

Modul 5: Súčasnú požiadavky na prevádzku a užívanie historických budov

Mikrobiologická prítomnosť v interiéri a exteriéri historických objektov (huby, plese, riasy, atď. – podmienky rastu, spôsob sanácie)

Domenico Pangallo

1. – 3. júl 2026, Academia Istropolitana Nova, Svätý Jur

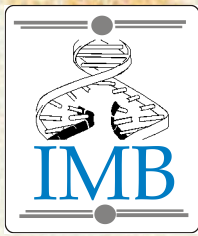




University of MESSINA
ITALY (SICILIA)



Comenius University
BRATISLAVA, SLOVAKIA



Institute of
Molecular
Biology, SAS



Department
of Microbial Ecology

Laboratory
of Environmental
and Food
Microbiology



- Non-invasive sampling
- Sample treatment
- Extraction of DNA and RNA
- Development of suitable DNA/RNA extraction protocols from small amount of samples
- Investigation of environmental microbiota by culture-dependent and culture-independent approaches
- **Study of microbial degradative and deterioration activities**
- Assessment of antimicrobial and cytotoxicity of various materials and chemicals
- **Investigation of the biodegradative microbiota in environments and important cultural heritage items**
- Characterization of the autochthon microbiota in traditional food
- Detection of food-borne pathogens by molecular methods
- Detection of non-declared components in food by DNA analysis

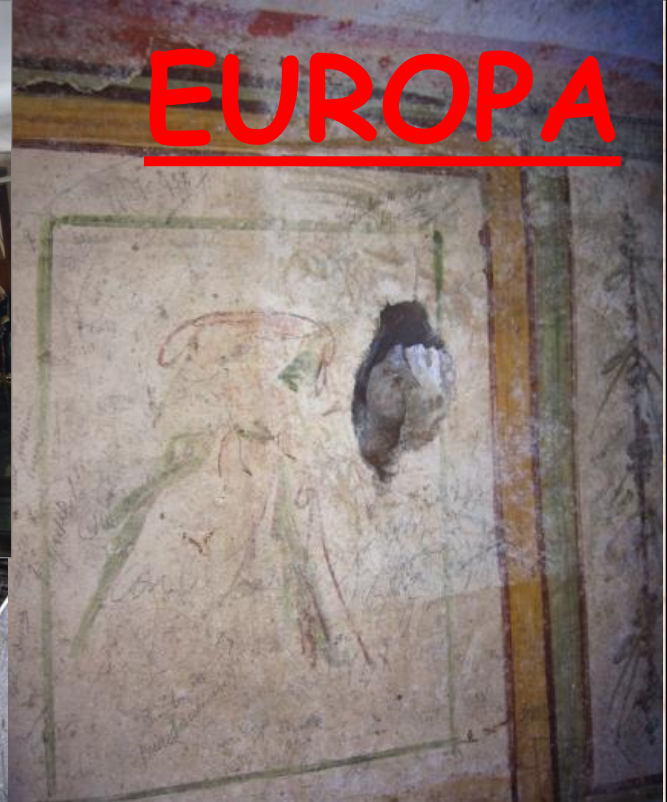
- **Reklama na našu prácu a našu skupinu.**
- **Chcem vám ukázať nejaký výskum, ktorý na Slovensku nie je taký známy.**
- **Možno si nakoniec položíte otázku, či by sa mikrobiológia v tomto kontexte mala brať naozaj vážne.**
- **Mohli by sme vám ponúknuť nejaké riešenia.**



