Deoxyribonucleic Acid Base Composition and Numerical Taxonomy of Yeasts in the Genus *Trichosporon*

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SUMMARY

Of the 49 strains of *Trichosporon* yeasts studied, 40 were retained in the genus and assigned to 10 species. The others were transferred to the genera *Candida* and *Endomycopsis*. The DNA base composition was determined for a representative of each *Trichosporon* species. On the basis of guanine and cytosine content (% GC), the species were arranged into four groups: (a) about 55 % GC, *Trichosporon aculeatum*, *T. aquatile*, *T. cutaneum*, *T. inkin* and *T. pullulans*; (b) about 64 % GC, *T. eriose*, *T. fermentans* and *T. infestans*; (c) 59 % GC, *T. capitatum* and (d) 45 % GC, *T. penicillatum*.

For 25 strains of the genus, 81 characteristics were selected for inclusion in a numerical taxonomic analysis with the aid of a computer. The dendrogram of the similarity values arranged these strains into three major branches arising at the 66 % *S* level. Branch I includes *Trichosporon aculeatum*, *T. cutaneum*, *T. infestans*, *T. inkin* and *T. pullulans*. Branch II is limited to *T. aquatile*. Branch III includes *T. capitatum*, *T. eriose*, *T. fermentans* and *T. penicillatum*. Species arising from branch I at differing *S* levels are *T. aculeatum* at 68 % *S*, *T. pullulans* at 72 % *S*, *T. inkin* at 75 % *S* and *T. infestans* at 78 % *S*. The remaining strains in branch I belong to the *T. cutaneum* group.

INTRODUCTION

Determination of DNA base composition and the use of Adansonian analysis have been shown to be of considerable value in bacterial taxonomy (Marmur, Falkow & Mandel, 1963; Sneath, 1964). Recently the application of these methods has been beneficial in studying the relationship among yeasts and other fungi. Storck (1966) determined the DNA base composition for 14 species of fungi representing 11 genera; Stenderup & Leth Bak (1968) did the same for 18 species of *Candida*. Nakase & Komagata (1968) and Meyer & Phaff (1969) reported the DNA base composition of yeast in several genera. Storck, Alexopoulos & Phaff (1969) determined the DNA base composition for 12 strains of the genus *Cryptococcus*, six strains of the genus *Rhodotorula* and 15 strains of the genus *Sporobolomyces*. Nucleic acid homologies were determined for species of *Candida* (Leth Bak & Stenderup, 1969) and for species of *Saccharomyces* (Bicknell & Douglas, 1970). Kocková-Kratochvílová (1969) outlined the relationships for several species of *Saccharomyces* by applying Adansonian analysis.

The present paper deals with the DNA base composition of 10 valid species belonging to the yeast genus *Trichosporon* and an Adansonian analysis of 25 strains of yeasts placed in this genus by various authors. The genus *Trichosporon* includes those yeasts in which sexual spores have not been found but which form both buds and arthrospores. The latter arise by the fragmentation of a mycelium and are arranged in a zigzag pattern. The members of this group have a variety of ecological origins and show wide ranges in the ability to use carbohydrates and L-amino acids as carbon sources (Hedrick & Dupont, 1968a).

METHODS

Organisms used. The organisms studied (Table 1) were either isolated by the authors from fresh waters (IIT, Illinois Institute of Technology strains) or were obtained from culture collections, including American Type Culture Collection (ATCC), Rockville, Maryland; Centraalbureau voor Schimmelcultures (CBS), Baarn, The Netherlands; Gulbenkian Institute of Science Culture Collection (IGC), Oeiras, Portugal; and Northern Regional Research Laboratories (NRRL), Peoria, Illinois. All cultures were reclassified according to the principles of Wickerham (1951) and Lodder & Kreger-van Rij (1952). Stock cultures were maintained and stored as slants on yeast extract-malt extract (YM) agar, which contained (g./l.): glucose, 10; peptone, 5; yeast extract, 3; malt extract, 3; and agar, 20. All strains were grown at 25°, except *Trichosporon pullulans* which was incubated at 10°.

