EXPERIMENTAL GENETICS

THE NATURE OF ALKALI PRODUCERS OF THE INTESTINAL GROUP OF BACTERIA

(UDC 576.851.48.095.5:576.8.098)

K. D. Rybakova

Department of Experimental Genetics (head, Active Member AMN SSSR Professor N. N. Zhukov-Verezhnikov), Institute of Experimental Biology (Director, Professor I. N. Maiskii)
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 61, No. 4, pp. 106-109, April, 1966
Original article submitted July 13, 1964

According to the current classification the group of so-called alkali producers includes variants of Gram-negative bacteria capable of fermenting carbohydrates.

The saccharolytically inert alkali producers were first obtained under laboratory conditions by V. D. Timakov and D. G. Kudlai [7-9, 11]. The authors cultivated Escherichia coli in a synthetic medium in which the only source of organic nutrition were heat-killed bacteria of other pathogenic species of this group, in particular, paratyphoid (Breslau) or typhoid bacilli [7, 8]. However, the nature of these atypical variants has not been elucidated.

The purpose of the present study was to elicit the genetic relationship of our obtained variants of alkali producers with the original lysogenic strain E. coli K-12 (λ).

EXPERIMENTAL METHOD

The experimental material was a lysogenic culture of E. coli K-12 (λ) which had been subjected to the effect of space factors in experiments on the Vostok-2 spaceship [1, 2, 4, 5]. An analysis of this culture after returning from space revealed an increase of dissociates [6]. In the investigated culture, along with the usual round, large, semi-transparent colonies which do not differ from the colonies of the control specimens that remained on earth, were found small atypical colonies which appreciably differed from the initial culture in morphological and biochemical characters. In connection with this it was of great interest to study in more detail the properties of these atypical subcultures. Upon repeated subculturing most of them acquired the form of colonies typical for E. coli K-12 (λ), but about 6% of the subcultures continued to retain the modified form of the colonies. Therefore, they were of particular interest for a further study.

To check the enzyme activity of these subcultures and their mutants we used various sugars with bromothymol blue and Andrade's indicators. The nutrient requirements were studied by means of our perfected replica method with the use of minimal and selective media [5]. The antigenic properties were analyzed in the agglutination reaction with the use of immune serum against the original strain of E. coli K-12 (λ). To check the retention of the character of lysogeny by the investigated subcultures we used methods of demonstrating spontaneous and induced phage formation by means of the indicator strain E. coli C-85 [10]. Induction was effected by x-rays whose source was the RUM-7 apparatus (0.1-mm Al filter, current strength 15 mA, voltage 50 Kv, irradiation dose 1000 and 10,000 r). Furthermore, the subcultures and their variants were tested for sensitivity to phages of the group T(T1 – T7) and streptomycin by the usual method.

EXPERIMENTAL RESULTS

Unlike the initial strain E. coli K-12 (λ) the saccharolytically inert subcultures when inoculated on meat-extract agar produced after 24 h of growth small transparent colonies about 1 mm in diameter. Within 48 h their diameter increased to 2 mm and only after 72 h did they attain a size characteristic of the original culture that had grown for 18-24 h. In this case we noted small sectors of lysis in a number of colonies. Sometimes we detected colonies with secondary growth, i.e., on the surface of the primary colonies secondary "papillae" had grown.

TABLE 1. Biochemical Properties of Mutants (bromothymol blue indicator)

