ISSN 1064-2293, Eurasian Soil Science, 2006, Vol. 39, No. 2, pp. 211–217. © Pleiades Publishing, Inc., 2006.
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SOIL BIOLOGY

The Effect of Soil Properties on the Preservation and Reproduction of Listeria and Yersinia

M. L. Sidorenko[1], L. S. Buzoleva[2], and N. M. Kostenkov[1]

[1] *Institute of Biology and Soil Science, Far East Division, Russian Academy of Sciences, pr. Stoletiya Vladivostoka 159, Vladivostok, 690031 Russia*

[2] *Research Institute of Epidemiology and Microbiology, Siberian Division, Russian Academy of Medical Sciences, ul. Sel'skaya 1, Vladivostok, 690031 Russia*

Received March 31, 2004

Abstract—The physical and chemical properties of brown forest, brown podzolic, and urban (lawns of the city of Vladivostok) soils that affect the reproduction of bacteria *Listeria monocytogenes* and *Yersinia pseudotuberculosis* were studied. The results of the experiments suggest that a high content of exchangeable bases, domination of the fraction of humic acids bound with clay minerals and the fraction of fulvic acids in humus, and high soil temperature stimulate the reproduction of the studied bacteria in soils. A high humus content, a predominance of humates, and a low soil temperature have an inhibiting effect on bacteria.

DOI: 10.1134/S1064229306020128

INTRODUCTION

Soil is one of the ecological niches of *Listeria monocytogenes* and *Yersinia pseudotuberculosis*, where these bacteria are equal members of the microbial community [4, 7, 14, 17], although the mechanisms of their adaptation and forms of existence in soil ecosystems are studied insufficiently. Up to now, scientists considered the influence of soil on the reproduction of *Listeria monocytogenes* and *Yersinia pseudotuberculosis* bacteria without regard for the soil genesis and soil chemical and physical properties. It is obvious that the type of soil and different soil properties play a certain part in the reproduction of pathogenic microorganisms, because soils differ sharply in their humus content, concentrations of nutrients, pH, particle-size composition, and microbial associations.

In this relation, the goal of this work was to study the physical and chemical properties of particular soil types affecting the preservation and reproduction of *Listeria monocytogenes* and *Yersinia pseudotuberculosis* in these soils. Investigation of this problem will give a further elucidating glimpse into the regularities of the distribution and reproduction of these bacteria in soils of different genesis.

OBJECTS AND METHODS

The samples of brown forest (forest area) and brown podzolic (arable land, cabbage field) soils distributed mostly in the Sikhote-Alin foothills and in the Khanka–Razdol'naya Plain, and of urban soils (the lawns in the city of Vladivostok) taken from the upper (0–10 cm) horizon were used as the study objects. The choice of these soils was not accidental: the data of Gershun [3, 4] suggest that most isolations of *L. monocytogenes* fell on forest soils; Kolesnikova [5], and Somov and Litvin [14] pointed to the great infection of arable soils used for growing cabbage with *Y. pseudotuberculosis* bacteria [5, 14].

In the experiments, we used the reference strains of *L. monocytogenes* (P, A, K, 10CN, 4B, and 1/2A) and *Y. pseudotuberculosis* (H-557, 282, 512, 907, H-2781, H-3515). The listeria strains were received from the All-Russia State Control Institute of Veterinary Preparations (Moscow); The yersinia strains were obtained from the Museum of the All-Russia Center for Yersenioses and Pseudotuberculosis (Research Institute of Epidemiology and Microbiology, Siberian Division, Russian Academy of Medical Sciences, Vladivostok).

The following parameters were determined in the soil samples: the humus by Tyurin, the group composition of humus by Tyurin in modification by Ponomareva and Plotnikova, the actual acidity, the exchangeable acidity, the hydrolytic acidity, the exchangeable bases (Ca^{2+} and Mg^{2+}), the cation exchange capacity, and the base saturation [1, 11]. The preparations of humic acids were obtained according to the commonly adopted method [12].