Table 1. *Trichosporon* yeasts studied

Name of yeast	Culture collection no.
<i>T. aculeatum</i> Phaff et Miller et Shifrine, 1956	NRRL YB 4297
<i>T. aquatile</i> Hedrick & Dupont, 1968	IIT 9, NRRL Y 7051
	IIT, 50, NRRL Y 7053
<i>T. atlanticum</i> Siepmann et Hohnk, 1962	IIT K 30
<i>T. behrendii</i> Lodder et Kreger-van Rij, 1952	CBS 2452
<i>T. beigelii</i> (Kuchenmeister et Rabenhorst) Vuillemin, 1902	ATCC 14905
	ATCC 10267
<i>T. capitatum</i> Diddens et Lodder, 1942	NRRL Y 1487
5 strains from fresh water	IIT, 15, 17, 21, 23, 45
<i>T. cutaneum</i> (de Beurmann et Gougerot et Vaucher) Ota, 1909	NRRL 1490
14 strains from fresh water	ATCC 14254, ATCC 4155
	IIT 1, 6, 8, 10, 20, 24, 26,
	27, 31, 38, 43, 46, 47, 51
<i>T. cutaneum</i> var. <i>multisporum</i> (Cochet, 1940)	NRRL 1520
<i>T. cutaneum</i> var. <i>peneaus</i> Phaff et Mark et Williams, 1952	NRRL 2002
<i>T. diddensii</i> Phaff et Mark et Williams, 1952	NRRL 2000
<i>T. eriense</i> Hedrick et Dupont, 1968	NRRL 7052
<i>T. fermentans</i> Diddens et Lodder, 1942	NRRL Y 1492
<i>T. giganteum</i> Unna, 1896	ATCC 10266
<i>T. hellenicum</i> Vernona et Picci, 1958	CBS 4099
<i>T. infestans</i> (Mosses et Vianna) Ciferri et Redaelli, 1913	NRRL Y 1491
<i>T. inkin</i> (Oho) do Carmo Sousa et Van Uden, 1967	IGC 3727
<i>T. loboii</i> Batista et Campos et Oliveira, 1963	CBS 5286
<i>T. lodderi</i> Phaff et Mark et Williams, 1952	NRRL Y 2001
<i>T. margartiferum</i> (Stautz) Buchwald, 1931	CBS 2537
<i>T. maritimum</i> Siepmann et Hohnk, 1962	IIT K 29
<i>T. penicillatum</i> do Carmo Sousa, 1965	IGC 3716
<i>T. piscium</i> Siepmann et Hohnk, 1962	IIT K 28
<i>T. pullulans</i> (Lindner) Diddens et Lodder, 1942	NRRL 1485, NRRL 2519
<i>T. sericeum</i> (Stautz) Diddens et Lodder, 1931	NRRL Y 1523
<i>T. undulatum</i> Windisch, 1952	CBS 2546

DNA extraction. The yeasts were grown for 24 h. in YM broth and lyophilized; 30 g. of dried yeast were suspended in 120 ml. of a solution of 0.15M-NaCl + 0.1M-Na ethylenediamine tetra-acetate + 2 % sodium dodecyl sulphate + 1 % mercaptoethanol at pH 8.0. The suspension was poured into a 1 l. beaker and exposed to chloroform vapours from a large cotton plug during incubation at 37° for 24 h. This treatment lysed enough of the yeast cells to release sufficient quantities of DNA for the base determination procedures for all organisms except *Trichosporon aquatile* and *T. eriense*. Eight alternate freezing and thawing

treatments in liquid nitrogen altered the cells of these species for adequate lysis by the above method.

DNA purification. The DNA was concentrated and purified by the method of Marmur (1961), as modified by Meyer & Phaff (1969). The DNA was precipitated by ethanol and collected by spooling the fibrous precipitate onto a glass rod.

Estimation of DNA base composition from absorbancy ratios. Fredericq, Oth & Fontaine (1961) estimated the DNA base composition from the ratio of extinction values ($E_{260}:E_{280}$) measured at 260 nm. and at 280 nm. De Ley (1967) compared the results obtained by this method with the thermal denaturation method of Marmur & Doty (1962) and derived an empirical equation for an approximation of the % guanine + cytosine (% GC)

$$\% \text{ GC} = 168.6 - 87.4(E_{260}/E_{280}).$$

For this method, the purified DNA was precipitated with isoamyl alcohol and redissolved in 5 ml. 0.1N-acetic acid to give an extinction value of 0.3 to 0.5 at 260 nm. when measured against 0.1N-acetic acid in a recording Beckman DB spectrophotometer. The % GC was calculated from the above equation.

