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THE STRUCTURE OF BACTERIAL CELL CYCLE AND AGE STRUCTURE OF BACTERIAL POPULATIONS

Study of synchronous and asynchronous cultures of Bacillus megaterium, Bacillus thuringiensis and Bacillus licheniformis has shown that the duration of chromosomal DNA replication (period C) is proportional to the generation time, and time between two cycles of the DNA replication (known as period I). The duration of period C is nearly constant and makes up from 0.5 to 1.0 hour at the variations of the generation time from 1.5 to 2.75 hours.

The duration of period B (the time between the termination of the cell division and initiation of DNA replication), and period D (the time between the termination of DNA replication and initiation of cell division) were experimentally revealed as stochastic parameters.

The theoretical model of the bacterial cell cycle and the age structure of bacterial population was suggested. The main points of this theory are that periods C and I may be stochastically disposed in the division cycle of individual cells and a sum of duration of C- and I-periods is equal to generation time. The data calculated from the theoretical model were confirmed by the experimental data of flow cytofluorometrical analysis of the age structure of synchronous and asynchronous cultures of the bacilli.

Key w o r d s : bacteria, cell cycle, population, age structure, flow cytofluorometry

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Microbial populations are heterogenous, systems. Heterogeneity is determined by the existence of temporal (historical), physical, chemical and biological (genetic) relations between the elements of microbial systems [15]. One type of the heterogeneity of microbial populations is the age heterogeneity or distribution of the cells in various phases of the cell cycle [15, 16].

An analysis of the age heterogeneity of bacterial population's is complicated by the absence of synchronization between the division cycle of the bacterial cell and the replication cycle of the chromosomal DNA [2. 6, 10]. Therefore the position of the prokaryotic cell in some point of the division cycle cannot be used for characterization of the cell age.

According to the Helmstetter and Cooper model of the time of the replication fork movement from ori C to the point of termination of the chromosomal DNA replication (period C) is a constant value. Period D is the time between the termination of the chromosomal DNA replication and cell division. The duration of period D is a constant value. Therefore the DNA synthesis in the cell can be observed on some replication forks, and the number of replication forks depends on generation time [7]. The interrelations of the periods of the cell division cycle and the DMA replication cycle may be named as the structure of the cell cycle.

Experimental studies of the structure of the bacterial cell cycle demonstrated the dependence of the duration of period C on the generation time [20, 24] and the great variety of the duration of period D [4, 13]. Periods B and I [12] have been also distinguished in the structure of the bacterial cell cycle. Period B is the time between cell division and initiation of DNA replication, and period I is the time between two DNA replication cycles (or between two initiations of synthesis of the chromosomal DNA).

The periods B, C and D were proposed to be the analogues of phases G1,S and M of mitotic cycle and the structure of the bacterial cell cycle as analogue of the eukaryotic cell cycle [8, 9]. We consider that it is possible only under the existence of synchronization between cell division and DNA replication cycles but that is not a specificity of prokaryotic cel! i[2, 6, 10].

The structure of the prokaryotic cell cycle is not enough studied in comparison with the data about the structure of eucaryotic cell cycle. The generally accepted model of the procaryotic cell cycle is not yet composed. This is due to insufficient experimental study of the bacterial cell cycle and age structure of bacterial populations. The age structure of bacterial population is the distibution of cells by their DNA content. The age structure of bacterial population depends on the structure of cell cycle. Therefore the studies of these structures must be carried out in complex.

Our work is aimed to study the structure of the cell cycle and age structure of bacterial population.

Materials and methods. The objects of our study were as follows: ***Bacillus megaterium, Bacillus thuringiensis*** HI4, ***Bacillus licheniformis*** from the Collection of Bacilli of the Institute of Microbiology and Virology, National Academy of Sciences, Ukraine. Bacteria were cultivated in ANK.UM-2 fermenter (Special Bureau of Biological Equipment, Puschino, USSR). The working volume of fermenter was 1 at a temperature of 30 °C, medium pH 7.0. Bacilli were cultivated on the media of the following composition (gxl~'): glucose, 10.0; Na_2P04, 3.0; K_2HPO_4, 3.0; NaCl. 2.0; $MgS_{04}*_7IT_{20}$ — 0.1; peptone — 0.5 (for ***B. thuringiensis***); sucrose — «8.5; KH_2PO_4 — 1.5; K_2HPO_4 — 3.5; NaCl—10; NHUC1—1.0; KC1—35*10-\ Na_2S0_4 —0.3; $MgCl_2*5H_20$ — 4.26 (for ***B. licheniformis)]*** ethanol—0.5; NH_4N0_3 —2.0; $MgCl_2$ —0.1; KH_2P0_4 —3.0; K_2HPO_4 — 7.0 (for ***B. megaterium).***

