Induction of Respiration-deficient Mutant of Saccharomyces cerevisiae by Pinacyanol

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The induction of a respiration-deficient mutant (RD mutant) of yeast was efficiently produced when cells were allowed to multiply in the presence of acriflavine or related dyes (2, 9). It has been well-established that acridine dyes interact with deoxyribonucleic acid (DNA) by intercalating between the base pairs of double-stranded DNA (6, 7). The intercalation model could explain satisfactorily the mutation mechanism, such as addition or deletion of nucleotides produced by these dyes (1).

By measuring the flow dichroism of dyes in double-stranded DNA solution, Kodama, Tagashira, and Nagata (4, 5) observed that pinacyanol interacted with DNA by being oriented perpendicular to the base of DNA. This was in contrast to acridine dyes which were oriented parallel to the base of DNA. Based on the striking difference in the way of interaction of pinacyanol with DNA from that of acridine dyes, the mutagenicity of pinacyanol to induce the cytoplasmic RD mutant of yeast was investigated.

The respiring haploid yeast, Saccharomyces cerevisiae RbQ2C (α, R, hist+, lys+, ρ+) (3), was inoculated at the initial concentration of 10^4 cells per ml in the medium containing glucose (2%), Difco proteose peptone (1%), and Difco yeast extract (1%) added with pinacyanol at 2 or 4 μg per ml. Pinacyanol, purchased from Tokyo Chemical Industries Co., was purified with an alumina column. Incubation was carried out aerobically with reciprocal shaking at 37°C. The cell number was determined at intervals of 2 hr by colony counting, and the percentage of RD mutants was determined by the triphenyltetrazolium chloride overlaying technique of Ogur, John, and Nagai (8).

Although pinacyanol, at the concentration of 2 μg/ml, did not give any inhibitory effect on the growth rate of yeast, it efficiently induced an RD mutant (Fig. 1 and 2). With the incubation for 14 hr in the presence of pinacyanol, about 70% of the total colonies consisted of RD mutants which could not reduce triphenyltetrazolium.

RD mutant cells showed a slightly lesser growth rate than did wild cells. Some cells of this haploid are in clusters consisting of several cells. If one of the cells in the cluster is an RD mutant and the rest are wild, the colony raised from such a cluster would be diagnosed as wild, since the growth of wild cells overcomes that of RD mutants on the agar plate. Considering these factors, the real percentage of RD mutants should be expected to be higher than 70%.

![Fig. 1. Growth curves. (▲) Control; (●) Pinacyanol, 2 μg/ml; (○) pinacyanol, 4 μg/ml.](http://jb.asm.org/)

The crosses of 20 strains of these RD mutants with chromosomal RD mutants of opposite mating type, RbO8D (a, r, hist+, lys−, ρ+) or RbO3B (a, r, hist+, lys+, ρ+), constantly yielded hybrid cells (a/α, R/r, ρ−) which could respire and grow on lactate medium. This and the high frequency of RD mutant induction, as described before, indicate that pinacyanol-induced RD mutants were of the ρ− type.

We conclude that pinacyanol induces cytoplasmic RD mutants of yeast as efficiently as acridine dyes, although the mechanisms of inter-
action with DNA of the two agents are quite different.

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