and non-fermentative, and pseudomycelial and non-mycelial yeasts. For the following associated species the matching is sufficiently close ($S_{sm} \geq 90$ %) to suggest synonymy, or a perfect/imperfect relationship: *C. beechii*/*T. torresii*, *C. guilliermondii*/*Pichia guilliermondii*, *C. foliarum*/*T. silvatica* (included in *C. norvegensis* and *T. magnoliae* groups respectively), *C. muscorum*/*T. fragaria*, *Hansenula capsulata*/*T. molischiana*, *K. delphensis*/*T. glabrata*/*T. inconspicua*. The suggested synonyms *C. foliarum* and *T. silvatica* not only belong to different genera owing to the primitive mycelium of *C. foliarum*, but differ in ability to assimilate nitrate.

Other matchings in the 80 to 90 % range, and therefore not close enough to suggest synonymy, may nevertheless be of interest. *Torulopsis candida*, itself showing a considerable range of variation within the description of the species, is similar to *C. glabrosa* and *C. guilliermondii*, but is probably the imperfect form of one or both species *Debaryomyces hansenii* and *D. marama* (van Uden & Vidal-Leiria, 1970). *Torulopsis stellata* shows some similarity to *Saccharomyces rosei*, but is not sufficiently close to confirm van Uden & Vidal-Leiria's (1970) suggestion that *S. rosei* and *T. stellata* are synonymous. Members of the *S. rosei* group (Campbell, 1972) are equally closely related to *T. colliculosa* (S_{sm} approximately 85 %); GC, p.m.r. and serological results (Nakase & Komagata, 1971*a, b*; Gorin & Spencer, 1970; Campbell, 1971; Tsuchiya, Fukazawa & Kawakita, 1965) confirm a close relationship.

Main groups of Candida and Torulopsis species. Although the type of habitat from which the species were isolated was not considered in the analyses, most groups of species in Figs. 1 and 2 show some ecological similarity.

The *C. valida*, *C. norvegensis* and *C. zeylanoides* group comprises 27 non-fermentative species, or species which ferment only glucose, usually weakly. These species have been isolated from a wide range of habitats. Many are associated with spoilage of foods and drinks; others are mammalian or insect parasites. *Candida krusei*, *C. lambica*, *C. norvegensis* and *C. zeylanoides* have been isolated from both types of habitat. The 27 species show a strong resemblance to the *P. membranaefaciens* group of species, e.g. *C. krusei* and *C. valida* are imperfect forms of *P. kudriavzevii* and *P. membranaefaciens* respectively (van Uden & Buckley, 1970). The evidence in this paper of a relationship between the species of the group is supported by the similarity of the p.m.r. spectra: many of the species were examined by Gorin & Spencer (1970) and belong mainly to p.m.r. groups 1, 9, 15 and 18. GC proportions (Nakase & Komagata, 1971*c*) are very close in the members of the various subgroups of the cluster, e.g. *C. lipolytica* and *C. rugosa* (48.3 to 50.7 %), but over the whole group the GC proportion rises from 34.4 % (*C. diversa*) to 55.9 % (*C. zeylanoides*) (Nakase & Komagata, 1971*c*).

The *C. albicans* group comprises ten species related at $S_{sm} \geq 85$ %, and a further seven species (*C. maritima* subgroup) related at S_{sm} 80 % to *C. albicans*. With the exception of *C. sake*, all species of the group are parasitic on humans or animals, although not necessarily in association with obvious disease. Most species of the group ferment a range of sugars, but none assimilate nitrate as nitrogen source. Numerical analyses of this group have consistently shown that the species merge as a cluster of overlapping species, as with *C. lusitanae* and *C. sake* in Fig. 1 (e.g. Kocková-Kratochvílová *et al.* 1967; Shechter *et al.* 1972).

The *C. boleticola* group comprises 11 species mutually related at 75 to 85 %. There appears to be little ecological significance in this group; the members have been isolated from a wide range of habitats. Except for *C. friedrichii*, they are non-fermentative.

The *C. diddensii* group comprises 15 species associated with insects, although *C. diddensii*, *C. membranaefaciens* and *C. tenuis* have also been isolated from other environments. All species of this group are weakly or non-fermentative.

