ECOLOGY

Population Characteristics of the Factors of Persistence of *Escherichia coli* Isolated from Different Ecological Niches

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The range of antilysozyme activity (the sign of persistence) of *Escherichia coli* clones from subpopulations isolated from natural and artificial ecosystems is established. It is shown that heterogeneity of the microorganism population reflects the ecological status of its habitat. Therefore, it can be used as a test-indicator in biomonitoring and as an index in the standardization of eubiotics.

Key Words: E. coli; eubiotic; population analysis; persistence

The population method has been widely used in epidemiology for prediction of epidemics [1], in experimental research for evaluation of persistence and elimination of the causative agent [5], and in environmental studies [6].

The factors of microorganism persistence, which reflect the flexibility of the adaptation mechanisms of a given population, may be employed to solve ecological, hygienic, and biotechnological problems [2].

The aim of this study was to explore the possibility of using the population method for assessing the habitat of microorganisms and for standardization of cultures used for the production of eubiotics.

MATERIALS AND METHODS

E. coli was selected because it is an ubiquitous microorganism with a high adaptive potential. Model subpopulations and subpopulations isolated from natural (human body and open water reservoir) and from man-made (sewers) ecosystems were used. All

Department of Microorganism Persistence, Institute of Ecology and Genetics of Microorganisms, Ural Division of the Russian Academy of Sciences, Orenburg escherichia isolated from different econiches possessed typical biological characteristics. The enterobacteria were isolated from human intestine with due consideration for their intracellular parasitism [4]. *E. coli* serogroups O-20, O-111, O-75, O-128, and O-114 were isolated from patients with enteric infections. Typical *E. coli* whose serotype could not be determined with the standard diagnostic kit were isolated from normal subjects, from the Ural river at the site of water intake (clean zone) and waste disposal (contaminated zone), and from sewers.

E. coli (strain M-17) contained in the preparation Colibacterin were employed as a model microorganism.

The subpopulations were cloned by serial dilutions and inoculations, so that no more than 100 colonies grew in each dish. Population analysis of the antilysozyme activity (ALA) of *E. coli* was performed by the method of imprints [8]. Antilysozyme activity was assayed as described elsewhere [3].

RESULTS

In the subpopulation which was isolated from patients and consisted of E. coli of different sero-

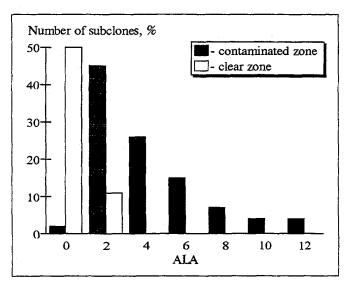


Fig. 1. Subpopulation of E. coli isolated from human organism.

groups, 94.3% clones displayed ALA ranging from 0 to 12 μ g/ml (Fig. 1). Figure 1 shows that clones with ALA of 1-2 μ g/ml (22%), 5-6 μ g/ml (24%), and 7-8 μ g/ml (25%) predominated. Clones without ALA (33%) and low ALA (1-2 μ g/ml, 13%, and 3-4 μ g/ml, 18%) predominated in the subpopulation of *E. coli* isolated from normal subjects. The range of ALA variability in the subpopulation of *E. coli* isolated from normal subjects was 1.2-fold lower compared with that in the subpopulation isolated from patients. Thus, *E. coli* subpopulation isolated from patients was more heterogeneous than that isolated from heathy subjects.

In the subpopulation of *E. coli* isolated from the Ural river, ALA variability depended on water purity (Fig. 2). Clones without (51%) and with minimal ALA (1-2 μ g/ml, 37%) predominated among escherichia isolated at the site of water intake. The variability of the subpopulations isolated at the site of waste disposal was 2.5-fold higher: 1.2 μ g/ml (45%), 3-4 μ g/ml (27%), 5-6 μ g/ml (15%), 7-8 μ g/ml (7%), and 9-10 μ g/ml (3%). Thus, heterogeneity of *E. coli* subpopulations isolated from contaminated water was higher than that of microorganisms circulating in the clean zone.

The distribution of clones according to ALA was virtually the same. A similar regularity was observed in the *E. coli* subpopulations isolated from sewers; however, the distribution according to ALA was different: 1-2 μ g/ml (61%) and 3-4 μ g/ml (22%). In 5, 9, 3, and 8% of clones ALA activity was equal to 0, 5-6, 7-8, and 9-10 μ g/ml, respectively.

In the *E. coli* subpopulation M-17 isolated form Colibacterin, all clones displayed ALA: 1 μ g/ml (80%) and 2 μ g/ml (20%). Thus, the pattern of ALA

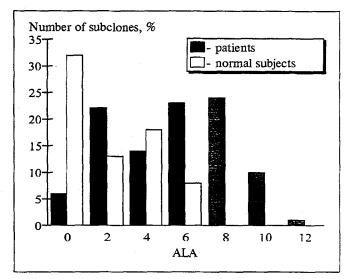


Fig. 2. Subpopulation of E. coli circulating in an open water reservoir.

distribution was virtually homogeneous. Homogeneity of the subpopulation may be indicative of a high biological density of the *E. coli* culture in the preparation. On the other hand, this may account for the difficulties encountered upon treatment of dysbiotic states with eubiotics, since a homogeneous culture markedly lowers the adaptive potential of the entire population of microorganisms.

Thus, population analysis of *E. coli* isolated from different ecological niches objectively reflects the population rearrangements in specific habitat.

This study shows that population analysis can be used for sanitary and hygienic evaluation of the habitat. Water contamination leads to an increase in the number of clones exhibiting ALA.

Heterogeneity of microbial population in terms of ALA can be used as a reference test in the production of eubiotics..

REFERENCES

- V. D. Belyakov, D. B. Golubev, G. D. Kaminskii, and V. V. Tets, *Autoregulation of Parasitic Systems* [in Russian], Leningrad (1987).
- O. V. Bukharin, Zh. Mikrobiol., Suppl., 4-13 (1994).
- O. V. Bukharin, B. Ya. Usvyatsov, A. P. Malyshkin, and N. V. Nemtseva, *Ibid.*, No. 2, 27-28 (1984).
- O. V. Bukharin, B. Ya. Usvyatsov, N. V. Nemtseva, et al., Byull. Izobret., No. 27 (1984).
- O. V. Bukharin, B. Ya. Usvyatsov, and N. V. Sheenkov, Zh. Mikrobiol., No. 2, 178-180 (1993).
- E. Wainert, R. Walter, T. Wetzel, et al., Biological Indicators of Contamination of the Surface Ecosystems [in Russian], Moscow (1988).
- N. V. Nemtseva and I. A. Misetov, in: Persistence of Bacteria [in Russian], Kuibyshev (1990), pp. 44-50.
- 8. J. Lederberg and E. M. Lederberg, J. Bacteriol., 63, No. 3, 399-406 (1952).