Research Article

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Remote In Vivo Scanning of Gut Microflora Genomes Using Gravitational Mass Spectrometry

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ABSTRACT

The gravitational mass spectroscopy (GMS) method was used to remotely study the gravitational noise (GN) from the distant molecular energy order of atomic nuclei clusters (ANC) in the microflora of the gastrointestinal tract (GIT) of a 72-year-old probant. GN from ANC – the genomes of the most important bacteria were registered in the stomach, small intestine, umbilical cord, rectum, and also in urine. The energy characteristics of the genomes of the most important bacteria of the gastrointestinal tract were studied: E-coli, Bacteroides fragilis NCTC 9343, Prevotella melaninogenicus, Bacteroides oralis, Enterobacter sp., Clostridium perfringens, Staphylococcus aureus, as well as Bacteriophage T4 (Escherichia virus) and Bacteriophage, vB_BfrS_NCTC. Signals of genomes in different conformations have been detected, providing insight into the activity of bacteria in real time and the space they occupy in the gastrointestinal tract. The distribution of bacteria in the gastrointestinal tract is not uniform, and the serotype composition of bacteria in GIT is not uniform either. Genomes, as ANC in the studied ensemble of masses from 50 Da to 3.4 billion Daltons, are subject to the general energy of the ensemble of masses in this interval. Minimization of potential energy in the ensemble forces the fragmentation of genomes and their reorganization, which in turn leads to the emergence of many bacterial serotypes. It has been shown that the penetrating ability of bacteria depends on both their shape and size, and on the activity of Bacteroides fragilis, which causes stretching of the intercellular space in the tissues of the gastrointestinal tract and, further, gas gangrene. The activity of this bacteria is controlled by the Bacteriophage, vB_BfrS_NCTC. The reason for genomes entering the genitourinary system has been established. A model of the process of bacterial infiltration from the gastrointestinal tract into the ureter tissues has been given.

Keywords

GMS, Genomes; Non-invasive diagnostics; Stomach; Intestine; Urine; Infiltration; Bacteriophages.

Introduction

Diagnostics of bacteria in the gastrointestinal tract (GIT) requires sampling and time for their expensive analysis. We have developed a method for remote and operational analysis of the state of bacterial genomes as atomic nuclei clusters (ANC). ANCs oscillate and thereby generate gravitational noise (GN), which can be recorded. The GSN can be used to determine both the mass of the ANC and the nature of its interaction with its surroundings [1,2]. The latter is very important in terms of understanding the activity of the bacterial genome, as its "brain", for example, operationally in the gastrointestinal tract in real time and *in vivo*. It is also of interest to understand the reasons for the appearance of bacteria in the human genitourinary system. In this regard, an attempt to experimentally detect the genomes of the main bacterial representatives of the microflora from the human gastrointestinal tract in the urine of a sampler was of interest.

The aim of this work was non-invasive, remote recording of the activity of the genomes of a number of bacteria and their interaction with their surroundings in the gastrointestinal tract and in human urine *in vivo*.

Material and Method

The object of the study was the gastrointestinal tract of probant-72 (72 years old), with suspected pathology in the gastrointestinal tract, *in vivo*, Figure 1

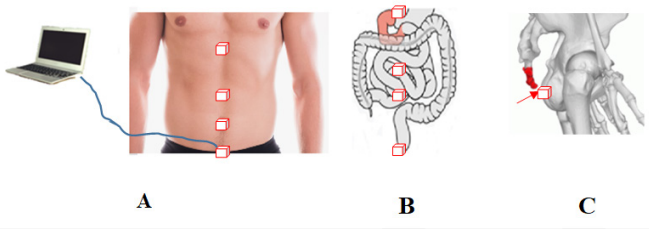


Figure 1: A - places of touch of the sensor (10 seconds) of the skin opposite the examined areas of the gastrointestinal tract. The sensor is indicated as a cube. **B** - a picture of the tract indicating the scanning locations of gravitational noise (GN) from the ring genomes of bacteria. An example of scanning the rectum (maximum proximity of the sensor to the intestine under the coccyx) is given on the right (**C**).

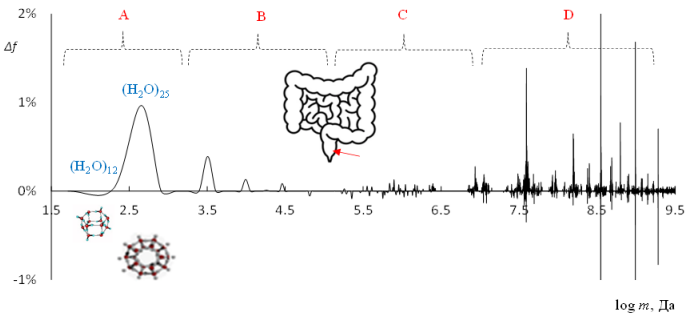


Figure 2: Example of a general GMS spectrum from the *rectal* GN. **A** – areas of oscillations of molecular clusters and domains; **B** – protein coils and their associates, nucleosomes; **C** – genes and solenoid structures in the chromosome topology; **D** – genomes, sub micellar, micellar and supra micellar structures. The model of the basic water cluster [13] was kindly provided by Prof. Lenz [14], the hypothetical cluster of 25 molecules is taken from [15]. The main characteristics of this ensemble of ANC: N = 2495, M_{GMS} = 706,544,939 Da, D_c = 28%.

Gravitational mass spectroscopy (GMS) was used to analyze ensembles of clusters of atomic nuclei (ANC) in the long-range molecular energy order of the ANC [1]. The GMS sensor was applied to the scanning location (Figure 1) and gravitational noise (GN) from ANC oscillations (clusters, domains, balls, nucleosomes, genomes, genes, solenoid coils, and micellar structures) was recorded in the mass range from 50 to 3.6 billion Daltons. The genomes of the following bacteria were diagnosed: *Bacteroides fragilis* NCTC 9343 [3], *Prevotella melaninogenicus* [4], *Bacteroides oralis* [5], *Enterococcus faecalis* [6,7], *Enterobacter sp.* [8], *E-coli* [9], *Clostridium perfringens* [10], and *Staphylococcus aureus* [11]. GMS spectra were obtained using the first Zubow equation [1,2]. Positive values of Δf are characteristic of loose forms of ANC, they reflect the energy fraction of ANC in the entire ensemble of the studied cluster of atomic nuclei, negative values of Δf reflect the same, but for dense conformations of ANC, Figure 2. Scanning time is 10 s. Statistical analysis was carried out by studying ~2 thousand GMS spectra. The spectrum in Figure 2 is given to understand the starting situation. The absence of free water molecules in the gum tissue was indicated by the signals of anhydrides of the Ti plasmid (R *radiobacter* strain R58), the bacterium (*Agrobacterium tumefaciens*) of which entered the food chain [12]. N is the number of types of molecular clots in the GMS spectrum (number of signals), $M_{GMS} = \sum(m \cdot \Delta f)$ is the average molecular mass of clusters in Daltons (Da), and D_c is the proportion of dense, potential energy-rich clusters (abs($-\Delta f/\sum\Delta f$)).

Results and Discussion

Let's start with *E-coli*, the signals of circular DNA of the bacterial genome in the GMS spectrum are detected for a large number of serotypes in the stomach, Figure 3. Let's observe the state of only one genome with a mass of 2,983,311,025 Da (4,639,675 bp) [16] in the gastrointestinal tract.

As can be seen from Figure 3**A**, the *stomach* contains masses equivalent to the serotypes of the genome masses of this bacterium. The DNA rings oscillate in both dense ($-\Delta f$) and loose conformations (Δf). Dense conformations indicate an inactive, conserved state of the molecule.

