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Restriction Analysis of DNA Cyanophages and Plasmids of Cyanobacteria

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Annotation

The given work is devoted to an actual question of modern microbiology-comparative study of cyanophages and characteristics of their interaction with host cells.

Modern microbiologic, physiologic- bio chemical, molecular-biologic and electric-microscopic investigational methods were used in the work and original data possessing fundamental and attached significance were gained.

The most important contribution to modern microbiology is the detection of two new types of cyanophages (N-5TиS-9T), lyzing checkered filamentous and unicellular cultures of cyanobacteria, representatives of Nostoc and Synechococcus family. These cyanophages differ from wel-known cyanophages by their characteristics and have wide distribution in the nature.

As a significant contribution in the sphere of cyanophages we can consider the determination of morphology of virion sizes, determination of the types of nucleic acids, molecular mass, content of GC-pair.

Sensitivity and resistance of virions to some physical-chemical factors were studied, their specificity and parameters of lytic cycle of cyanophages were determined.

Key words: Cyanobacteria, cyanophages, DNA, RNA, ferments of endonucleases, nucleic acids,

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plasmids.

All cyanophages described so far (more than 50 strains) consist of one type of nucleic acid. The genome of all cyanophages has double-stranded DNA, but differs in nucleotide composition and molecular weight [1,2], according to the molecular weight of the genome, all the studied cyanophages are divided into three groups: 1) Cyanophages LPP-1, LPP-2, LPP-3A, A- 2, 5-1, S-2L, N-2, N-3T, NP-1T and NPC-1T (with a molecular weight of DNA up to 30 Maltons); 2) Cyanophages N-1 and others (41-45 Malton); 3) Cyanophages SM-1 and AS-1M (with a molecular weight of DNA of which 52–62 Maltons, respectively). In this regard, it was interesting to find out some features of the DNA of the studied cyanophages N-5T and S-9T,[3].

Nucleic acid preparations isolated from purified cyanophagevirions (Fig. 1) N-5T and S-9T have absorption spectra typical of DNA with a maximum at 259 nm and a minimum at 232 nm. The ratio of their optical density at 260 nm to optical density at 280 nm was not lower than 1.76, and the ratio 260/230 was not lower than 1.6.,[4].

Identification of the type of nucleic acids of the cyano-phage under study was carried out both by color reactions and by sensitivity to enzymatic and alkaline hydrolysis. After incubation with 0.75 N NaOH for 18 hours at 37 ° C and after incubation with RNAase, the nucleic acid remained in acid-soluble form. The DNA preparation is hydrolyzed by DNAase and gives a positive reaction with diphenylamine. These data and the results of subsequent experiments on restriction enzyme analysis indicate that the nucleic acids of the studied cyanophages N-5T and S-9T belong to the deoxyribose type,[5].

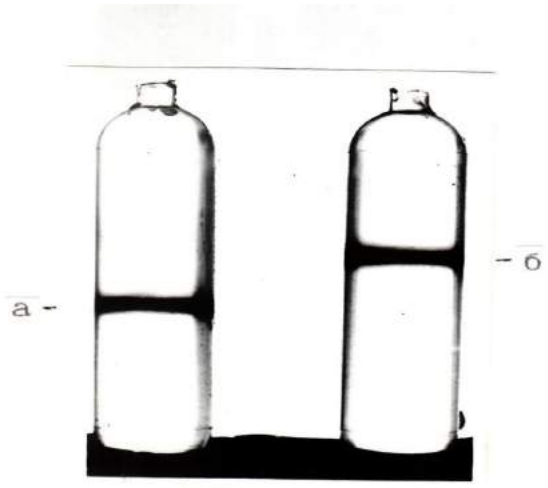

Fig. 1. Particle distribution of cyanophages in a stepwise gradient of sucrose density: a - N - 5T, b - S -9T

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The secondary structure of DNA was investigated by thermal denaturation in solutions of various ionic strengths -1 x SSC and 0.1 x SSC. Decreasing the ionic strength of the solvent by one order of magnitude lowers the melting temperature of DNA of the studied cyanophages by 16.9 - 14.9, practically without affecting other parameters of thermal denaturation. The content of HC pairs in the preparations, determined by the melting curves in 0.1 x SSC buffers, is almost completely identical for each of the studied DNA and is equal to 65.42% for the cyanophage DNA N-5T, and for DNA S-9T - 66 , 84%, [6]. Equilibrium centrifugation in the concentration gradient of CsCI of the native cyanophage DNA made it possible to obtain information on both its primary and secondary structure (Fig. 2).