The flotation was used for synchronization of cultures [17, 26, 27]. Synchronization of bacterial proliferation was provided by separation from the population of the quickly flotating cells (5—10% of the total biomass). This fraction was used as inoculum. For its isolation 1 liter of microbial suspension was passed through the laboratory flotator for 10 min. The flotator was the cylinder with the volume of 100 ml. The air (0,2 1/min) was dispersed in the flotator resulting in foam formation. The exhausted foam was the fraction of the quickly flotating cells. The index of synchronization of the batch cultures was calculated by (lie changes of cell concentration. Our method of synchronization ensures the index of synchronization from 0.55 to 0.70 [26].

"lo determine the DNA content in the cells they were dyed by ethidium bromide (Serva) solution with prehydrolysis of RNA and protein by the known method [14]. The histograms of the distribution of 20 thousand of cells by DNA content (intensity of red fluorescense) or by cell size (intensity of forward scattered light) were obtained using FACStarPius flow cytofluorometer (Becton Dickinson). The fluorescence of cells was excitxd by argon laser with wave length of 488 nm. To measure the red fluorescence of propidium iodide-DNA complex, a 590 nm barrier filler was used in front of the red channel of photomultiplier. Forward light scatter was measured at angles between 1.5° and 19 to the illuminating beam. The light scatter is correlated approximately with cell volume [!4|. To determine the protein content in the cells they were dyed with fluoresceine isothiocyanate (green fluorescence) without prehydrolysis of RNA and protein [14].

Results of investigations and discussion. Synchronous cultivation of Bacillus megaterium. The changes in the concentration of cells and quantity of DNA in the samples from three independent synchronous cultures of ***'Bacillus megaterium*** are shown in Fig. 1. The generation time varied from 1.75 to 2.5 hours. These variations can be caused by differences in duration of the flotative separation of population and period from

separation to inoculation. Data on the structure of the cell cycle are shown in Table 1. The duration of periods B and D varied as stochastic valu-'s, but duration of period C linearly correlated with the generation time. The duration of period 1 is nearly constant value and makes up from 1.00 to 1.25 hours.

Synchronous cultivation of Bacillus thuringiensis. The changes **in** the concentrations of cells and quantity of DNA in the samples from four independent synchronous cultures of *Bacillus thuringiensis* are shown in Fig. 2. The generation time, as in experiments with *B. megaterium,* varied