On the contrary, loose forms indicate that external interactions with their surroundings dominate in DNA, there is an intensive exchange of information, the genome controls the bacterium, and that is, the bacterium is in an active state. Serotype with a mass of 2,983,311,025 Da is not active in the *stomach*. In the middle part of the *abdomen*, the GN from this serotype (**B**) indicate a practical equality of dense and loose forms of DNA genome rings, the signals mutually compensate each other. Here, there is a weak manifestation of bacterial activity. The same can be said about the state of the *E-coli* genome in the *umbilical cord* of the sampler (**C**). In the serotype with a mass of 2,983,311,025 Da, intramolecular interactions prevail. The bacterium is not active. The practical equality of dense and loose ring conformations of this serotype is also found in the *rectum* (**D**). The situation changes dramatically when analyzing GN in the *urine* of the sampler (**E**). Loose conformations of the bacterial DNA ring reliably dominate in the *urine*; good conditions are created for it here and it is active. How a genome with a mass of almost 3 MDa penetrates from the *intestine* into the urinary system can be understood only by taking into account the active work of the membrane proteins of the body's cells (tetraspannins, **F**), forcibly pushing lymph into the intercellular space [19]. The signal from the active gene (**G**) may become the answer to understanding the mechanism by which large, torpedo-shaped bacterial cells enter the urine of the sampler. In the tissues of the genitourinary system, the gene, for example, of integrin is activated when there is a deficiency of this protein, which is lost (tribochemical) when tetraspannins [19] work as a "plunger pump" (**F**). Integrins, in dense conformations, were constantly detected in the *urine* of the sampler. Since the intercellular distance, under normal conditions, is small for pushing torpedo-shaped bacteria between cells, there must be a mechanism for stretching the space between cells. Such a mechanism may be the activity of bacteria causing gas gangrene in the *intestine* [20], for example, *Clostridium perfringen* (Figure 6), the genomes of various serotypes of which were found in the intestine of the sampler. We will consider the mechanism of this phenomenon at the end of this message, after analyzing all the data

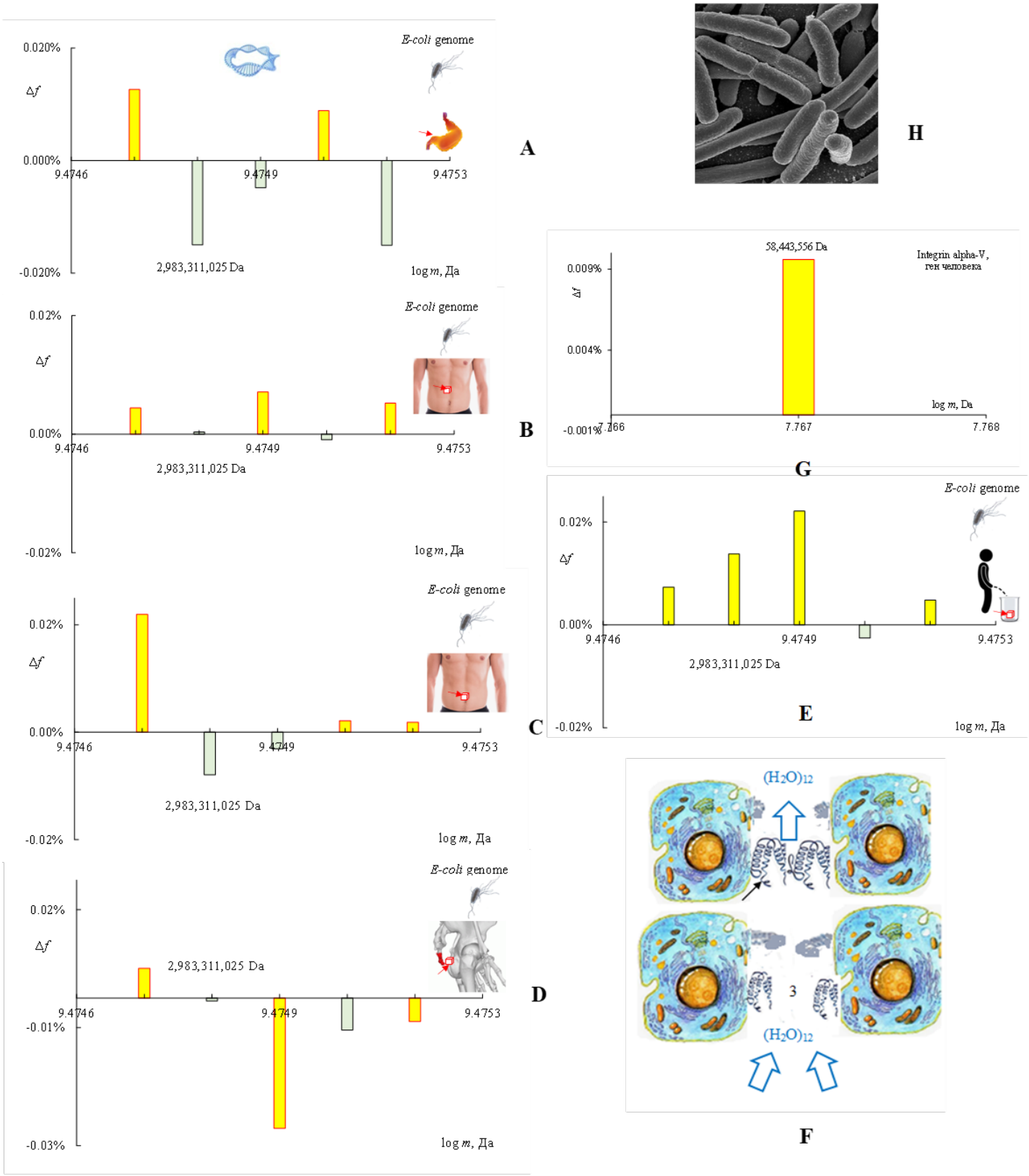


Figure 3: **A** – fragments of the GMS spectra in the region of oscillations of the *E-coli* genome with a mass of 2,983,311,025 Da, *gaster*, **B** – the same as **A**, but in the *linea alba* region, **C** – the same as **A**, but in the *umbilicus* region, **D** – the same as **A**, but in the *rectum* region. **E** – the same as **A**, but in the *urine* of the probant. **F** – model of intercellular pushing of water by membrane proteins. **G** - signal from an activated human gene (*urethra* wall of the probant), responsible for the production of RNA for the subsequent synthesis of integrin V [17]. The integrin is indicated by the arrow. **H** - a photograph of a bacterial colony is taken from [18].

on other genomes of the bacteria studied here.

Figure 4 shows fragments of the GMS spectra in the area of oscillations of the *Bacteroides fragilis* NCTC 9343 genomes in the gastrointestinal tract and *urine* of the sampler. As can be seen from the spectra, the bacterial genome signals were absent in all samples, with the exception of the region opposite the small *intestine*. In this region, the gene weakly interacts with its surroundings, barely reaching very low Δ*f* values of ~0.004% in the GN ensemble of the ANC in the bacterial cytoskeleton, and controls its activity. The weakness of the signal indicates that the bacteria are not comfortable in the microflora of the small *intestine*. Some factors prevent its activity. These factors may be bacteriophages, Figure 11.