DNA molecules of cyanophages N-5T and S-9T are localized by individual peaks in areas with a density of 1.730 g / cm3 and 1.731 g / cm3, respectively (Table 1). The percentage of pairs of -HC, calculated by floating density and T.pl., is quite good for the DNA of both cyanophages, which is typical for double-stranded DNA with a standard set of nitrogen bases,[7,8].

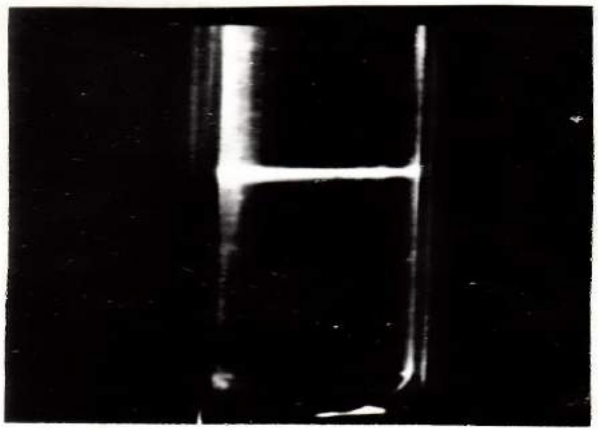

Fig. 2. The distribution of DNA cyanophage N-5T in the density gradient of Hoechst - CsCI

Table. 1. Physicochemical parameters of DNA of cyanophages and cyanobacteria

Parameters	Cyanophages		Cyanobacteria	
	S-9T	N-5T	S. elongates	N. linckia
Mp DNA (1xSSC), C	94.74	95.12	87.42	86.28
Mp DNA (0,1xSSC), C	77.84	80.22	72.52	71.36
G + C (determined by Tm)	66,84	65,42	44,19	41,41

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Floating density of DNA CsCI g / cm -3	1,731	1,730	1,705	1,703
G + C (determined by floating density% in CsCI)	72.44	71.42	44.90	43.87
Molecular mass (kbp)	48,8	44,3	-	-

Electron microscopic nucleotide analysis showed that the DNA molecules of cyanophages N-5T and S-9T isolated from virions are linear, have open ends (Fig. 3.4). The number of DNA pairs of cyanophages N-5T and S-9T is 44.3 and 48.8 kb, respectively (Table 1).