To study the growth the kinetics of listeriosis and pseudotuberculosis microbes in different media (soil samples, humin preparations, extracts of humic acids) at the temperatures of 20–22°C and 4–6°C, bacterial cultures washed out three times in physiological solution were used: the bacterial cultures were introduced to the tested media at the rate of 100 cells per 0.1 ml of the medium. The number of bacterial cells per 1 ml of suspension was determined according to the optical standard of turbidity. The study was carried out under

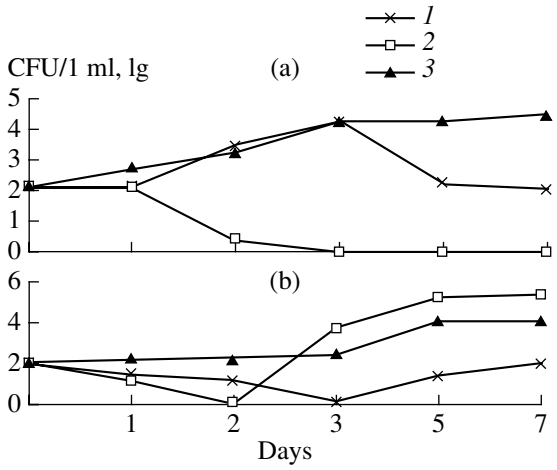

Fig. 1. Dynamics of reproduction of *L. monocytogenes* strains P (a) and K (b) in different soils under 20–22°C. Here and in Fig. 2: (*1*) brown podzolic soil, (*2*) brown forest soil, (*3*) urban soil.

periodical cultivation. To build the growth curve, the dynamics of the bacteria number was determined by the way of periodic inoculations with 0.1 ml of inoculate (from a particular serial dilution) into differentiation-diagnostic media (yeast agar for Listeria and Serov's medium for Yersinia). The stages of the growth curves and the maximum concentrations of bacteria were determined by way of counting the number of colonies (colony-forming units, CFU) grown on Petri dishes [6].

To carry out an experiment on determination of the number of viable cells of *L. monocytogenes* and *Y. pseudotuberculosis* in the soil ecosystems, suspensions of soil samples were prepared that contained 1 g of soil per 100 ml of physiological solution. The suspensions were subject to steaming for sterilization 20 min a day for 3 days under the temperature of 100°C with flowing steam, then they were inoculated with daily cultures of Listeria and Yersinia in the concentration 10^3 CFU/ml (according to the turbidity standard). The initial dose in the control inoculation from the soil suspension comprised 100 CFU/ml. In all, five variants of the experiment were studied in triplicate under temperatures of 20–22°C and 4–6°C.

The reproduction of bacteria on the humic preparations from the soils was studied in the following way. Special media composed of distilled water and 0.02% humic preparation [2] were prepared preliminary and sterilized with flowing steam 20 min a day for 3 days under 100°C. The prepared sterile media in the tubes were inoculated with daily cultures of *L. monocytogenes* and *Y. pseudotuberculosis* in the amount 10^3 CFU/0.1 ml. The cultures were incubated under the temperatures 4–6°C and 20–22°C. Solid nutrient media were inoculated during 15 days with daily intervals. In all, 120 variants of the experiment were studied. The study was carried out in triplicate.

The following method was used to reveal the effect of soil humus composition on the reproduction of listeriosis and pseudotuberculosis microbes. Daily cultures of Listeria and Yersinia were introduced to the tubes with media. The media were prepared with distilled water by adding the particular extracts of humic acid or fulvic acid fractions (depending on the variant of the experiment) in concentration 0.02% [2]. The ready-made media were sterilized with flowing steam 20 min a day for 3 days under 100°C. Then, they were inoculated in tubes with daily cultures of *L. monocytogenes* and *Y. pseudotuberculosis* in the amount 10^3 CFU/0.1 ml. Then, Petri dishes with proper media were inoculated periodically from these tubes with 0.1 ml of suspension, and the number of colonies per dish was counted. In all, 420 variants of the experiment were carried out under the temperature 20–22°C and 420 variants of the experiment were carried out under the temperature 4–6°C. The study was carried out in triplicate.

