The coenzyme Q system in the classification of the ascosporogenous yeast genus *Dekkera* and the asporogenous yeast genus *Brettanomyces*

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Fourteen strains of the genera *Dekkera* and *Brettanomyces* were examined for the coenzyme Q system. Without exception they contained the Q-9 system. The results are discussed from the taxonomic point of view.

INTRODUCTION

With respect to their physiology, representatives of the ascosporogenous yeast genus *Dekkera* and of its asporogenous counterpart *Brettanomyces*, are characterized by producing large amounts of acetic acid through an incomplete oxybiontic dissimilation of carbohydrate. Furthermore, both genera show a so-called "negative Pasteur effect" ("Custers effect"), i.e. a stimulation of fermentation by molecular oxygen (Wikén et al., 1961; Scheffers and Wikén, 1969).

These properties have been used as taxonomic criteria (van der Walt, 1970*a*, *b*). In previous papers we stated that the coenzyme Q system is very useful for classifying yeasts and yeast-like organisms (Yamada and Kondo, 1972*a*,*b*,*c*, 1973; Yamada et al., 1973*a*,*b*,*c*, 1976*a*,*b*, 1977).

This paper deals with the significance of the coenzyme Q system[a] in the taxonomy of organisms which belong to the genera *Dekkera* and *Brettanomyces*.

[a] The abbreviations used for coenzyme Q or ubiquinone in this paper are: Co-Q, coenzyme Q; Q-*n*, coenzyme Q with *n* isoprene units in the side chain, e.g. Q-9.

Materials and Methods

Microorganisms and cultivation

The yeast strains used in this study are listed in Table 1. The cultures were cultivated as described previously (Yamada and Kondo, 1973). However, the psychrophilic strains of *Brettanomyces nanus* nom. nud. were cultured at room temperature.

Extraction and purification of coenzyme Q

The Co-Q was extracted from intact cells and partially purified by preparative thin layer chromatography (Yamada and Kondo, 1973).

Determination of the coenzyme Q system

Reversed phase paper chromatography was used for the preparation of Co-Q. Mass spectrometry was applied for the determination of the Co-Q system (Yamada and Kondo, 1973).

Reagents and chemicals

Authentic preparations of the Co-Q series (Q-6 to Q-10) were obtained as described in our preceding papers (Yamada and Kondo, 1973; Yamada et al., 1973*a*,*b*,*c*, 1976*a*,*b*). Other reagents and chemicals were commercial preparations (Nakarai).

Table 1. The coenzyme Q system in the genera *Dekkera* and *Brettanomyces*.

Species and strain	Other remarks	Co-Q system
D. bruxellensis CBS 74	ATCC 36234, IFO 1590, type	Q-9
D. intermedia CBS 4914	ATCC 36235, type	Q-9
B. abstinens CBS 6055	ATCC 22341, IFO 1589, type	Q-9
B. anomalus CBS 77	ATCC 10559, NRRL Y-1415, IFO 0796, type	Q-9
B. bruxellensis CBS 72	ATCC 10560, NRRL Y-1411, type	Q-9
B. claussenii CBS 76	ATCC 10562, NRRL Y-1414, IFO 0627, type	Q-9
B. custersianus CBS 4805	IFO 1585, type	Q-9
B. custersii CBS 5512	IFO 1586, neotype	Q-9
B. intermedius CBS 73	IFO 1587, type	Q-9
B. lambicus CBS 75	ATCC 10563, NRRL Y-1413, IFO 0797, type	Q-9
B. naardenensis CBS 6042	ATCC 22075, IFO 1588, type	Q-9
B. nanus nom. nud.	Scheffers (1966)	
CBS 1945		Q-9
CBS 1955		Q-9
CBS 1956		Q-9

CBS, Centraalbureau voor Schimmelcultures, Delft, The Netherlands; IFO, Institute for Fermentation, Osaka, Japan; ATCC, American Type Culture Collection, Rockville, Maryland, U.S.A.; NRRL, ARS Culture Collection, Northern Regional Research Center, U.S. Department of Agriculture, Peoria, Illinois, U.S.A.

Results and Discussion

The strains examined in the genera *Dekkera* and *Brettanomyces* were found to have Q-9 without exception (Table 1). This indicates that these two genera are, in this respect, taxonomically homogeneous. They have the Q-9 system in common with representatives of the genera *Debaryomyces*, *Schwanniomyces*, *Lodderomyces*, *Lipomyces*, *Wickerhamia*, *Endomyces* and *Metschnikowia*, and with some strains in the genera *Pichia* and *Schizosaccharomyces* (Yamada et al., 1973*b*,*c*, 1976*a*,*b*, 1977). However, the genera *Dekkera* and *Brettanomyces* differ in the Co-Q system from representatives of the genera *Hansenula* (Q-7 and Q-8), *Saccharomyces* (Q-6), *Kluyveromyces* (Q-6), *Nadsonia* (Q-6), *Saccharomycodes* (Q-6), *Hanseniaspora* (Q-6), *Kloeckera* (Q-6), *Nematospora* (Q-6) and from the majority of the strains in the genera *Pichia* (Q-7 and Q-8; some strains Q-9) and *Schizosaccharomyces* (Q-10; some strains Q-9).

Van der Walt (1963, 1970*a*) suggested that the genera *Dekkera* and *Brettanomyces* and the genera *Hanseniaspora* and *Kloeckera* would be derived from a common progenitor, based on the facts that all four genera utilize nitite slowly in low concentration, that some species of the ascosporogenous genera *Dekkera* and *Hanseniaspora* produce hat-shaped, easily liberated ascospores, and that *Hanseniaspora valbyensis* is able to form notable amounts of acetic acid. Fiol and Billon-Grand (1978) proposed several phylogenetic lines among the species of the genera *Dekkera*, *Brettanomyces*, *Hanseniaspora* and *Kloeckera* on the basis of activities of intracellular glycosidases and nitrate and nitrite reductases.

Spencer and Gorin (1969) determined the proton magnetic resonance spectra of mannans of several *Brettanomyces* species. They accommodated the *Brettanomyces* species in subgroup b of group X or the *Saccharomyces microellipsodes* group. These *Brettanomyces* species have the spectra with signals at τ 4.30 and τ 4.40, which are common to *Kluyveromyces lodderi*, *Hanseniaspora osmophila* and *Saccharomyces carlsbergensis*. These three species have coenzyme Q-6 (Yamada et al., 1976*a*,*b*; unpublished data).

Having tested strains of all the known species of the genera *Dekkera* and *Brettanomyces*, we have found that their Co-Q system differs considerably from that of the genera *Hanseniaspora* and *Kloeckera*. Despite the similarity of the production of hat-shaped ascospores which are easily liberated from asci, the difference from Q-9 to Q-6 tends to exclude the possibility that these genera are closely related (Fig. 1).

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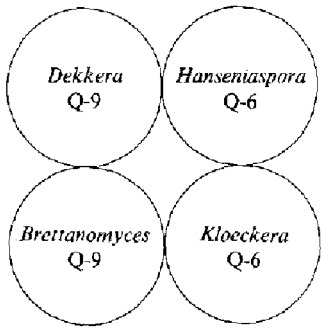

Fig. 1. Schematic representation of the coenzyme Q system in the genera *Dekkera*, *Brettanomyces*, *Hanseniaspora* and *Kloekera*. These genera are divided into two groups, namely, the Q-9 and the Q-6 possessing organisms.

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