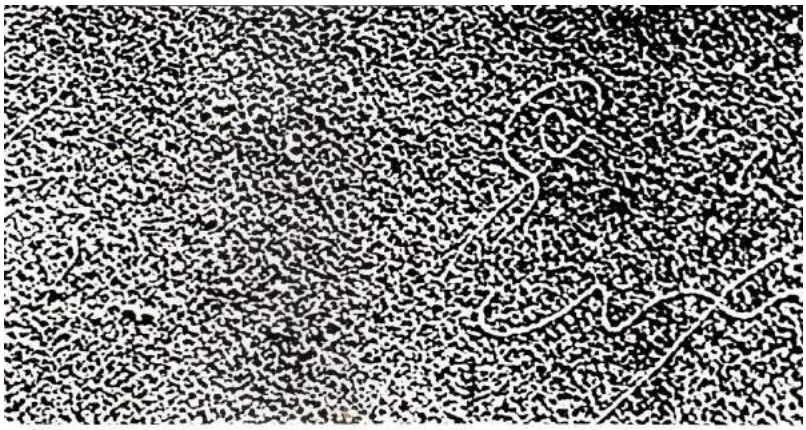

Figure 3. Electron micrograph of DNA cyanophage N-5T Uranyl acetate x 50 LLC

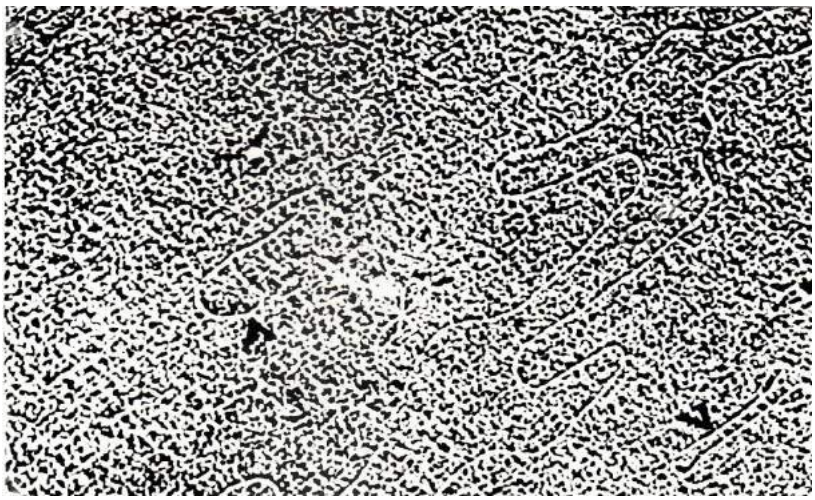

Figure 4 Electron micrograph of cyanophage S-9T DNA Uranyl acetate x 50 LLC

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We also characterized the gene of cyanobacteria - the host of the studied cyanophages (Fig. 5,). In the cells of the cyanobacteria N. linckia - N59 and S. elongatus - N58, in addition to DNA and RNA, low molecular weight plasmid-like DNAs are found (Fig. 5, lanes 2,3) spectrophotometry, a positive reaction with diphenylamine, sensitivity to DNAase and the absence of the effect of hydrolysis with alkali and RNAse, we identified cyanobacteria nucleic acids as DNA,[9].

The distribution in the linear density gradient of CsCI DNA of the studied species of cyanobacteria is presented in Fig. 9.

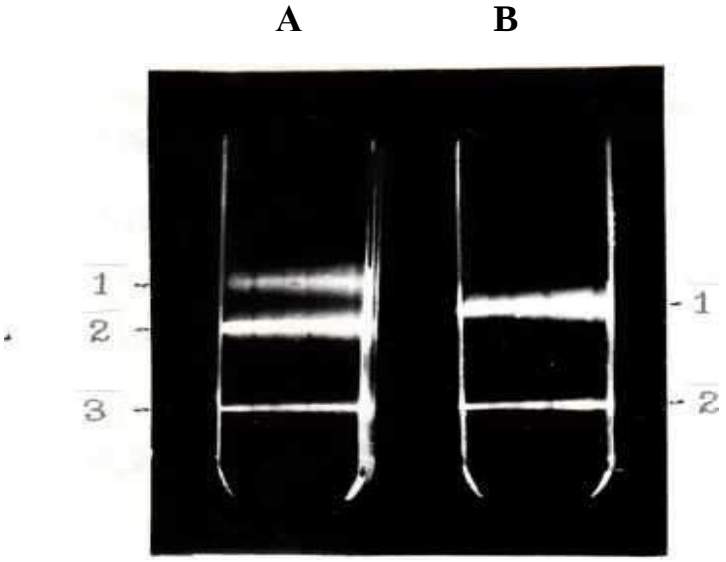

Fig. 5. DNA distribution of cyanobacteria S.elongatus-N58 (a) and N.linckia-N59 (b) in the density gradient of Hoechst-CsCl

S.elongatus-N58 **N.linckia –N 59**

A - 1-minor component **B - 1-native DNA**

A - 2-native DNA **B - 2-DNA plasmids**

A - 3-DNA plasmids

Fig. 6. Electron micrograph of DNĶ cyanobacteria Nostoc linckia- N59 x

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50,000

Fig. 7. Electron micrograph of DNA cyanobacteria Synechococcus elongatus - N 58 x 50 000

