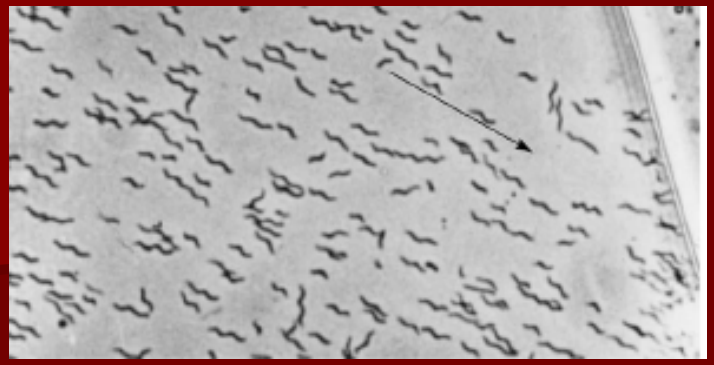
BACTERIAL MAGNETOSOMES

Magnetospirillum sp. strain AMB-1



Anežka Džarová

supervisor: Dr M. Timko

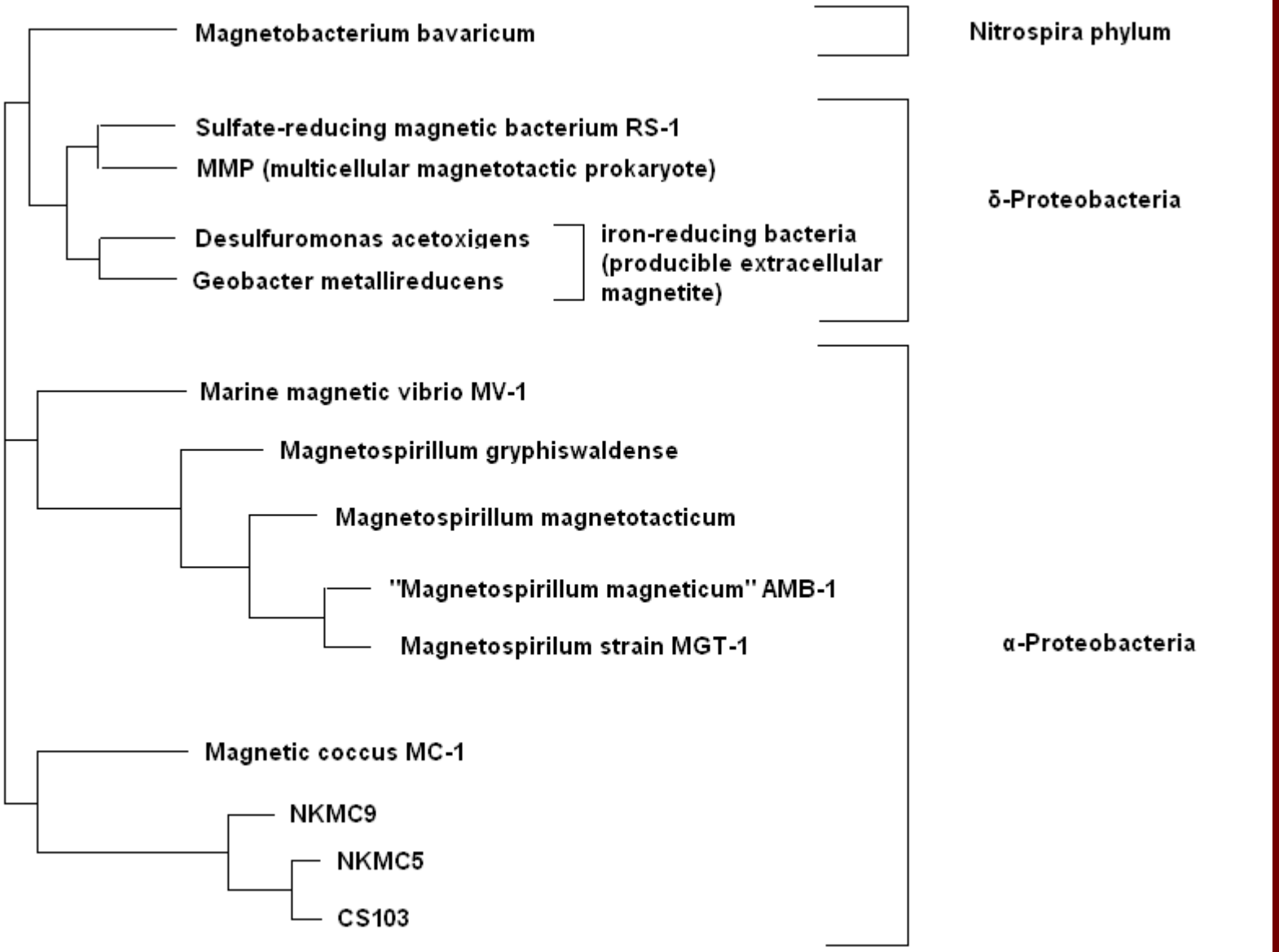
Our research interests in the section of the magnetism:

- preparation magnetic particles :

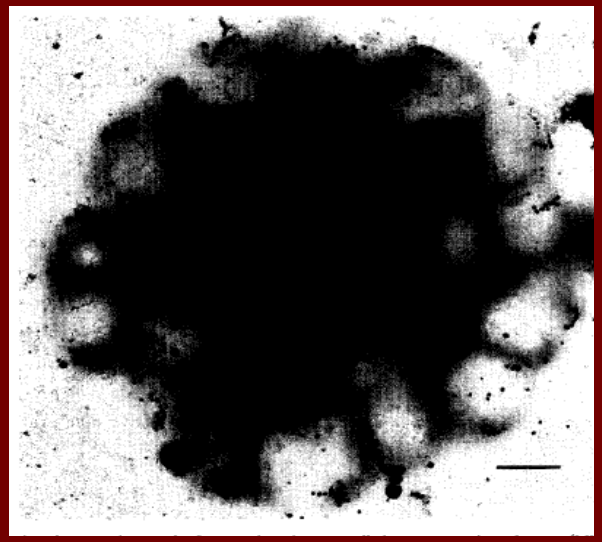
biomineralizations (natural mag. particles)