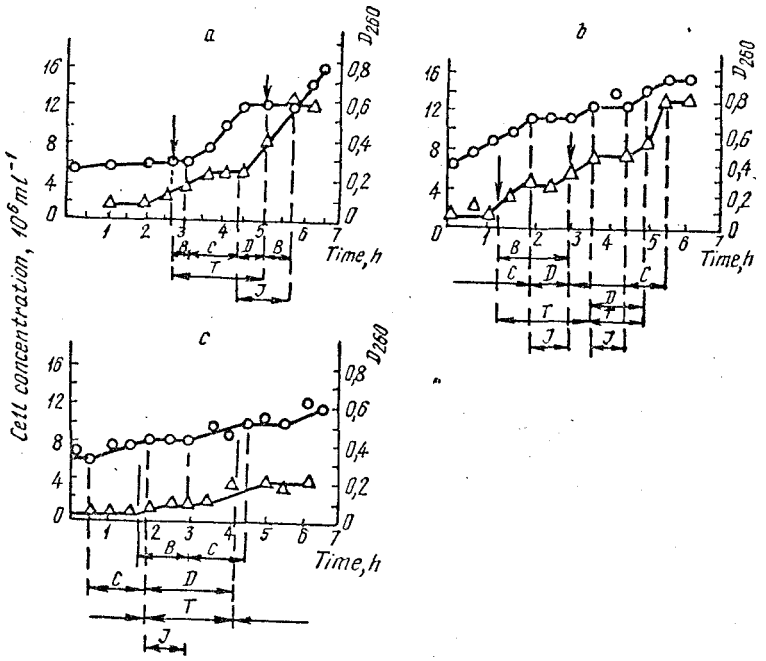

Fig. 1. The changes of DNA content in sample (marked by circles) and concentration of cells (marked by triangles) during three experiments *(a—c)* on synchronous cultivation of *Bacillus megaterium.* $D_{=60}$ — the content of DNA in sample (as optical density units). The durations of periods B, C, I and generation time *(T)* are indicated at the bottom.

from 2.0 to 3.0 hours. Data on the structure of the cell cycle are shown in [Vole ***2.*** There is no strict succession of periods B, C, D, in synchronous cultures of ***B. thuringiensis.*** The duration of periods B and D varied as stochastic values but durations of periods C and I were close to constant values'. The sum of the durations of these periods was equal to generation time.

Cytofluorometric analysis of the flotative fraction of bacterial population. Tnt cell distributions of the quickly flotating fraction of ***B. tru-ritig.ensis*** population and the cells of initial population **by** the DNA content ^intensity of red fluorescence) were nearly identical (Fig. 3, ***a).*** The distributions of the cells of quickly flotating fraction of population and those of intact population–by size (intensity of side scattered light) and

T a b l e 1. The duration of the periods of *B. megaterium* cell cycle

Generation time (T), h	The duration of the periods, h			
	B	C	D	1
1.75	1.75	0.50	1.00	1.00
2.00	0.00	1.00	1.50	1.00
2.50	1.25	1.50	2.25	1.00
2.50	0.25	1.50	0.75	1.25

protein content (intensity of green fluorescence) were distinguished between ihen:selves (Fig. 3 6, c). The quickly flotating cells are smaller and have less protein content than those of intact population. Similar results were obtained in ehperiments with 2B. licheniformis (Fig. 4).

These data witness that the quickly flotating cells are newly-divided cells. Therefore the use of the quickly flotating cells as inoculum guaranteed JHe synchronisation of the bacilli division.

Cytofluorometric analysis of the synchronous cultures of bacilli. The curves of the dist-ibutions of the cells by DNA content for synchronous cultu'o ol *B. thuritigiensis* have various forms (Fig. 5). The curve with