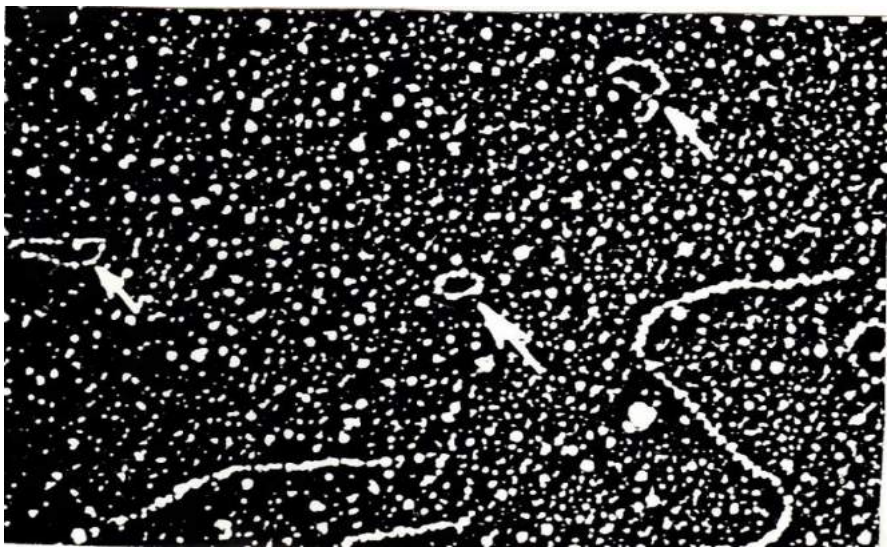

Fig. 8. Electron micrograph of plasmid DNA of cyanobacteria Nostoc linckia- N59 x 50,000

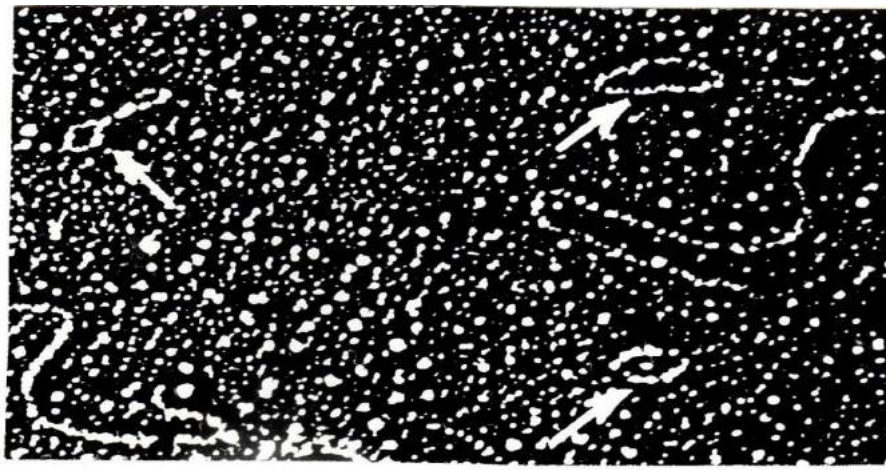

Fig. 9. Electron micrograph of plasmid DNA of cyanobacteria Synechococcus elongatus - N 58 x 50 000

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As seen from Fig. 6.7. The DNA of the bacteria species studied by us in a linear density gradient of CsCl shows a multimodal distribution pattern. An electrophoretic analysis of the molecular masses of the individual DNA fractions showed that the lower DNA fractions from Fig. 5, (AZ and B2) correspond to plasmid cyanobacteria. UN.linckia-59 DNA fractions \ B 1 according to electrophoretic mobility and floating density corresponds to chromosomal DNA.

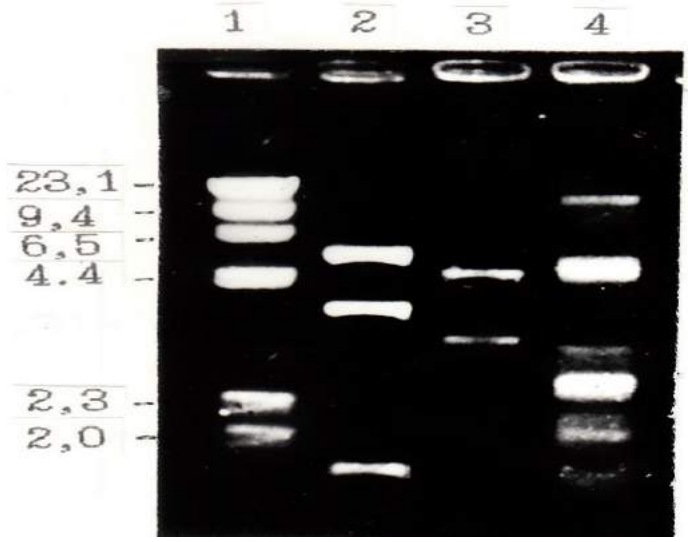

fig. 10 Electrophoretic separation of cyanobacteria plasmids
1. Hind III - DNA fragments of phage λ
2. Native plasmid S.elongatus-N58
3. Native plasmid N.linckia N59
4. EcoRI - DNA fragments of phage λ