Figure 5 shows fragments of the GMS spectra in the region of oscillations of the *Enterococcus_faecalis* genome. In the *stomach* and *rectum*, in the conformations of circular DNA, loose genomes that actively interact with their surroundings dominate. Their signals are weak, but even in this case, they should be understood

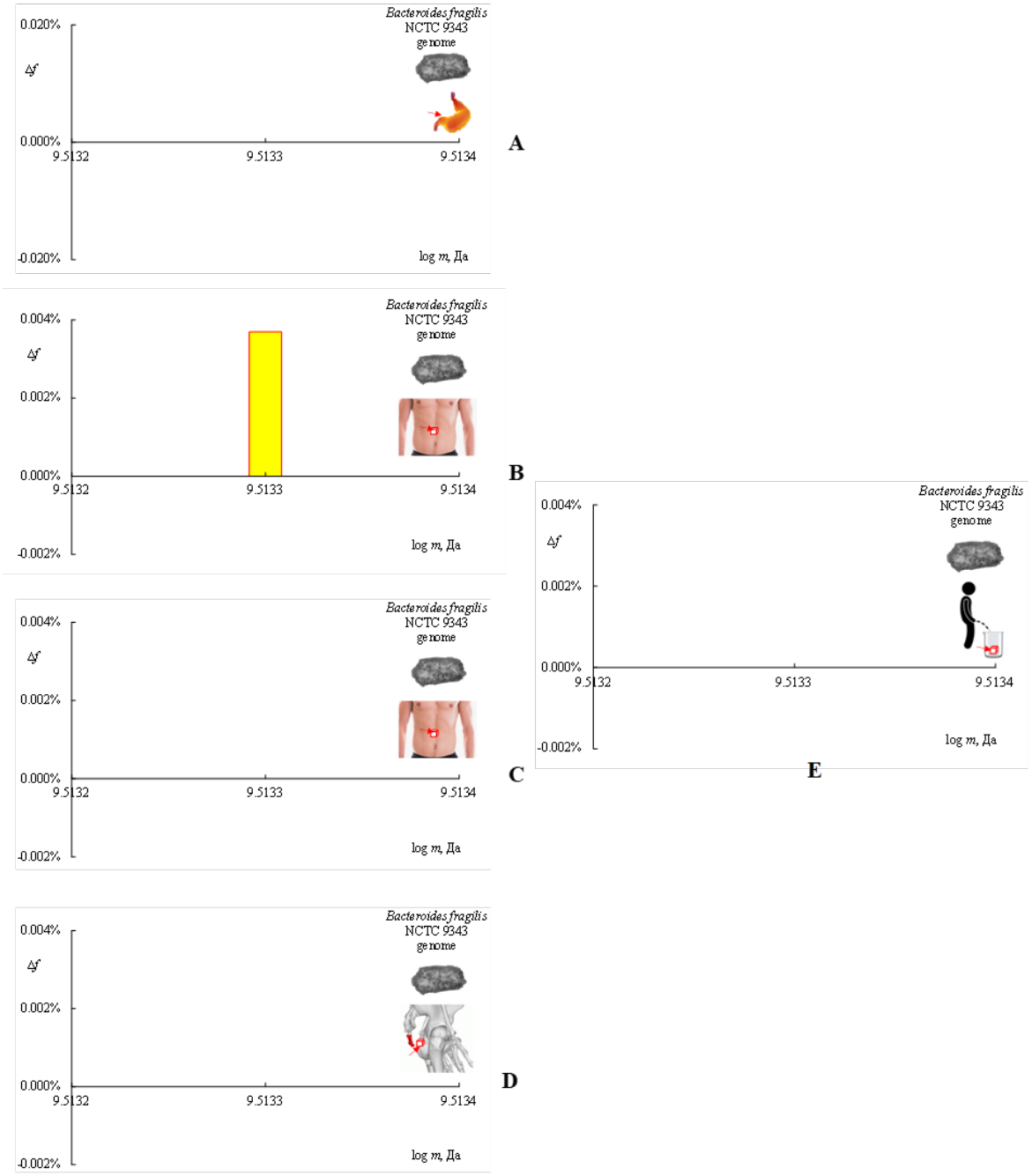


Figure 4: A – fragments of the GMS spectra in the region of oscillations of the *Bacteroides fragilis* NCTC 9343 genome with a mass of 3,260,604,775 Da [21], *gaster*, **B** – the same as **A**, but in the *linea alba* region, **C** – the same as **A**, but in the *umbilicus* region and **D** – the same as A, but in the *rectum* region. **E** – the same as **A**, but in the *urine* of the sampler. A photograph of the bacteria taken from [3].

