IRSTI: 68.05.45 DOI: 10.52301/1684-940X-2024-2-31-35

PROLIFERATION DYNAMICS OF MICROBIAL CELLS OF BINARY CULTURES IN GERM-FREE (STERILE) SOIL AND FIELD CONDITIONS OF THE BAIKAL REGION

***Ts. D-Ts. Korsunova [1], R.B. Haydapova [2], E.E. Valova [3]**
[1]Institute of General and experimental biology, Siberian branch of the Russian Academy of Sciences, Ulan-Ude, Republic Buryatiya, Russia
[2]Federal state health institution «Centre for hygiene and epidemiology in the Republic of Buryatia», Ulan-Ude, Republic Buryatiya, Russia
[3]Geography and land of the Buryat State University, Ulan-Ude, Republic Buryatiya, Russia
**zinakor23@yandex.ru*

Summary

Currently, the issues surrounding the ecology of soil microorganisms are gaining an increasing amount of practical importance. The issue of the contamination of the environment by chemical and biological agents is located in close proximity to them. Therefore, it is of utmost significance to investigate the ecology of both the soil pathogens and the microflora that are antagonistic to them, as this inhibits the life activities of the pathogens. While conducting research on the ecology of soil microorganisms, it is essential to take into consideration the location of a specific region, the functional characteristics of biological properties that establish the conditions for the formation of microbial coenoses, the structure of microbial cenosis, and the enzymatic activity of soils in specific districts of Buryatia.

Keywords*: soil microorganisms, soil pathogens, antagonistic microflora, Baikal region, binary cultures*

Introduction. The microbial association involved in the normal cenosis, create a «microbial landscape». There are different types of ratios between prevailing and subtypes of microorganisms: apathetic, synergetic, antagonistic; and each type is biologically justified, because in the end, it is necessary to implement a kind of «automatic regulation» of the complex symbiotic relationships formed during a long evolution between macro - and microorganisms. Between true parasitism and authentic saprophytism there is a group of intermediate relations, which can be called antagonistic and associative.

Special attention deserves one of the types of relations - antagonism of microbes. The study of this phenomenon represents a vast field of studies to determine its biological essence and practical use of the most active antagonists to inhibit the development of the sensitized pathogenic bacteria [1].

Activity and the possibilities of antagonistic microbes are still very poorly investigated. Little is known about the nature and the way of formation of antibiotic substances and less is known about their mechanism of action [3].

Materials and methods. For bacteriological examination of soil samples of the following soil types were selected: cryogenic meadow chernozem soil (arable land), cryogenic meadow soil (arable land), cryogenic grey forest soil (wild land), cryogenic grey forest soil (wild land), cryogenic grey forest soil (forest), farinaceous calcareous chernozem selected in Eravninskiy and Bichurskiy areas [4].

In laboratory experiments we used avirulent and virulent strains isolated from different natural sources, and Museum culture from the Museum of living cultures of the Department of Microbiology, Virology and Ice BSAA named after V. R. Filippov: Bac. antracis STI, Bac. antracis pieces 55, Bac. cereus, Bac. 89 PCs. megaterium, Bac. pseudoanthracis, Bac. L2 subtilis, E. coli 25922 PCs, List. monocytogenes PCs 1219, St. albus, St. aureus, S. typhimurium pieces 79, 79 B. mycoides, B. mesentericus 70. The study of morphological, cultural, tinctorial, biochemical and pathogenic properties of microbial cultures has been conducted by methods of General Microbiology (Birger

M. O., 1983; Gerhard F., 1983). For light microscopy, microscopes MBI–6, ICBM, illuminator OI–19 were used. To study tinctorial properties of microorganisms smears stained by gram, Romanovsky-gimsa, Trujillo. The character of growth was studied in liquid and solid nutrient media, pigmentirovanie (MPA, MPB, KAA, environments, gissa, Endo, Levin, Ploskireva, bismuth-sulfite agar).

For identification and differentiation of microbial cultures there biochemical properties were studied. A system of indicator papers (SIB) was used for identification the microorganisms of the Enterobacteriaceae population of the Gorky Research Institute of Epidemiology of the Ministry of Healthcare [5,6].

Antagonistic interaction between microorganisms was determined by several methods: 1) method of agar blocks (Gauze, G. F., 1958), 2) the method of line sowing 3) the method of hole sowing.

Experimental data were processed by the method of variation statistics on the Chair of computer science and technology of the BSAA named after. V. R. Filippov.

Results and discusion. The results of the study showed that for cultures Bac. pseudoanthracis and Bac. megaterium, E. coli and St. aureus, Salm. typhimurium and St. aureus, E. coli and Bac. pseudoanthracis the maximum yield of microbial mass was after one month of cultivation, and a significant number of binary culture cells belonged to the first culture - antagonist. For such crops as Salm. tiphimurium and Bac. megaterium, E. coli and St. albus, St. albus and Bac. cereus, E. coli and Bac. cereus, St. aureus and Bac. megaterium the maximum number of cells was observed after the second month of the experiment.