In the composition of S. elongatus-58 DNA, in addition to the chromosomal iplasmid DNA, minor DNA components (A-1) were found with a floating density of 1.701 g / cm_3. Analogous results were obtained by series of other authors in the study of DNA of other species of bacteria [10].

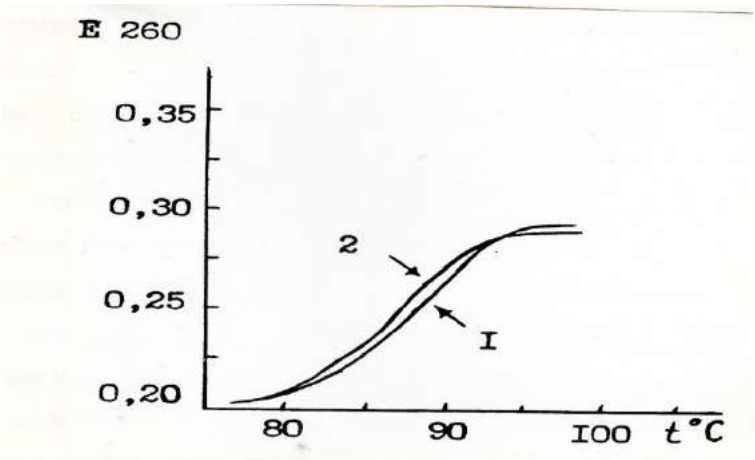

Fig. 11. Thermal melting curves of cyanophage S 9T (1) and N-5T (2) DNA into a 1.0 x SSC buffer

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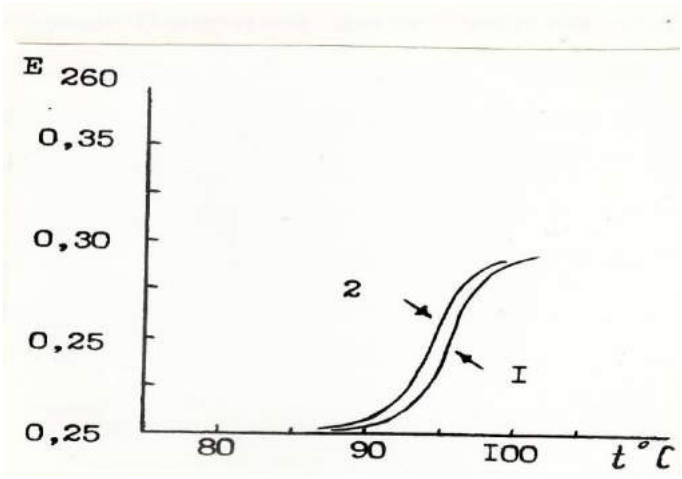

Fig. 12. Thermal melting curves of the DNA of the cyanobacteria S.elongatus (1) and N.linkia (2) in buffer l, 0xSSC

The thermal melting curves of N.linckia-59 DNA of the studied cyanophages and ichyanobacteria are shown in Fig. 11 and .12, from which it is seen that all the preparations of DNA of cyanophages and cyanobacteria studied by the DNA have only one point of loss.

According to the nucleotide composition, the DNA of the studied cyanophages belong to the pronounced GC type, and the DNA of the cyanobacteria of the species studied by us is of the AT type. The number of HC pairs in the DNA of N.linckia-59 culture is 41.41% (calculated by the melting point), and the calculated floating density is 43.87%. GC-steam S.elongatus -58 - 44.90%, respectively.

Over the past few years, ferments of restriction endonucleases are intensively used for selective fragmentation of molecules of DNA in studying different genomes and construction of recombinant molecules of DNA in Genetic engineering. This kind of specific endonucleases(enzymes) are able to recognize definite nucleotide sequences (sites) on double-stranded DNA and to cut phosphoriethide bonds of DNA within these consequences (or in some distances from them). It results in split of DNA molecules into fragments, number and size that are characteristic to each DNA(genome) and restriction enzyme, [11, 12].

