Research of Impact of IMCS* Structures on the Stability of Experiments Using Molecular Biology Methods.

- Microbial culture inoculation and growing in artificial media.
- Stable DNA purification from various materials (cultured microbial media, soils, agarose gel).
- Improving quality of electrophoresis reactions.
- Successful amplification and restriction by molecular genomic and physical methods.

The study was performed using system adapters

Translighter Super Translighter Blaga

Structural Impact on DNA Purification

The impact of structures was evaluated in an experiment with the use of fulvic acids and glucose sent to our genomic technologies laboratory from a soil institute in the Netherlands.

DNA purification from fulvic acid and glucose substrates has remained a difficult, laborious task. Nobody from European specialists was willing to take on this challenge. Having a well-equipped technical base, our laboratory decided to take the risk. Our attempts were repeatedly unsuccessful for two months. But in February 2014, we received the first positive results of this experiment. This success, as subsequent research has shown, can be clearly attributed to the influence of the structures (Translighter Super mainly and Translighter Blaga).

Below are the results of DNA purification with structures and without (Fig. 1, Fig. 2, Fig. 3).

Fig. 1 Raw DNA extract electrophoresis gel in agarose gel. Without the adapters.

No purified DNA fragment is visible in Fig. 1. On the left hand side, all you can see is a standard scale of markers, benchmark fragments of various lengths.

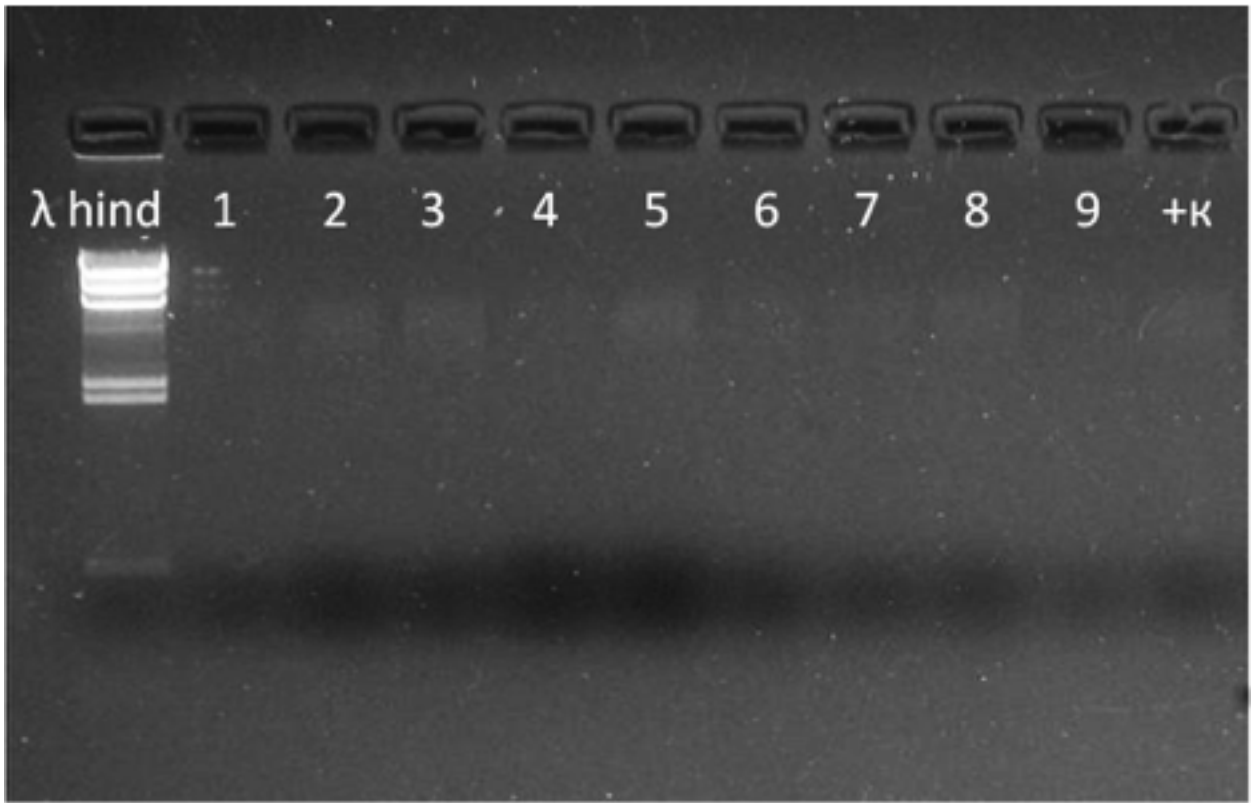

Fig. 2 Raw DNA extract electrophoresis gel in agarose gel. Translighter Blaga. In the upper part of the figure (across from the brightest part of the marker scale) you can see DNA fragments visible in electrophoresis. They are not very distinct but, nevertheless, they are there.