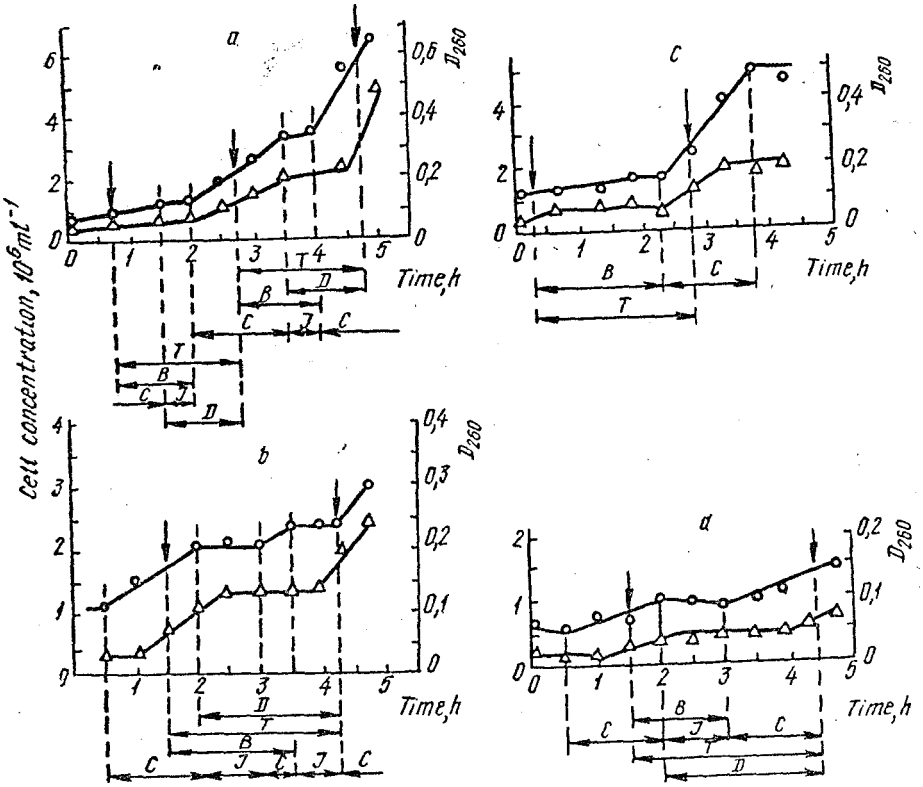

Fig. 2. The changes of DNA content in sample (marked as circles) and concentration of cells (marked as triangles) in four experiments *(a—d)* on synchronous cultivation of *Bacillus thuritigiensis*. Other marks as in Fig. 1.

one maximum (Fig. 5, ***a, b)*** changed into the curve with one maximum and plateau (Fig. 5, c), and the curve with two maxima (Fig. 5, ***d).*** Then 'his curve changed into the curve with one maximum (Fig. 5, ***e—h)*** and rl.is maximum can be as narrow peak or a wide one (Fig. 5, ***i).***

There were two forms of cells distributions by DNA content during syncivonuus cultivation of ***B. licheniformis.*** The first form of the cu-vc has one i 'aximum (Fig. 6, ***a—d).*** The second form of the curve, has one maximum and plateau (Fig. 6, ***e — i).*** The same forms of the distribution

T a b l e 2. The duration of the periods of *B. thuringiensis* cell cycle

Generation time (T). h	The duration ol the periods, h			
	B	C	D	I
2.00	1.25	1.50	1.25	0.50
2.00	1.25	—	1.25	0.50
2.50	2.00	1.50	0.00	—
2.75	2.00	1.50	2.25	1.00
3.00	1.50	1.50	2.50	1.00

N o t e : «—» — no data.

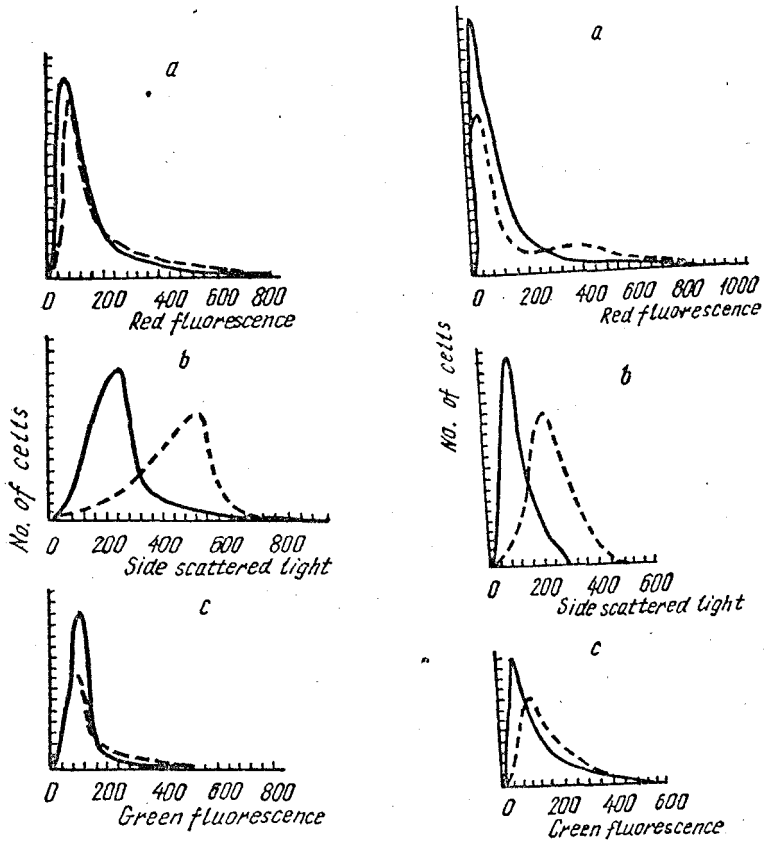

Fig. 3. The distributions of the *Bacillus thuringiensis* cells by DNA content *(a)*, size *(b)* and protein content *(c)*. Solid line corresponds to the quickly flotating cells and the dotted line corresponds to the cells of intact population.