Determination of DNA base composition by thermal denaturation. Each sample of DNA was dissolved in 0.15M-NaCl + 0.15M-Na citrate, pH 7 (SSC) to give a solution containing approximately 20 µg. DNA/ml. according to the method of Burton (1956). The extinction of each sample was measured at 25° at 260 nm. against SSC; the temperature was then quickly raised to within 10° of the estimated T_m . The spectrophotometer was equipped with thermospacers heated at a constantly increasing rate by a circulating hot water bath containing ethylene glycol + water (1:3). The rate of temperature increase was 0.2°/min. as monitored by a previously calibrated thermosensor immersed in the covered blank cuvette. The reliability of the method was checked by comparing the measured T_m value of *Escherichia coli* DNA with that given in the literature. The T_m values represent the midpoints of the extinction temperatures corrected for thermal expansion. The mean base composition (% GC) was calculated from the equation of Marmur & Doty (1962):

$$\% \text{ GC} = (T_m - 69.3)/0.41.$$

Numerical Taxonomy and Features. The numbers given to the features and the strains with their parameters as described by these features are summarized in the Appendix. The types of tests used are described below; the features number is given in parentheses. Each feature was coded for computer analysis according to the Adansonian principles of numerical taxonomy as developed by Sneath (1962). The primary aim was to group strains into clusters composed of related strains. The similarity index, % *S*, was calculated using the following equation:

$$\% S = n_s \times 100/(n_s + n_d),$$

where n_s is the number of similar matches and n_d is the number of dissimilar matches. The program for the IBM 360/40 computer was developed by the Computation Centre at Illinois Institute of Technology.

Over 100 features were determined for each of the 25 strains of *Trichosporon* but only 81 were incorporated into the data table as tests in which all strains gave (+) or (−) results were omitted. The latter included: (i) utilization tests – glucose (+), ethanol (+), inulin (−), 8L-amino acids (+) as a carbon source, and cysteine and cystine (−) as a source of C and N; and (ii) sensitivity to antimicrobial agents: nystatin (+) and tributon chloride (+).

Growth rate (features 1 to 3). Extensive growth of the test organism in glucose (0.5 %, w/v)

in Difco yeast nitrogen base (0.67 %, w/v) (YNB) medium after incubation for 3 days (1), 7 days (2) or 14 days (3). If feature 1 was (+), features 2 and 3 were scored as 'no comparison' and indicated by (*). If feature 2 was (+), feature 3 was scored as an (*).

Colony characteristics (features 4 to 9). These features were observed after 7 days incubation on the defined agar medium of Wickerham (1946). The characters recorded: (a) sheen – shiny (4), intermediate between shiny and matt (5) or matt (6); (b) topography – smooth (7), intermediate between smooth and rough (8) or rough (9).

Pellicle formation (feature 10). Film formation after growth on glucose–YNB broth.

Growth at different temperatures (features 11 to 14). Good growth at 4° (11), 15° (12), 25° (13) and 37° (14) was recorded.

Table 2. *DNA base composition of valid species in the genus Trichosporon*

Species	% GC	
	From E_{280}/E_{260}	From T_m *
<i>T. aculeatum</i> NRRL YB 4297	54.9	55
<i>T. aquatile</i> NRRL Y 7051	55.1	55
<i>T. capitatum</i> NRRL Y 1487	60.1	59
<i>T. cutaneum</i> NRRL Y 1490	54.5	55
<i>T. ericense</i> NRRL 7052	62.4	64
<i>T. fermentans</i> NRRL Y 1492	65.0	64
<i>T. infestans</i> NRRL 1491	65.9	64
<i>T. inkin</i> IGC 3727	57.6	57
<i>T. penicillatum</i> IGC 3716	44.9	45
<i>T. pullulans</i> NRRL Y 1485	56.7	55

* T_m values represent averages of at least two determinations; the greatest difference between the two values was less than 1°.

Fermentation of glucose and galactose (features 15 to 16). The accumulation of CO₂ within insert-tubes during growth in a medium containing 0.75 % sugar (w/v) and 0.45 % (w/v) Difco yeast extract. Fermentative activities recorded were glucose (15) and galactose (16).