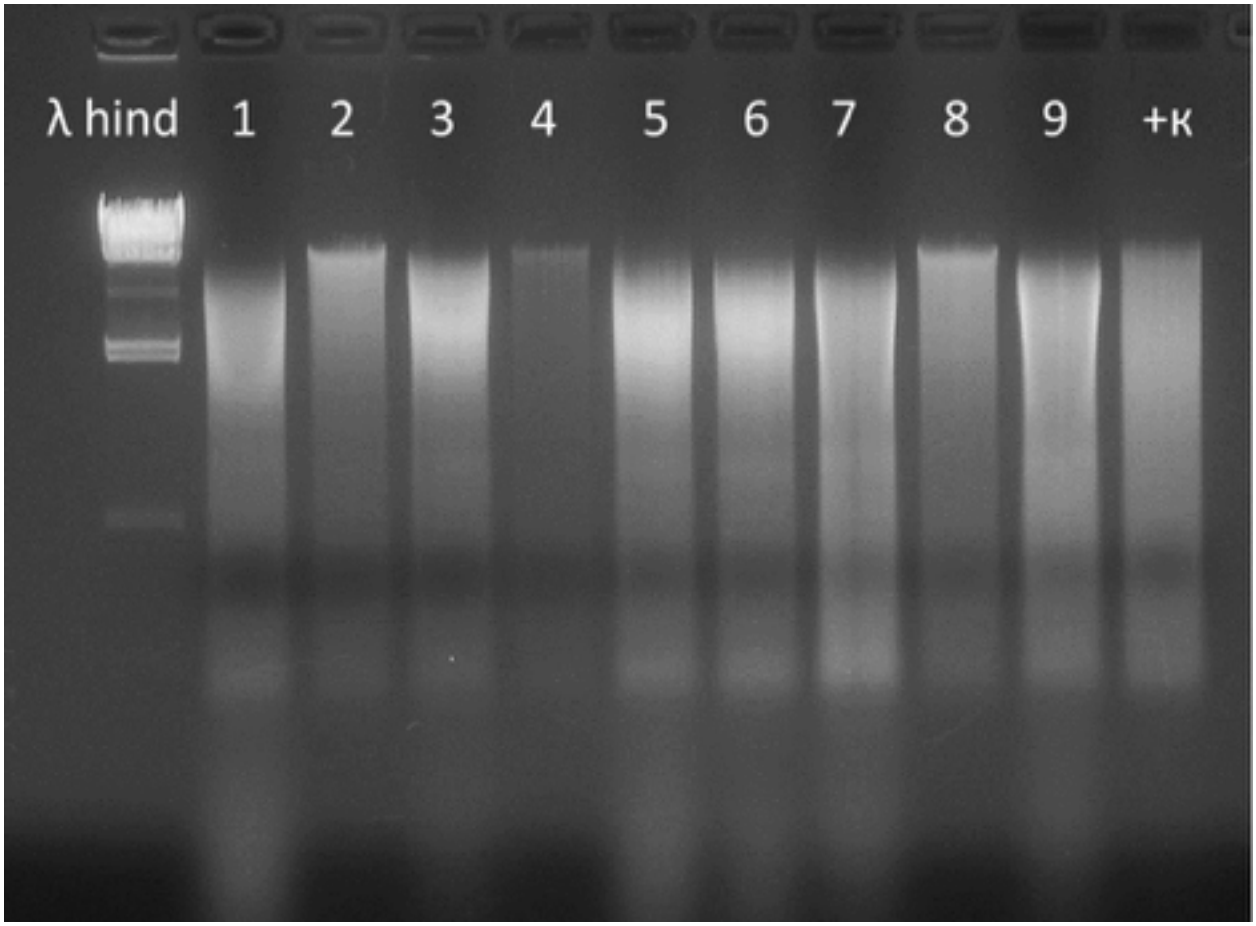

Fig. 3 Raw DNA extract electrophoresis gel in agarose gel. Translighter Super. In this figure, trails of DNA fragments of various lengths are visible clearly. DNA concentration in the solution is shown by the intensity of glow. Further research can be carried out with a solution of such intensity; fragments can be excised from the gel, amplified and sequenced.

