A comparison of the killer character in different yeasts and its classification

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The interactions between 20 killer yeasts of various genera and species were examined. Ten distinct groups were recognised with respect to killer activity and 10 distinct groups with respect to resistance to killer action. Using both killing and resistance phenotypes, 13 classes of killer yeast were found. With the exception of Torulopsis glabrataNCYC 388, non-Saccharomyces strains of yeast were not killed by a member of the genus Saccharomyces.

The killer character of the 3 killing groups of Saccharomyces identified could be cured by treatment with cycloheximide or incubation at elevated temperature and the effectiveness of these procedures was indicative of the category of killer yeast examined. Killer yeasts not belonging to the genus Saccharomyces could not be cured of their activity. Double-stranded ribonucleic acids were extracted only from Saccharomyces spp. and the molecular weights of the species present were a function of the killer class to which a strain belonged.

By an analysis of the effects of proteolytic enzymes, temperature and pH on killer activity and by gel chromatography of crude preparations of killer factors, the toxins of different killer classes were shown to be biochemically distinct. However all toxins had certain properties in common consistent with there being a protein component essential to killer action.

INTRODUCTION

The killer character in Saccharomyces cerevisiae was first identified by Bevan and Makower (1963). Killer yeasts of the genus Saccharomyces and of other genera secrete toxins (killer factors) which are lethal to sensitive cells (Woods and Bevan, 1968; Bussey, 1972; Bussey and Skipper, 1975; Philliskirk and Young, 1975). The genetic determinant of killer activity in the laboratory strains of the genus Saccharomyces studied is a double-stranded RNA (dsRNA) molecule. Three species of dsRNA have been detected in extracts of killer yeast.
The species of lowest molecular weight (M) is associated with killer activity and killer-cured strains (unable to produce active killer factor) lack this dsRNA. Furthermore, strains carrying suppressive plasmids lack the M species but contain a species (S) of lower molecular weight than M (Vodkin, Katterman and Fink, 1974; Tzen, Somers and Mitchell, 1974).

In partially purified form, the killer factor secreted by laboratory strains is reported to be of high molecular weight and to contain both carbohydrate and protein, active only in a narrow range of pH, and is inactivated by temperatures in excess of 20°C and by proteolytic enzymes (Woods and Bevan, 1968; Bussey, 1972).

Evidence that structurally different killer factors may occur is provided by (1) studies of Torulopsis glabrata (Bussey and Skipper, 1976), (2) interactions between different killer yeasts (Naumov and Naumova, 1973) where a killer strain of wine yeast killed a laboratory killer yeast and vice versa and (3) from the finding that filtrates of different killer yeast cultures showed different pH optima in respect of their killer activities (Philliskirk and Young, 1975).

Killer yeasts have not been classified according to their activities although Wickner (1976) has suggested the terms K₁ and K₂ to describe the killing phenotypes of the laboratory killers and the wine strain killer yeast respectively. He further suggests the terms R₁ (resistant to K₁) and R₂ (resistant to K₂) to denote the resistant phenotypes of these strains.

Classification of inhibitory or toxic substances may be achieved in several ways e.g. (1) by determining their spectrum of activity against sensitive organisms; (2) by testing their activity against mutants resistant to one or more substances (as for colicinogenic determinants; Davies and Reeves, 1975); (3) by examining the cross-reactivity of the producing strains (as for killer character in Ustilago; Puhalla, 1968); and (4) by a structural analysis.

In the case of killer character in yeasts the most appropriate technique would appear to be an examination of cross-reactivity (interaction between strains) since it is technically simple and enables both patterns of killer activity and resistance to activity to be determined.

We have selected 20 of the killer yeasts identified by Philliskirk and Young, (1975) and determined the interactions between them. In addition the strains have been compared by their response to killer-curing treatments and by an analysis of dsRNA content. An indication of the structural relationships between the various killer factors has been obtained by examining the effects of proteolytic enzymes, temperature and pH on culture filtrates and by gel chromatography of concentrated culture filtrates.