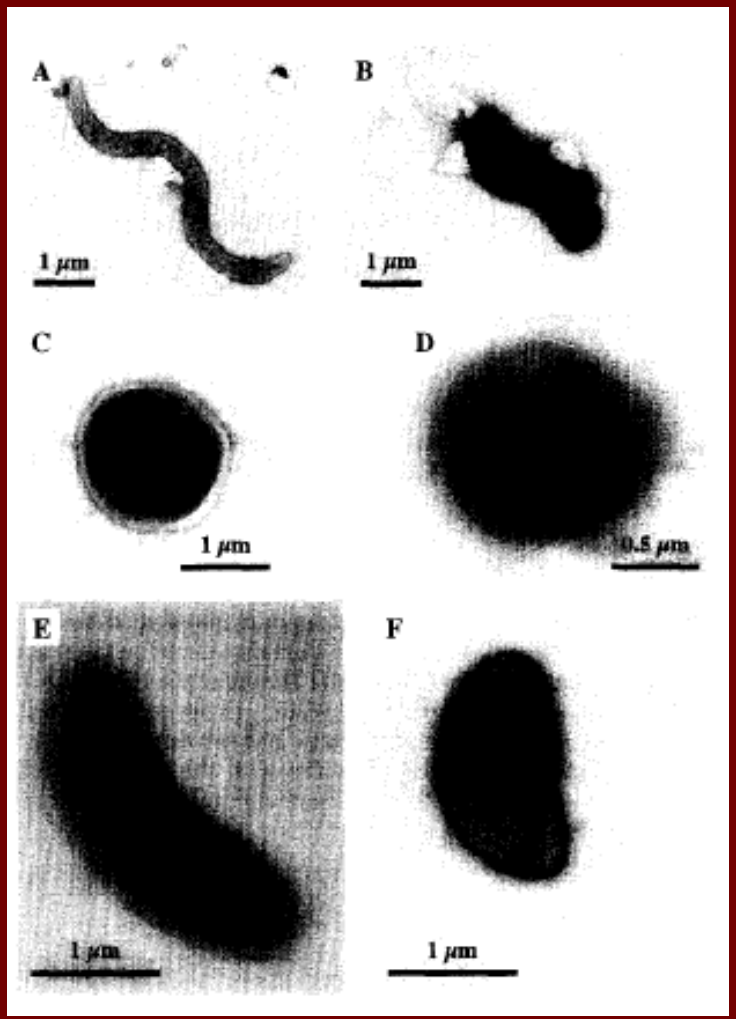
- a new biological magnetic particles (magnetosomes) was found as a product of biomineralization process from magnetotactic bacteria. The encapsulation of magnetosomes within the organic membrane provides a natural coating, which ensures superior dispersibility of the Fe_3O_4 particles and provides excellent target for immobilization biologically active substances

Dendrogram


Magnetobacterium bavaricum
Nitrospira phylum
Sulfate-reducing magnetic bacterium RS-1
MMP (multicellular magnetotactic prokaryote)
δ-Proteobacteria
Desulfuromonas acetoxigens
Geobacter metallireducens
iron-reducing bacteria (producible extracellular magnetite)
Marine magnetic vibrio MV-1
Magnetospirillum gryphiswaldense
Magnetospirillum magnetotacticum
"Magnetospirillum magneticum" AMB-1
Magnetospirilum strain MGT-1
α-Proteobacteria
Magnetic coccus MC-1
NKMC9
NKMC5
CS103

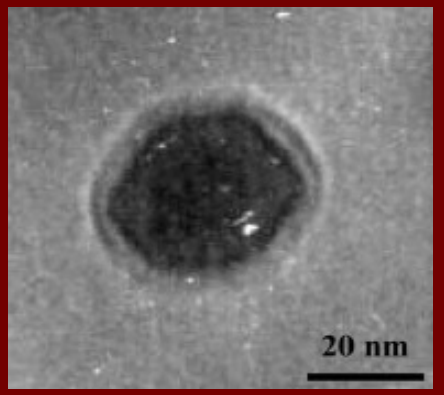
- Blakemore (1975)
- they are constituents of natural microbial communities in sediments and chemically stratified water columns and a broad diversity of morfological forms has been found in many marine and freshwater habitats





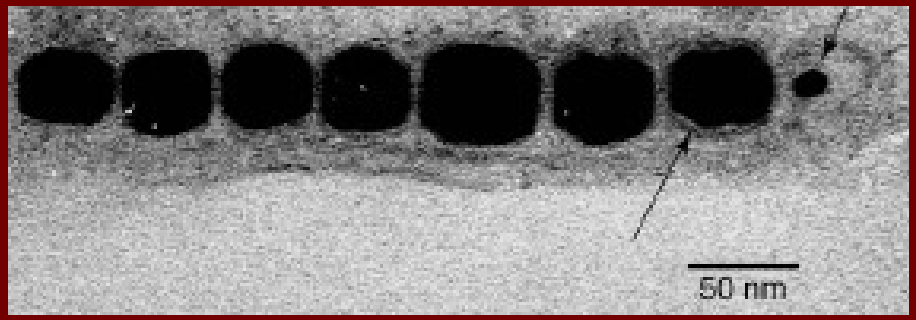
- MTB orient and migrate along geomagnetic field lines – this ability is based on intracellular magnetic structures known as –

  MAGNETOSOMES



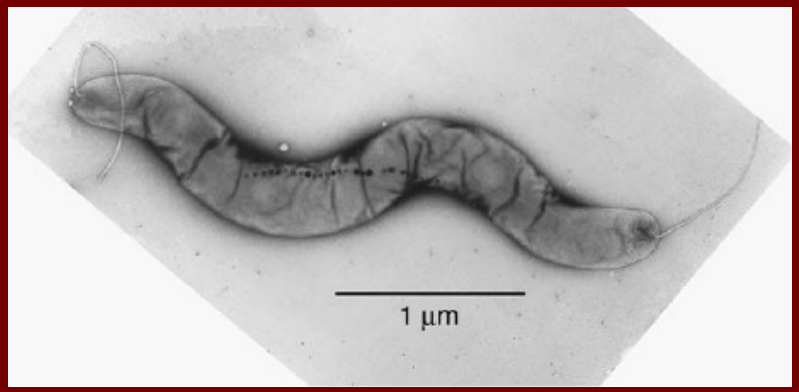
[an intracellular single-magnetic-domain crystal of a magnetic iron mineral (the magnetite) to be enclosed by a membrane (a lipid bilayer admixed with proteins) the membrane is intracellular and may be connected to the cytoplasmic membrane]

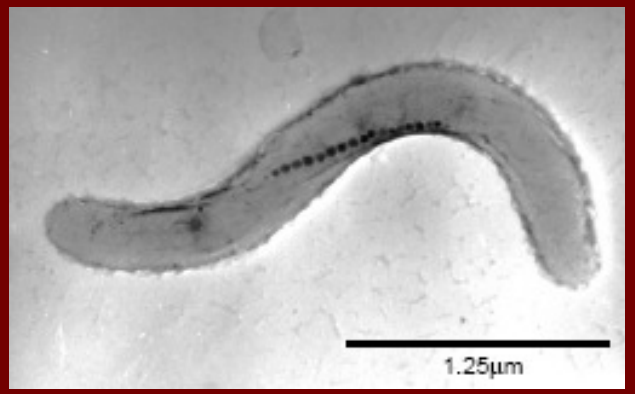
- AMB-1 have single chain of magnetosomes longitudinally traverses the cell