Carbohydrate utilization (features 17 to 44). A positive result is the utilization of one of 29 carbohydrates after incubation for 21 days. Each carbohydrate solution was added to YNB to give a final carbohydrate concentration of 0.5 % (w/v). The carbohydrate and the YNB solutions were sterilized by filtration.

L-Amino acids utilized as nitrogen sources (features 45 to 55). The methods for media preparation and growth measurements were those of Hedrick & Dupont (1968a).

L-Amino acids used as carbon and nitrogen sources (features 56 to 71). Media and methods were identical to those in preceding tests, except glucose was omitted.

Utilization of nitrate (feature 72). Two sets of agar media were inoculated with each yeast: one medium contained Difco yeast carbon base plus 0.78 g. KNO₃/l. and 1.5 % Noble's washed agar (1928); the other medium contained no added nitrate. Equal growth on both media was scored as (–); greater growth on nitrate agar was recorded as (+).

Splitting of arbutin (feature 73). Samples (9 ml.) of arbutin (0.5 %, w/v) containing Noble's agar (1.5 %) were autoclaved, and after cooling to 50° 1 ml. of sterile YNB (6.7 %, w/v) and 0.2 ml. of a saturated solution of FeCl₃ (sterilized by filtration) were added. The formation of a dark purple-brown zone around yeast growth within 21 days was recorded as (+) (Lodder & Kreger-van Rij, 1952).

Sensitivity to antimicrobial agents (features 74 to 81). Yeasts grown for 24 h. in glucose YNB broth were added to 10 ml. YM agar at 50°; this mixture was overlaid upon a base

of Noble's agar (1.5 %) in a Petri dish. Paper discs containing different antimicrobial agents were placed upon the hardened overlay and incubated for 72 h. Inhibition of growth around the discs was considered a (+) test.

RESULTS

DNA base composition. As indicated in Table 2, the GC content in the DNA of five species is in the range of 55 to 57 % and for four species it is in the range 59 to 64 %. Separated distinctly from these two groups is *Trichosporon penicillatum* with 45 % GC in the DNA. Although Nakase & Komagata (1968) reported a 54 % GC for *T. pullulans* and 59 % GC for *T. cutaneum* DNA, we find a value of 55 % for both species (based on the melting-point method). The different values from the two investigations for *T. cutaneum* could very well be due to strain differences in this heterogeneous species.

Fig. 1. Dendrogram derived from computed *S* values for 25 strains of *Trichosporon* yeasts. Numbers are those assigned to each strain in our laboratory.

Numerical taxonomy. The groupings obtained by using the *S* values from the computed analysis of the taxonomic data are presented in the dendrogram (Fig. 1). Three branches arise at the 66 % *S* level. Branch I includes *Trichosporon aculeatum*, *T. cutaneum*, *T. infestans*, *T. inkin* and *T. pullulans*. Branch II is restricted to *T. aquatile*. Branch III includes *T. capitatum*, *T. erienze*, *T. fermentans* and *T. penicillatum*.

Species which split off from branch I at differing levels of similarity are *Trichosporon aculeatum* at 68 % *S*, *T. pullulans* at 72 % *S*, *T. inkin* 75 % *S*, and *T. infestans* at 78 % *S*. The remaining yeasts of this branch are those in the *T. cutaneum* group. The sorted similarity matrix table for all the strains, with the *S* values shown as percentages, is given in Table 3.

The differing characteristics of the yeasts in each branch are presented in the Appendix.

Table 3. Sorted *S* value matrix table for 25 strains of *Trichosporon yeasts*

S is expressed as a percentage. Numbers 1 to 25 inclusive refer to number assigned to yeast strain; see Appendix and Fig. 1.