Also, a significant proportion of cells for each binary culture is the culture of the antagonist. After five months of experiment set the allocation of the suppressed culture from Bac. pseudoanthracis and Bac. megaterium, Salm. typhimurium and Bac. megaterium, E. coli and St. albus, St. albus and Bac. cereus, E. coli and Bac. cereus, E. coli and Bac. pseudoanthracis has been entirely ceased (Table 1).

Table 1. Proliferation dynamics of microbial cells of binary cultures in germ-free soil (nx106).

MONTH	*Bac.pseudoanthracis* и *Bac. megaterium*		*S. typhimurium* и *Bac. megaterium*		*E.coli* и *St. albus*		*E. coli* и *St. aureus*		*St. albus* и *Bac. cereus*		*S. typhimurium* и *St. aureus*		*E.coli* и *Bac.pseudoanthracis*		*E.coli* и *Bac. cereus*		*St. aureus* и *Bac. megaterium*	
1	6	5	7	6	10	7	12	10	12	10	15	9	15	12	5	5	16	7
2	5	3	8	6	17	9	10	5	17	10	12	5	6	2	7	8	12	10
3	6	2	5	3	15	5	10	3	16	5	12	5	16	8	13	1	15	5
4	4	3	4	1	15	1	12	2	12	1	18	3	18	2	12	1	13	2
5	3	0	5	0	10	0	8	1	8	0	15	2	12	0	14	0,5	12	0,5
6	10	0	6	0	8	0	10	1	10	0	8	0	3	0	10	0	12	0

Thus, there has been observed the inhibition of population growth of suppressed culture in the competition for nutrient substrates, where the microbe-antagonist dominated. In all cases when the isolation of suppressed culture was stopped the quantitative decline in the number of cells of the germ-antagonist was observed at the same time [3].

While studying microbial binary cultures (Bac. pseudoanthracis and Bac. megaterium, E. coli and St. aureus, Salm. typhimurium and St. aureus, E. coli and Bac. pseudoanthracis, Salm. typhimurium and Bac. megaterium, E. coli and St. albus, St. albus and Bac. cereus, E. coli and Bac. cereus, St. aureus and Bac. Megaterium) in the

Table 2. Population dynamics of binary cultures in field conditions [nx10^6]

MONTH	*Bac.pseudoanthracis* и *Bac. megaterium*		*S. typhimurium* и *Bac. megaterium*		*E.coli* и *St. albus*		*E. coli* и *St. aureus*		*St. albus* и *Bac. cereus*		*S. typhimurium* и *St. aureus*		*E.coli* и *Bac.pseudoanthracis*		*E.coli* и *Bac. cereus*		*St. aureus* и *Bac. megaterium*	
1	3	4	1.5	2	3	3	5	3	5	4	3	1	3	2	5	3	3	2
2	2	3	1.5	1	1	1	3	2	3	1	2	1	6	3	1	1	3	1
3	3	2	2	1	2	1	3	2	3	1	1	1	4	2	2	1	1.5	1
4	4	1	4	2	1	0	2	1	2	1	3	0	3	1	4	1	3	0.5
5	3	0	2	1	3	0	4	1	3	0	2	0	4	0	3	0,5	3	0,5
6	3	0	1.5	1	1	0	4	1	2	0	1	0	3	0	2	0.5	1	0.5

field the selection of the bacteria was different. The number of microbes depended on climatic conditions.

For the winter-spring period the number of microbial cells of the test sites had a tendency to increase associated with the increase in temperature of the environment [7]. And at the same time the opposite trend was observed for decreasing the size and lack of growth in summer. This is due to activation of biochemical processes in the soil, which had a negative impact on the growth and reproduction of microbes. At the same time, the quantitative characteristics of these cultures under field conditions confirm the results of number in terms of binary cultures in sterile soil (Table 2).

Conclusion. An investigation into the growth patterns of microbial cells in two -species cultures in sterile soil and in natural settings in the Baikal region provides valuable knowledge about the behaviour and relationships of microorganisms in different habitats. Binary cultures in sterile soil exhibited rapid initial growth as a result of the absence of competitive and antagonistic microbial communities. This facilitated the examination of inherent growth patterns and interactions among the matched microbial species [8,9,10].

On the other hand, the conditions in the field were more intricate and competitive, which had a substantial impact on the growth and spread of microorganisms [11]. The native microbial populations and environmental conditions, such as temperature, moisture, and nutrient availability, influenced the growth and interaction patterns found in the binary cultures significantly. The associations between introduced and native microorganisms were characterised by antagonism and synergy, resulting in distinct proliferation results compared to sterile circumstances.

These findings emphasise the significance of context-specific conditions in investigations of microbial ecology. Although sterile soil experiments allow for the manipulation of variables to study basic interactions, field research provide a more authentic view of microbial behaviour in actual environments. The study emphasises the importance of combining laboratory and field methods to gain a thorough understanding of microbial dynamics. This understanding is crucial for implementing agricultural practices, managing the environment, and conducting bioremediation in the Baikal region and other areas.