Listeriosis and pseudotuberculosis microbes were cultivated in the following media: nutrition agar (pancreatic hydrolyzate of sprat 17.9 g, agar 11.2 g, sodium chloride 7.7 g, distilled water up to 1 l, pH 7.3 ± 0.2); yeast agar with glucose, nalidixic acid, and trypaflavine (sodium chloride 5.5 g, hydrolyzate of fodder yeast 12 g, agar 12.5 g, distilled water up to 1 l, glucose 2 g, nalidixic acid 20 mg, trypaflavine 30 mg, pH 7.2–7.4), Serov's medium (nutrition agar 3.2 ml, glucose 0.5 g, urea 0.1 g, ammonium molibdate 0.1 g, soda (Na_2CO_3) 0.08 g, 30% solution of bile, 2 ml, 1% solution of crystal violet 0.1 ml, 1.6% solution of Congo-red 0.9 ml, distilled water 100 ml).

RESULTS AND DISCUSSION

The dynamics of listeria reproduction in different soil types was studied at the first stage of research. It was found that the preservation and reproduction of *L. monocytogenes* and *Y. pseudotuberculosis* in soils depended on the type of soil as well as on the biological properties of the bacteria.

The substrates of urban and brown podzolic soils had positive effects on the reproduction of most strains (A, P, 10CN, 4B, and 1/2A) of listeria. The reproduction of the listeriosis microbe reached the value of 4.5 lg in urban soil and 4.2 lg in brown podzolic soil, whereas the death of bacteria was observed in brown forest soil already on the third day. The strain K of *L. monocytogenes* was an exception (Fig. 1); it reproduced better in brown forest (5.4 lg) and urban (4.1 lg) soils as compared with the brown podzolic soil (2 lg).

The strain 907 of *Y. pseudotuberculosis* reproduced well in urban and brown podzolic soils, reaching 5.3 and 5.2 lg respectively (after adaptation) at the third day. Practically, it did not reproduce in the brown forest soil. A similar regularity was observed for the strains 282, 512, H-557, H-3515 of Yerisinia. Bacteria of strain H-2781 *Y. pseudotuberculosis* reproduced in the brown

forest soil (4.3 lg) and practically did not reproduce in the urban and brown podzolic soils as can be seen from Fig. 2.

It should be noted that both lLsteria and Yersinia reproduced actively under the temperature 20–22°C, and slow growth of a bacterial culture was observed under 4–6°C.

It was revealed that soil acidity being an abiotic environmental factor effected the reproduction of *L. monocytogenes* and *Y. pseudotuberculosis* in the studied soils. The pH range 6.6–7.4 (the acidity of urban and brown podzolic soils is usually in this range) appeared to be optimal for the growth of these bacteria, and this fact is in agreement with the data presented in the works of Gershun [4], and Maksimenkova and Litvin [8], because both Listeria and Yersinia have growth optimums in this range of pH. For example, Gershun [4] experimentally determined that Listeria reproduce well under pH 6.7. The pH range from 6.0 to 8.0 is optimal for the growth of Yersinia in soils according the data of Maksimenkova and Litvin [8]. The departure of the pH value from these values is destructive for the bacteria. Hence, both Listeria and Yersinia reproduce better in soils with the acidity close to neutral.

The acidity of brown forest soil (pH 5.8) was the least suitable for these bacteria, so most strains practically did not reproduce in this soil. Additionally, the data of Table 1 show that the brown forest soil had higher values of the exchange and hydrolytic acidity than the urban and brown podzolic soils and this had an effect on the base saturation. The brown forest soil not only had an extremely low pH for Listeria and Yersinia reproduction but also the least value of the exchangeable bases (37.5 mg-equiv/100 g).

It was found out that the cation exchange capacity (CEC) of the brown podzolic soil exceeded those of the other studied soils. This soil contained greater amounts of calcium and magnesium ions, which are vitally important for normal growth and reproduction of Yersinia and Listeria [13]. Consequently, brown podzolic soil is favorable for reproduction of Listeria and Yersinia, because it has optimal values of the pH and base saturation and contains sufficient amounts of calcium and magnesium ions.

