Occurrence of auxotrophic variants among bacteria of Enterobacteriaceae family isolated from water objects of the north-west of the Murmansk region

E A Zatsarinrnya1, V D Kalchugina2, N V Kolupaeva1,

1 Scientific laboratory of evolutionary ecology, Ryazan State University named by S. A. Esenin, 46 Svobody st, Ryazan, 390000, Russia
2 Institute of Environmental and Agricultural Biology (X-Bio), University of Tyumen, 6 Volodarskogo St., 625003 Tyumen, Russia

E-mail: microbiog@mail.ru

Abstract. In the last decade, interest in the problem of survival of bacteria with the loss of biosynthetic genes in environments with low nutrient content has increased markedly. The phenomenon of auxotrophy among bacteria living in nutrient-rich environments is well studied. However, information about the requirements of growth factors for saprophytic microorganisms is presently inadequate. The question of the ability of auxotrophs to survive in out-organism environments remains open. The results of the study show that both the representation of Enterobacteriaceae and the prevalence of auxotrophic forms among them varied significantly in the waters of the northwestern Murmansk region. The rivers Menikkajoki, Kolosjoki and Kuvernerinjoki are characterized by the highest enterobacteria concentrations. Thermotolerant coliform bacteria were not found in the samples. The occurrence of auxotrophic variants of Enterobacteriaceae was quite high - 48.5% (n=163). Auxotrophs most often were isolates of Enterobacter spp. (28.4%) and Citrobacter freundii (21.6%), rarely – Hafnia alvei, Klebsiella mobilis, Klebsiella oxytoca а Citrobacter koseri.

1. Introduction
Over the past decade, there has been a marked increase in interest in the question of the survival of bacteria lacking biosynthetic genes in low-nutrient environments [1, 2, 3, 4]. Loss of biosynthetic genes is the cause of auxotrophy, i.e. the requirement for growth factors in the environment. The phenomenon of auxotrophy among bacteria living in nutrient-rich environments (pathogens, endosymbionts, lactic acid bacteria) has been well studied [2]. However, information about the requirements for growth factors of saprophytic microorganisms is currently inadequate [4, 5, 6]. The question of the ability of auxotrophs to survive in non-organizational environments remains open.

It is well known that the loss of biosynthetic genes can lead to many factors that remain poorly understood [7, 8]. In a number of papers that consider the survival of auxotrophic and prototrophic variants, it is emphasized that the survival of auxotrophs could be significantly influenced by the genetic background of bacteria formed in specific environmental conditions. [9]. Based on this information, the assessment of the presence of auxotrophs in water bodies in the territories with different degrees of anthropogenic influence is an active area of research.
The aim of this research is to assess the occurrence of bacterial variants lacking biosynthetic genes (auxotrophs) among isolates of the Enterobacteriaceae family inhabiting surface water bodies of the northwestern Murmansk region.

2. Materials and methods
Water sampling for bacteria isolation of Enterobacteriaceae family was performed in accordance with the general requirements of GOST R 31942-2012. Water samples were taken from a depth of 10-15 cm from the surface and placed into sterile 500 ml glass vials.

Water samples were taken in September 2016 from 6 rivers in the northwestern part of the Murmansk region: Shuonijoki, Kuvernerinjoki, Kotsel’joki, Kolosjoki, Namajoki, Menikkajoki.

2.1. Bacterial counts, isolation and species identification
We used the membrane filtration to count the number of members of the family Enterobacteriaceae. First, water samples were filtered through 0.45 μm membrane filters (Vladisart, Russia). The filters were placed on Endo agar. The plates were incubated for 24-48 h at 37°C. Species identification of isolates was carried out using a test-system for rapid biochemical identification of enterobacteria "Rapid Entero 200 M" (Institute of epidemiology and microbiology named after Pasteur, St.-Petersburg, Russia), which is based on microvolume technology using liquid differential medium.

2.2. Identification of auxotrophs.
Identification of auxotrophic variants was carried out on the minimum agar due to the inability to grow [10]. The minimum agar had the following composition: 300 ml of 2% water agar, 100 ml salt concentrate (NH₄Cl - 20 g, NH₄NO₃ - 4 g, Na₂SO₄ - 8 g, K₂HPO₄ - 12 g, KH₂PO₄ - 4 g, MgSO₄ × 7 H₂O – 0.4 g, distilled water - 1000 ml) and 4 ml of 20% glucose solution. The plates were incubated for 24-48 h at 37°C.

3. Results
The results of the study show that the representation of Enterobacteriaceae and the prevalence of auxotrophic forms among them in the rivers of the North-West of the Murmansk region varied significantly (table 1). The highest numbers of Enterobacteriaceae were observed in the rivers Menikkajoki, Kolosjoki and Kuvernerinjoki. Thermotolerant coliform bacteria were not found in any of the examined streams. The quality of water in all these rivers meet the existing sanitary norms.