Substrate specificity of some restriction enzymes is showed in N.I.Matvienko's review (1979)[13]. In that review a number of bacteria were listed from which restriction enzymes, names of ferments, enzyme site recognized by ferments and the number of such sites in standard DNA phagal were distinguished. Besides, internucleotide bonds torn by ferments are showed by arrow symbols (table 2). In the given table enzymes of endonucleases used by us in studying DNA cyanophages and plasmids of cyanobacteria and their substrate specifity are listed. The experiment results i.e. hydrolysis of DNA cyanophages and plasmid of cyanobacteria with the above-mentioned restriction enzymes are presented in the table 2, and in figures 13, 14 and 15.

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Restriction endonucleases used in studying DNA cyanophages, plasmids of cyanobacteria and their subtracted specifity

Table 2.

Restriction enzyme	Ferment source (denomination of microorganisms)	Recognizable consequences	Number of restriction sites of DNA phage lambda
Bam HI	Bacillus amyloliguefaciens H	G' GATCC	5
Bsp RI	Bacillus species	GG' CC	10
Hind III	Haemophilusinfluenzae RD	A'AGCTT	6
Msp I	Moraxella species	C'CGG	50
EcoR I	Escherichia coli RY 13	C'AATTC	5
EcoR V	Escherichia coli	GAFATC	21
Pst I	Providenciastuartii	CTGGA'G	18
Sau3A	Staphylococcus aureus3A	'GATC	50
Sma I	Serratiamarcesgens	CCC'GGG	3
Sal I	Streptomyces albus	G'TCGAC	2

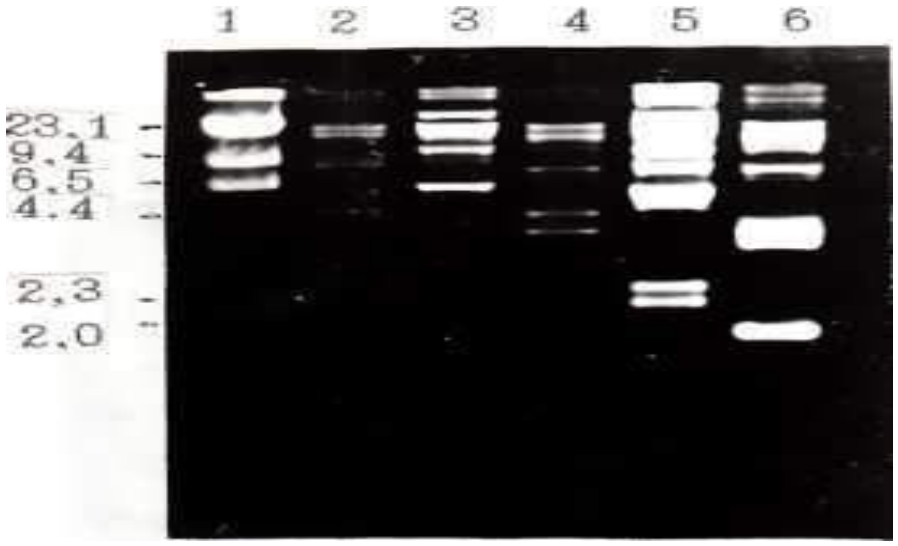

Fig.13. Electrophoretic division of fragments that are formed under the influence of different restriction enzymes on DNA cyanophage S-9T.

1. HindIII-fragments of DNA phage lambda , 2.Bam HI, 3. Hind III, 4.Msp I, 5.Pst I, 6.Sau3A- fragments of DNA-cyanophages S-9T