E. coli	Dulcitol	Maltose	Sucrose	Sorbitol	Raffinose	Arabinose	Glucose	Mannitol	Lactose	Rhamnose	Xylose	Galactose
K-12 (control)	–	AG	–	AG	–	–	AG	AG	AG	AG	AG	AG
No. 7	SA	SA	A	SA	A	SA	–	A	–	A	A	SA
No. 7/1	A	SA	A	–	A	A	A	SA	SA	A	A	SA
No. 7/2	A	SA	A	–	A	–	SA	–	SA	A	A	SA
No. 7/3	A	SA	A	–	A	–	SA	SA	SA	A	A	SA
No. 7/4	A	SA	A	A	A	A	–	SA	SA	A	A	SA
No. 7/5	A	SA	A	A	A	A	SA	A	A	A	A	SA
No. 7/6	A	SA	A	A	A	A	SA	A	–	A	A	SA
No. 7/7	A	SA	A	A	A	A	–	A	A	A	A	SA
No. 7/8	A	SA	A	A	A	A	A	A	A	A	A	SA
No. 7/9	A	SA	A	A	A	SA	–	–	–	A	A	SA
No. 7/10	A	SA	A	A	A	SA	A	A	–	A	A	SA
No. 7/11	A	SA	A	A	A	A	–	A	SA	A	A	SA
No. 7/12	A	SA	A	A	A	A	–	A	SA	A	A	SA
No. 7/17	–	SA	A	A	A	A	–	A	SA	A	A	SA
No. 7/18	A	SA	A	SA	A	A	–	SA	SA	A	A	SA
No. 7/19	A	SA	A	A	A	A	–	A	SA	A	A	SA
No. 7/20	A	SA	A	SA	A	A	–	A	SA	A	A	SA
No. 7/21	A	SA	A	A	A	A	–	SA	SA	A	A	SA
No. 7/22	A	SA	A	SA	A	A	–	A	SA	A	A	SA
No. 7/23	A	SA	A	A	A	A	–	SA	SA	A	A	SA
No. 7/26	A	SA	A	A	A	A	–	A	SA	A	A	SA

Designations: A) alkalization of Hiss medium; SA) slight alkalization of medium; –) sugars not utilized; AG) acid and gas form upon fermentation of sugars.

TABLE 2. Sensitivity of Isolated Mutants to Phages of Group T and Streptomycin

E. coli	Phage filtrate								Strepto-myocin
	T1	T2	T3	T4	T5	T6	T7	λ	
K-12(λ) control	+	+	+	+	+	+	+	–	S
No. 7	–	–	–	–	–	–	–	–	S
No. 7/1 mutant	–	–	–	–	–	–	–	–	S
No. 7/2 "	–	–	–	–	–	–	–	–	S
No. 7/3 "	–	–	–	–	–	–	–	–	S
No. 7/4 "	–	–	–	–	–	–	–	–	S
No. 7/5 "	–	–	–	–	–	–	–	–	S
No. 7/6 "	–	–	–	–	–	–	–	–	S
No. 7/7 "	–	–	–	–	–	–	–	–	S
No. 7/8 "	–	–	–	–	–	–	–	–	S
No. 7/9 "	–	–	–	–	–	–	–	–	S
No. 7/10 "	–	–	–	–	–	–	–	–	S
No. 7/11 "	–	–	–	–	–	–	–	–	S
No. 7/12 "	–	–	–	–	–	–	–	–	S
No. 7/17 "	–	–	–	–	–	–	–	–	S
No. 7/18 "	–	–	–	–	–	–	–	–	S
No. 7/19 "	–	–	–	–	–	–	–	–	S
No. 7/20 "	–	–	–	–	–	–	–	–	S
No. 7/21 "	–	–	–	–	–	–	–	–	S
No. 7/22 "	–	–	–	–	–	–	–	–	S
No. 7/26 "	–	–	–	–	–	–	–	–	S

Designations: +) phage lyses culture; –) no lysis; S) "streptomycin-sensitive" culture.

TABLE 3. Neutralization Reaction of Phage-Particles Obtained from Mutant Cultures of Lysogenic Strain E. coli K-12 (λ) by Serum against Phase (λ)

Phage filtrate	Average number of phage particles		Neutralization constant
	expt.	control	
λ (E. coli K-12)	$4.7 \cdot 10^7$	$199 \cdot 10^7$	7.4
Mutant (E. coli No. 7/1)	$51.5 \cdot 10^1$	$1121 \cdot 10^1$	6.3
Mutant (E. coli No. 7/7)	$3.5 \cdot 10^1$	$125.7 \cdot 10^1$	7.1

TABLE 4. Agglutination Reaction of Investigated Cultures with Serum Obtained from Rabbits Immunized by Normal E. coli K-12 (λ)

E. coli	Serum dilution							
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1280
K-12 (λ)	+	++	++	++	+	+	±	±
No. 7	+	+	+	±	–	–	–	–
No. 7/18	+	+	±	–	–	–	–	–
No. 7/26	+	+	±	±	–	–	–	–
(Control)	–	–	–	–	–	–	–	–

Designations: ++) different degree of positive reaction; ±) doubtful reaction; –) negative.