	13	12	11	7	9	10	18	8	5	4	25	15	21	14	19	20	23	24	1	2	3	16	17	6	22
13	100																								
12	97	100																							
11	97	83	100																						
7	90	93	93	100																					
9	90	94	94	88	100																				
10	90	94	94	88	94	100																			
18	89	91	91	94	91	88	100																		
8	87	90	90	92	89	86	89	100																	
5	89	86	86	86	83	83	86	83	100																
4	86	82	82	84	81	81	86	83	96	100															
25	83	79	79	79	80	82	80	79	79	80	100														
15	76	80	80	81	84	80	80	80	78	80	84	100													
21	69	73	73	76	75	73	75	77	70	72	78	64	100												
14	70	74	74	71	76	76	70	73	69	71	74	81	81	100											
19	68	64	64	68	64	61	68	67	72	74	78	74	69	66	100										
20	71	71	73	73	72	72	74	75	70	69	74	69	68	65	52	100									
23	64	68	66	65	65	70	63	64	57	58	64	63	60	63	54	55	100								
24	63	67	67	65	68	72	64	65	57	58	65	66	61	66	50	56	92	100							
1	60	62	63	64	62	63	65	69	56	56	70	66	63	66	63	63	64	65	100						
2	57	61	61	63	60	63	63	64	64	63	66	66	63	65	58	63	56	55	65	100					
3	49	49	49	48	52	51	52	53	55	54	47	52	52	56	41	49	45	47	89	100					
16	63	65	65	66	66	66	60	65	64	65	64	61	52	54	55	63	42	43	59	65	100				
17	62	60	60	66	67	61	68	68	69	69	69	62	52	54	61	52	44	45	54	60	56	100			
6	40	42	42	40	37	44	45	41	45	46	50	49	48	53	58	54	43	43	59	58	54	68	76	69	100
22	52	50	50	49	49	51	52	48	56	57	55	53	50	49	55	57	41	42	51	57	49	51	70	69	100

In summary: species in branch I (as a group) can use 14 more carbohydrates than can those in branch III; strains in branch II can use seven more carbohydrates than can those in branch III.

DISCUSSION

Forty-nine strains were studied and 40 were identified as valid *Trichosporon* yeasts. *Trichosporon atlanticum*, *T. diddensii*, *T. hellenicum*, *T. lodderi*, *T. malassiezi*, *T. margaritifera*, *T. maritimum* and *T. piscium* produced neither arthrospores nor sexual spores, but did form a pseudomycelium. These characteristics are typical for members of the genus *Candida*. *Trichosporon anglicum*, *T. behrendii* and *T. sericeum* formed ascospores in addition to a true or false mycelium and therefore belong to the genus *Endomycopsis*. All other strains formed arthrospores, but none formed sexual spores when cultured on several different types of media known to induce spore formation; these yeasts are, therefore, valid strains of the genus *Trichosporon*. In addition to the 14 strains of *T. cutaneum* isolated from fresh water and three standard cultures obtained from culture collections, the following strains should be considered synonymous with *T. cutaneum*: *T. beigelii*, *T. figueirae*, *T. giganteum*, *T. lobo*i and *T. undulatum*. This conclusion is in agreement with Carmo-Sousa (1970), out data from this and previous investigations disagree with her conclusion that *T. infestans* and *T. cutaneum* are synonymous. Hedrick & Dupont (1968*b*) supplied evidence supporting their opinion that *T. infestans* should be maintained as a separate species. This decision is strengthened by the differences in the DNA base composition of these two organisms: *T. cutaneum*, 55 % GC, and *T. infestans*, 64 % GC.

The base composition of the DNA of a sufficient number of yeasts has been determined to warrant a brief comparison of this character for several genera. The 15 species studied by Meyer & Phaff (1969), representing sporogenous yeasts or their corresponding imperfects, had GC contents of 34.9 to 48.3 %. These figures are representative of the lower range of values for the GC content of DNA, as are the values for 15 of 18 species of *Candida* (Stenderup & Leth Bak, 1968), where the average % GC for the 18 species was 43.3 and the range was 35.1 to 57.6 % GC. Nakase & Komagata (1968) reported the DNA base composition of 62 yeasts and yeast-like organisms; these included selected species of several genera. The % GC in the sporogenous yeasts such as *Endomycopsis*, *Saccharomyces*, *Pichia*, and *Hansenula* ranged from 26 to 42.5. The ranges in % GC for some of the asporogenous yeasts were 34 to 52 for *Torulopsis*, 32 to 59 for *Candida*, 46 to 56 for *Cryptococcus*, and 47 to 64 for *Rhodotorula*. Storck *et al.* (1969) reported the % GC content for the genera *Cryptococcus*, *Rhodotorula*, and *Sporobolomyces*. For *Cryptococcus* the average was 55.7 (range 49.0 to 65.5), for *Rhodotorula* the average was 57.9 (range 52.5 to 70.0), and for *Sporobolomyces* the average was 59.9 (range 51.5 to 65.0).