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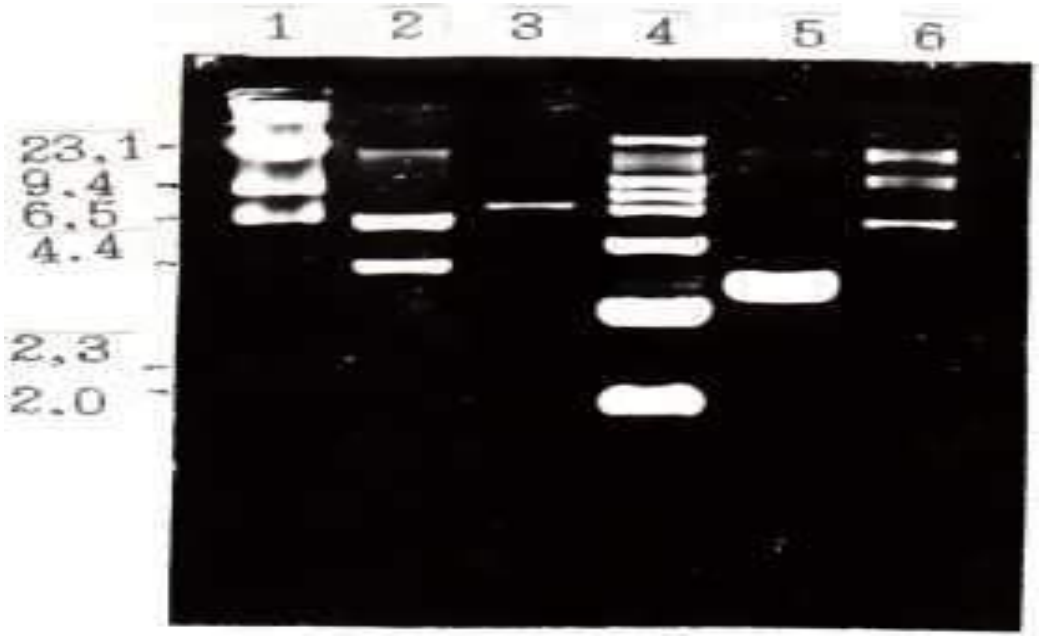

Fig. 14. Electrophoretic division of fragments formed under the influence of different restriction enzymes on DNA cyanophages N-5T. HindIII- fragments of DNA phage lambda, 2. Bam HI, 3. Hind III, 4. Msp I, 5. Pst I, 6. Sau 3A- fragments of DNA of cyanophages N-5T

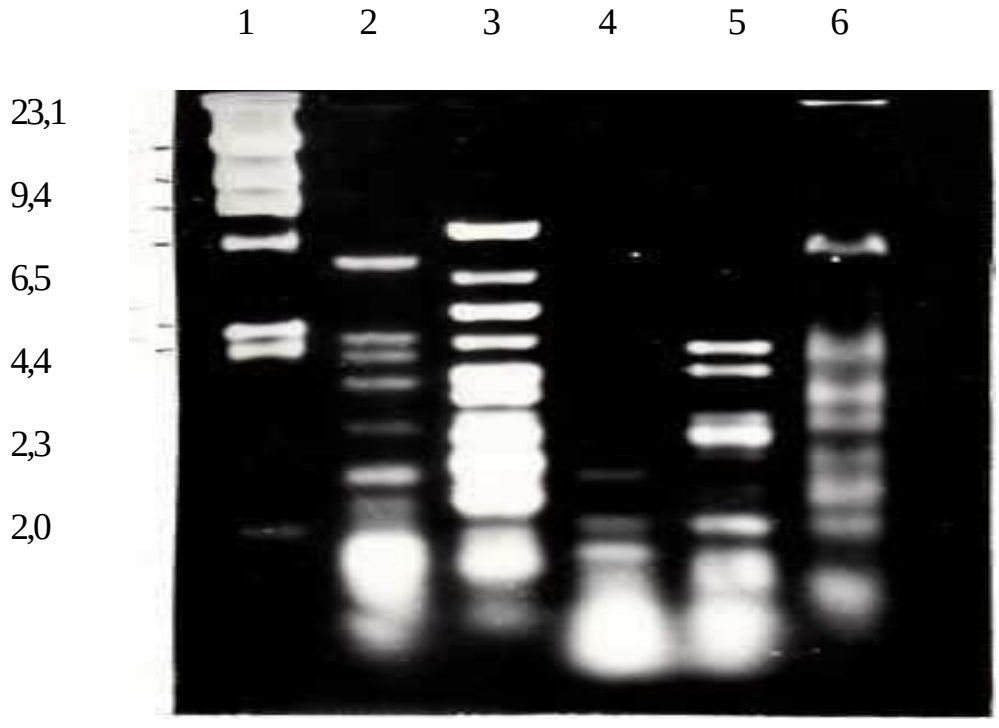

Fig.15. electrophoretic division of fragments formed under the influence of restriction enzyme on plasmids of cyanobacteia N.linkia-59 and S. elongatus-58. 1.HindIII – fragments of DNA phage ʎ, 2.BamHI – fragments pNl-59. 3.BamHI–fragments pSE-58, 4. HindIII–fragments pNl-59, 5.HindIII–fragments pSE 58 . 6.EcoRY–fragments of

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DNA phage ʎ.