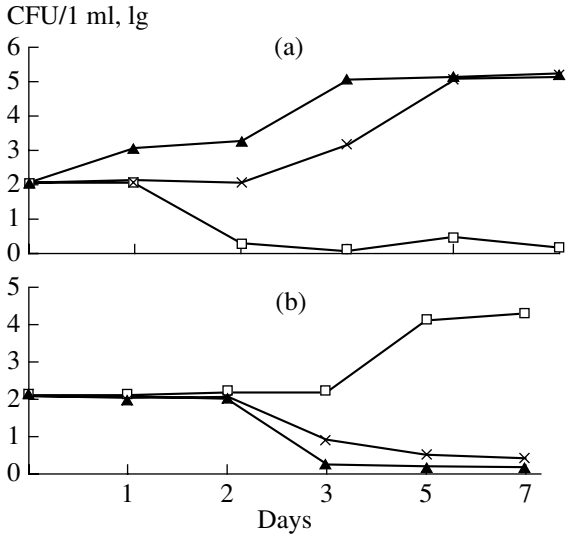

Fig. 2. Growth of *Y. pseudotuberculosis* strains 907 (a) and H-2781 (b) in different soils under 20–22°C.

Since the physicochemical and chemical properties did not allow explaining the reproduction of pathogenic bacteria in urban soil, its humus composition was studied. The content of total carbon reached 4.5% in the urban soil, in the brown podzolic soil it was 2.6%, and in the brown forest soil it was 1.1% (Table 2). It could be assumed that the composition of the organic substances in the soil has an effect on the listeriosis and pseudotuberculosis microbes in the soil ecosystems.

Urban soil had the most optimal content of humic and fulvic acids (Table 2). For example, Buzoleva [2] demonstrated that Yersinia reached maximum reproduction in the solutions with a low concentration (0.02%) of humic acids; higher concentrations had an inhibiting effect on the bacteria reproduction. It should be noted that the sample of the urban soil contained half as much of the first aggressive fraction of fulvic acids as the other two soils. It is obvious that this fact had a positive effect on the growth and reproduction of the pathogenic bacteria in this environment, because the fulvic acids of this fraction can inhibit the growth of bacteria. This could be probably explained by the fact that the first aggressive fraction of fulvic acids is connected with mobile sesquioxides, which adversely

Table 1. Physicochemical and chemical properties of soils

Soil	pH		Exchange acidity	Hydrolytic acidity	Cation exchange capacity	Exchangeable bases		Base saturation, %
	H_2O	KCl	mg-equiv/100 g of soil			Ca^{2+}	Mg^{2+}	
Brown forest	5.85	5.00	3.25	7.20	44.70	30.3	7.5	83.9
Brown podzolic	7.36	6.36	1.15	1.74	80.74	65.1	14.2	97.8
Urban	6.60	5.58	1.53	2.88	45.88	35.2	8.1	93.7

Table 2. Group composition of humus (percentage of total soil carbon)

Soil	C, %	Humic acids				Fulvic acids					$C_{ha} + C_{fa}$	C_{ha}/C_{fa}	Humin
		1	2	3	Σ	1a	1	2	3	Σ			
Brown forest	1.1	12.73	13.63	15.54	41.90	5.45	1.82	24.54	8.90	40.71	82.61	1.1	17.39
Brown podzolic	2.6	6.11	25.95	16.84	48.90	5.72	5.35	3.05	14.84	28.96	77.86	1.7	22.14
Urban	4.5	3.10	9.73	16.37	29.20	2.21	2.29	13.50	2.88	20.88	50.08	1.4	49.42

Note: Σ means sum total of fractions.

influence the surface structures of bacteria and make it difficult for the exchange processes in the latter.

All soils had humus of fulvate-humate type (C_{ha}/C_{fa} = 1–2). However, the humus in the brown forest soil contained a greater amount of fulvic acids (C_{ha}/C_{fa} = 1) than the brown podzolic soil (C_{ha}/C_{fa} = 1.7) or the urban soil (C_{ha}/C_{fa} = 1.4), and this might be unfavorable for the reproduction of Yersinia and Listeria. The total amount of fulvic acids in the brown forest soil was 1.4 times higher, and that of the second fraction of fulvic acids was 8 times higher than in the brown podzolic soil.