Fig. 4. The distributions of the *Bacillus licheniformis* cells by DNA content *(a)*, size *(b)* and protein content *(c)*. Other marks as in Fig. 3.

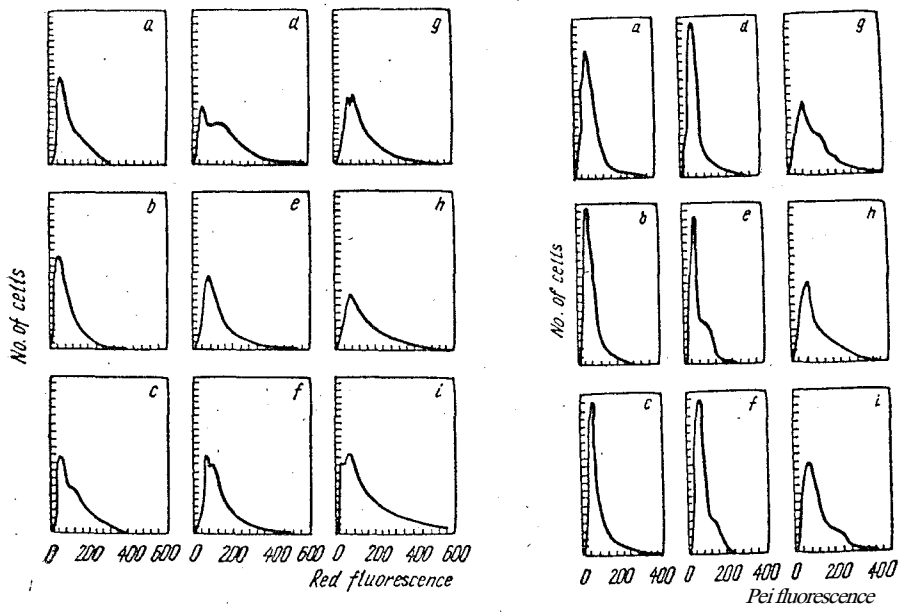

Fig. 5. The distributions of *Bacillus thuringiensis* cells by DNA content at synchronous cultivations, *a* — cells of inoculum; from *b* to *i* — cells 30, 60, 90, 120, 150, 180 and 210 minutes after inoculation.

Fig. 6. The distribution of *Bacillus tichenijormis* cells by DNA content at synchronous cultivation. Other marks as in Fig. 5.

of the cells by DNA content were observed during synchronous cultivation of *B. megaterium.* There were the curves with one maximum (Fig. 7,. *a, c,* n and one maximum and plateau (Fig. 7, *b, d—h)..*

A theoretical model of the age structure of bacterial population. The exper.mef ts with synchronous cultivations of bacilli have shown the variety r-f the distributions of the cells by DNA content, i. e. the variety of the'forms of age structure of the bacterial populations. We have proposed the theoretical model of the bacterial cell cycle for understanding this variety. The main point of this theory is the distinguishing of four classes of cells in bacterial populations. There are B-cells. with one chromosomal DNA set, Cl-cells with one to two chromosomal DNA sets, D-cells with two chromosomal DNA sets, C2-cells with the DNA content between two or four chromosomal DNA sets. Another points are that periods C and I may be stochastically disposed in the division cycle of individual cells, and' a sum of duration of C- and I-periods is equal to the" generation time.

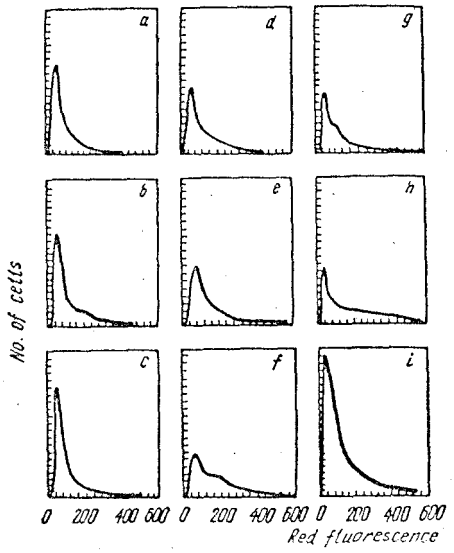

Fig. 7. The distributions of *Bacillus megaterium* cells by DNA content at synchronous cultivation. Other marks as in Fig. 5_

Fig. 8 illustrates the stochastic combinations of the dispositions of periods C and I in the division cycle of the individual bacterial cells at the various ratio of the duration of period C and generation time. The frequencies of B-, C1-, D- and C2-cells were calculated from this scheme. The calculation data are shown in Fig. 9.