Impact of the Structures on PCR.

Significant impact of the structures of PCR amplification has been established on the example of nine pairs of universal primers for 16S rRNA. It is evident that Translighter Super improves the course of PCR enhancing the concentration of the final product of amplification (Fig. 4, Fig. 5).

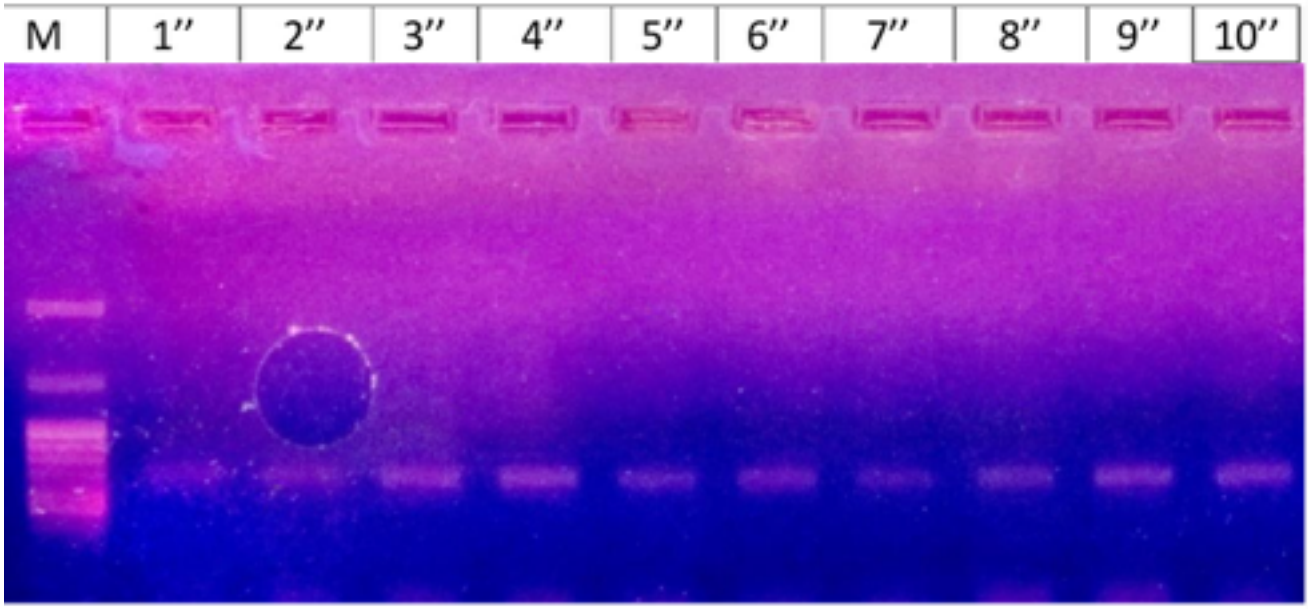

Fig. 4 PCR 16 S rRNA electrophoretogram of the bacterial complex of fulvic acids and glucose. Without the structures. This figure shows agarose gel with purified DNA fragments in the lower side. DNA gel fragments are then excised and used for sequencing (base sequencing and genetic code reading). Although all fragments are visible, their concentration in the gel is extremely low. That means that further research may show unstable results.