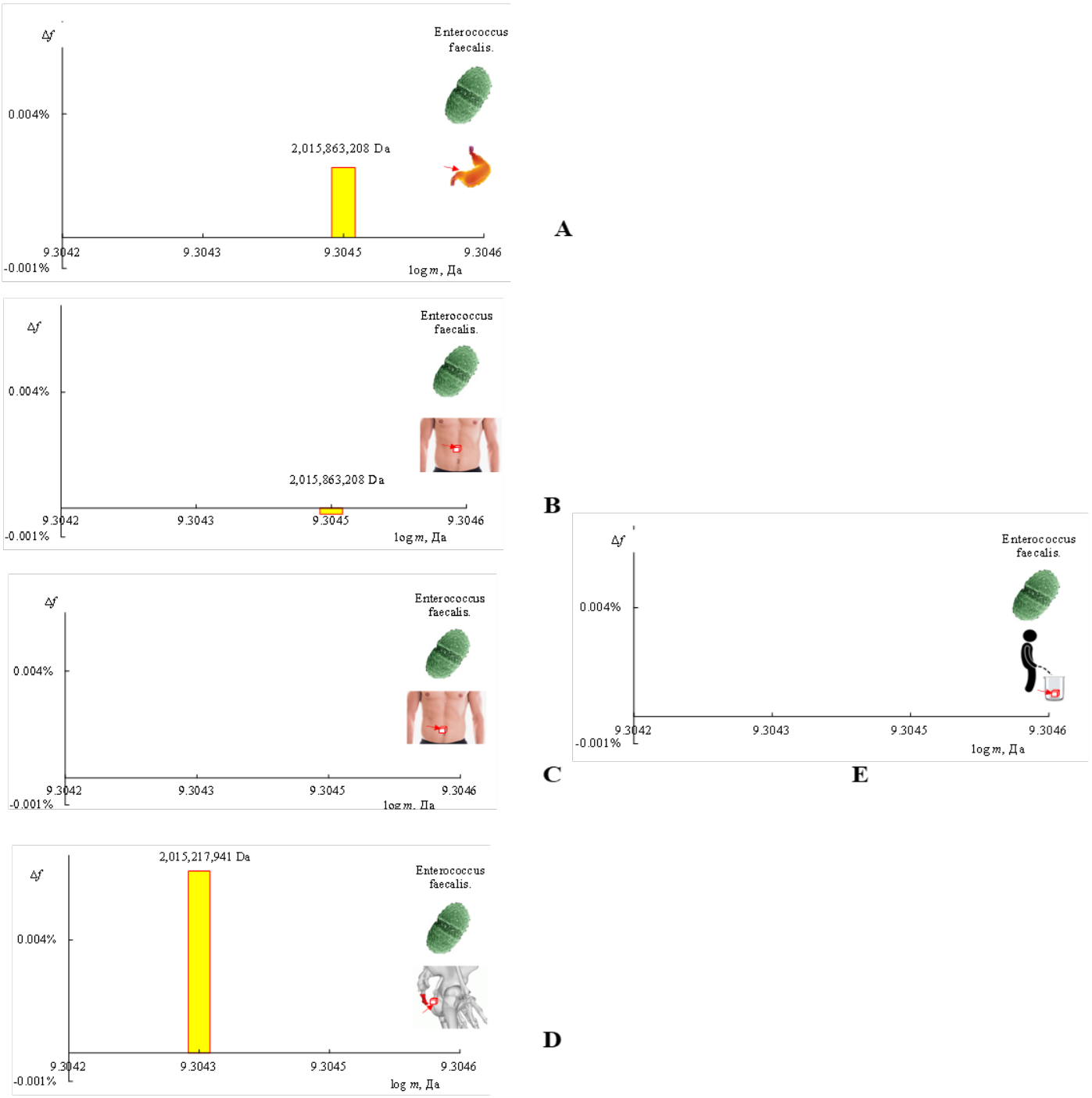


Figure 5: **A** – fragments of the GMS spectra in the region of oscillations of the *Enterococcus_faecalis* genome with a mass of 2,015,217,941 Da (3,134,087 bp) [22] and its strain with a mass of 2,015,863,208 Da, *gaster*, **B** – the same as **A**, but in the region of *linea alba*, **C** – the same as **A**, but in the region of *umbilicus* and **D** – the same as **A**, but in the region of *rectum*. **E** – the same as **A**, but in the *urine* of the tester. A photograph of the bacteria taken from [6].

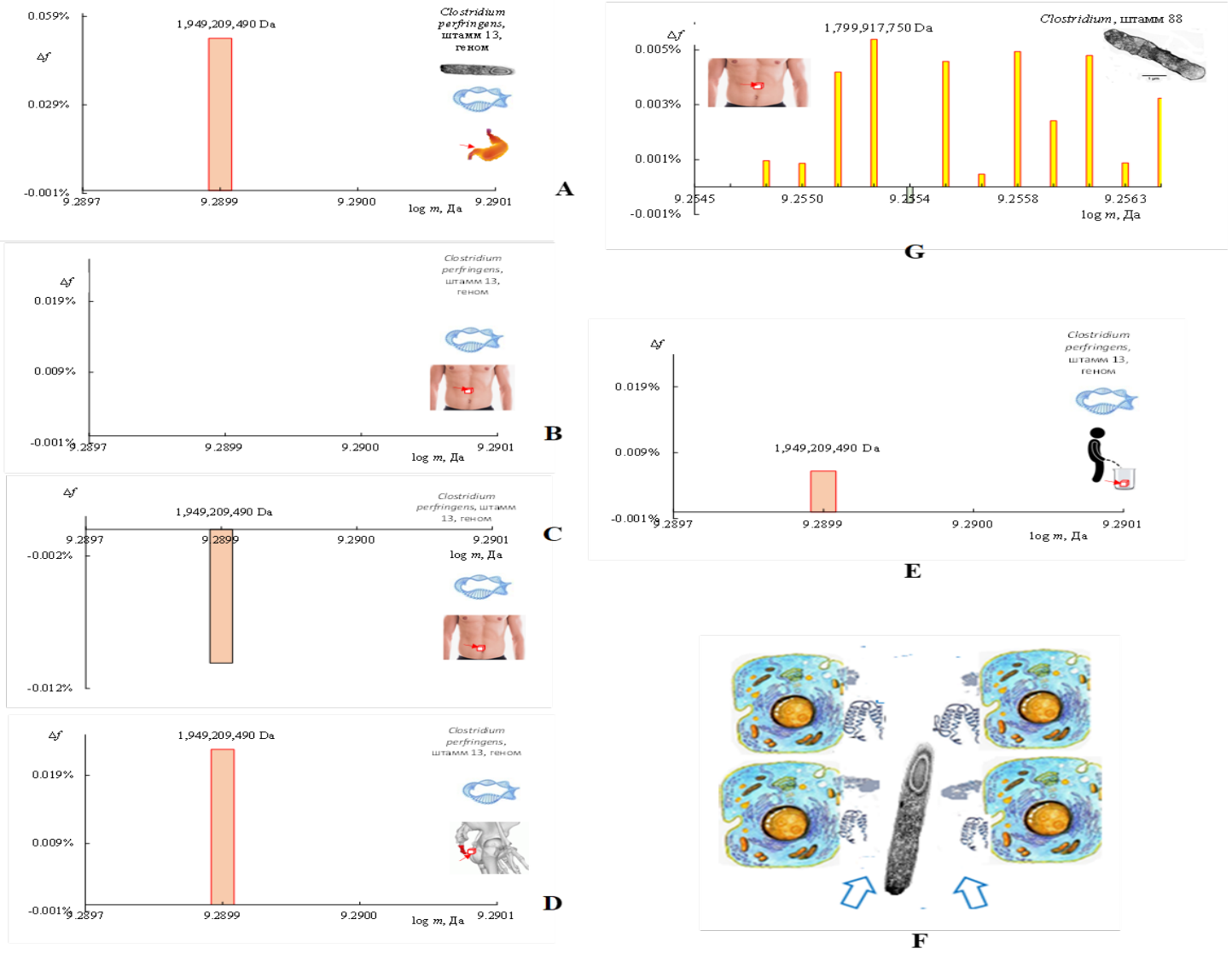


Figure 6: A – fragments of the GMS spectra in the region of oscillations of two-stranded circular DNA of the *Clostridium perfringens* genome with a mass of 1,949,209,490 Da (3,031,430 bp, ccDNA) [24], *gaster*, **B** – the same as **A**, but in the region of *linea alba*, **C** – the same as **A**, but in the region of *umbilicus* and **D** – the same as **A**, but in the region of *rectum*. **E** – the same as **A**, but in the *urine* of the probant. **F** – model of pushing a "torpedo" by tetraspannins in the intercellular space. **G** – the same as **A**, but in the *linea alba* region for strain 88 (2,799,250 bp), the photograph is taken from [23].