There was no significant difference between the total amounts of humic acids in the brown forest and brown podzolic soils. However, it is known that the humic acids of brown forest soil are weakly consumed in comparison with those of brown podzolic soil [2]. This might be the reason for the better reproduction of Yersinia and Listeria in the brown podzolic soil. Moreover, the brown podzolic soil was used for a long time in agriculture and was treated mechanically and chemically (with ameliorants). Therefore, the humic acids in this soil can be more easily available for bacteria, and the soil absorbing complex contains greater amounts of exchangeable bases, especially of calcium and magnesium ions.

As the bacteria studied differ in the structure of their cell walls (Listeria are Gram-positive, and Yersinia are Gram-negative [9]) and have surface structures of different chemical composition, the mechanisms of interaction of these bacteria with humic and fulvic acids can be different. Biological processes occurring in urban soil are more active than in brown forest and brown podzolic soils, and this results in the formation of humus acid fractions that are more available for microorganisms.

The preferable development of the studied bacteria in urban soil is explained by the composition of the humic acids more than by their amount. Humic acids are known to be characterized by heterogeneity and polydispersity [10].

Hence, the preservation and reproduction of Yersinia and Listeria in soils of different types are affected by the organic substances of the soils and the soil physicochemical properties as well as the biological properties of studied strains. The bacteria *L. monocytogenes* and *Y. pseudotuberculosis* reproduce the best in urban and brown podzolic soils, and this is explained by the optimal pH values in these soils and the lower concentrations of fulvic acids in comparison with brown forest soil. Additionally, brown podzolic soil has great amounts of exchangeable calcium and magnesium ions in its soil absorbing complex. Because the study of the composition of the humic and fulvic acids did not explain the preference of pathogenic bacteria for urban soil, the effects of different fractions of the humic and fulvic acids on the reproduction of Listeria and Yersinia should be studied separately.

To study the effect of the humus composition of the soil on the reproduction of different *L. monocytogenes* and *Y. pseudotuberculosis* strains, an experiment was carried out for which acid and alkaline extracts of humic and fulvic acids were obtained from the studied soils. The results of the experiment suggest that humic and fulvic acids possess biological activity toward the studied bacteria. It was determined that all the strains of Listeria and Yersinia used in the experiment reproduced 0.5–1.5 lg better under 20–22°C than under 4–6°C. It should be noted also that all the tested strains of Yersinia demonstrated very good growth in all the variants of the experiment and reproduced 1–2 lg more actively than Listeria. For example, the reproduction of *Y. pseudotuberculosis* reached 6 lg and *L. monocytogenes* 4.5 lg under 20–22°C, and 4.5 and 3.5 lg, respectively, under 4–6°C. It was found that particular extracts of the fractions of humic and fulvic acids had different effects on the reproduction of Listeria and Yersinia. These effects depended on the bacterium species, their strain characteristics, and the temperature of cultivation.

For example, the strain P of *L. monocytogenes* reproduced equally well in all the variants of the experiment under both temperatures of cultivation, and more intense growth was observed on the extracts of the fulvic acid fractions than on the extracts of the humic acid fractions (Fig. 3). The strain H-2781 of *Y. pseudotuberculosis* reproduced under a temperature of 4–6°C better on the extract of the second fraction of fulvic acids of the urban soil than on the extract of the second fraction of the humic acids.

It is difficult to say unambiguously which fraction extracts had a stimulating effect and which had an inhibiting effect on the development of the studied bac-

