Isolation of new methylotrophic species *Bacillus baksanea* from deep underground hot spring of Baksan Neutrino Observatory

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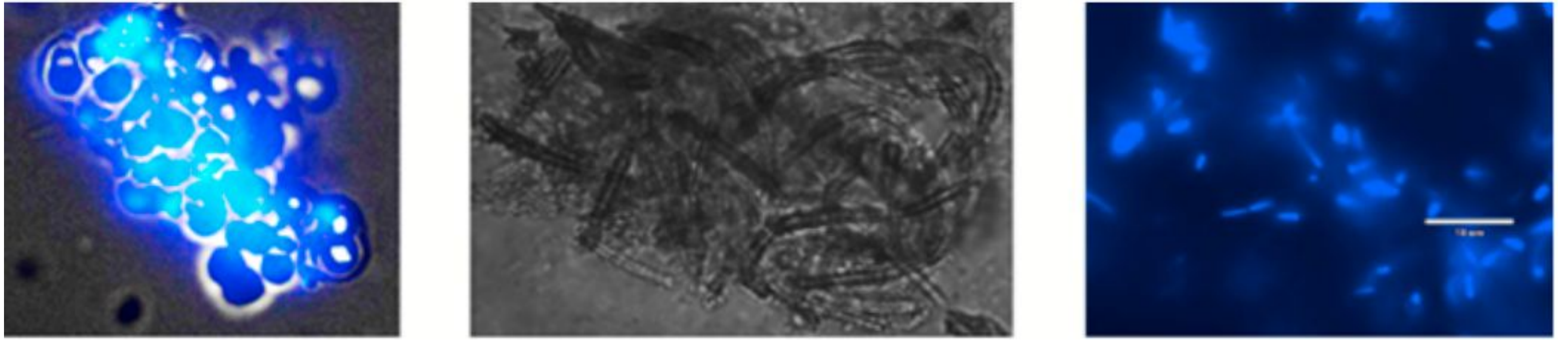

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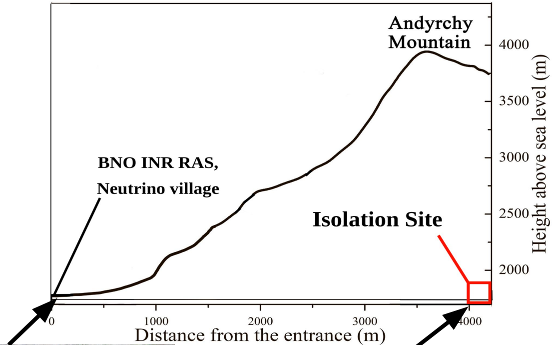
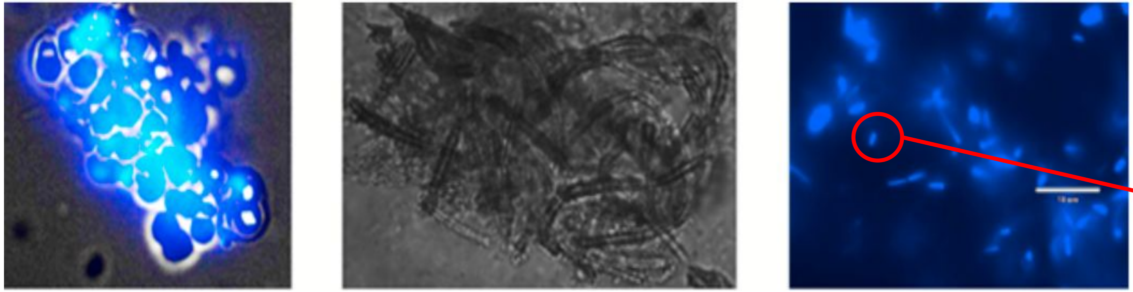
Isolation Site

Baksan Neutrino Observatory is situated about 22 km to the southwest of mountain Elbrus. There are hot water springs at the end of 4 km tunnel with unique microbial community of extremophiles.



Bacillus baksanea was isolated from that community. It is the only species that we we were able to cultivate in laboratory conditions.

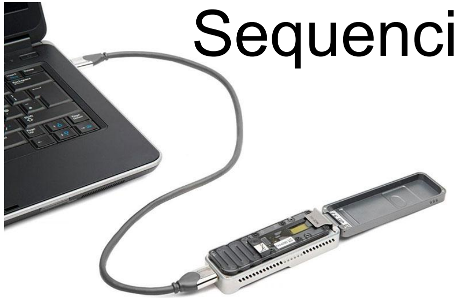


B. baksanea's DNA has been extracted and sequenced for further genomic and metabolic analyses.

Pictures represent snapshots of microbial community (left and central) and *B. baksanea* culture (right).