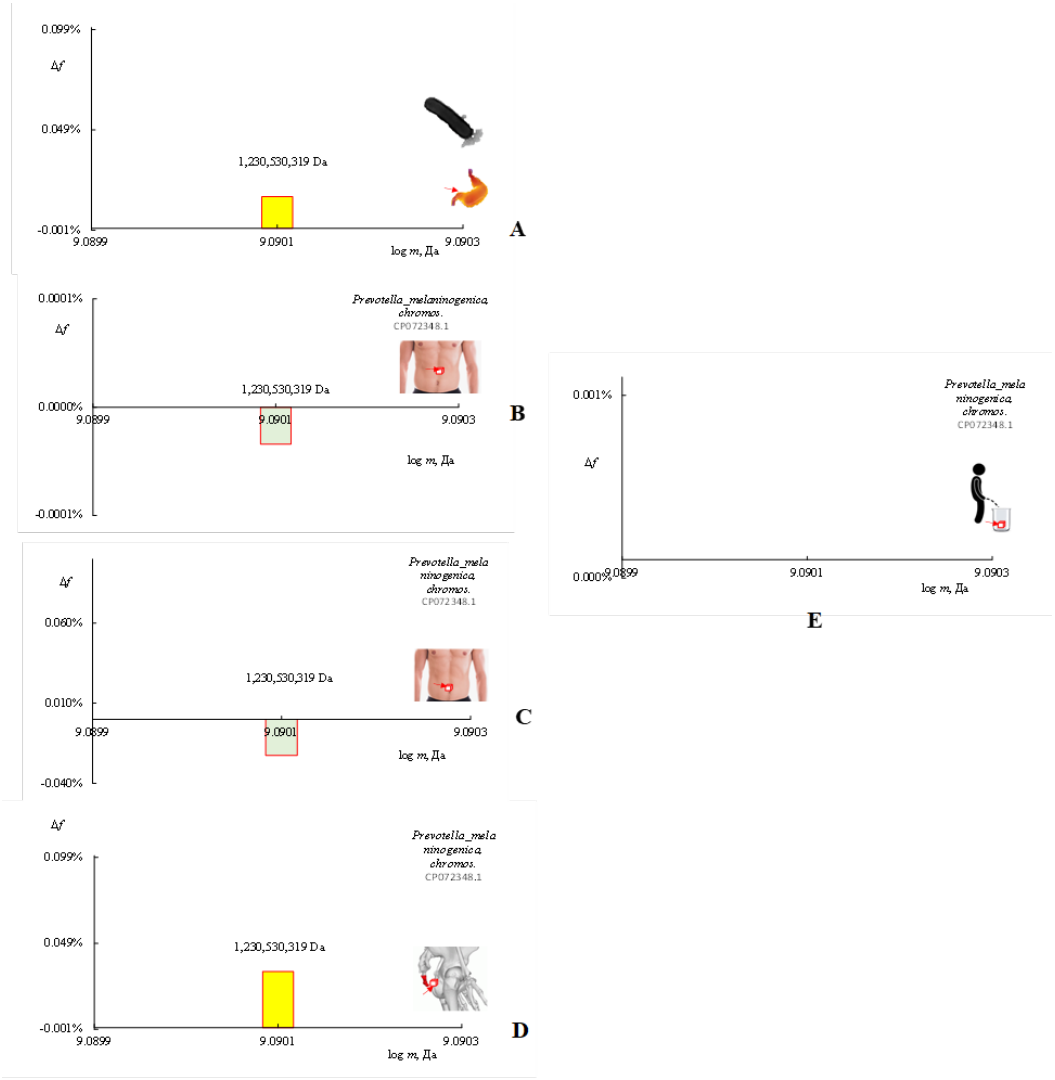


Figure 7: A – fragments of the GMS spectra in the region of DNA oscillations of the *Prevotella melaninogenicus* genome with a mass of 1,230,530,319 Da (1,913,733 bp, DNA) [26] in the *gaster*, **B** – the same as **A**, but in the *linea alba* region, **C** – the same as **A**, but in the *umbilicus* region and **D** – the same as **A**, but in the *rectum* region. **E** – the same as **A**, but in the urine of the probant. A photograph of the bacteria taken from [4].

as active control of the genome over all events in the bacterial cell; it feels good here. However, there are no genome signals in the *umbilical cord* or in the *urine* of the sampler. In the first case, this means that the micro biocenosis in the *umbilical cord* is not in favor of the bacterium, it is alien here and does not take origin, and in the second case, that the size of the bacterium (it is no longer a "torpedo" but a "barrel") does not allow it to penetrate through the intercellular space into the genitourinary system of the sampler's body.

The following figure 6 shows fragments of the GMS spectra in the region of oscillations of the *Clostridium perfringens* genome. The shape of the bacterium is clearly torpedo-shaped, the signals of the genome in a loose, active form are present only in the GN from the *stomach* (**A**) and *rectum* (**D**). In the region of the small *intestine* (**B**) there are no signals from this bacterium. However, in the *umbilical cord* (**C**), a strong signal from the genome with a mass of 1,949,209,490 Da was detected. It reflects a noticeable dominance of dense conformations in DNA rings, indicating suppression of the activity of the bacterium by external factors of its habitat.

In contrast, in the *rectum* (**D**) and *urine* of the sampler (**E**), the dominance of loose, active DNA conformations is noticeable. That is, this "torpedo-shaped" bacterium penetrates through intercellular channels from the gastrointestinal tract into the *urine* of the probant (**F**). Since this bacterium causes gas gangrene [23], leading to the distension of *intestinal* tissues by gases, the states of the genomes of some of its serotypes were analyzed. It turned out that strain 88 [24] with its series of serotypes is most active in the small intestine *linea alba*. Let us pay special attention to this area, in terms of regulating the activity of the strain by bacteriophages, Figure 11.

Figure 7 shows fragments of the GMS spectra in the region of oscillations of the *Prevotella melaninogenicus* genome. The activity of the genome and its bacteria in the *stomach* and *rectum* is high, loose forms noticeably dominate in the genome. However, in the region of the small *intestine* and *umbilical cord*, the activity

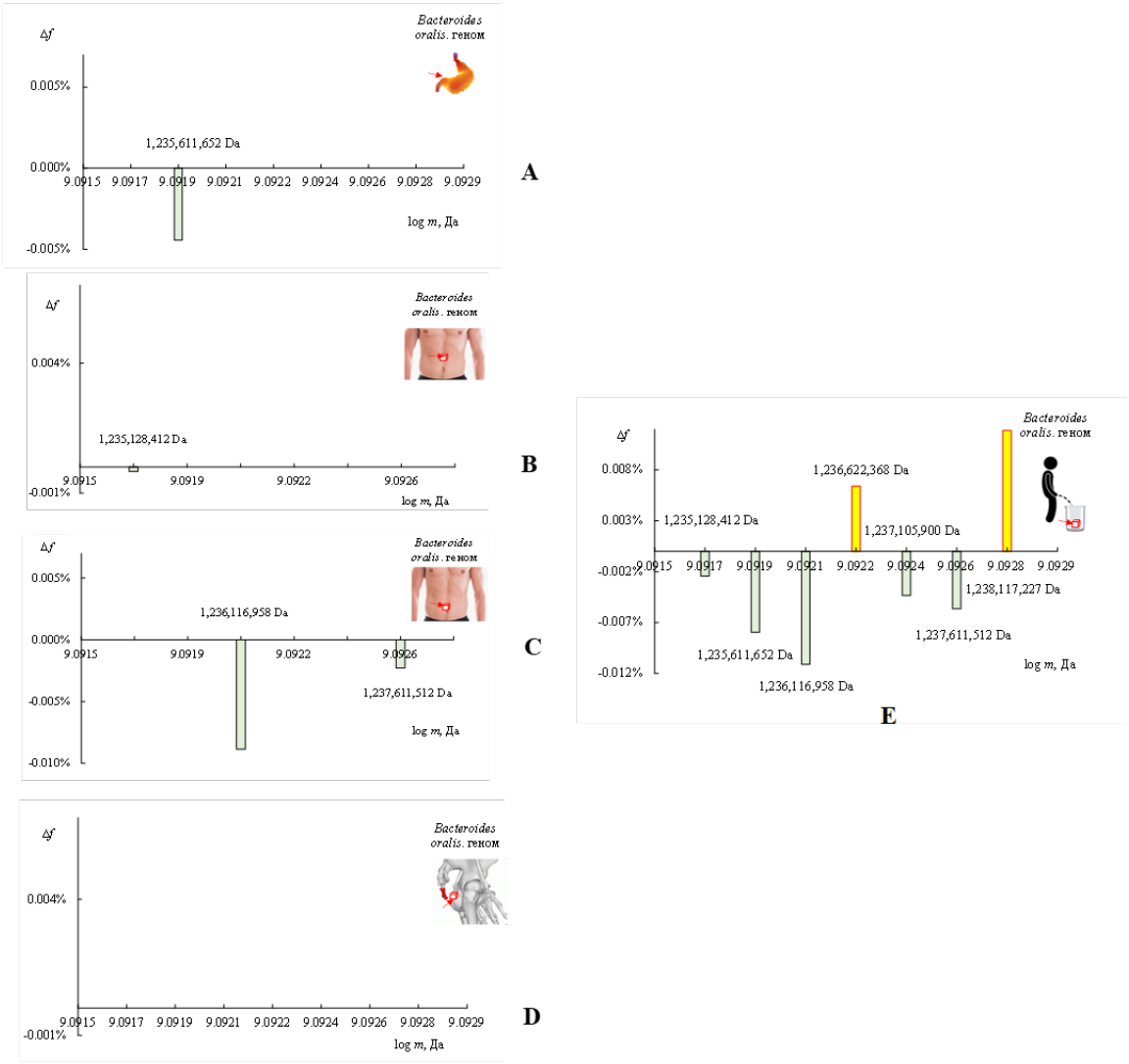


Figure 8: A – fragments of the GMS spectra in the region of DNA oscillations of the *Bacteroides oralis* genome with a mass of 1,235,128,412 Da (1,920,884 bp, DNA) [28] in the *gaster*, **B** – the same as A, but in the *linea alba* region, **C** – the same as **A**, but in the *umbilicus* region and **D** – the same as A, but in the *rectum* region. **E** – the same as **A**, but in the *urine* of the sampler.