As it is seen from Table 2 and presented figures, the ferments BamHIHindIII,MspI, PstI and Sau 3AI hydrolyzes DNA cyanophage S-9T to 6, 7, 7, 9, 8 fragments, and DNA cyanophage N-5T to 4, 2, 9, 2, 7 fragments, respectively, [14] Fragments BamHI and Hind III split plasmid DNA cyanobacteria N.Iinckia into 7 and 6 fragments, and DNA plasmid cyanobacteria into 11 and 8 fragments, respectively (fig. 15). Molecular mass of fragments were calculated using Hind III – fragments of DNA phage labda as a standard. Fragment sizes were expressed in units of thousand base pairs k.b.p. (Table 3.1.7.3). By the suggestion given in the literature (Nathans D, Smith H.O., 1975) calculations begin from the largest fragment,[15], denoted by capital letter - A of Latin alphabet and continuing their list, use the following letters of the alphabet or expressed by numbers. We denoted the fragments of DNA of experimented cyanophages and plasmids by numbers in or work, [16, 17, 18, 19, 20]. Comparison of the settlement of fragments in electrophoregrams p авнение i.e. molecular mass of fragments, points out that the figure of site division by genomes of investigated cyanophages and plasmids are not the same even in those cases when restriction enzyme brought in equal total number of fragments in DNA and plasmids.

Table 3.

The number of sites of restriction enzymes under the influence of specific endonucleases on cyanophages and cyanobacteria.

Denominatio n of cyanophages and plasmid	Number of sites of restriction enzymes				
	Bam HI	Hind III	Msp I	Pst I	Sau3A
S-9 T	6	7	7	9	8
N-5 T	4	2	9	2	7
pN. 1-59	7	6	-	-	-
pS.E-58	11	8	-	-	-

Table 4.

Molecular mass of fragments of single hydrolysis of DNA cyanophages and plasmid of cyanobacteria of restriction enzymes BamHI, k.b.p.

Sequential N- fragments	cyanophages		Plasmids	
	S-9T	N-5T	pNl-59	pSE-58
1.	23.0	22.8	2.5	3.6

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2.	9.2	11.3	1.4	1.7
3.	7.3	5.9	1.1	1.3
4.	4.3	4.3	0.6	0.7
5.	3.8		0.3	0.6
6.	1.2			0.5
7.				0.3
8.				
	48.8 k.b.p.	44.3 k.b.p.	5.9 k.b.p.	8.7 k.b.p.

The data of the table 3 points that the correspondence of sizes of DNA fragments of different cyanophages and plasmids are hardly present. The figure of division of sites of restriction enzymes into DNA molecules in each cyanophage and plasmids is individual; it can serve for identification of cyanophages and classification of plasmids, [16].

The results of works on restriction analysis of nuclein acids, of extracted and studied cyanophages and plasmids of cyanobacteria are presented in the table 4 and figures 13, 14 and 15.These data show the dissimilarity both in electrophoretic mobility of fragments in agarose gel and in the number of the same fragments.

The received data can be put in the basis of taxonomy of cyanophages and recommended as models for molecular-biological investigations and their possible utilization as vectors in the works of Genetic engineering.

Therefore, restriction analysis is a specific method that can be applied for the characteristics and identification of genomes and cyanophages. Accumulation of the materials of such analysis on the structure of DNA enables to determine the similarity and difference of different cyanophages of DNA and plasmids and cyanobacteria that can serve as the basis for further works on molecular biology of cyanophages, cyanobacteria and their plasmids.

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// Тез.докл. Всесоюзной конференции "Микробиологические и биотехнологические основы интенсификации растениеводства и кормопроизводства ". Секция П. Вирусы растений, живодных и микроорганизмов. - Алма-Ата. - 1990 д. - С. 77.

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