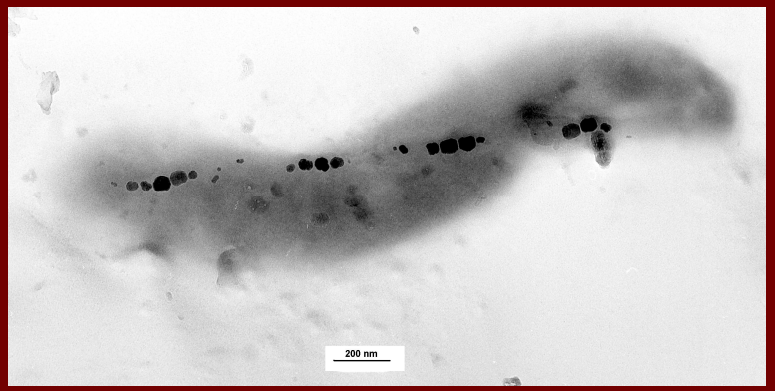
In my experiments magnetosomes were prepared from MTB strain Magnetotacticum Magnetospirillum :

- heterogeneous group of Gram-negative bacteria
- group of aquatic prokaryotes





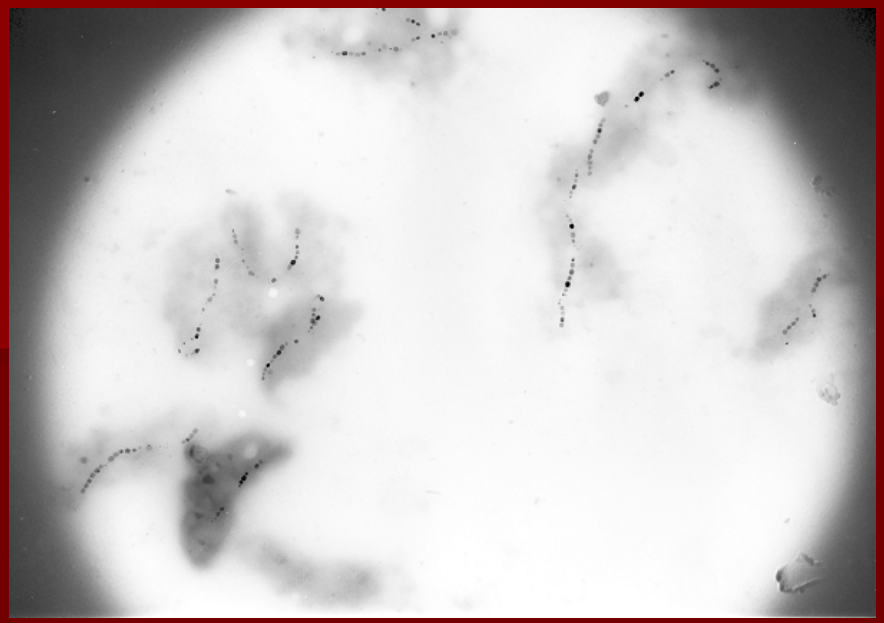
- strain AMB-1 is Gram-negative α-proteobacterium that more oxygen-tolerant

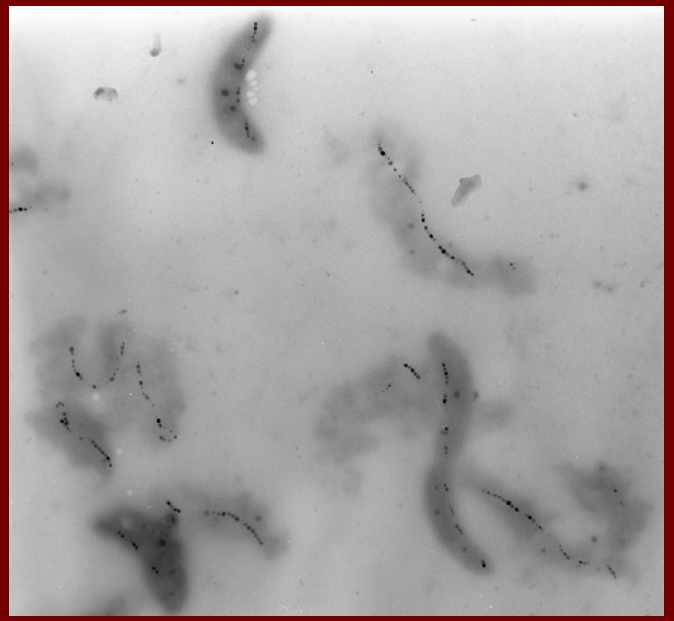


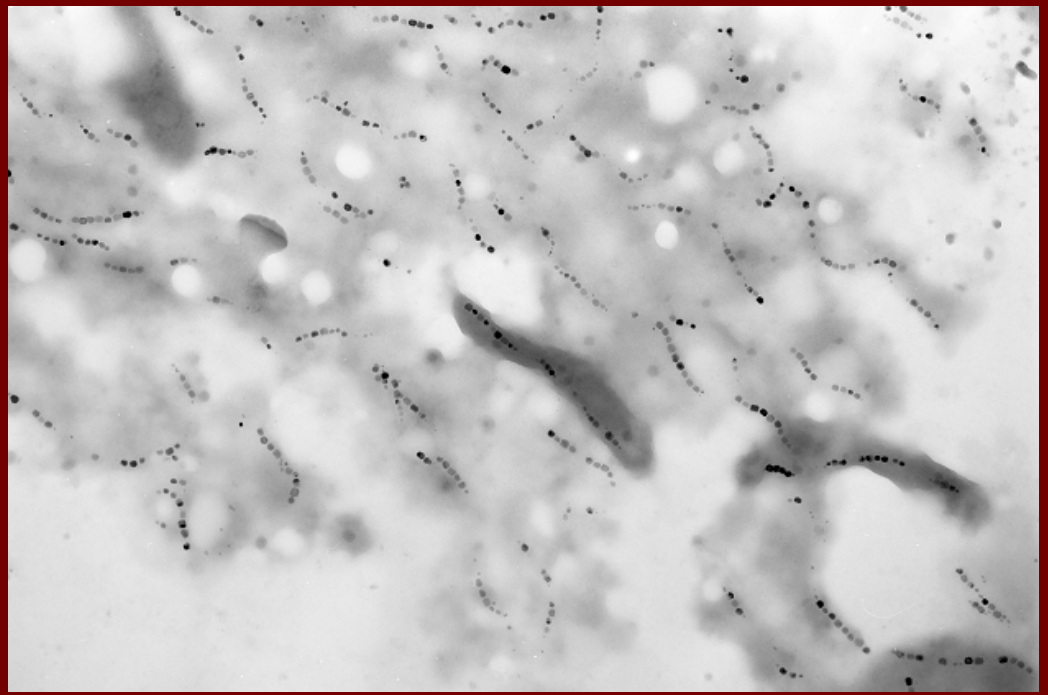
In our experiment for cultivation *Magnetotacticum Magnetospirillum* sp. AMB-1 we used medium consisted of (per 1 L medium):