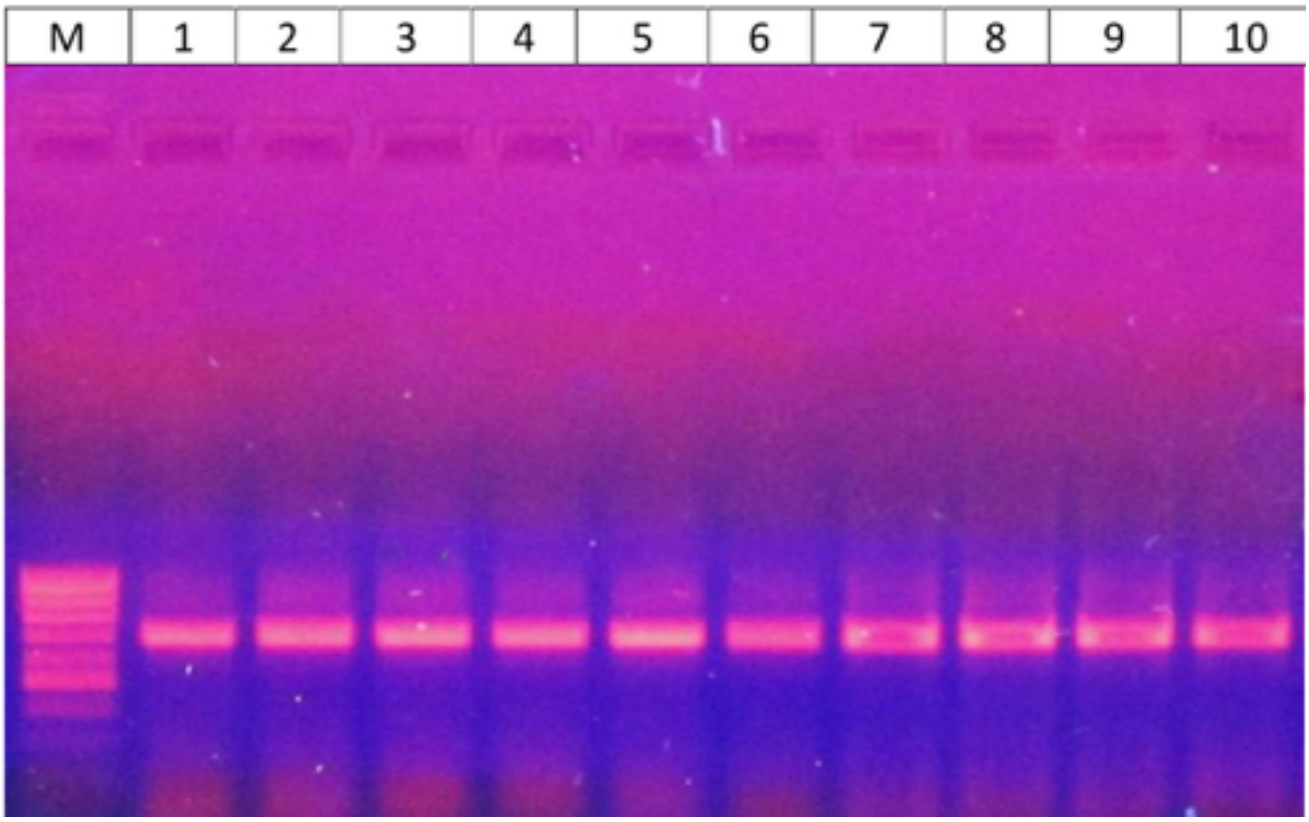

Fig. 5 PCR 16 S rRNA electrophoretogram of the bacterial complex of fulvic acids and glucose. Translighter Super. This figure represents agarose gel with clearly visible DNA fragments in the form of glowing stripes. Purification of the same initial DNA with the use of Translighter Super System Adapter gave a more stable PCR reaction and more efficient electrophoresis. As a result, a sufficient concentration of DNA fragments in a gel for further sequencing contain a minimum amount of impurities, as can be judged by distinct borders and strong glowing.

Evaluation of Structure and Dynamics of Microbial Communities With the Use of Structures

A genomic library of 16SrRNA was obtained for each primer. Sequencing was performed with the use of Roche primers, in an automatic sequencer, with the use of Roche reagents in compliance with the manufacturer's protocol. Base sequences were deposited with GenBank.

Sequence alignment was performed in ClustalX software and genomic distances matrix and phylogenetic trees were built in MEGA5 software. Taxonomic identification of sequences was performed using RDPII server.

In Fig. 6, a clear distinction is visible between taxonomic diversity with and without the use of Structures. The Structures increase the number of identifiable microbial representatives and allow seeing clear differences between the samples. The best results here were obtained when all three structures were used at the same time.