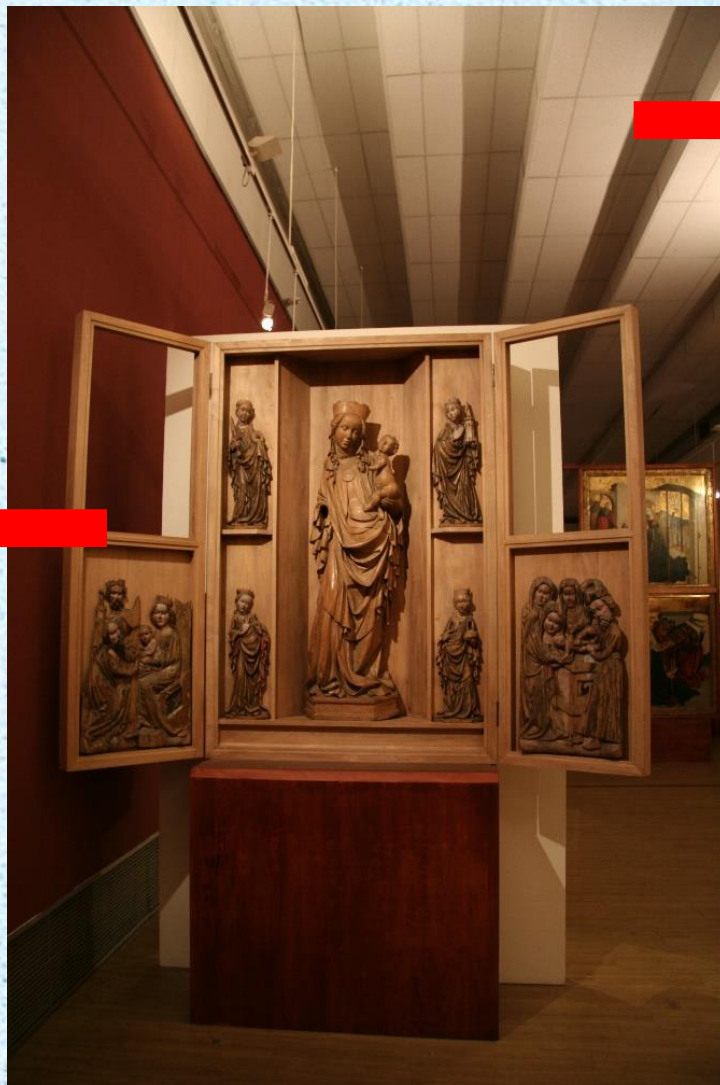
SLOVENSKO



BIODETERIORATION AND PRESERVATION IN ART, ARCHAEOLOGY AND ARCHITECTURE



PREDMET



PROSTREDIE

Pleseň ničí tisíce obrazov

• Dominik Hutko, Pravda 15.08.2017 07:00

Uložiť na Facebook

Páči sa mi to 187

21.03.2017, 06:55 | Správy | Domáce správy | Západné Slovensko

FOTO Ohrozené vzácne obrazy na Trenčianskom hrade: Máriu Teréziu ničí pleseň!

V prístavbe galérie sú len kuny a na stenách plesneň

Stavba stála 111 miliónov korún, na opravu treba milióny eur.

4. apr 2011 o 13:27 Martina Hilbertová

Múzejné zbierky v Michalovciach ohrozujú plesne a vlhkosť

Tohtoročné povodne a vlhký rok narobili problémy aj múzeám. Nadmerná vlhkosť spôsobuje tvorbu plesní.

3. dec 2010 o 0:00 Milan Potocký

Ničí vzácne Hložníkové obrazy plesneň?