Natural variability of the durations of the periods B, C, D and I were-also accounted in Fig. 9. Depending on the ratio of «time of C-period/generation time» the curves of the cell distributions by DNA content are the curves with two maxima (Fig. 9*, a, d),* curve with one maximum and plateau (Fig. 9, *b,* and curve with one maximum (Fig. 9, c). These theoretical types of age structure of the bacterial populations are displayed during the experimental analyses of the age structure of bacterial populations (Fig. 5—7).

The results of the studies of bacterial synchronous cultures confirm the absence of the coordination between the division cycle of bacterial cells and the cycle of the chromosomal DNA replication. The absence of coordination between these cycles is one of the fundamental distinctions-of the procaryotic and eucaryotic cells [6]. The absence of the coordination between the division cycle and the cycle of the chromosomal DNA replication explains the variability of the duration of periods B and D-revealed in the experiments with the synchronous cultivations of bacilli. The variability of the duration of the periods B and D was also observed by other authors [4, 5, 13, 18, 19]. Therefore it is expedient to exclude-the periods B and D from the analysis of the individual cell cycle. Meanwhile it is expedient to take into account the average duration of the periods B and D as the average parameters of the, procaryotic cell cycle.

According to our model of the bacterial cell cycle the duration of" the periods C and I are the most informative parameters of the cell cycle. Our experiments have shown that the duration of period I is close to constant. This constancy is known for *Escherichia coli* and shows that duration of period I is determined by the time necessary for formation of super-coiling and membrane-tied structure in the region of ori C for initiation; of DNA replication [11].

Thus the structure of the cycle of the chromosomal DNA replication. in bacterial cell is determined by the duration of periods C and I. The sum of these durations is equal to generation time. It is the regularity that connects the cell division cycle and DNA replication cycle of bacteria. The mechanism of this coupling is likely to be multiply determined [10].

The idea about the age structure of the bacterial population as the-ratio between B-, C1-, D- and C2-cells as well as the theoretical model.

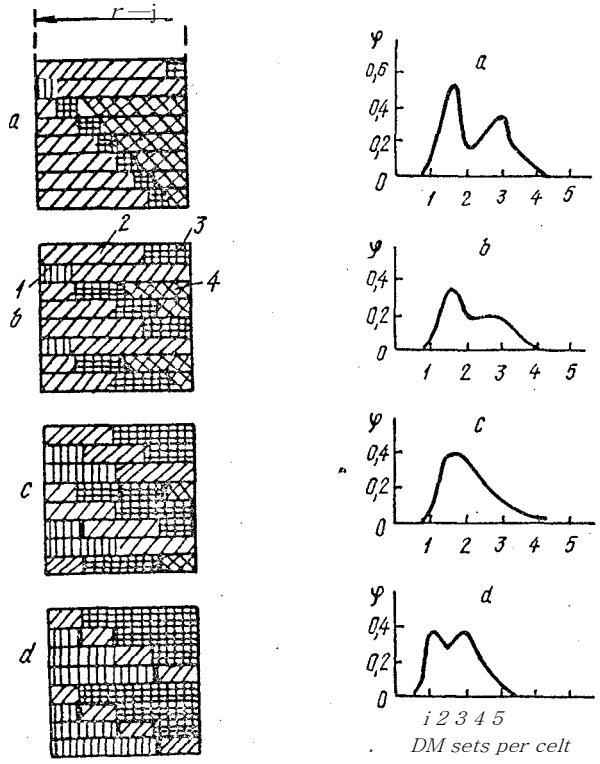

Fig. 8. The theoretical scheme of the possible combinations for the disposition of periods C and I in cell division cycle. The duration of period C makes up: 87,5 % of generation time *(a)*, 75% of generation time *(b)*; 50% of generation time *(c)*; 25% of" generation time *(d)*. *1*•—B-cells; *2* — C-cells; *3* — D-cells; *4* — C2-cells.

