Catalase-Negative *Escherichia coli* Isolated from Blood

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A catalase-negative variant of *Escherichia coli* was isolated from the blood of a patient with acute leukemia who had been treated with various antibiotics and gentamicin. This small-colony variant grew almost as actively under anaerobic conditions as its large-colony revertant or *E. coli* NIHJ JC-2. The variant was resistant to gentamicin, in contrast with the revertant. Streptomycin and hemin stimulated growth of the variant slightly. With repeated subculturing the variant tended to increase slightly in colony size with coincident recovery of weak catalase production. The possibility that such a variant may have been induced by gentamicin was indicated.

Infections due to small-colony variants of enterobacteria have been reported (3, 4, 7, 9), but most of the variants were auxotrophic mutants with specific growth requirements for various nutrients. We have recently isolated a catalase-negative variant of *Escherichia coli* from the blood of a patient with acute leukemia. As this strain showed some peculiar cultural characteristics, we took a little longer time than usual to clearly recognize it as an anaerobic variant of *E. coli*. The purpose of this paper is to describe a case and the laboratory problems involved in the cultural characterization of the strain.

MATERIALS AND METHODS

Case history. An 11-year-old boy with acute myelogenous leukemia of 7 months' duration developed a fever of unknown origin early in March 1975. He was placed first on cefazolin and gentamicin and then, 5 days later, on carbenicillin, gentamicin, and lincomycin in combination without defervescence. Both peripheral blood and bone marrow studies showed an almost complete replacement by blast cells. Antileukemic chemotherapy proved to be unsuccessful. On 20 May, his temperature rose to 40.3°C, with a pain in the left flank accompanied by short breathlessness, tachycardia, and hypotension. Two separate blood cultures grew gram-negative rods, subsequently identified as a catalase-negative variant of *E. coli*. Antibiotic therapy was switched to colistimethate alone. He died 4 days after the onset of septicemia.

Strains. Strain Sm was a stable small-colony variant isolated from the blood of the patient described above. On aged culture this strain developed a few colonies of the "normal" large type, designated strain La. *E. coli* NIHJ JC-2 was chosen for control purposes.

Culture media. The agar media used were heart infusion (HI) (Eiken), bromothymol blue-lactose (Eiken), and GAM (Nissui) agars. For preparing blood agar, HI agar was supplemented with 5% sheep blood.

bromocresol purple semisolid medium (Eiken) was used for sugar fermentation tests. The sugars tested were glucose, L-arabinose, lactose, sucrose, adonitol, dulcitol, D-mannitol, L-inositol, xylose, trehalose, rhamnose, D-fructose, and D-sorbitol, at 1% concentrations (wt/vol). All cultures were incubated at 37°C in this study.

Anaerobic culture. The anaerobic jar culture method was used, with an atmosphere at 80% N₂, 10% CO₂, and 10% H₂ in the presence of room temperature catalysts (anaerobic jar KJ-1, Tomy Seiko).

Identification of organisms. *E. coli* was identified by the conventional techniques of Cowan and Steel (1).

The catalase test was performed according to the description in the *Manual of Clinical Microbiology* (8), namely, by pouring 1 ml of a 3% solution of hydrogen peroxide over a slant culture as well as by emulsifying a small portion of the culture in 1 drop of 30% hydrogen peroxide on a glass slide. The test for iron-porphyrin compounds was performed on a piece of o-tolidine-impregnated filter paper (Hema-Combistix). The test colony was smeared onto the filter paper moistened with distilled water.

Estimation procedures. To estimate colony size, diameters of 20 well-isolated colonies were measured, after 24 h of incubation, under a wide-field microscope (profile projector model 6C, Nikon), and the results were averaged. For viable cell counts, bacterial counts of 3 colonies of the average size were made by the conventional pour-plate method, and the results were averaged. Growth in liquid media was measured in a Klett-Summerson photometer equipped with a red filter, using 10 ml of GAM broth (Nissui) without aeration and 5 ml each of GAM and HI broths with sufficient aeration.

Susceptibility test. Minimum inhibitory concentrations of various antibiotics were determined by the agar dilution method standardized by the Japan Society of Chemotherapy (5), but GAM agar was substituted for HI agar.

Preparation of somatic (O) antisera. Prepara-

tion of O antisera was described elsewhere (2). Cultures heated at 100°C for 1 h were used in tube agglutination and absorption. The standard tube agglutination techniques were used.

RESULTS

Gram stain. Gram-stained strain Sm did not show any difference in shape or size from strain La or the control *E. coli* when grown on HI agar.

Colonial morphology. Strain Sm formed smooth, tiny colonies like those of streptococci after 18 to 24 h of incubation on various agars. It grew better under anaerobic conditions (Fig. 1), but not in an atmosphere of air plus 5 or 10% CO₂. Reversion to the large-colony form was found only in aerobic cultures.

Biochemical reactions. Except for a negative catalase reaction, strain Sm showed the same biological properties as strain La. Strain La differed from *E. coli* NIHJ JC-2 in negative fermentation of sucrose. The revertant presented about the same catalase reaction as the control strain. The *o*-tolidine test was positive in both of the large-colony strains, but not in strain Sm.

Strain Sm was more susceptible than the others to 1% hydrogen peroxide (Fig. 2).

Estimation of growth. (i) Growth on solid media. Growth of strain Sm on HI and GAM agars became more active on shifting from aerobic to anaerobic conditions, whereas in the large-colony strains quite the opposite occurred (Table 1). Accordingly, strain Sm proved to be almost equal to strain La in anaerobic growth.