10 mL Wolfe's vitamin solution
5 mL Wolfe's mineral solution
0.68 g KH_2PO_4
0.848 g sodium succinate hexahydrate
0.575 g sodium tartrate dihydrate
0.083 g sodium acetate trihydrate
0.225 mL 0.2% (w/v) resazurin (aqueous)
0.17 g $NaNO_3$
0.04 g ascorbic acid
2 mL 0.01 M ferric quinate (quinic acid + $FeCl_3.6H_2O$)

- resazurin was added to media as colorimetric indicator of redox potential
- the pH was adjusted to 6.75 with NaOH
- this medium was prereduced under nitrogen for a period of 1 hour, using copper as a reducing agent, and was subsequently dispensed into culture tubes in an anaerobic hood
- inoculated tubes were incubated at 25°C for a period of 4 days



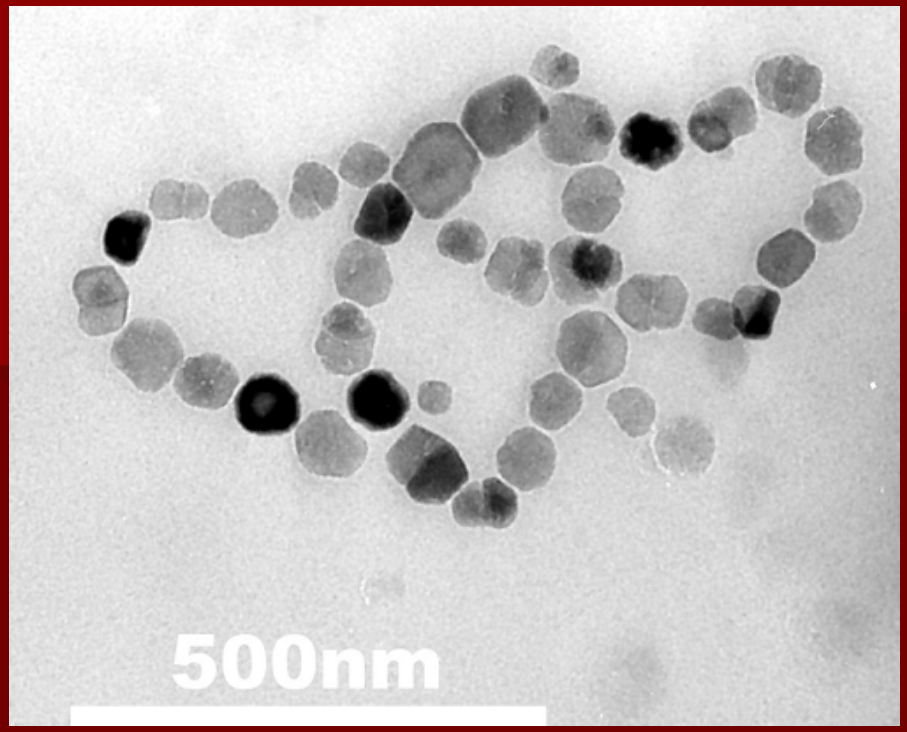


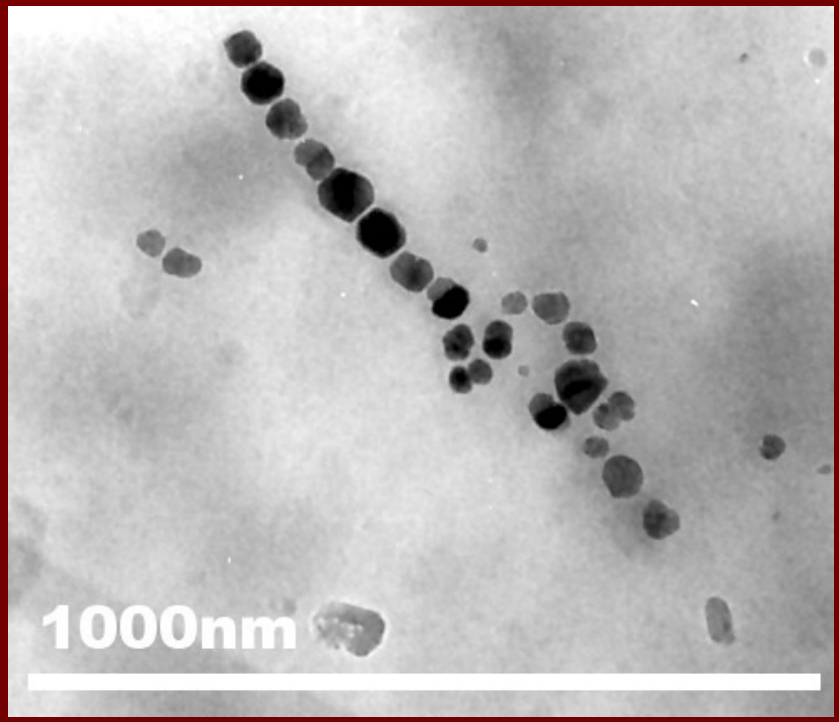


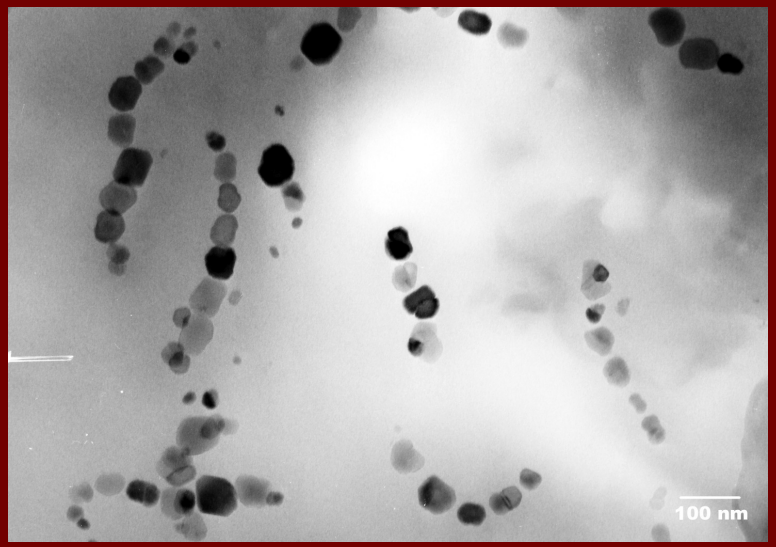
Techniques for the isolation and purification of magnetosome particles from *Magnetotacticum Magnetospirillum* species:

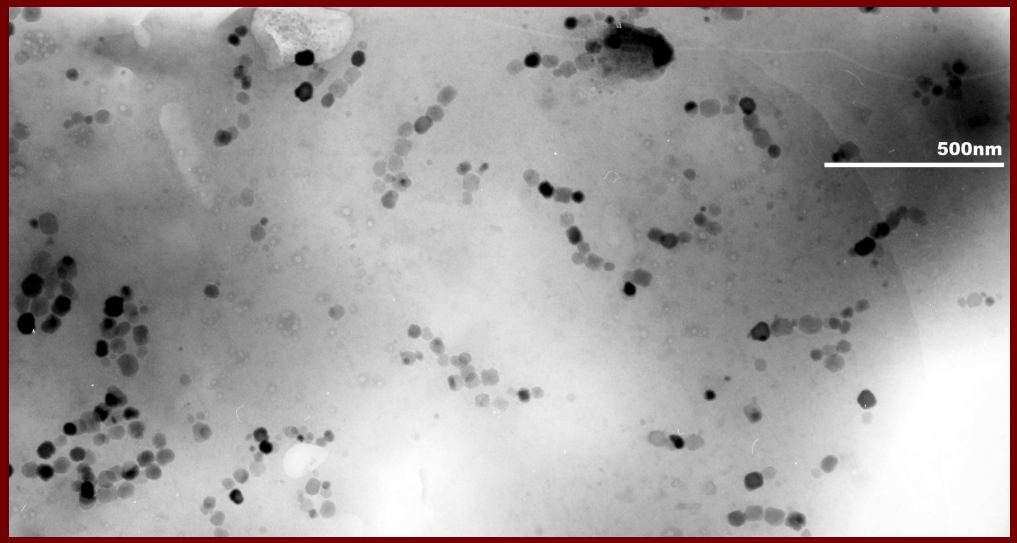
Karen Grünberg, Cathrin Wawer, Bradley M. Tebo, Dirk Schüler, *A large Gene Cluster Encoding Several Magnetosome Proteins Is Conserved in Different Species of Magnetotactic Bacteria*, App. Environ. Microbiol. 2001, 67(10):4573-4582

- are based on magnetic separation or a combination of a sucrose-gradient centrifugation and a magnetic separation technique


500nm

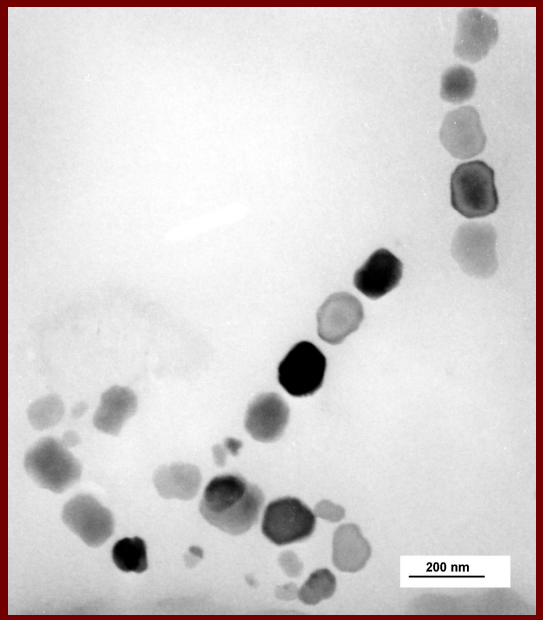

1000nm


100 nm


500nm

MAGNETOSOMES:

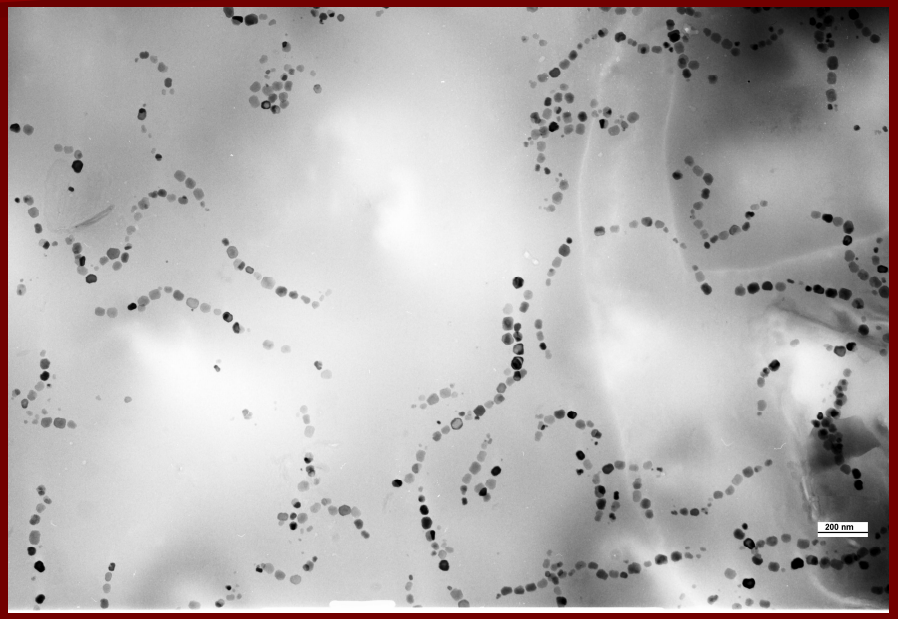
The prepared magnetosomes in our laboratory were examined by TEM , XRD, IČ, SEM, DLS (Dynamic light scattering) and magnetic measurement were examined by SQUID magnetometer Quantum Design:

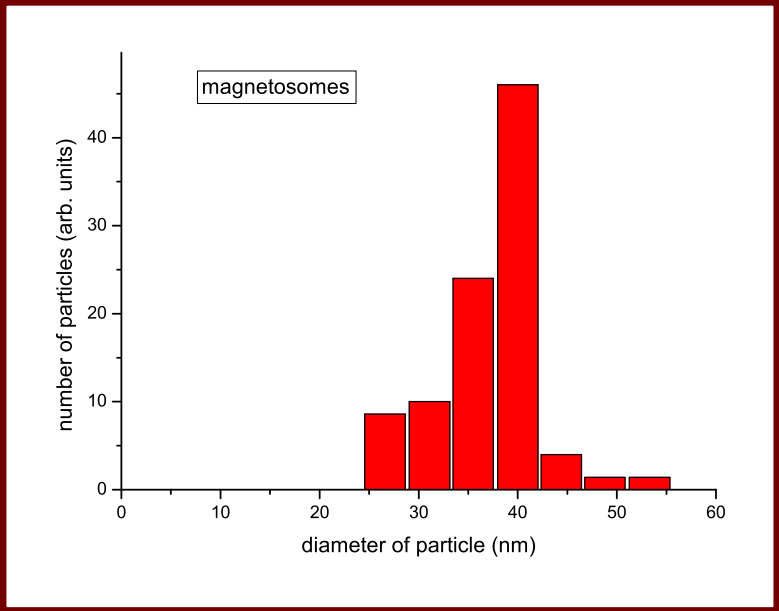


- comprise nanometer –sized, membrane-bound crystals (bacterial magnetic particles) of the magnetic iron minerals magnetite (Fe_3O_4)