References

1. Agapova R. B., Tsydypov V. C. Characteristics of the manifestations of antagonism at the opportunistic and pathogenic microorganisms during long-term storage.// Proceedings of the international conference, «Age physiology and pathology of agricultural animals», dedicated to the 90th anniversary of Professor V. R. Filippov. Ulan-Ude, 2003, P. 135-136.

2. Korsunova Ts. D-Ts., Agapova R. B., Korsunov V. A. Microbiological activity of cryoarid soils of the Baikal region. // Mate-

rials of the international scientific practical conference «Sustainable land management in extreme conditions». Ulan-Ude, 2003.P. 101-103.

3. Agapova R. B., Tsydypov V. C., Korsunova Ts. D-Ts. Antibiotic susceptibility of the microbial cultures isolated from various environmental objects. // Materials of the All -Russian conference of students and young scientists of agricultural universities of Russia. Yakutsk. 2003. P.28-30.

4. Anderson, T. H., & Domsch, K. H. (2010). Soil microbial biomass: The eco-physiological approach. Soil Biology and Biochemistry, 42(12), 2039-2043.

5. Bardgett, R. D., & van der Putten, W. H. (2014). Belowground biodiversity and ecosystem functioning. Nature, 515(7528), 505-511.

6. Griffiths, B. S., & Philippot, L. (2013). Insights into the resistance and resilience of the soil microbial community. FEMS Microbiology Reviews, 37(2), 112-129.

7. Insam, H., & Goberna, M. (2004). Use of Biolog for the Community Level Physiological Profiling (CLPP) of environmental samples. Molecular Microbial Ecology Manual, 1-2, 853-860.

8. Kandeler, E., & Gerber, H. (1988). Short-term assay of soil urease activity using colorimetric determination of ammonium. Biology and Fertility of Soils, 6(1), 68-72.

9. Kent, A. D., & Triplett, E. W. (2002). Microbial communities and their interactions in soil and rhizosphere ecosystems. Annual Review of Microbiology, 56, 211-236.

10. Rillig, M. C., & Mummey, D. L. (2006). Mycorrhizas and soil structure. New Phytologist, 171(1), 41-53.

11. Smith, P., et al. (2008). Climate change and soil microorganisms: A review of the impact of increased CO2 and temperature on microbial activity and community structure. Soil Biology and Biochemistry, 40 (3), 679-690.

Material received on 23.05.24

Зақымданбаған мәдениеттер екілік (стерильді) топырақ пен Байкал облысының саласындағы микробтық жасушалар таратпау динамикасы

Аңдатпа

Топырақ микроорганизмдерінің экологиясы мәселелері барған сайын практикалық маңызға ие болуда. Бұл мәселелер қоршаған ортаның химиялық және биологиялық ластану проблемасымен тығыз байланысты. Мұнда топырақ фитопатогендерінің, сондай-ақ олардың өмір сүруін тежейтін антагонистік микрофлораның экологиясын зерттеу маңызды мәселелер болып табылады. Белгілі бір аймақтағы топырақ микроорганизмдерінің экологиясын зерттеуде микробоценоздардың қалыптасу жағдайлары, микробтық ценоздың құрылымы, Бурятияның жекелеген аудандарындағы топырақтың ферментативтік белсенділігі ашылатын биологиялық қасиеттерін бағалаудың функционалдық сипаттамасы маңызды рөл атқарады.

Түйінді сөздер: *топырақ микроорганизмдері, топырақ патогендері, антагонистік микрофлора, Байкал аймағы, бинарлы дақылдар*

Материал баспаға 23.05.24 түсті

Динамика численности роста микробных клеток бинарных культур в стерильной почве и в полевых условиях Байкальского региона

Аннотация

Все большее практическое значение приобретают вопросы экологии почвенных микроорганизмов. С ними тесно связана проблема химического и биологического загрязнения окружающей среды. Важным вопросом здесь становится изучение экологии как самих почвенных фитопатогенов, так и антагонистической микрофлоры, подавляющей их жизнедеятельность. Немаловажное значение имеет место в изучении вопросов экологии почвенных микроорганизмов конкретного региона функциональная характеристика оценки биологических свойств, в которых раскрываются условия формирования микробоценозов, структура микробного ценоза, ферментативная активность почв отдельных районов Бурятии.

Ключевые слова: *почвенные микроорганизмы, почвенные патогены, антагонистическая микрофлора, Байкальский регион, бинарные культуры*

Материал поступил в редакцию 23.05.2024

Authors' contribution. The contribution is distributed as follows:

Korsunova Ts.D.-Ts. – conducting an experiment and collecting information, analyzing the results. Corresponding author. Compliance with all required publication deadlines, correct completion of *documentation*, filling out information about all authors of the work, preparation of research.

Haydapova R.B. – conducting an experiment and collecting information, analyzing the results, their interpretation, work on selecting literature and introductions.

Valova E.E. – management of the article, formulation of the idea and goals of the study, monitoring the conduct of research activities, compliance with ethical standards of the publication process, formation of the design concept.

Disclosure statement. The authors declare that there are no conflicts of interest to disclose in this article.