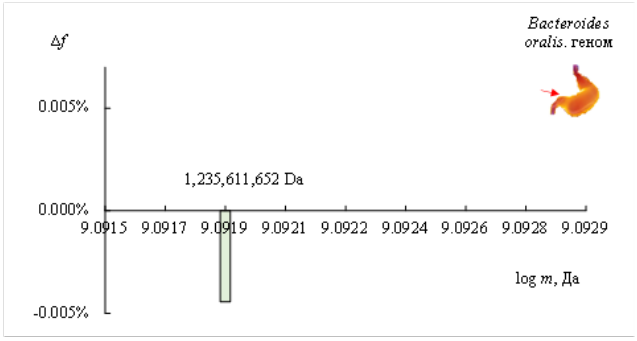
of the bacteria is suppressed, dense conformations prevail in the genomes. Apparently, the high activity of bacteriophages plays a role here. However, the signals of the genome of this bacterium are absent in the urine of the probant. The bacterium is very small and although it has an almost torpedo-shaped form [25], it apparently has difficulty passing through the intercellular space in the areas of the small *intestine* and *umbilical cord*, where its activity is suppressed.

On the other hand, the possibility of it getting into the *urine* from the *rectum* area should be high, but this is not the case. It is also known that these bacteria, forming associates, do not have steric opportunities to penetrate the *ureter*. Here, in any case, additional research is required to understand the details of the problem.

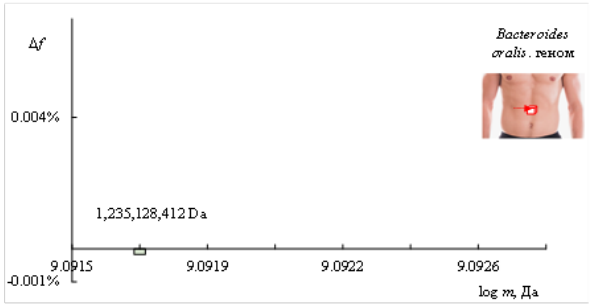
Figure 8 shows fragments of the GMS spectra in the area of oscillations of the *Bacteroides oralis* genomes. Here, completely opposite results were obtained. The bacterial genome is present in all studied areas of the gastrointestinal tract (except the *rectum*) and *urine*. The genome is generally inactive. The bacterium is also inactive. However, in the area of oscillations of the genome, there are signals from other genomes with masses close to this bacterium.

It seems that intra genomic processes occur in bacteria, such as "jumping genes" [27], in search of a new thermodynamic equilibrium of DNA rings with a minimum of potential energy in the ANC ensemble.

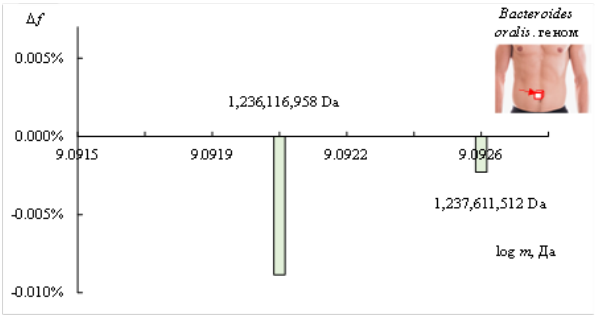
Another reason may be the binding of the genome into complexes with proteins that inactivate its oscillations and that are destroyed in the urine. The spectrum (**E**) reveals a number of signals from both dense and loose conformations of the genomes of this bacterium. That is, having got into the urine, the individualization



A



B



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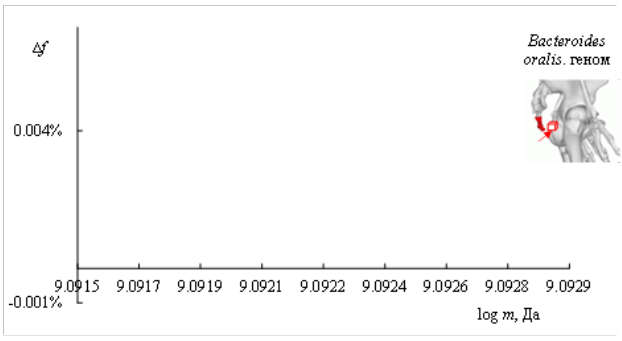

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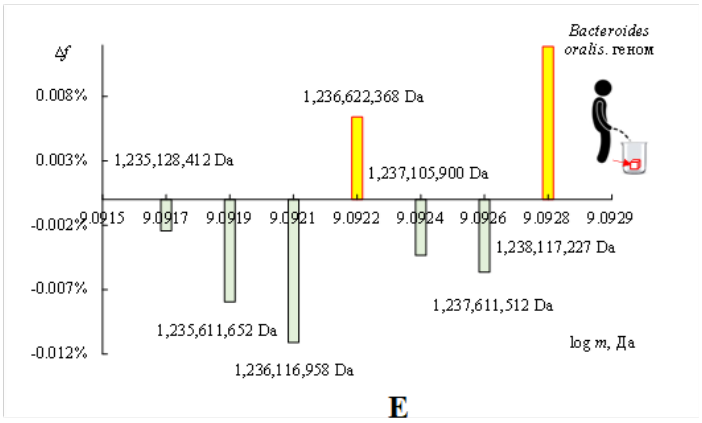

E

Figure 8: **A** – fragments of the GMS spectra in the region of DNA oscillations of the *Bacteroides oralis* genome with a mass of 1,235,128,412 Da (1,920,884 bp, DNA) [28] in the *gaster*, **B** – the same as A, but in the *linea alba* region, **C** – the same as **A**, but in the *umbilicus* region and **D** – the same as A, but in the *rectum* region. **E** – the same as **A**, but in the *urine* of the sampler.