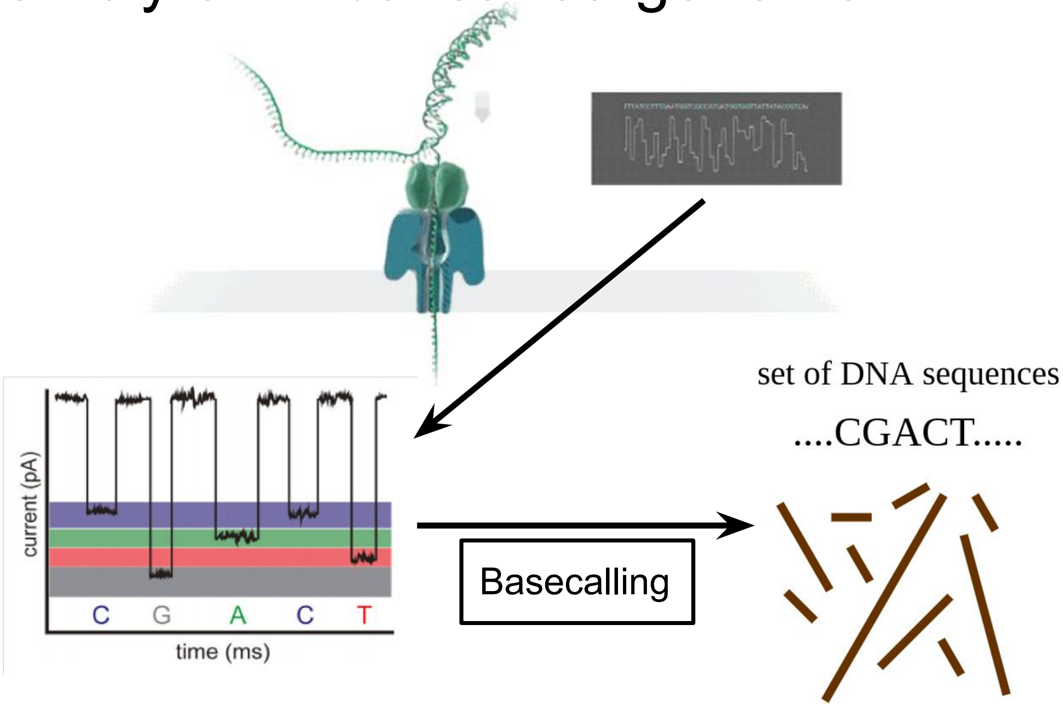
Pictures: Baksan Neutrino Observatory site (https://www.inr.ru/bno/), map of Caucasus (https://www.grida.no/resources/7628)

Sequencing and assembly of *B. baksanea* genome



Oxford Nanopore® sequencing technique is based on ion current registration through nano-sized protein pore while DNA molecule traverses through the pore at the same time.

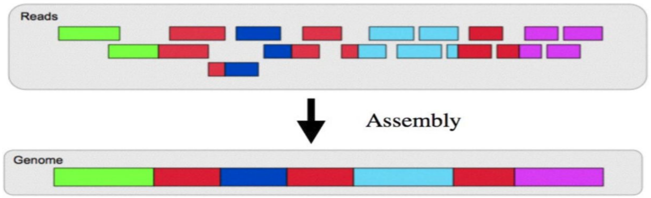
Basecalling is a process ofdetermining of type nucleotide by neural network processing of ion current versus time plot.



set of DNA sequences

....CGACT.....