The subcultures produced a very slight homogeneous turbidity 24 h after inoculation on meat-extract broth. After 48 h the turbidity appreciably increased, and an annular protein formed on the test tube. All subcultures grew best at 25-30°. Growth of the culture was much worse in a thermostat (37°). The bacterial cells of these subcultures were short Gram-negative rods.

By means of the replica method and selective media a high frequency of biochemical mutations (about 3%) was noted for the saccharolytically inert cultures. All isolated mutants were incapable of synthesizing methionine. A study of the enzyme activity of these subcultures and the biochemical mutants isolated from them revealed that they can not utilize sugar and cause a pronounced alkalization of the peptone media with carbohydrates (Table 1).

Unlike the starting strain E. coli K-12 (λ) all alkali producers and their mutants are resistant to bacteriophages of group T and the temperature phage (λ) (Table 2). However, despite the many outward characters, the subcultures causing alkali production retained the capacity to produce the temperate phage (λ) both spontaneously and when exposed to x-rays. Here the serological properties of the spontaneous and induced bacteriophages isolated from these cultures are analogous to the properties of the temperature phage produced by the initial culture of E. coli K-12(λ). Table 3, shows the serological properties of certain bacteriophages isolated from the indicated subcultures. As is apparent from Table 3, the neutralization constant of the temperate phage (λ) isolated from E. coli K-12 (λ) is close to the neutralization constant of bacteriophages isolated from subcultures No. 7/1 and 7/7.

Subcultures No. 7, 7/26, and 7/18 were used to study the serological properties; the remaining subcultures were not used since they produced spontaneous agglutination upon preparing the bacterial suspension in a physiological salt solution.

We see from Table 4 that the subcultures of the alkali producers and the biochemical mutants isolated from them react in the agglutination reaction with the antiserum against the initial E. coli K-12 (λ), although in a somewhat smaller titer. They retained sensitivity to streptomycin.

These data indicate that the initial strain for the isolated alkali producers was E. coli K-12 (λ) having such a stable kinetic character as lysogeny.

The production of alkali, as we have already noted [3], is apparently associated with the profound change of the initial culture of E. coli K-12 (λ) not only as a consequence of mutation but also probably as a result of correlative variability.

LITERATURE CITED

1. N. N. Zhukov-Verezhnikov, I. N. Maiskii, V. I. Yazdovskii, et al., in book: Problems of Space Biology [in Russian], 1, Moscow (1962), p. 118.
2. N. N. Zhukov-Verezhnikov, I. N. Maiskii, V. I. Yazdovskii, et al., in book: Problems of Space Biology [in Russian], 2, Moscow (1962), p. 140.
3. N. N. Zhukov-Verezhnikov, I. N. Maiskii, A. P. Pekhov, et al., Vestn. AMN SSSR, No. 12 (1962), p. 49.
4. N. N. Zhukov-Verezhnikov, I. N. Maiskii, V. I. Yazdovskii, et al., in book: Aviation and Space Medicine [in Russian], Moscow (1963), p. 185.
5. N. N. Zhukov-Verezhnikov and A. P. Pekhov, Bacterial Genetics [in Russian], No. 15, Moscow (1963), p. 104.
6. N. N. Klemparskaya, in book: Artificial Earth Satellites [in Russian], No. 15, Moscow (1963), p. 104.
7. D. G. Kudlai, Zh. mikrobiol., No. 6 (1954), p. 32.
8. D. G. Kudlai, Variability of Bacteria of the Intestinal Group [in Russian], Moscow (1954).
9. D. G. Kudlai, in book: Multi-Volume Handbook on Microbiology, Clinical Aspects and Epidemiology of Infectious Diseases [in Russian], 1, Moscow (1962), p. 473.
10. A. P. Pekhov, N. I. Rybakov, and B. A. Mishchenko, Labor. delo, No. 4 (1963), p. 3.
11. V. D. Timakov, Izv. AN SSSR, Seriya biol., No. 2 (1952), p. 36.