The DNA base composition for the asporogenous *Trichosporon* species are in the higher ranges of those reported for other yeasts. The general affinities of the *Trichosporon* species, based on the % GC in the DNA, may be expressed as follows. Cluster A (about 55 % GC): *Trichosporon cutaneum*, *T. aculeatum*, *T. aquatile*, *T. inkin*, and *T. pullulans*. Cluster B (about 64 % GC): *T. eriense*, *T. fermentans*. *Trichosporon capitatum* is intermediate between these two groups with 59 % GC, and *T. penicillatum* is distinctly different with 45 % GC.

The sexual stages of *Trichosporon* yeasts have not been elucidated; however, Carmo-Sousa (1969) found asexual endospores in *Trichosporon capitatum*, *T. fermentans* and *T. cutaneum*. Banno (1967) showed that *Rhodotorula glutinis* (DNA with 64 % GC) has a life-history resembling the Ustilaginales of the basidiomycetes and established the genus *Rhodosporidium* for this species. Newell & Fell (1970) and Newell & Hunter (1970) have

described similar life-histories for other *Rhodotorula* species; these also belong to the genus *Rhodotorula*. Fell, Statzell, Hunter & Phaff (1969) have shown that some species of *Candida*, including *Candida scottii* (DNA with 58.5 % GC), form teliospores which germinate into sporidia, and established a genus *Leucosporidium* to accommodate these species. Meyer & Phaff (1970) predict that yeasts, whose DNA has about 50 to 70 % GC, should have sexual stages similar to other yeasts in the ustilaginous group of fungi.

With certain exceptions, there was agreement in the taxonomic affinities of the *Trichosporon* species as determined by computer analysis and the DNA base composition results. Branch I in the *S* similarities dendrogram included *Trichosporon cutaneum*, *T. aculeatum*, *T. inkin*, *T. pullulans* and *T. infestans*. Cluster A of the base composition results included all branch I organisms except *T. infestans*, which has a distinctively higher % GC, and *T. aquatile*, which forms branch II of the dendrogram. The species in branch III of the *S* dendrogram were *T. eriense*, *T. fermentans*, *T. capitatum* and *T. penicillatum*. Cluster B included the first two mentioned yeasts in branch III and *T. infestans*. The % GC for *T. capitatum* DNA is somewhat lower than that for the others in cluster B. The % GC for *T. penicillatum* is considerably lower and separates this organism from the other species in cluster B.

After the completion of this study two new species, *Trichosporon fennicum* (Sonck & Yarrow, 1969), *T. melibiosaceum* (Scott & van der Walt, 1970) and a new variety, *T. cutaneum* var. *atlanticum* (Goto, Sugiyama & Iizuka, 1969), have been described. These organisms were not included in this investigation.

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APPENDIX

Summary of the morphological and physiological characteristics of selected strains of Trichosporon yeasts

Each roman numeral: designation for a dendrogram branch.

Each arabic numeral: designation for a yeast strain.

