Phenethyl Alcohol Sensitivity in Escherichia coli

HAKOBU NAKAMURA

Biological Institute, Faculty of Science, Konan University, Kobe, Japan

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In the present communication, it is shown that phenethyl alcohol can bring about a nondividing, nonlethal state in a phenethyl alcohol-resistant strain of E. coli. When a phenethyl alcohol-sensitive strain was tested under the same conditions, this state did not occur. Strain characteristics, preparation of culture media, and bacterial-mating techniques were described in preceding papers (H. Nakamura, J. Bacteriol. 90:8, 1965; J. Bacteriol. 92:1447, 1966).

The standard phenethyl alcohol-sensitive strain, 18/1042, was obtained as a spontaneous mutant of a wild-type female strain of E. coli K-12. The standard resistant strain, N90, was obtained by mating a resistant Hfr strain of E. coli K-12, W1895, with 18/1042. Phenethyl alcohol to be used for the experiment was distilled under low pressure and sterilized by membrane filtration.

![Figure 1](http://jb.asm.org/)

**FIG. 1.** Changes in viable-cell number of phenethyl alcohol- and acriflavine-resistant strain N90. Level of pH in the medium: (A) 6.2, (B) 7.2, (C) 8.0. Percentage of phenethyl alcohol concentration is indicated on each curve.

Cells of an overnight culture of 18/1042 and N90 were washed three times with saline (0.85% sodium chloride solution) and inoculated in liquid broth media adjusted to pH 6.2, 7.2, and 8.0 by phosphate buffer. Shaking for 30 min at 37°C was followed by addition of phenethyl alcohol and incubation for several hours. It was found that the inhibitory effect of phenethyl alcohol on cell division was greater at higher pH, and the sensitive 18/1042 strain was more sensi-
tive to phenethyl alcohol than the resistant N90 strain at any pH.

Freshly grown cells of both strains were washed with saline and inoculated in pH-controlled broth containing different concentrations of phenethyl alcohol with an inoculum of about $5 \times 10^7$ cells per milliliter. Cultures were shaken in a water bath at 37°C. Cells were sampled at intervals and plated on agar to determine the number of viable cells. Figure 1 shows that in N90 the cell count did not change at phenethyl alcohol concentrations of 0.26 and 0.24% at pH 6.2 and 7.2, respectively; at pH 8.0, phenethyl alcohol did not bring about a nondividing, nonlethal state. With 18/1042, as represented in Fig. 2, a phenethyl alcohol concentration which inhibited cell multiplication without impairing viability was not found at any pH.

The phenethyl alcohol-sensitive 18/1042 strain also is sensitive to acriflavine and other basic dyes, whereas the phenethyl alcohol-resistant W1895 strain is resistant to the basic dyes. The hybrid, N90, which inherited the acriflavine resistance property of W1895 also inherited its resistance to phenethyl alcohol. All other 18/1042 hybrids which have inherited the property of acriflavine resistance from W1895 also have been found to be resistant to phenethyl alcohol. The possibility that both phenethyl alcohol resistance and acriflavine resistance are controlled by the same gene, or closely linked genes, remains to be investigated.

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