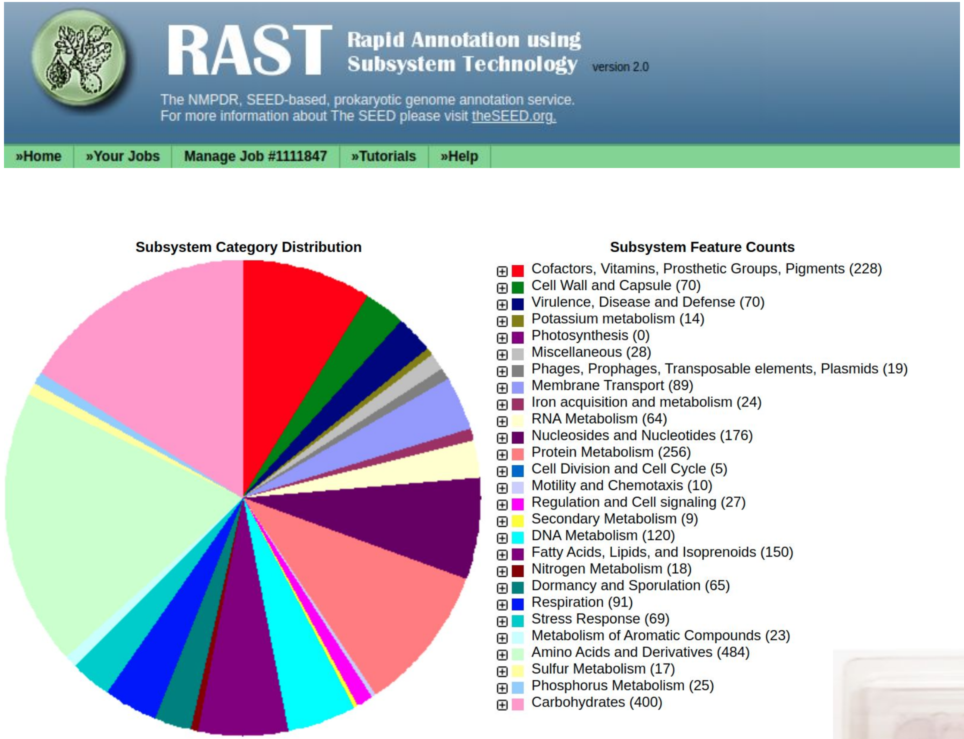
Basecalling



After sequencing one ends up with DNA sequences called **reads.** To obtain the genomic DNA reads must be assembled into one (in case of bacteria) continuous molecule.

Pictures: gif image (http://en.biomarker.com.cn/nanopore-sequencing), article about nanopore sequencing on Ksivalue (https://ksivalue.com/nanoporeru), lecture on bioinformatics by Jared Simpson from the Ontario Institute for Cancer Research (https://www.youtube.com/watch?v=5wvGapmA5zM&ab_channel=BioinformaticsDotCa), article about nanopore sequencing (https://www.medgadget.com/2015/09/nanopore-minion-novel-dna-sequencing-device-diagnosis-treatment-guidance-urinary-tract-infections.html)

Gene annotation and metabolic tests of *B. baksanea*



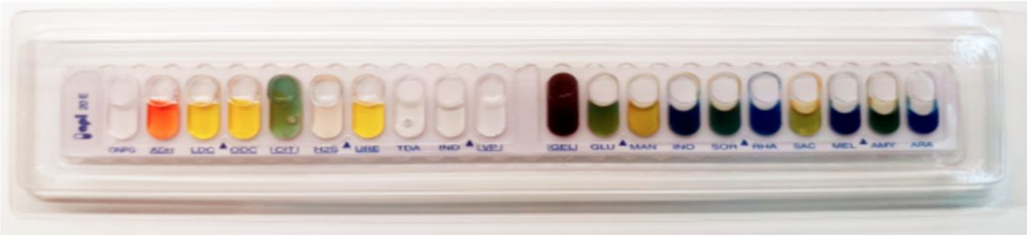
The NMPDR, SEED-based, prokaryotic genome annotation service.
For more information about The SEED please visit theSEED.org.

Subsystem Feature Counts

⊞ Cofactors, Vitamins, Prosthetic Groups, Pigments (228)
⊞ Cell Wall and Capsule (70)
⊞ Virulence, Disease and Defense (70)
⊞ Potassium metabolism (14)
⊞ Photosynthesis (0)
⊞ Miscellaneous (28)
⊞ Phages, Prophages, Transposable elements, Plasmids (19)
⊞ Membrane Transport (89)
⊞ Iron acquisition and metabolism (24)
⊞ RNA Metabolism (64)
⊞ Nucleosides and Nucleotides (176)
⊞ Protein Metabolism (256)
⊞ Cell Division and Cell Cycle (5)
⊞ Motility and Chemotaxis (10)
⊞ Regulation and Cell signaling (27)
⊞ Secondary Metabolism (9)
⊞ DNA Metabolism (120)
⊞ Fatty Acids, Lipids, and Isoprenoids (150)
⊞ Nitrogen Metabolism (18)
⊞ Dormancy and Sporulation (65)
⊞ Respiration (91)
⊞ Stress Response (69)
⊞ Metabolism of Aromatic Compounds (23)
⊞ Amino Acids and Derivatives (484)
⊞ Sulfur Metabolism (17)
⊞ Phosphorus Metabolism (25)
⊞ Carbohydrates (400)