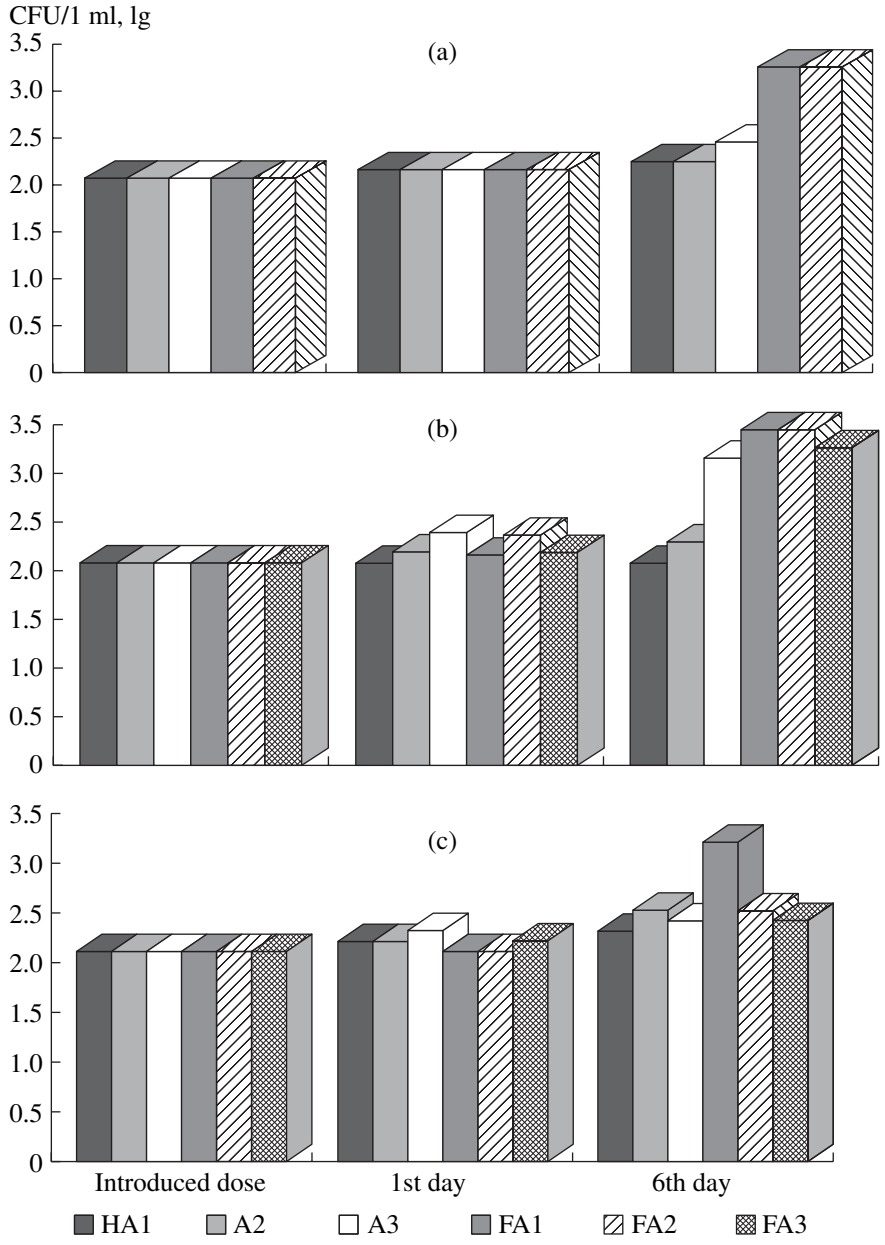

Fig. 3. Growth of *L. monocytogenes* strain P on different fractions of humic acids under 4–6°C: (a) brown podzolic soil, (b) brown forest soil, and (c) urban soil.

teria. Because similar extracts of fractions of humic and fulvic acids from different soils differ in composition, the results were ambiguous. In some cases, Listeria and Yersinia grew and reproduced better in the media with fulvic acids than in the media without them; this phenomenon can be explained by the fact that fulvic acids in comparison with humic acids contain less benzenepolycarboxylic acids in their nucleus and a greater number of chains with polypeptide bonds in the peripheral part of the molecule; i.e., they are more hydrophilic, hence, more available for bacteria. Fulvic acids have a lower molecular weight than humic acids, contain less carbon and nitrogen, and the nitrogen of fulvic acids is mostly hydrolyzable; i.e., it is confined mostly to the aliphatic part of the molecule and is, therefore, more available for microbes. For example, Orlov [10] noted that fulvic acids are one of most available for the microbe groups of soil humus and because of this are quickly utilized by microorganisms.