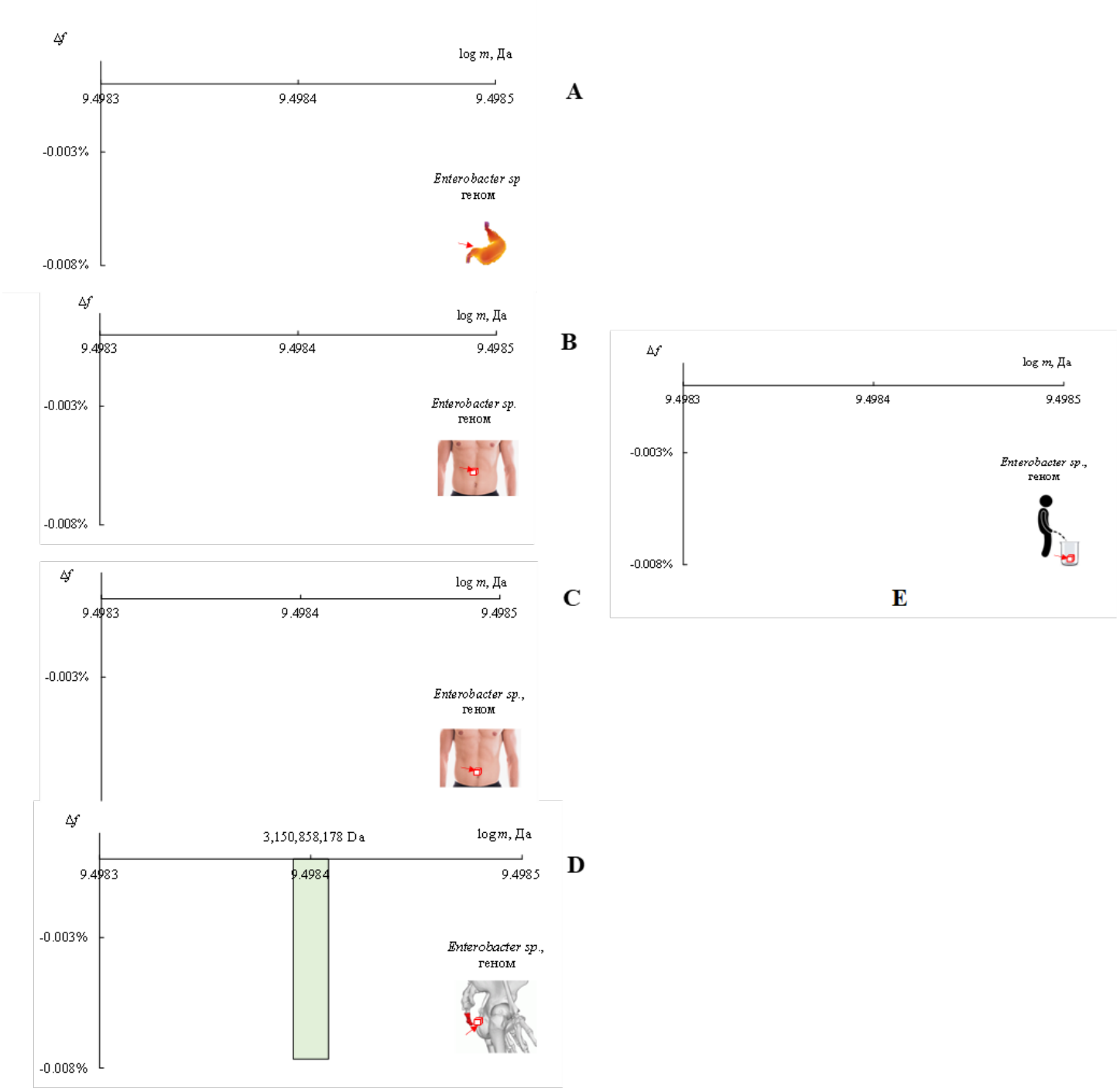

Figure 9: **A** – fragments of the GMS spectra in the region of DNA oscillations of the *Enterobacter sp*. genome with a mass of 3,150,858,178 Da (4,900,246 bp, DNA) [29] in *gaster*, **B** – the same as **A**, but in the *linea alba* region, **C** – the same as **A**, but in the *umbilicus* region and **D** – the same as **A**, but in the *rectum* region. **E** – the same as **A**, but in the *urine* of the probant.

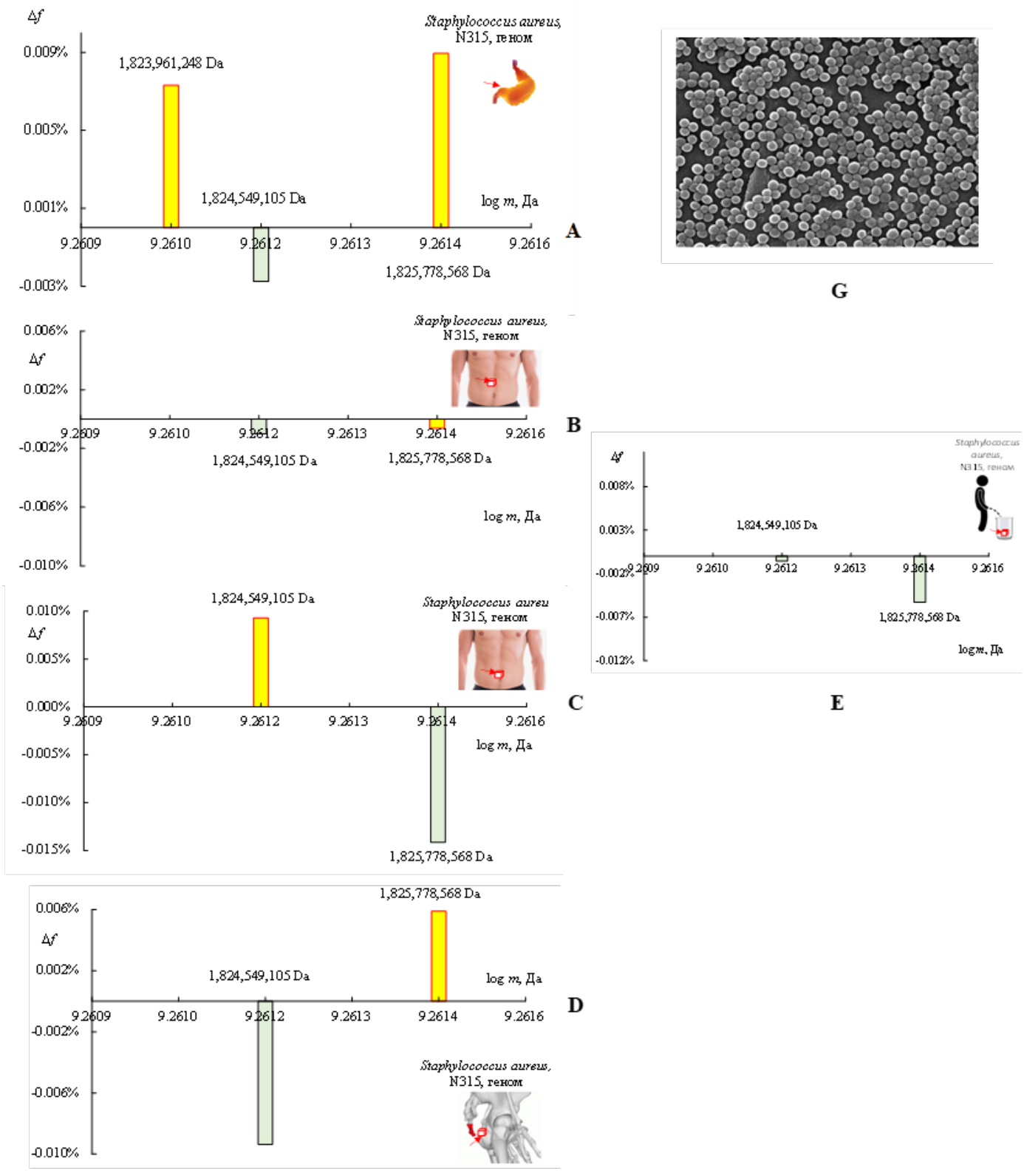

Figure 10: **A** – fragments of the GMS spectra in the region of DNA oscillations of the *Staphylococcus aureus* genome with a mass of 1,825,778,567 Da (2,839,469 bp, DNA) [30], in the *gaster*, **B** – the same as **A**, but in the *linea alba* region, **C** – the same as *A*, but in the *umbilicus* region and **D** – the same as **A**, but in the *rectum* region. **E** – the same as **A**, but in the urine of the probant, **G** – a photograph of the bacterial colony taken from [31].

of the bacterial genomes in the new habitat occurs. Thus, remote diagnostics of *Bacteroides oralis* genomes in the gastrointestinal tract requires additional analysis of the state of the bacterial DNA in the urine.