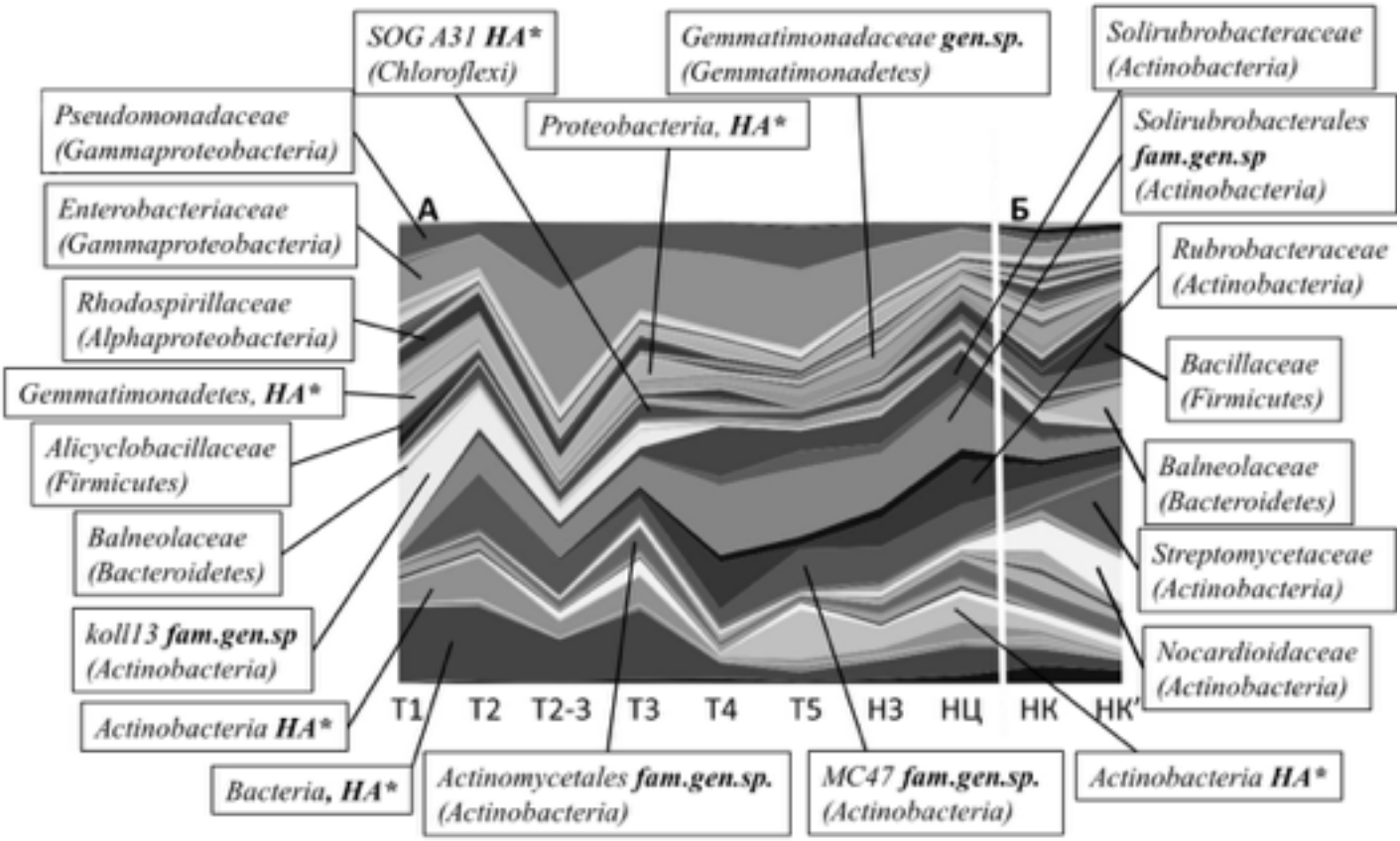

Fig. 6 Taxonomic diversity of microbial communities (at the level of a family) under conditions A – with Structures (Т1-НЦ) and B – without Structures (НК-НК'). HA - HA – unattributed sequence on the order level.

Experiments with Structures were performed only on other samples such as soils, plants, lenses, human blood, mycorrhiza etc. (Kazakhstan, Ukraine, Finland, Russia). We received amazing results proving once more that Structures have a significant impact on the results of experiments at different stages. Such data need time for processing into a report.

All previous experiments were performed at the molecular genetics level. During all stages of the experiment, relevant equipment was used. At this moment, it can be stated that Structures have a positive impact on functioning of devices. In order to study further the direct impact of Structures on living cells and organisms we have planned and carried out a number of additional experiments focused specifically

on this issue. At this moment, experiments with living cells are in progress and their results will be presented at the beginning of the following month (July 2014).

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