• Renáta Jaloviarová, Jakub Prokeš, Pravda 29.05.2017 19:14

Uložiť na Facebook

Páči sa mi to 145

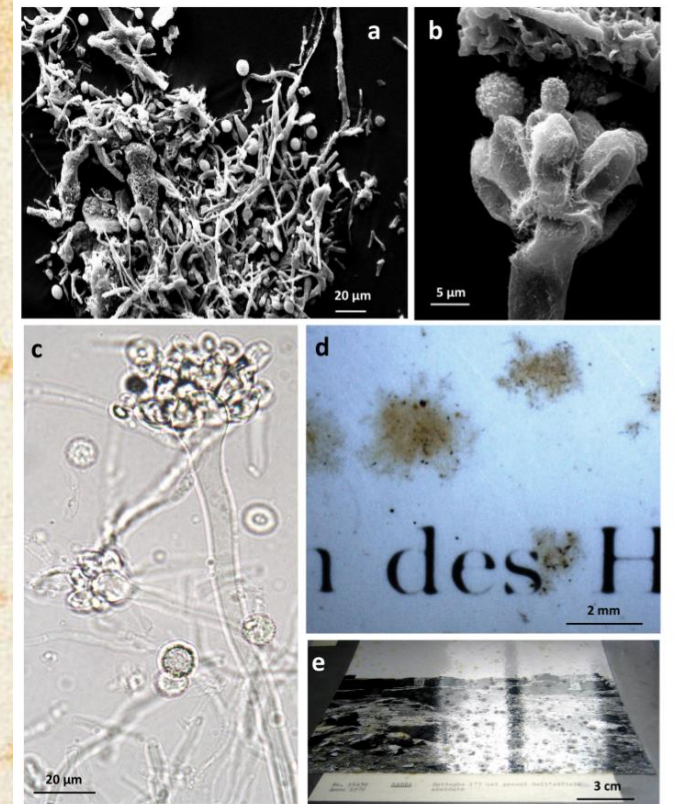
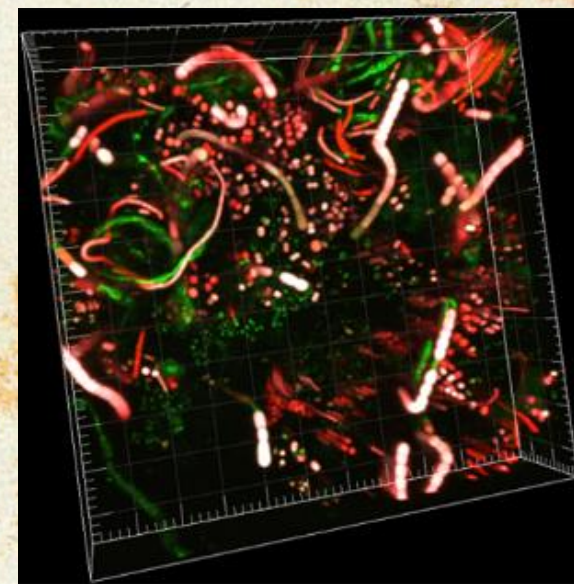
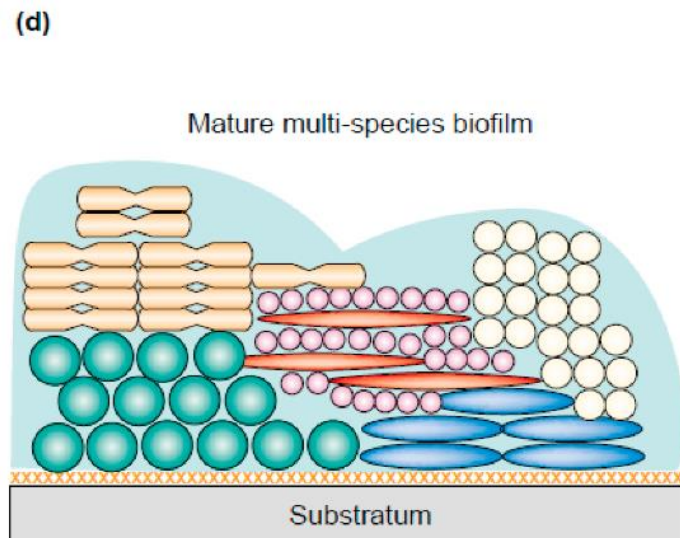
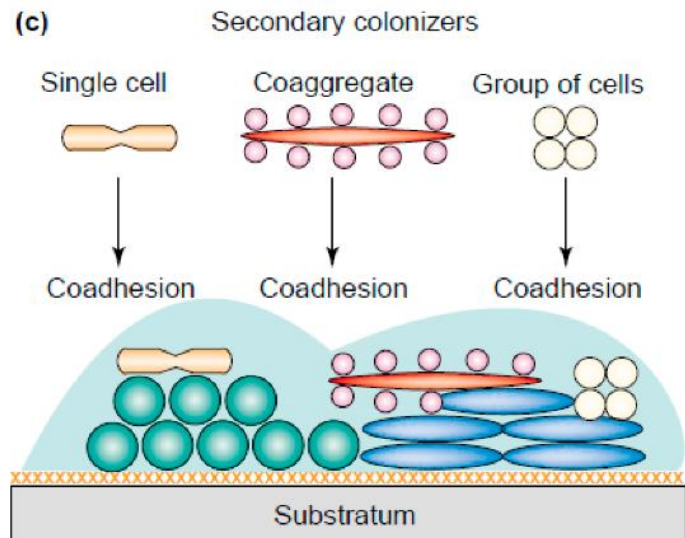
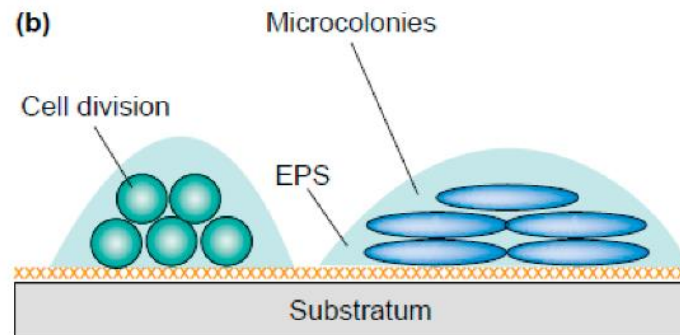