	* No comparison											II		III			
	I																
	<i>T. aculeatum</i> YB 4297	<i>T. pullulans</i> Y 1485, Y 2519	<i>T. inkin</i> IGC 3727	<i>T. infestans</i> Y 1491	<i>T. cutaneum</i> Y 1490	<i>T. cutaneum</i> IIT 10	<i>T. cutaneum</i> IIT 13	<i>T. cut. v. multisporum</i> Y 1520	<i>T. cut. v. peneaus</i> Y 2002	<i>T. lobi</i> = <i>T. cut.</i> CBS 5286	<i>T. aquatile</i> Y 7051, Y 7053	<i>T. capitatum</i> Y 1487	<i>T. ericense</i> Y 7052	<i>T. fermentans</i> Y 1492	<i>T. penicillatum</i> Y IGC 3716		
Features with their numbers	1	23, 24	20	19	7	10	13	14	15	21	2, 3	6	16	17	22		
Days required for good growth:																	
1. 3 days	+	—	+	+	+	—	+	+	+	+	+	—	+	—	+		
2. 7 days	*	—	*	*	*	+	*	*	*	*	+	+	*	+	*		
3. 14 days	*	+	*	*	*	*	*	*	*	*	*	*	*	*	*		
Colony sheen and topography:																	
4. Shiny	—	—	—	+	—	—	+	—	—	—	—	—	—	—	—		
5. Intermediate between shiny and matt	—	—	+	—	—	—	—	—	—	+	—	—	—	+	—		
6. Matt	+	+	—	—	+	+	—	+	+	+	+	+	+	+	+		
7. Smooth	—	—	—	+	—	—	—	—	—	—	—	—	—	+	+		
8. Intermediate between smooth and rough	+	—	+	—	—	—	—	—	—	+	—	+	+	—	—		
9. Rough	—	+	—	—	+	+	+	+	+	+	+	—	—	—	—		
Miscellaneous features:																	
10. Pellicle	+	+	+	—	+	+	+	+	+	+	+	+	—	—	+		
11. Good growth at 4°	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—		
12. Good growth at 15°	—	+	—	—	—	—	—	—	—	—	—	—	—	—	—		
13. Good growth at 25°	+	—	+	+	+	+	+	+	+	+	+	+	+	+	+		
14. Good growth at 37°	—	—	+	+	—	—	+	—	+	+	—	+	—	+	+		
15. Glucose fermentation	+	—	—	—	—	—	—	—	—	—	—	—	—	+	+		
16. Galactose fermentation	—	—	—	—	—	—	—	—	—	—	—	—	—	+	+		
Use of carbohydrates as carbon source:																	
17. Galactose	—	+	+	+	+	+	+	+	+	+	+	+	+	+	+		
18. L-Sorbose	—	+	—	—	—	+	+	+	—	—	—	—	+	+	+		
19. Sucrose	+	+	+	—	+	+	+	+	+	+	+	—	+	+	—		
20. Maltose	+	+	+	+	+	+	+	+	+	+	+	—	—	—	—		
21. Cellobiose	+	+	+	+	+	+	+	+	+	+	+	—	+	+	—		
22. Trehalose	+	+	+	—	+	+	+	+	+	+	+	—	—	—	—		
23. Lactose	—	+	+	+	+	+	+	+	+	+	+	—	—	—	—		
24. Melibiose	—	+	—	—	—	+	+	+	+	—	+	—	—	—	—		
25. Raffinose	—	+	—	—	+	+	+	+	—	—	—	—	—	—	—		
26. Melezitose	—	+	+	—	+	+	+	—	—	+	—	—	—	—	—		
27. Soluble starch	+	+	+	—	—	—	—	+	—	—	+	—	—	—	—		
28. D-Xylose	—	+, —	+	—	+	+	+	+	+	+	+	—	+	+	+		
29. L-Arabinose	—	+	—	—	+	+	+	+	+	+	+	—	—	—	—		
30. D-Ribose	—	—	+	+	+	+	+	+	+	+	+	—	—	—	—		
31. L-Rhamnose	—	+	—	+	+	+	+	+	+	+	—	—	—	—	—		
32. Glycerol	+	—	+	—	+	+	+	+	+	+	—	+	+	+	+		
33. Erythritol	—	+	+	+	+	+	+	+	—	—	+	—	+	—	—		
34. Ribitol	+	—	—	+	+	+	+	+	+	+	—	—	—	+	—		
35. Galactitol	—	—	—	+	+	+	+	—	—	+	—	—	—	—	—		