In the final part of the dendrogram (Fig. 1), *C. chiropterorum*, *C. diffluens* and *C. melibiosica* are shown to be related at only 55 to 65 % to the rest of the genus *Candida*, casting doubt on their validity as *Candida* species. However, there are no properties which obviously distinguish them from other *Candida* species. Otherwise, the remaining species of *Candida* are sufficiently closely related ($S_{sm} \geq 65$ %) to be considered as members of a single genus.

There is no obvious association of ecological types in the main groups of the genus *Torulopsis*. On the other hand, subgroups of the larger groups are often associated with a

particular environment: *T. apis* and *T. gropengiesseri*, which are probably synonymous, are both associated with insects, as are *T. apicola*, *T. bombycola* and some strains of *T. magnoliae*. *Torulopsis fructus* and *T. musae*, related at S_{sm} 95 %, were both isolated (Nakase, 1971c) from bananas and represent strains of the same species.

Computerized identification. Standard descriptions were prepared for computerized identification of *Candida* and *Torulopsis* species using the same order of characters as for identification of species of *Saccharomyces* and *Kluyveromyces* (Campbell, 1973b), and *Brettanomyces*, *Debaryomyces*, *Endomycopsis*, *Hansenula* and *Pichia* (unpublished results). Although 58 characters had been used for identification of *Saccharomyces* and *Kluyveromyces* species, insufficient information was available for inclusion of the final eight characters in descriptions of the other genera. Existing data on serological properties, hydrolysis of tributyrin, clearing of chalk, and tolerance of high concentrations of ethanol were incomplete and were therefore not used.

Each distinct species was represented by at least one standard description for identification. Each group of 2 to 4 species related at $S_{sm} \geq 90$ % in Figs. 1 and 2 was regarded as a single valid species, to be represented by a single description. In the larger clusters, including *C. albicans*, *C. diddensii* and *T. glabrata*, descriptions differing by 15 % or more (cf. Campbell, 1973b) were prepared by trial and error. In those preliminary tests all matches over 85 % were reduced by eliminating one of the species of the pair, or by altering the descriptions to reduce the matching coefficient to ≤ 85 %; all adjustments were made within the recognized range of properties of the species. When species were combined as synonyms, an average description was prepared to represent the new species. Thus, *T. apis* and *T. gropengiesseri* were re-defined as one valid species (*T. gropengiesseri*, by the normal rules of priority) but the mean standard description did not coincide exactly with the description of *T. gropengiesseri sensu* van Uden & Vidal-Leiria (1970). In this respect the system differs from that of Barnett & Pankhurst (1974), who provided a description of every physiological type which could occur within the standard description of the species; in our system only a single description of a hypothetical mean organism is normally required (Campbell, 1973b).

The extension of the identification scheme from *Saccharomyces* and *Kluyveromyces* to other genera introduced the difficulty of distinguishing species related at $S_{sm} > 85$ %, but of different genera. After some attempts to remove or adjust descriptions of *Candida* and *Torulopsis* species to avoid close matching with species of perfect genera, such a system was rejected as both misleading and unnecessary. In our present system, within each genus no species are closer than S_{sm} 85 %, but higher matching coefficients are tolerated between species of different genera. Normally, a culture is identified as the species whose standard description it matches most closely. Closely related descriptions of perfect and imperfect yeasts introduced the possibility of two valid highest matches in different genera. Thus, if the best matching coefficients are, for example, *Candida krusei* 96 % and *P. kudriavzevii* 93 %, the culture is reported simply as *C. krusei*/*P. kudriavzevii* (cf. Beech *et al.* 1968). Higher matching with the imperfect species, as in the example, does not necessarily indicate an asporogenous yeast. Subsequent examination for spores may be performed if desired, but is not included in the computer identification.

Based on the analyses described above and in earlier papers (Campbell, 1973a, b), a selection of mean standard descriptions was prepared for identification of the genera *Brettanomyces* (including *Dekkera*), *Candida*, *Endomycopsis*, *Hansenula* (including *Citeromyces* and *Pachysolen*), *Pichia* (including *Debaryomyces* and *Wingea*), *Saccharomyces* (including *Kluyveromyces*) and *Torulopsis*. The system has given good agreement with identifications by Lodder (1970) and Barnett & Pankhurst (1974). Since the system is still

in the process of modification and improvement the descriptions of species are not presented here, but a list of the present computer descriptions of species is available on request.

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