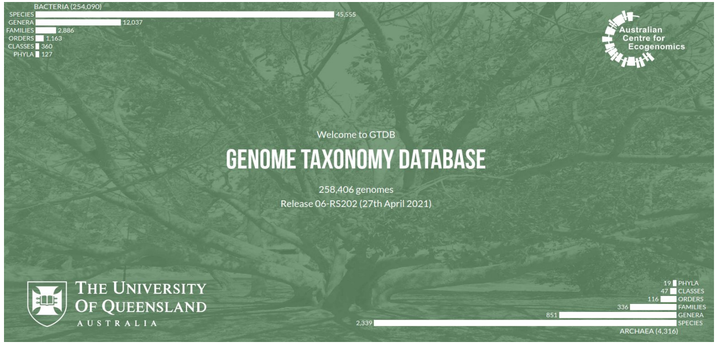
Metabolic features of *B. baksanea:*

1. C_1-compounds utilization (methanol, formaldehyde, methylamines)
2. Inability to utilize most part of surface-common substrates
3. Riddance of heavy ions (cobalt, cadmium, zinc, copper)
4. Resistance to termo- and osmotic stresses.



Phylogenetic analysis and future work

Phylogenetic analysis infers that studied bacteria is a new species of extremotolerant organism for which name *Bacillus baksanea* is suggested. It is most related to *Cytobacillus oceanisediminis,* also extremophylic bacteria, inhabiting marine sediments.

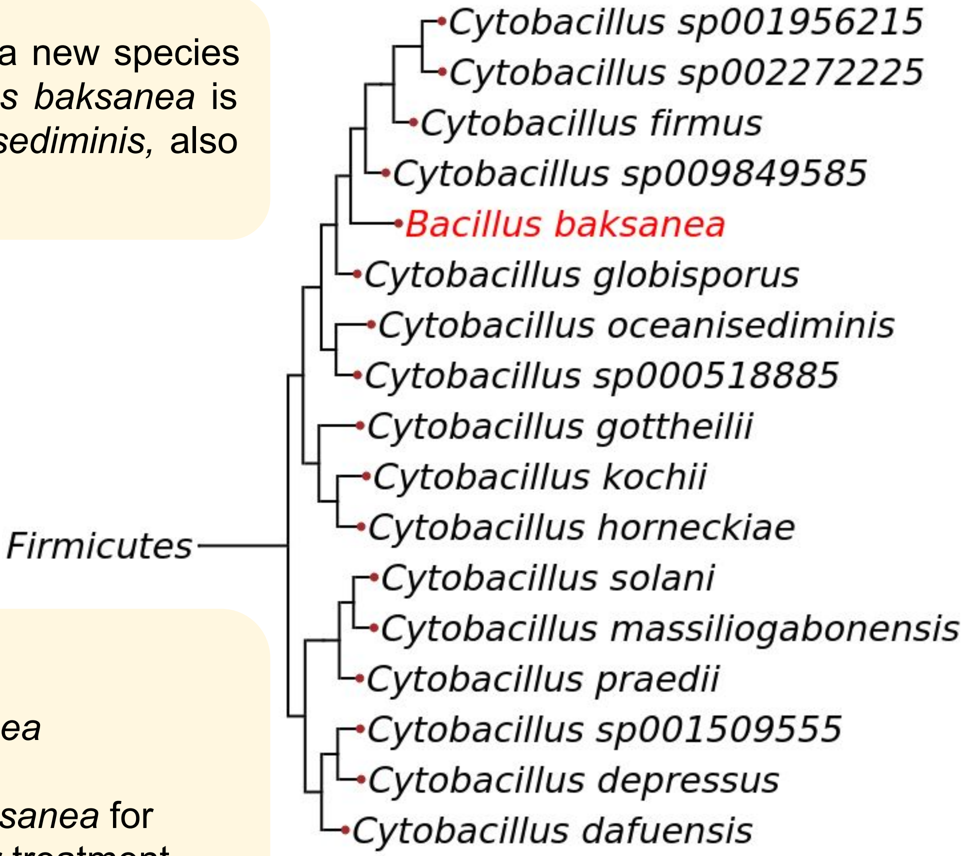


Welcome to GTDB

GENOME TAXONOMY DATABASE

258,406 genomes

Release 06-RS202 (27th April 2021)



Future plans:

1. Further metabolic characterization of *B. baksanea*
2. Characterization of bacteriophage
3. Assessment of biotechnological value of *B. baksanea* for methanol utilization and heavy metal ions water treatment

Thank you for your attention!