



## Sample: Biochemistry - Investigation of Newly-explored Species

We should obtain the pure culture of those new bacteria species. Firstly, we should identify the method that would allow us to make evidence of the proper bacterial types isolation. We could use methods of DNA staining in order to visualize the ovoid structure inside the cells. It would help us to identify the proper colonies after the application of selection. Secondly, selective medium must be applied to favor the growth of our bacteria only. Selective medium must provide all required nutrients in accordance to our bacterium carbon and energy sources, and electron donors. To avoid heterogeneity of bacterium population spread-plate and streak-plate techniques should be used. A solid colony represents a pure culture, so can be proceed to further investigation after staining and microscopy.

Specimen prepared from our bacteria should viewed under transmission electron microscope to investigate ultrathin structure of our new structure.

To identify the type of proteins and nucleic acid we have to extract them out the cells and purified. Nucleic-acid binding proteins should be positively charged. Therefore they can be purified by ion-exchange chromatography. A negatively-charged support (cation exchanger) will bind proteins with an overall positive charge.

Proteome of our new species can be analyzed by two-dimensional electrophoresis. Moreover, we can take bacterium, which is morphologically similar to our bacteria, or bacterium from the same habitat, but without discovered new structure. Proteins in this known bacterium can be resolved by two-dimensional electrophoresis to compare with our unknown bacterium proteome. In this way we can discriminate proteins unique to our bacterium. Particular attention should be paid to proteins with high isoelectric point, which mean high content of basic amino acids.

Further we can extract protein of interest from the gel and determine its mass with mass spectrometry. With the help of peptide mass fingerprinting it could be possible to identify our protein, if this protein is in fingerprint databases of course. If our protein was not found in databases, we can sequence it. Primary structure then can be align with primary structures of known proteins from databases with help of bioinformatics tools. Such bioinformatics analyses provides us with information about our proteins' orthologs. Thus it can help us to clarify place of our species in taxonomy.

The main group of DNA-binding proteins in eukaryotic cells are histones. Histones are very conservative proteins. That's why we have a chance that our proteins will be recognizable by commercially available antibodies to histones. So immunoprecipitation can be applied to extract proteins. Purity of such protein sample is very high. We have to rid of antibodies and protein can be sequenced. Commercially available fluorescent tag antibodies can applied to visualized new structure in our bacteria.

Description of nucleic acid as long chains give me reasons to assume that this is DNA. RNA has more complex secondary and tertiary structure. Thus, we should extract DNA from bacteria and sequenced it. And similarly to protein sequences analysis – bioinformatics tools can provide us with helpful information to define the closest relatives and origin of our species.

Besides, knowing of nucleic acid sequence make possible to manipulate with bacterium genome with the help of gene engineering tools. Gene that encode our protein can be inserted into vector, so a lot of pure protein of interest can be obtained. From pure protein crystals can be grown to undergo x-ray crystallography to provide us with data about protein's tertiary structure. Nuclear magnetic resonance (NMR) spectroscopy is another one method for elucidating three-dimensional structure.



“Long chains of nucleic acid” – this is description of DNA molecule, thus this is genetic material of the cell. “Nucleic acid wrapped around a protein” – a description of the nucleosome – the basic unit of DNA packaging in nucleus of eukaryotic cells. Thus, we can speculate, that found new species is a transitional form between prokaryotic and eukaryotic organisms. And found new structure is the new way of packaging of genetic material, different from the arranging of bacterial genetic material and closer to eukaryotic DNA packaging.

#### References

Lansing M. Prescott (2002). Microbiology. (L. M. Prescott, J. P. Harley, D. A. Klein). McGraw-Hill Higher Education

J.M. Berg, J. L. Tymoczko, L. Stryer (2002) Biochemistry (Fifth ed.) New York: W. H. Freeman