TRANSMISION ELECTRON MICROSCOPY

- the mean size of our magnetosomes estimated from TEM was 34 nm





SCANNING ELECTRON MICROSCOPY

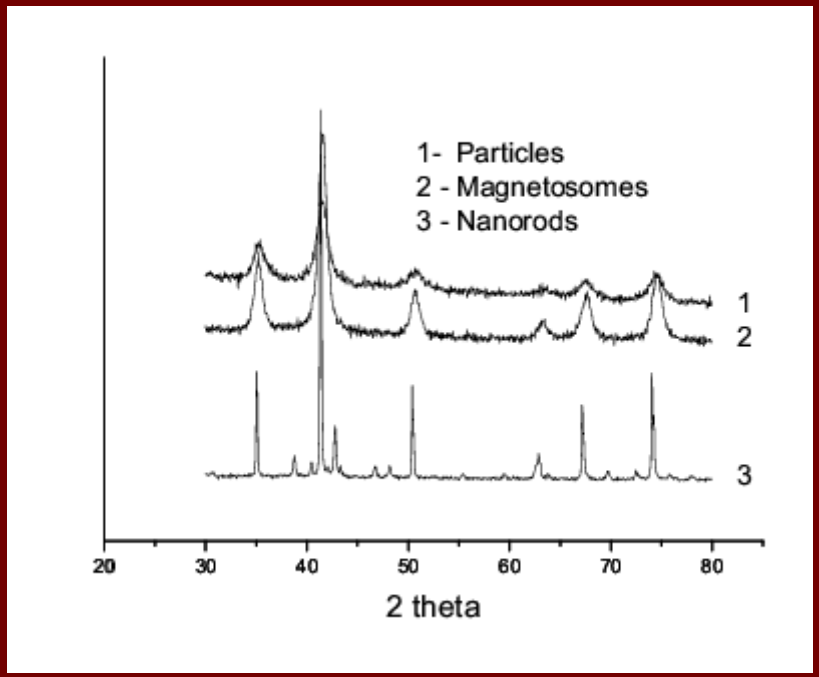
- the mean size of our magnetosomes estimated from SEM was 35 nm

X-RAY DIFFRACTION

- the mean size of our magnetosomes estimated from and XRD 37 nm

SCHERRER EQUATION:

$$D = 0.9\,\lambda / \beta \cos\theta$$

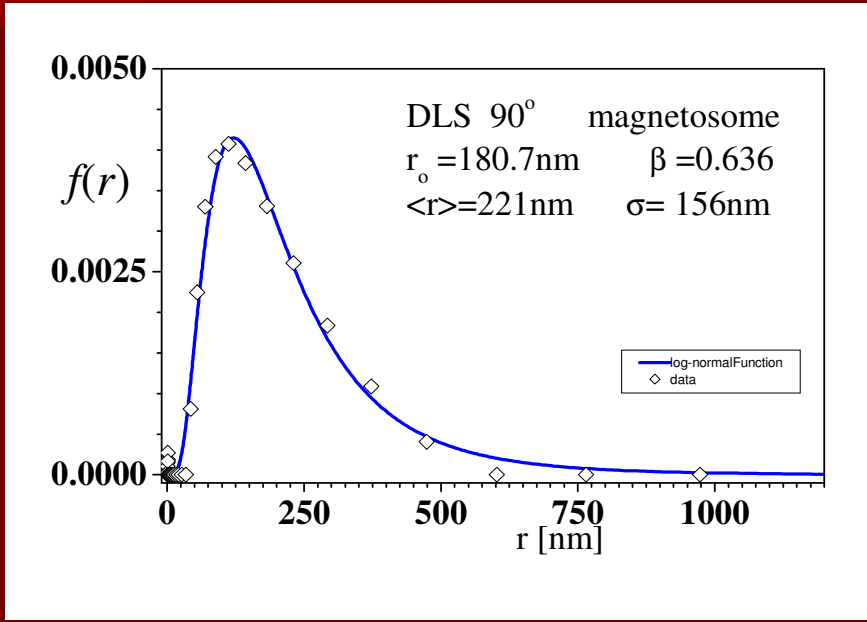


λ - wavelenght of the incident X-ray

Θ - diffraction angle

β - full-width at half-maximum of corresponding diffraction peaks

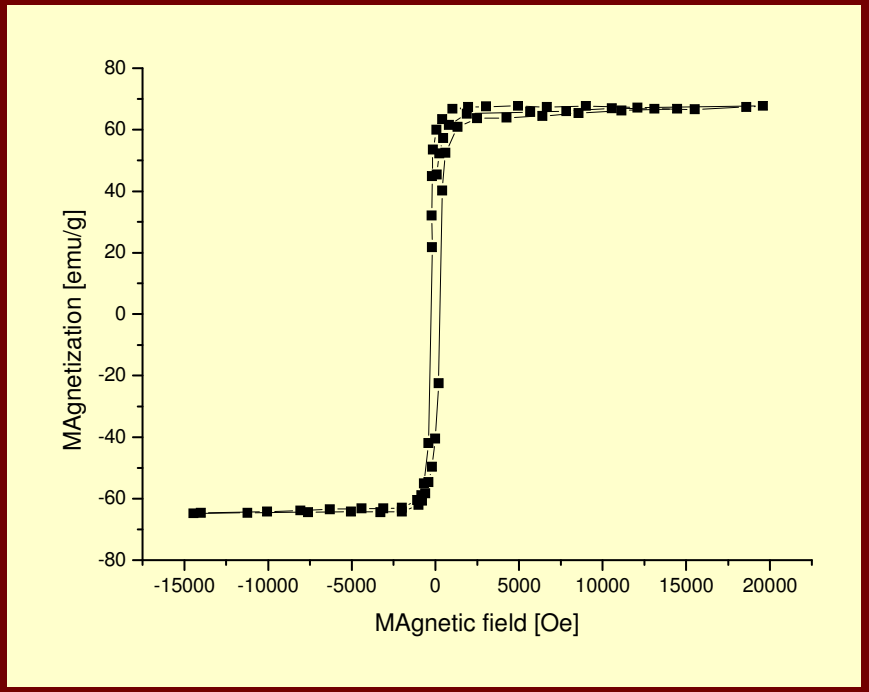
DLS (Dynamic light scattering)



Magnetic properties were examined by SQUID magnetometer Quantum Design:

- the saturation magnetization of the magnetosomes was estimated to be 62 emu/g what is smaller than for chemically synthetized magnetite 75 emu/g at room temperature due to presence of nonmagnetic organic layer

- the curve of field dependence of magnetization at 293 K exhibited the remanence of 21 emu/g



- coercivity of 185 Oe what is connected with fact that the mean diameter (34 nm) is larger than critical size for transition from superparamagnetic to ferrimagnetic behaviour