The highest number of total coliform bacteria was observed in the river Menikkajoki. The concentration of Enterobacteriaceae here was 64.22 CFU/100 ml, and the species diversity was represented by Enterobacter spp., C. freundii and K. ascorbata. In the Kolosjoki river, the number of enterobacteria varied greatly in different areas. The largest number of Enterobacteriaceae isolates was isolated in the upper reaches of the river (59.3 CFU/100 ml), the smallest being found in the lower parts (2.42 CFU / 100 ml) (table 1). The Kolosjoki river was characterized by a wide variety of coliform species. Isolates of Enterobacter spp. were found in the water samples from the river, along with samples of Klebsiella oxytoca and Klebsiella mobilis. Samples of Providencia alcalifaciens, Citrobacter freundii, Hafnia alvei, Kluyvera ascorbata were rare here. The enterobacteria concentration was one of the lowest (1.14 CFU/100 ml) in the river Kotsel’joki, which is a tributary of Kolosjoki. Of the species, only Enterobacter spp was detected.

Enterobacteria isolated from river Namajoki, are characterized by high species diversity. The average number of bacteria in this family was 1.42±0.90 CFU/100 ml (0.71±0.63 CFU / 100 ml in the estuary). Isolates of Citrobacter freundii and Citrobacter koseri prevailed among enterobacteria isolated from the river Namajoki. Providencia alcalifaciens, Hafnia alvei, Kluyvera ascorbata and Enterobacter spp were also present. In samples from the estuary area Namajoki C. freundii were found, but Enterobacter spp was the predominant species.

Among all the surveyed rivers enterobacteria were quantitatively most evenly distributed in samples of the river Shuonijoki, where their population was on average 3-4 CFU/100 ml on different
sections of the river. Isolates of *Citrobacter freundii* predominate in the species composition in the river Shuonijoki whereas *Kluyvera ascorbata* was the predominate species in samples taken from the estuary section of the river. The number of this group of bacteria reached 31,69 CFU/100 ml in the river Kuvernerinjoki. This is slightly higher than the average number of enterobacteria in the study area. The species composition was presented to *K. mobilis, H. alvei, C. freundii* and *Enterobacter spp*.

Analysis of the distribution of auxotrophic isolates of enterobacteria in the waters of the Murmansk region shows that the occurrence auxotrophic microorganisms is substantial, representing slightly less than half of the number of all isolated strains (48.5% n=163). Auxotrophic variants of *Enterobacteriaceae* were isolated from almost all rivers. The exception was the river Namajoki. Auxotrophs were dominant over prototrophic bacteria in the rivers Shuonijoki, Kuvernerinjoki and Menikkajoki (table 1). Analysis of the species of the detected auxotrophs isolates shows that the largest number of them belonged to the genera *Enterobacter* and *Citrobacter*, which include about a third of all isolated isolates (figure 1). The remaining four genera had a relatively uniform distribution (about 10%). Thus, the species distribution of auxotrophic variants repeats the representation of these species in the general analyzed sample. From our results it follows that it is impossible to say that some of the genera are more predisposed to the appearance of auxotrophs among the members of this genera.

### Table 1. Bacteria count, sample, content of auxotrophs in the surveyed rivers.

| River                  | Enterobacteriaceae concentration, CFU/100 mL | N analyzed isolates | N (%) auxotrophs |
|------------------------|---------------------------------------------|-------------------|-----------------|
| Kolosjoki              |                                             |                   |                 |
| Target 1-upper current | 59.30±5.78                                  | 16                | 34 (45.3)       |
| Target 2-average current | 21.90±6.46                                 | 18                | 11 (68.7)       |
| Target 3-in the settlement of Nickel | 43.90±6.54                             | 26                | 10 (55.5)       |
| Target 4 - the mouth of the river | 2.42±1.17                                | 15                | 6 (23.0)        |
| Shuonijoki             |                                             |                   |                 |
| Target 1-upper current | 4.42±1.59                                  | 14                | 11 (78.5)       |
| Target 2-lower current  | 3.14±1.34                                  | 11                | 8 (72.7)        |
| Namajoki               | 11.69±2.56                                  | 25                | 0 (0.0)         |
| Kotsel’joki            | 1.14±0.81                                   | 3                 | 3 (100.0)       |
| Kuvernerinjoki         | 31.69±4.23                                  | 20                | 13 (65.0)       |
| Menikkajoki            | 64.22±6.01                                  | 15                | 9 (60.0)        |
| Total                  |                                             | 163               | 79 (48.5)       |
Figure 1. Species composition of auxotrophs among the isolates of the Enterobacteriaceae family isolated from six rivers of the North-West of the Murmansk region (N=79).

4. Conclusions
The results of the study showed that auxotrophic bacteria of the family Enterobacteriaceae occurred quite often in northern rivers. According to the results of growth experiments, auxotrophs have an advantage in growth rate compared to prototrophic variants in the presence of the necessary growth factors [2]. It was found that auxotrophic variants were more common in areas with low river flow velocity. Auxotrophic variants have been shown to be more able to survive in environments with poor growth factors only in the presence of adequate metabolic exchange between microorganisms [2]. Thus, their more frequent detection in areas with a slow flow can be explained by better conditions for aggregation by microorganisms, which, accordingly, contributes to the exchange of growth factors between participants in such "communities".

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