ISOLATION OF AUXOTROPHIC MUTANTS FROM CULTURES OF PATHOGENIC SEROTYPE Escherichia coli O111:B4:H2 TREATED WITH N-METHYL-N'-NITRO-N-NITROSOGUANIDINE

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UDC 576.851.48.095.5.095.18

By treatment of cultures of the pathogenic serotype Escherichia coli 0111:B4:H2 with nitrosoguanidine, auxotrophic mutants with different requirements of nutrient factors were obtained. The biochemical properties of some mutants were modified. The greatest changes were observed in the antigenic structure of the mutants: some of them retained certain antigens of the original strain, while others lost them all.

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Many workers have reported the isolation of auxotrophic mutants of Escherichia coli and other microorganisms induced with N-nitrosoguanidine [1-11], but it is not yet known whether auxotrophic mutants induced by this mutagen can be isolated from cultures of pathogenic serotypes of E. coli. Yet such mutants are of great interest for the study of mutagenesis of pathogenic bacteria.

The object of this investigation was to isolate auxotrophic mutants from cultures of the pathogenic serotype E. coli O111:B4:H2 after treatment with N-methyl-N'-nitro-N-nitrosoguanidine and to determine the serological properties of the isolated mutants.

EXPERIMENTAL METHOD

Streptomycin-sensitive cultures of the pathogenic serotype E. coli O111:B4:H2 were used for the isolation of auxotrophic mutants.

The selective medium consisted of a minimal medium of the following composition: 20 g NH_4Cl, 4 g NH_4NO_3, 8 g anhydrous Na_2SO_4, 12 g K_2HPO_4, 4 g KH_2PO_4, 0.4 g $MgSO_4 \cdot 7H_2O$, 15 g Difco agar, 4 g glucose, and 1000 ml distilled water, pH 7.2.

Cultures of the pathogenic serotype E. coli O111:B4:H2 were grown in 30 ml meat-peptone broth for 6 h. Next, to 9.5 ml of the broth culture 0.5 ml of a freshly prepared solution of nitrosoguanidine was added, so that the final concentration of mutagen was 50 μg/ml. The treatment was carried out with aeration for 1 h at 37°. The same cultures but without the addition of the mutagen were used as the control. The cultures treated with nitrosoguanidine were washed twice with physiological saline. The residue thus obtained was suspended in 5 ml meat-peptone broth and seedings of 1 ml were added to five tubes containing the same broth, after which they were grown for 18-20 h at 37°. The culture was diluted to 10^{-6}, and seedings of 0.1 ml were then plated out on meat-peptone agar and incubated for 18-20 h at 37°. When from 90 to 120 discrete colonies per plate had grown, they were transferred by the replica technique [9] to dishes containing minimal agar. After growth for 18-20 h in an incubator, the colonies which did not grow on the dishes with minimal agar were seeded on agar slopes, from which subcultures were taken on minimal agar in order to purify the isolated auxotrophic mutants and to determine their requirements. The nutrient requirements of the isolated mutants were identified by Holliday's method [8].

Laboratory of Genetics of Vaccine Strains, Tashkent Research Institute of Vaccines and Sera, Ministry of Health of the USSR. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 69, No. 2, pp. 110-112, February, 1970. Original article submitted July 14, 1969.

TABLE 1. Requirements of Nutrients by Isolated Auxotrophic Mutants

No. of auxotrophic mutant	Growth factor															
	tryptophan	histidine	methionine	phenylalanine	cysteine	ornithine	thymine	thiamin	leucine	proline	valine	tyrosine	guanine	isoleucine	alanine
2 108								+							
5		+	+								+			+	
6			+								+				
9,65,69															
16,24,25, 27,28,71															
72,77					+										
19								+				+			
22,56,60, 85,89,33,			+												
26,29,82						+									
31				+					+			+			
47	+				+										
49	+											+			
55			+							+		+	+		
58,80									+						
59		+													
66			+				+								
70			+		+			+							
91	+														
93										+					
101			+				+								+
106	+							+			+				
107												+			
109											+				

Note. + Denotes that amino acid is essential.

EXPERIMENTAL RESULTS

As a result of this work, 55 auxotrophic mutants of the pathogenic serotype E. coli O111:B4:H2 were isolated. In their morphological and cultural properties they were indistinguishable from the original strain.

The results of the investigation showed (Table 1) that 27 of the mutants were mono- and 13 were polyauxotrophic. The character of the nutrient requirements of 15 mutants could not be determined by tests with the growth factors used. Five of the 13 polyauxotrophic mutants were dependent on three or more of these growth factors.

A study of the biochemical properties of the isolated mutants showed that with the exception of three mutants which did not decompose maltose, all were indistinguishable in the fermentation properties from the original strain.

The serologic properties of the original strain and of the isolated mutants also were investigated by the agglutination test (linear in test tubes, and on slides), using an absorbed homologous type-specific OB serum. These tests showed that the original strain O111:B4:H2 is agglutinated by homologus OB serum in high titers on slides and in tubes; 30 of the 55 mutants gave a positive agglutination reaction on slides only, and 25 were not agglutinated at all by this serum; 11 of the 20 mutants were agglutinated in the slide agglutination test in titers of 1:200-1:400 only with a living culture and gave a negative reaction with a heated culture.

It was thus possible to isolate auxotrophic mutants, induced by means of N-nitrosoguanidine, from the pathogenic serotype E. coli O111:B4:H2. However, under these conditions considerable changes were observed in the serological properties of the mutants, in the form of a decrease in their antigenic activity.

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