Figure 9 shows fragments of the GMS spectra of *Enterobacter sp*. genome oscillations. A very weak signal from genome oscillations occurs only from the GN region from the rectum (**D**). The genome is dominated by dense, inactive conformations of DNA rings. The activity of bacteria is suppressed and their concentration is extremely low. It is possible that the high ability of these bacteria to form strong associates is an obstacle to their entry into the urine *via* intercellular traffic.

Figure 10 shows fragments of the GMS spectra in the region of oscillations of *Staphylococcus aureus* genomes. Since the bacterium has many serotypes, we will consider only those that lie in the region of the genome with a mass of 1,825,778,567 Da. The bacterium is found in human tissues in the form of large stable associates, but there are also many individual spherical serotypes. As can be seen from (**A**), in the GN emanating from the *stomach* region there are three signals of genomes with masses of 1,823,961,248; 1,824,549,105 and 1,825,778,568 Da. The first and third are in loose, active conformations, while in the second genome dense forms dominate. In the small *intestine* region (**B**), the first signal has disappeared, and in the second two a weak dominance of dense DNA structures is detected. In the *umbilical cord* region (**C**), the first signal is again absent, the second indicates the dominance of loose, active genomes, in the third they are strongly suppressed dominance of dense conformations in DNA rings. In the rectum region (**D**), the first signal is absent, and the second and third have exchanged conformations, in the second dense forms of rings dominate, and in the third loose forms of rings. Only bacteria with the second and third genomes enter the urine (*E*), both in dense conformations, the signals of which are very weak.

In conclusion, let us consider the state of the bacteriophage *Bacteriophage*, vB_BfrS_NCTC, which is a counterpart of the pathogenic bacterium *Bacteroides fragilis*, which causes gas gangrene, Figure 11. The state of bacteriophage activity is important for understanding the energetic contributions of bacterial genomes, especially pathogenic ones, such as ANC in a large ensemble of masses, in the intestinal microflora. The Figure shows that the activity of the bacteriophage genome and the diversity of its serotypes are especially high in the small *intestine* (**A**) and *rectum*. All genomes actively interact with their surroundings. Since the genome in the capsid is in a dense conformation, and the spectra signals indicate loose forms, they should be understood as active introduction of the virus genomes into the bacterial cells, where they acquire loose forms. That is, in **A** and **C**, the virus is at work. In the spectrum (**B**), the virus is mainly in the capsid. It is not active here. So, the bacteriophage is active in the small *intestine* and thus suppresses the activity of the pathogenic bacterium, Figure 6. Here it checkmates it. This is the factor that manifested itself in Figure 6 **B**. In the *umbilical cord*, the virus is apparently in a waiting mode and only checkmates the bacterium and suppresses its activity (Figure 6**C**).

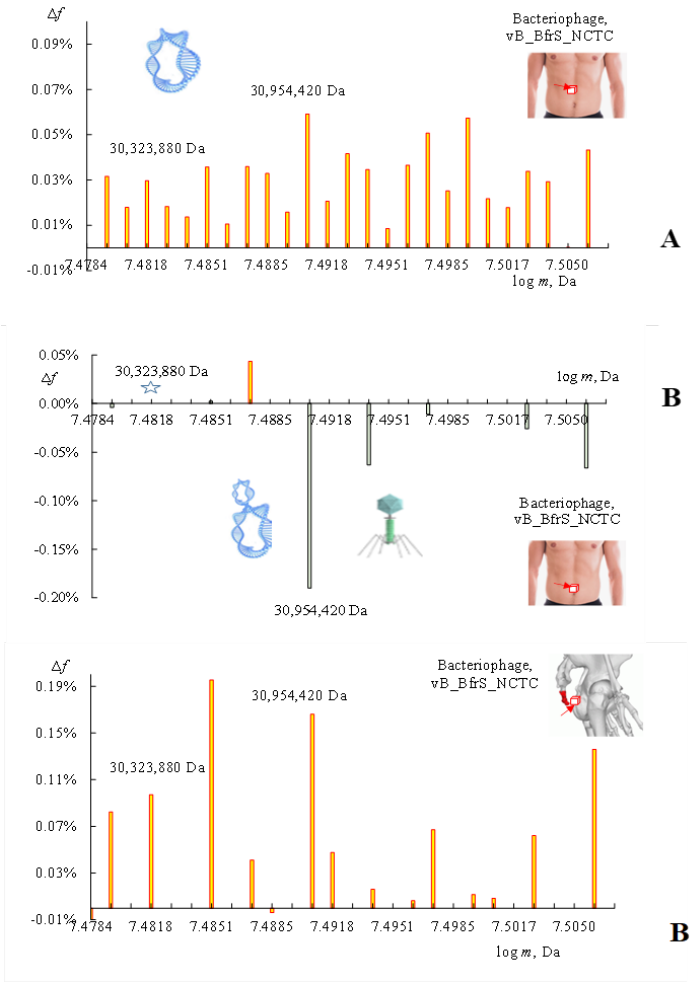

Figure 11: A – fragments of the GMS spectra in the region of DNA oscillations of bacteriophage genomes: *Bacteriophage*, vB_BfrS_NCTC and in the *linea alba* region, **B** – the same as **A**, but in the *umbilicus* region and **C** – the same as **A**, but in the *rectum* region.

Signals from oscillations of the bacteriophage T4 genome [32] were not detected in all GN samples in the gastrointestinal tract.

Conclusion

The GMS method allows for prompt non-invasive diagnostics of the state of bacterial and viral genomes in the gastrointestinal tract *in vivo*.

Bacteria are distributed differently in different areas of the gastrointestinal tract.

The activity of bacteria in the gastrointestinal tract is different.

There are no free water molecules in the tissues of the human body and in the gastrointestinal tract. Ring structures of DNA of bacteria in the gastrointestinal tract can rearrange and adapt to the requirements of minimizing the potential energy of the entire ensemble of clots of masses from 50 Daltons to 3.4 billion Daltons.

Membrane proteins play an important role in the processes of entry of gastrointestinal bacteria into the lymphatic system of the body. The process can be stimulated by the activity of pathogenic bacteria in the gastrointestinal tract.

In this case, membrane proteins wear out and enter the intercellular surroundings and then into the urine.

The penetration of bacteria from the gastrointestinal tract into the lymph is affected by their shape, size and ability to form stable colonies.

The activity of pathogenic bacteria in the gastrointestinal tract is regulated by specific bacteriophages.

Bacteriophages influence the entry of bacteria into the lymph and then into the urine.

The *umbilical cord* acts as a storage facility for bacteria and bacteriophages.

The genomes of bacteria and viruses, as elements of a large ensemble of oscillating masses, are forced to forcibly minimize their potential energy, within the framework of the general minimization of the energy of a large ensemble of pendulum masses.

The genomes of bacteria in the gastrointestinal tract, as physical oscillators, arise and are resolved by the gravitational noise of the Universe (see 3D Chladni effect) [33].

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