I

	I										II	III				
	<i>T. aculeatum</i> YB 4297	<i>T. pullulans</i> Y 1485, Y 2519		<i>T. inkin</i> IGC 3727	<i>T. infestans</i> Y 1491	<i>T. cutaneum</i> Y 1490	<i>T. cutaneum</i> IIT 10	<i>T. cutaneum</i> IIT 13	<i>T. cut. v. multisporum</i> Y 1520	<i>T. cut. v. penaeus</i> Y 2002	<i>T. lobi</i> = <i>T. cut.</i> CBS 5286	<i>T. aquatile</i> Y 7051, Y 7053	<i>T. capitatum</i> Y 1487	<i>T. erlense</i> Y 7052	<i>T. fermentans</i> Y 1492	<i>T. penicillatum</i> Y IGC 3716
Features with their numbers	1	23, 24	20	19	7	10	13	14	15	21	2, 3	6	16	17	22	
36. D-Mannitol	+	+	+	-	-	+	+	-	-	-	-	-	+	+	-	
37. D-Glucitol	+	+	+	-	+	+	+	+	-	-	-	-	-	-	-	
38. α -Methyl-D-glucoside	+	+	+	-	+	+	+	+	-	-	+	-	+	-	-	
39. Salicin	+	+	+	-	+	+	+	-	-	-	-	-	+	-	-	
40. DL-Lactic acid	-	+	+	+	+	+	+	+	+	+	+	-	+	+	+	
41. Succinic acid	-	+	-	+	+	+	+	+	+	+	+	+	+	+	+	
42. Citric acid	-	-	-	+	+	+	+	+	+	+	-	-	-	+	+	
43. <i>D</i> -Inositol	-	+	+	+	+	+	+	+	+	+	-	-	-	+	-	
44. D-Glucosamine	+	+	-	+	+	+	+	+	+	+	-	-	-	+	-	
Use of L-amino acids as nitrogen source:																
45. Aspartic acid	+	+	+	+	+	+	+	+	+	-	-	+	-	+	+	
46. Cysteine	-	+	+	+	+	+	+	+	+	-	-	+	-	+	-	
47. Cystine	-	-	+	-	+	+	+	-	-	-	-	+	+	+	+	
48. Glycine	+	+	+	+	+	+	+	-	+	-	-	+	+	+	+	
49. Histidine	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
50. Lysine	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	
51. Methionine	+	+	+	-	+	+	+	-	+	-	-	-	+	+	-	
52. Phenylalanine	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
53. Serine	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
54. Threonine	+	-	+	+	+	+	+	+	+	+	+	+	+	+	-	
55. Tryptophan	+	+	+	+	+	+	+	+	+	+	+	-	+	+	+	
Use of L-amino acids as nitrogen and carbon source:																
56. Alanine	-	+	+	-	+	+	+	+	+	+	+	+	-	+	+	
57. Arginine	-	-	-	-	+	-	+	-	+	-	-	-	-	+	+	
58. Aspartic acid	-	+	+	-	+	+	+	-	+	+	-	-	+	+	+	
59. Glutamic acid	-	+	+	+	+	+	+	-	+	+	+	-	+	+	+	
60. Glycine	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	
61. Histidine	-	-	-	-	+	+	+	-	+	+	-	+	-	+	+	
62. Isoleucine	-	-	+	-	+	+	+	-	+	+	+	-	+	+	+	
63. Leucine	-	-	+	+	+	+	+	+	+	+	+	-	+	+	+	
64. Lysine	-	-	+	-	+	+	+	+	+	-	-	+	-	+	+	
65. Phenylalanine	-	-	-	-	+	+	+	+	+	-	-	+	-	+	+	
66. Proline	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	
67. Serine	-	+	+	-	+	+	+	-	-	-	+	+	-	+	+	
68. Threonine	-	-	-	-	+	+	+	+	+	-	-	+	-	+	+	
69. Tryptophan	-	-	-	-	+	+	+	-	+	-	-	-	-	+	-	
70. Tryosine	-	-	-	-	+	+	+	-	-	-	-	-	-	+	-	
71. Valine	-	-	+	-	+	-	+	+	+	+	+	-	-	+	+	
72. Use of KNO ₃ as N	-	+	-	-	-	-	-	+	+	+	-	-	-	+	+	
73. Splitting of arbutin	+	+, -	+	-	+	-	+	-	-	-	-	+	-	+	-	
Sensitivity to antimicrobial agents:																
74. Gantrisin	-	-	+	-	-	-	-	-	-	-	-	+	+	+	+	
75. Elkosin	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	
76. Madribon	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	
77. Sulphadiazine	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	
78. Sul-spansion	-	-	-	-	-	-	-	-	-	-	-	+	+	-	+	
79. Mandelamine	-	-	-	-	+	-	-	-	+	-	-	-	+	-	+	
80. Polymyxin B	-	+, -	-	+	-	-	-	-	-	-	-	-	-	-	-	
81. Sulphamerazine	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	