The extract of the first aggressive fraction of fulvic acids engaged our attention. The data of Tables 1 and 2 suggest a similarity in composition of the soil absorbing complex in the brown forest and urban soils, and the latter soil contained half as much of the first aggressive fraction of fulvic acids as the brown forest soil. This fact was probably the cause of the inhibition of growth

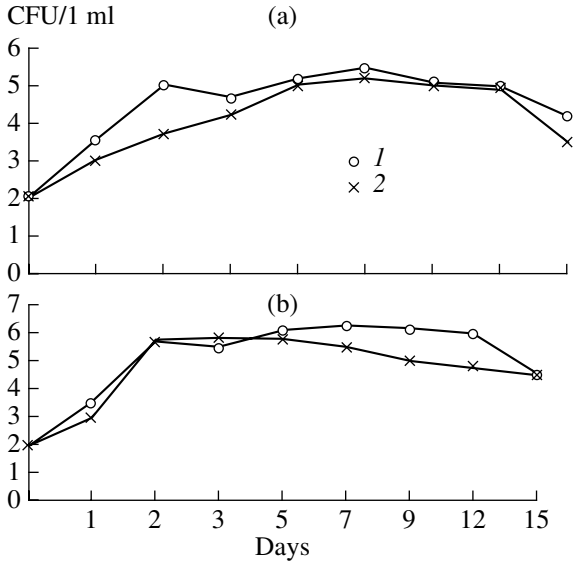

Fig. 4. The curves of bacterial growth on humus preparations from soil: (a) *L. monocytogenes* strain 10 CN; (b) *Y. pseudotuberculosis* strain 512; (*1*) ammonium humates of brown podzolic soil, (*2*) ammonium humates of brown forest soil.

and reproduction of different *L. monocytogenes* and *Y. pseudotuberculosis* strains in the brown forest soil.

The pH values in all extracts of the fractions of humic and fulvic acids were in the range 6–8. Only the media with the extract of the first aggressive fraction of the fulvic acids, where the pH was 2.5–2.8, were the exception. It was noted already that the optimal values for the development of *L. monocytogenes* and *Y. pseudotuberculosis* are in the range 6–8. Because of this, the acidity did not influence the preservation and reproduction of Listeria and Yersinia in the variants of the experiment without the extract of the first aggressive fraction of fulvic acids. The adverse effect of the first aggressive fraction of fulvic acids in this experiment is explained by the low pH value unfavorable for the reproduction of *L. monocytogenes* and *Y. pseudotuberculosis.*

The dynamics of the Listeria and Yersinia reproduction was studied in the preparations of humic acids in the form of ammonium humate. The results of the experiment suggest that *L. monocytogenes* and *Y. pseudotuberculosis* reproduced practically equally on the humates from the brown forest soil and the brown podzolic soil (Fig. 4). Both Listeria and Yersinia reached maximum reproduction on the humates of brown podzolic soil (5.4 and 6 lg respectively), and these values are comparable with those obtained for the reproduction of these bacteria on laboratory nutrient media.

Hence, it resulted from the experiments that the total humus content, its fraction composition, its acid–alkaline conditions, and the temperature regime of the soil environment, as well as the strain characteristics of the bacteria, influenced the existence of Listeria and Yersinia in the soils.

CONCLUSIONS

(1) It was determined that urban soils with a low content of humic acids had the optimal conditions for reproduction of *L. monocytogenes* and *Y. pseudotuberculosis.* The reproduction of Listeria and Yersinia in brown podzolic soil was determined by the presence of increased amounts of calcium and magnesium in the soil adsorption complex necessary for normal reproduction of microorganisms.

(2) The temperature affects the reproduction of listeriosis and pseudotuberculosis microbes in soil. Both listeria and Yersinia reproduce more intensely under the temperature of 20–22°C, whereas the intensity of their reproduction decreases under 4–6°C.

(3) Humic and fulvic acids are biologically active with respect to the reproduction of *L. monocytogenes* and *Y. pseudotuberculosis* in all the soils studied. Particular extracts of the fractions of humic and fulvic acids from every soil type had different effects on the reproduction of these bacteria.

(4) Reproduction of Listeria and Yersinia on the fractions of the humic acids depended on the strain of the studied bacterium and the environmental temperature. All the Listeria strains reproduced more intensely on the fractions of fulvic acids. Such regularity was not observed in the Yersinia. Fraction 3 of the humic acids was most active as relative to the growth of the studied bacteria irrespectively of the soil type.

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