(ii) Growth in liquid media. Strain Sm grew almost as rapidly as the large-colony strains in GAM broth without aeration, whereas it grew

poorly in well-aerated GAM broth, as noted especially in the first 2 h (Fig. 3). However, strain Sm grew fairly rapidly during the next 2 h, probably because the oxidation-reduction potential of GAM broth supplemented with sufficient reductants, even if well-aerated, would have become lower concomitantly with its growth.

Such a difference was more evident in well-aerated HI broth.

Effect of streptomycin and hemin. Aerobic growth of strain Sm was stimulated to some extent by addition of streptomycin or hemin (Fig. 4 and 5).

Antibiotic susceptibilities. Minimum inhibitory concentrations of various antibiotics for strain Sm were as follows: ampicillin, cefazolin, streptomycin, and chloramphenicol, ≥ 100 μ g/ml each; kanamycin and tetracycline, 50 μ g/ml

FIG. 2. Susceptibility to 1% hydrogen peroxide, 24 h of incubation on bromothymol blue-lactose agar.

FIG. 1. Colonies on HI agar after 24 h of incubation.

TABLE 1. Growth (24-h incubation) under aerobic and anaerobic conditions

Strain	Conditions	Growth and medium			
		Diameter (mm)		Cell count	
		HI agar	GAM agar	HI agar	GAM agar
Sm	Aerobic	0.4	0.7	1.6×10^6	1.2×10^7
	Anaerobic	1.5	1.9	3.7×10^7	1.1×10^8
La	Aerobic	4.0	4.4	1.3×10^9	2.6×10^9
	Anaerobic	1.8	2.6	7.5×10^7	2.9×10^8
NIHJ JC-2	Aerobic	4.0	4.8	1.5×10^9	2.0×10^9
	Anaerobic	1.7	2.6	1.0×10^8	3.2×10^8

FIG. 3. Growth in liquid media.

FIG. 4. Effect of streptomycin on growth of strain Sm, 48 h of incubation on HI agar. SM, Streptomycin, 500 $\mu\text{g}/\text{ml}$. OD of cylinder cup, 8.0 mm.

each; gentamicin, 25 $\mu\text{g}/\text{ml}$; and colistin, 0.39 $\mu\text{g}/\text{ml}$. In contrast, strain La was susceptible to gentamicin (minimum inhibitory concentration, 6.25 $\mu\text{g}/\text{ml}$), but otherwise the revertant dis-

played about the same susceptibility pattern as strain Sm.

Antigenic constitution. By means of cross-absorption, the somatic (O) antigen of strain Sm was found to be quite identical with that of strain La. Both aerobic and anaerobic cultures of these strains showed the same agglutination titers to the corresponding antisera.

Effect of continuous transfer. With repeated subculturing over 2 years after its isolation, strain Sm tended to form slightly larger colonies (Fig. 6). Growth was stimulated notably on blood agar, which contains large amounts of catalase. Coincidentally with a gradual increase in colony size, its catalase reaction proved to be weakly positive.

DISCUSSION

This is the first isolation of a catalase-negative variant of *E. coli* from blood that we know of. The variant described here behaved as actively under anaerobic conditions as if it were the normal wild-type strain. In this sense our strain

may as well be designated an anaerobic variant of *E. coli*.

The variant exhibited the same biological properties as the large-colony revertant except for a negative catalase test. Poor growth in air, therefore, may be due to susceptibility to hydrogen peroxide. As shown in a negative *o*-tolidine test, the catalase production of strain Sm was very much impaired on its primary isolation, but it was gradually restored as the colony size be-

FIG. 5. Effect of hemin on growth of strain Sm, 24 h of incubation on bromothymol blue-lactose agar. X, X-disk (Eiken) containing 10 µg of hemin, 6.5 mm in diameter.

came larger through continuous transfer. This fact indicates that the catalase production may not have been completely suppressed, but was too weak to detect with the method used.

The patient reported here had been treated with gentamicin in combination with one or two other antibiotics for a long time before the onset of septicemia. Li et al. (6) have recently described a gentamicin-induced anaerobic variant of *Serratia marcescens* that acquired resistance to gentamicin and lacked various enzyme activities, but not catalase activity. Its revertant was identical with the parent in both biochemical reactions and antibiotic susceptibility pattern. Therefore, it seems reasonable to suspect that gentamicin would be involved in the production of anaerobic variants of enterobacteria through its inhibition of protein synthesis. Eventually, we may be dealing with an *in vivo* phenomenon similar to that described by them.

The small-colony variant showed a phenomenon similar to streptomycin dependence. Streptomycin may have caused misreading at the ribosome level. Our patient had not been treated with this drug. Hemin, the prosthetic group of catalase, stimulated growth to some extent. It is known that this iron compound has a catalase activity in itself (10). Exact definition and significance of these effects remain to be clearly elucidated.

In the future, patients undergoing gentamicin

FIG. 6. Effect of continuous transfer, 24 h of incubation on blood agar. (A) Strain Sm, maintained at -80°C since about 1 year after the isolation from the blood. (B) The same strain, repeatedly subcultured over 2 years after the isolation. (C) Strain La.

treatment may develop such infections at an increasing frequency. If this should occur difficult problems involved in the diagnosis and treatment of such infections might arise in laboratory and clinical medicine.

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