Cultivation *Magnetotacticum*
Magnetospirillum sp. AMB-1:


26.3.2009 pred naočkovaním
2009 3 26


20.3.09 1ml
po 3 dňoch
(23.3.2009)
2009 3 23


20.3.09 1ml
po 4 dňoch
2009 3 24


20.3.09 1ml
po 6 dňoch (26.3.2009)
2009 3 26

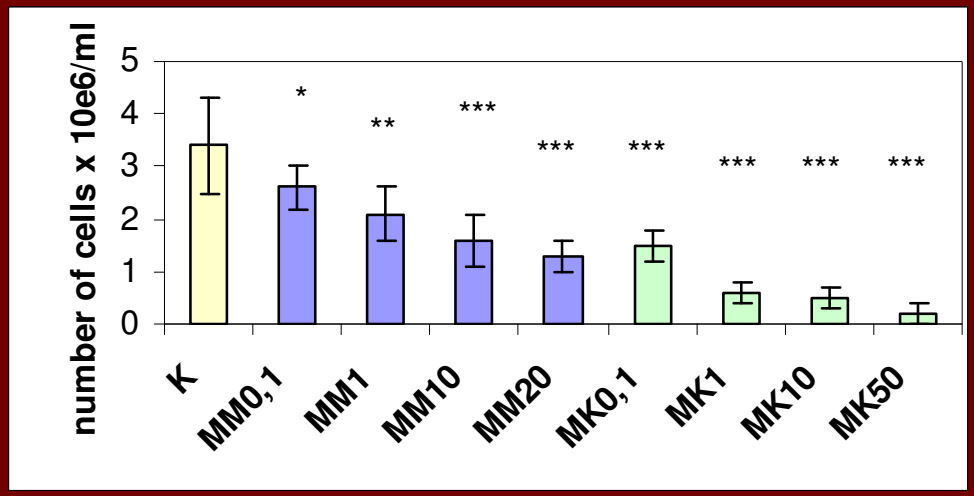
10 mL Wolfe's vitamin solution
5 mL Wolfe's mineral solution
0.68 g KH_2PO_4
0.848 g sodium succinate hexahydrate
0.575 g sodium tartrate dihydrate
0.083 g sodium acetate trihydrate
0.225 mL 0.2% (w/v) resazurin (aqueous)
0.17 g $NaNO_3$
0.04 g ascorbic acid
2 mL 0.01 M ferric quinate (quinic acid + $FeCl_3.6H_2O$)


+0,5 ml WMS
12.3.09
1,5 ml
+ 0,5 ml WVS
12.3.09
2 ml
+WMS
po 6 dňoch
+ WVS
2009 3 18


+ 0,5 ml FQ
12.3.09
2 ml
+ 1 ml FQ
12.3.09
2 ml
+ 1 ml FQ
12.3.09
2 ml
po 6 dňoch
+ FQ 1/0,5ml
2009 3 18

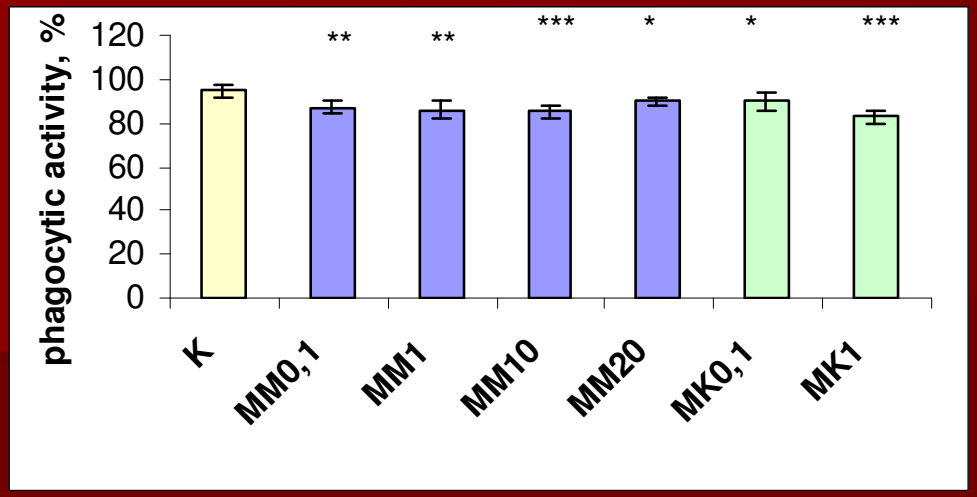

12.3.09
2 ml
12 3 09
2 ml
12.3.09
2,5 ml
12.3.09
2,5 ml
12.3.09
3,5 ml
2 ml
2,5 ml
3,5 ml
12.3.09
2 ml
12.3.09
2 ml
1 ml
po 6 dňoch
2009 3 18

The influence of magnetite nanoparticles on human leukocyte activity

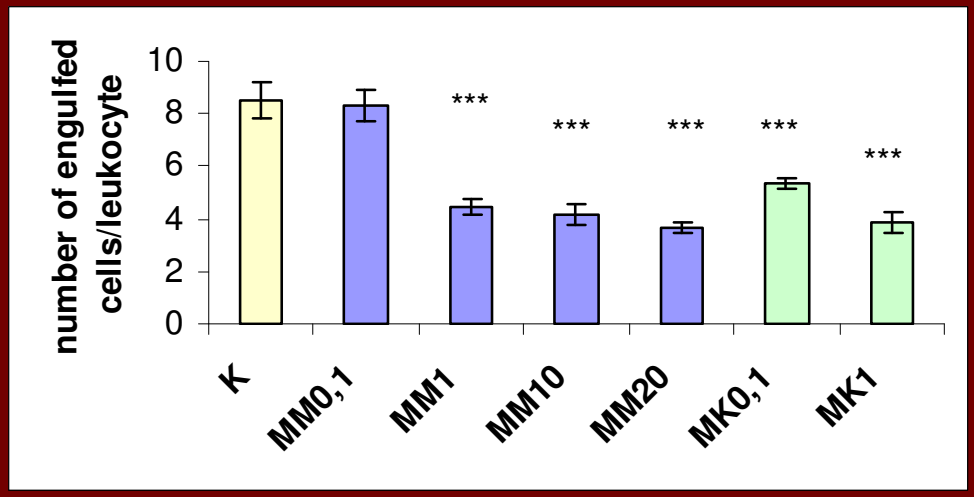


Number of leukocytes/ml for incubation in CO_2 at 37°C

K – control sample
MM – sample of leukocytes with magnetosomes (bacterial magnetite nanoparticles) of concentration: 0,1, 1, 10, 20 µg/ml
MK – sample of leukocyte with synthetic magnetic nanoparticles of concentration: 0,1, 1, 10, 50 µg/ml

