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The Expanding World of Ballistosporous Yeasts: Isolation, Taxonomy and Molecular Phylogeny

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Ballistosporous yeasts have been considered as a special, minor group of yeasts. Recent studies, however, revealed that ballistosporous yeasts are not a special group of yeasts but ballistosporous aspect of various kinds of basidiomycetous yeasts having different phylogenetic origins. The expanding world of ballistosporous yeasts is discussed from several aspects of systematics.

Isolation of unknown species from natural environment

The 3rd edition of "The Yeasts, a Taxonomic Study" published in 1984 includes only 19 species of ballistosporous yeasts (*Sporidiobolus* 4, *Sporobolomyces* 7, *Bullera* 6, *Fibulobasidium* 1, and *Sirobasidium* 1). Since two species of *Sporobolomyces* are the same with those of telemorphic genus *Sporidiobolus* and the culture of *Bullera grandispora* was lost, practically 16 species are known in this group of yeasts at that time.

In recent ten years, we carried out extensive isolation study of ballistosporous yeasts from natural environment and described 36 new species including new genera. In addition to these species, 15 additional new species were reported by several researchers. Accompanied by the increase of the number of species, the heterogeneity of taxonomic properties also increased and the revision of the taxonomic system is required for the clarification of taxonomy of ballistosporous yeasts.

The mode of vegetative reproduction

The productivity and morphology of ballistospores have been employed as important taxonomic criteria. A question has arisen, however, on the significance of these criteria by the isolation of yeasts having the different combinations of the mode of conidiogenesis which have not been reported in ballistosporous yeasts, and by chemotaxonomic and molecular phylogenetic studies including the analysis of the nucleotide sequence of small subunit ribosomal RNA/DNA (SSU rRNA/DNA).

For a long time, ballistosporous yeasts are considered to reproduce by budding yeast cells and ballistospores but not to reproduce by any other kind of conidia. Recent studies revealed that several ballistosporous yeasts produce stalked conidia but lack budding yeast cells, and some others produce three kinds of conidia, ballistospores, stalked conidia and budding yeast cells. Ballistosporous ability is easily lost during the maintenance. It is very or completely lacks in certain strains even if in fresh isolates (Nakase & Suzuki, 1985). Probably, every basidiomycetous yeast has the ability to produce budding yeast cells, stalked conidia and ballistospores, when suitable conditions are provided (Nakase et al., 1991).

Mol % G+C of nuclear DNA

Basidiomycetous yeasts usually have G+C values of nuclear DNA in the range of 50 to 70 mol %. Several species are known to have G+C values from 46 to 50 mol %. An exceptionally low G+C value of 38.8-40.4 mol % was found in *Bullera dendrophila*. Recently, however, we isolated three undescribed species of *Bullera* having low G+C values of 41.7-42.3, 44.5 and 44.7 mol %, from plants collected in New Zealand. They were described as respective new species, *B. mrakii*, *B. hanna*e and *B. huiaensis* (Hamamoto & Nakase, 1996). This fact suggests that the low G+C value found in *B. dendrophila* is not an exceptional case but may spread over basidiomycetous yeasts.

Phylogenetic analysis of nucleotide sequence of SSU rRNA/DNA

The analysis of nucleotide sequence of ribosomal RNAs/DNAs provides important informations on the phylogenetic relationship among microorganisms. In the case of basidiomycetous yeasts, such studies were carried out by Guého et al. (1989, 1990), Yamada et al. (1989-1990), and Fell et al. (1995).

We examined the partial nucleotide sequence of fifty species of ballistosporous yeasts and a related genus at positions 1451-1618 (in *Saccharomyces cerevisiae*) of SSU rRNA and drew phylogenetic trees by using neighbor-joining method (NJ) of Saitou and Nei (1987) for ballistosporous and several non-ballistosporous yeasts (Nakase et al., 1993).

Ballistosporous yeasts are clearly separated into two groups, a group of xylose-containing species and that of xylose-lacking species in the cells. This fact suggests the primary importance of the presence or absence of xylose in the cells in the taxonomy of yeasts as stressed by Weijman & Golubev (1987).

Recently we determined the complete sequence of almost all of species of ballistosporous yeasts including the yeast-like genus *Tilletiopsis* and the filamentous genera *Tilletia* and *Tilletiaria*, and analyzed phylogenetically by NJ, maximum likelihood and parsimony methods (Takashima et al., 1995; Suh & Nakase, 1995; Suh et al., 1996; Takashima & Nakase, 1996; Suh et al., 1996; Suh et al., 1996; Hamamoto et al., in preparation).

The species of basidiomycetes and their anamorphs were placed in three major clusters in the phylogenetic trees based on the SSU rDNA sequences. We found that basidiomycetous yeasts were placed in all of these groups. Among them, ballistosporous yeasts were placed in groups 2 (Hymenomycetes) and 3 (Urediniomycetes) and ballistosporous yeast-like fungi *Tilletiopsis* species were placed in group 1 (Ustilaginomycetes).

Any clear correction was not found between the phylogenetic trees and the mode of conidiogenesis except for the genus *Kockovaella* in which ballistospores, stalked conidia and budding

yeast cells are produced. For example, species of *Bullera* do not constitute a group on the phylogenetic tree but widely distribute beyond the boundary of the taxonomic rank genus together with species in *Cryptococcus*. It is well-known that strains of *Bullera* cannot be distinguished from strains of *Cryptococcus* when they lose ballistosporous ability. The analysis of the nucleotide sequence of SSU rRNA suggests that the mode of conidiogenesis has less taxonomic significance than hitherto believed.

The genus *Sporobolomyces* is clearly differentiated from the genus *Tilletiopsis* in the phylogenetic tree based on SSU rDNA sequences. Sometimes, however, it is difficult to distinguish these two genera based on traditional taxonomic criteria such as morphology of ballistospores and hyphae. Recently, we found that the neutral sugar composition of purified cell walls clearly discriminate these two genera (Takashima & Nakase, in preparation).

As discussed by Wejman & Golubev (1987) in the case of *Bullera* and *Sporobolomyces*, boundaries of taxonomic group of higher rank, which are defined based on the chemotaxonomic analysis, are independent from criteria used in older studies.

Conclusion

The world of ballistosporous yeasts are expanding year and year and the systematic study of ballistosporous yeasts will be integrated into the mainstream of the systematics of basidiomycetous yeasts and related basidiomycetous filamentous fungi including mushrooms. The significance of traditional criteria is decreasing and molecular approaches are now essential for the further progress of the taxonomy of these yeasts. In addition, continuous isolation study of new strains from natural environment is essential for the healthy development of systematics of ballistosporous yeasts.