Note

Mutants of Yeast *Saccharomyces cerevisiae* Producing Large Amounts of the Flavor Components Isobutyl Alcohol and Isoamyl Alcohol

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Isobutyl alcohol (iso-BuOH), isoamyl alcohol (iso-AmOH), and their acetates have fruity flavors and are very important aroma components in various alcoholic beverages. The majority of these compounds are produced by yeast during fermentation. iso-BuOH and iso-AmOH are thought to be formed from α -ketoisovalerate and α -ketoisocaproate respectively by decarboxylation and reduction.¹⁾ These α -keto acids are formed from the sugar substrate *via* the pathway of branched-chain amino acids synthesis.¹⁾

In our study, mutants which could produce large amounts of both iso-BuOH and iso-AmOH were obtained by selecting β -(2-thiazoryl)-DL-alanine (2TA)-resistant

mutants of *Saccharomyces cerevisiae*.

S. cerevisiae Kyokai no. 7 (K-7), which is a diploid strain used for *sake* production, and *S. cerevisiae* SK-16 (*MAT α CAN^S1⁺*) and X2180-1A-901 (*MAT α can^R1*), which are haploid strains preserved in our laboratory, did not grow with 2 mg/ml of 2TA on YNB agar (0.67% Difco Yeast Nitrogen Base without amino acids, 2% glucose, and 2% agar). This growth inhibition was reversed by the addition of 1 mM L-valine or 1 mM L-leucine. This suggests that 2TA is an antimetabolite of branched-chain amino acids. Strains K-7 and SK-16 were mutated with 3% ethyl methanesulfonate at 30°C for 1 hr by the method of Lindegren *et al.*²⁾ and spread on YNB agar containing 2 mg/ml of 2TA. The colonies grown on the plates were isolated as 2TA-resistant mutants. Forty colonies appearing on the plates were examined for their production of iso-BuOH and iso-AmOH by cultivation in a test tube containing YNB liquid medium (5% glucose) for 3 days at 30°C with shaking. These alcohols were measured with head space gas chromatography by the method of Yoshizawa.³⁾ The production of iso-BuOH and iso-AmOH by those mutants and their parents are shown in Fig. 1. Mutant K-7-21 derived from K-7 and mutant SK-16-20 derived from SK-16 produced both iso-BuOH and iso-AmOH ten-fold compared to their parents. Since no amino acids were contained in the culture medium initially, these alcohols might be produced by *de novo* synthesis from glucose.

The genetics of one of the mutants, SK-16-20, was analyzed by the standard procedure of Spencer *et al.*⁴⁾ None of the diploids derived from crosses between the resistant mutant SK-16-20 and the sensitive strain X2180-1A-901 showed either tolerance to 2TA (2 mg/ml)

Fig. 1. Production of Isobutyl Alcohol and Isoamyl Alcohol by Mutants Resistant to 2TA (2 mg/ml) Derived from Strains K-7 and SK-16.

(○), mutants derived from strain k-7; (●), strain K-7 (wild type); (□), mutants derived from strain SK-16; (■), strain SK-16 (wild type). Isobutyl alcohol and isoamyl alcohol were measured after cultivation in YNB liquid medium (glucose 5%) for 3 days at 30°C with shaking.

Table I. TETRAD ANALYSIS OF CROSSES BETWEEN THE 2TA-RESISTANT MUTANT SK-16-20 AND THE SENSITIVE STRAIN X-2180-1A-901

Phenotype	Segregation pattern				
	4+ : 0—	3+ : 1—	2+ : 2—	1+ : 3—	0+ : 4—
2TA+ : 2TA— ^a	2	5	3	0	0
Can+ : Can— ^b	0	0	10	0	0
Mat a : Mat α ^c	0	0	10	0	0

^a Growth on YNB agar containing 2 mg/ml of 2TA.^b Growth on YNB agar containing 0.2 mg/ml of canavanine.^c Identified by mass mating with the standard a- and α -type strains X2180-1A and X2180-1B, respectively.

or overproduction of these alcohols in YNB liquid medium. This indicates that the mutation is recessive. Ten asci from the diploid obtained by crossing between mutant SK-16-20 and strain X2180-1A-901 were dissected. Tetrad analysis indicated that this mutant had more than two nuclear genes conferring 2TA-resistance (Table I). It might be suggested that several enzymes in the pathway for branched-chain amino acids, such as α -acetolactate synthase or β -isopropylmalate synthase were desensitized to feedback inhibition or repression by branched-chain amino acids. These enzyme levels are now under investigation. Among these segregants, no evidence was found for linkage between 2TA-resistance and the ability to overproduce these alcohols.

Mutants K-7-21 and SK-16-20 accumulated three times the L-valine, L-leucine, and L-isoleucine in the cells that their parents did when they were grown in the YNB liquid medium (data not shown). This suggests that altered regulation enhanced the biosynthesis of branched-chain amino acids and led the overproduction of iso-BuOH and iso-AmOH formed from α -ketoisovalerate and α -ketoiso-

caproate, respectively.

Fermentation for making *sake* and rice *shochu* was done on a 3-liter scale by the mutant K-7-21. These products contained two or three times more iso-BuOH, iso-AmOH, and their acetates than those by the parent, and had a fine quality in aroma and taste.

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