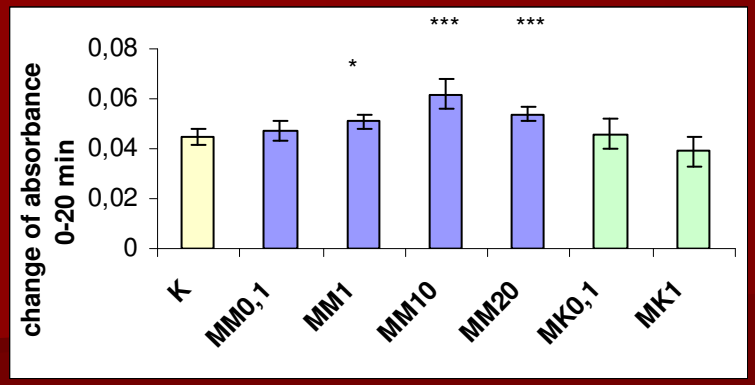


Number of phagocytosing cells from 100 phagocytes

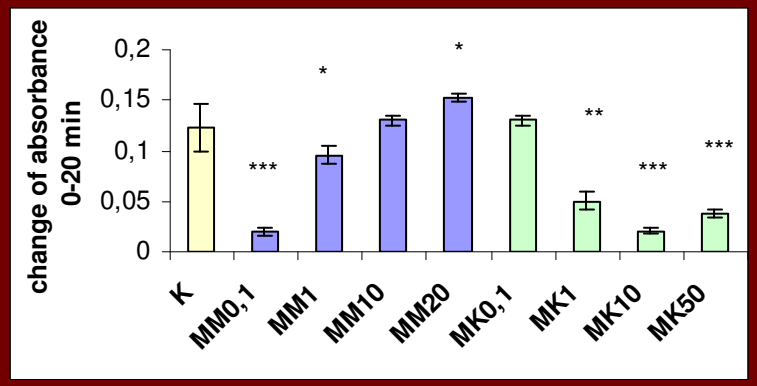


Number of engulfed cells per one leukocyte

K – control sample
MM – sample of leukocytes with magnetosomes (bacterial magnetite nanoparticles) of concentration: 0,1, 1, 10, 20 μg/ml
MK – sample of leukocyte with synthetic magnetic nanoparticles of concentration: 0,1, 1, 10, 50 μg/ml



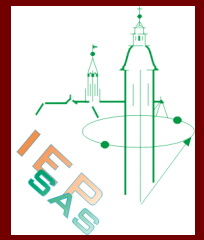
Lyzozyme activity expressed change of absorbance (410 nm) after 20 minutes of reaction



Peroxidase activity expressed change of absorbance (490 nm) after 20 minutes of reaction

K – control sample
MM – sample of leukocytes with magnetosomes (bacterial magnetite nanoparticles) of concentration: 0,1, 1, 10, 20 µg/ml
MK – sample of leukocyte with synthetic magnetic nanoparticles of concentration: 0,1, 1, 10, 50 µg/ml

CONCLUSION:



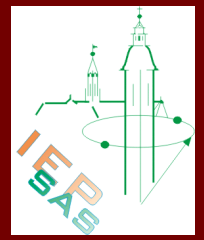
- successful cultivation and isolation
- magnetosome in MTB are arranged in straight chains
- after isolation from MTB those chains tend to form closed loops
- the mine size is 34 nm (TEM, SEM) or 37 nm (XRD)
- saturation magnetization of the magnetosomes Ms=62 emu/g
- coercivity Hc=185 Oe
- remanence Mr= 21 emu/g
- the both tested samples lysed (killed) leukocyte cells during incubation. From obtained results follow that spherical nanoparticles are more aggressive material than magnetosomes

PUBLICATIONS:

- TIMKO, Milan - DŽAROVÁ, Anežka - ZÁVIŠOVÁ, Vlasta – KOVÁČ, Jozef – ŠPRINCOVÁ, Adriana - KONERACKÁ, Martina - KOPČANSKÝ, Peter – TOMAŠOVIČOVÁ, Natália - GOJZSEWSKI, Hubert – SKUMIEL, Andrzej – JOZEFCZAK, Arkadiusz – VÁVRA, Ivo Magnetic Properties and Heating Effect in Bacterial Magnetic Nanoparticles. In ***Journal of Magnetism and Magnetic Materials***, 321 (2009) 1521–1524

- KOPČANSKÝ, Peter - TOMAŠOVIČOVÁ, Natália - KONERACKÁ, Martina - ZÁVIŠOVÁ, Vlasta - TIMKO, Milan - DŽAROVÁ, Anežka - ŠPRINCOVÁ, Adriana - ÉBER, N. - FODOR-CSORBA, K. - TÓTH-KATONA, T. - VAJDA, A. - JADZYN, Jan. Structural changes in the 6CHBT liquid crystal doped with spherical, rodlike, and chainlike magnetic particles. In ***Physical Review E***. ISSN 1539-3755, 2008, vol. 78, part 1, p. 011702-1-5.

- TIMKO, Milan - DŽAROVÁ, Anežka - KOPČANSKÝ, Peter - ZÁVIŠOVÁ, Vlasta - KONERACKÁ, Martina - KOVÁČ, Jozef - ŠPRINCOVÁ, Adriana - VACLAVÍKOVÁ, Miroslava - IVANIČOVÁ, Lucia - VÁVRA, Ivo. Magnetic Properties of Magnetite Formed by Biomineralization and Chemical Synthesis. In ***Acta Physica Polonica A***. ISSN 0587-4246, 2008, vol. 113, no. 1, p. 573-576.



- TIMKO, Milan - DŽAROVÁ, Anežka - KOVÁČ, Jozef – KOPČANSKÝ, Peter - GOJZSEWSKI,, Hubert – SZLAFEREK , Andrzej. Magnetic Properies of Bacterial Nanoparticles, In ***Acta Physica Polonica A****, 2009, vol 115, no. 1, 381-383*

- TIMKO, Milan - DŽAROVÁ, Anežka - ZÁVIŠOVÁ, Vlasta - KONERACKÁ, Martina - ŠPRINCOVÁ, Adriana - KOPČANSKÝ, Peter - KOVÁČ, Jozef - VÁVRA, Ivo - SZLAFEREK, A. Magnetic properties of bacterial magnetosomes and chemosynthesized magnetite nanoparticles. In ***Magnetohydrodynamics***. ISSN 0024-998X, 2008, vol. 44, no. 2, p. 113-120.

- DŽAROVÁ, Anežka - TIMKO, Milan - ŠPRINCOVÁ, Adriana - KOPČANSKÝ, Peter - KOVÁČ, Jozef - KONERACKÁ, Martina - VACLAVÍKOVÁ, Miroslava - VÁVRA, Ivo. Formation and magnetic properties of magnetosomes. In ***Materials Structure in Chemistry, Biology, Physics and Technology***. ISSN 1211-5894, 2008, vol. 15, no. 1, p. 10-12.

Thank you for your attention