Fig. 9. The theoretical types of the distributions of the bacterial cells by DNA content (the number of chromosomal set). The duration of period C makes up: 87,5 % of generation time *(a)*; 75% of generation time (*b*); 50% of generation time (*c*); 25% of generation time *(d)*. Greek letter «phy» marks the frequency of some type of the cells in the bacterial population.

of the bacterial cell cycle permitted us to reveal these types of the cell' distributions by DNA content.

These theoretical types were experimentally revealed under the synchronous cultivations of bacilli and confirmed by the results of the other' authors. Thus the curves with two maxima were observed for *Escherichia coli* K-12 in the medium with chloramphenicol [3] and for *Rhizobium meliloti* [21, 22]. The curves with one maximum and plateau were revealed for *Escherichia coli* B/r K/l grown in a chemostat [25] and *Rhizobium meliloti* [21, 22]. The curves with one wide maximum were observed in the experiments with *Escherichia coli* K-12, *Rhizobium japonicum* [21] and *Escherichia coli* HB 101 [23]. The existence of all theoretical types-of age structure of bacterial populations in nature is the evidence for our theoretical model of bacterial cell cycle. On the basis of these theoretical types one can develop the quantitative method of analysis of the age structure of bacterial populations. Though there were some attempts [23, 25, 28] this method is not yet developed.

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СТРУКТУРА КЛІТИННОГО ЦИКЛУ БАКТЕРІЙ ТА ВІКОВА СТРУКТУРА БАКТЕРІАЛЬНИХ ПОПУЛЯЦІЙ

Р е з ю м е

Вивчення синхронних і асинхронних культур *Bacillus'tnegateriutn, Bacillus thuringiensis* та *Bacillus Ucheniformis* показало, що тривалість періоду реплікації хромосомної ДНК (С-період) пропорцій на часу генерації, а час між двома циклами реплікації ДНК (що зветься I-періодом) май же постій ний та становить 0,5—1,0 год при варіації часу генерації від 1,5 до 2,75 год.

Клітинний цикл (період між клітинними поділами) та цикл реплікації ДНК не синхронізовані між собою. Тому тривалість В-періоду (час між термінацією клітинного поділу та ініціацією реплікації ДНК), а також D-періоду (час між термінацією реплікації ДНК та ініціацією клітинного поділу) виявляються як стохастичні параметри в експериментах по синхронному культивуванню.

Запропоновані теоретичні моделі клітинного циклу бактерій та вікові структури бактеріальних популяцій . Дані, що випливають з теоретичних моделей , узгоджуються з експериментальними даними проточного цитофлуорометричного аналізу синхронних та асинхронних культур бацил.

К л ю ч о в і с л о в а : бактерії, клітинний цикл, популяція, вікова структура, проточна цитофлуорометрія

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СТРУКТУРА КЛЕТОЧНОГО ЦИКЛА БАКТЕРИИ И ВОЗРАСТНАЯ СТРУКТУРА БАКТЕРИАЛЬНЫХ ПОПУЛЯЦИЙ

Р е з ю м е

Исследование синхронных и асинхронных культур *Bacillus megaterium, Bacillus thuringiensis* и *Bacillus Ucheniformis* показало, что длительность периода репликации хромосомной ДНК (С-период) пропорциональна времени генерации, а время между двумя циклами репликации ДНК (называемое обычно I-периодом) примерно постоянно и составляет 0,5—1,0 ч при вариации времени генерации от 1,5 до 2,75 часа.

Клеточный цикл (период между клеточными делениями) и цикл репликации хромосомной ДНК не синхронизованы между собой . Поэтому продолжительность В-периода (время между терминацией клеточного деления и инициацией репликации ДНК.), а также D-периода (время между терминацией репликации ДНК и инициацией клеточного деления) проявляются как стохастические величины в экспериментах по синхронному культивированию.

Предложены теоретические модели клеточного цикла бактерий и возрастной структуры бактериальных популяций . Данные, вытекающие из теоретических моделей , согласуются с экспериментальными данными проточного цитофлуорометрического анализа синхронных и асинхронных культур бацилл.

К л ю ч е в ы е с л о в а : бактерии, клеточный цикл, популяция, возрастная структура, проточная цитофлуорометрия

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