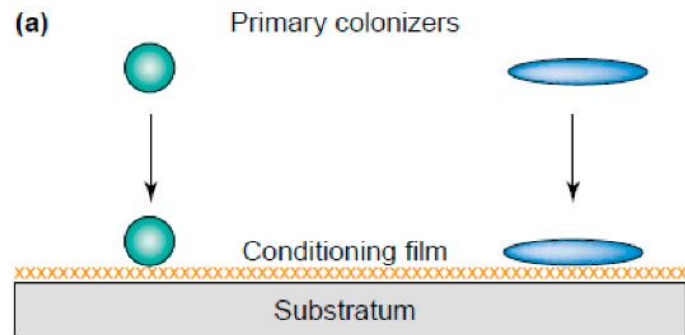
Škandál, aký nemá obdobu. Sú alebo nie sú poškodzované umelecké diela, ktorými jeden z najväčších slovenských umelcov, majster Vincent Hložník, prispel na Národný poklad?

OTÁZKA

NOVINKA

Baktérie alebo kvasinky?

BIOFILM – Mikrobiálne spoločenstva

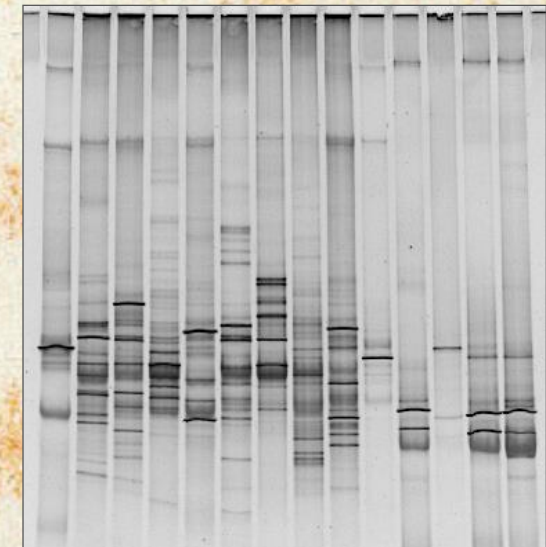
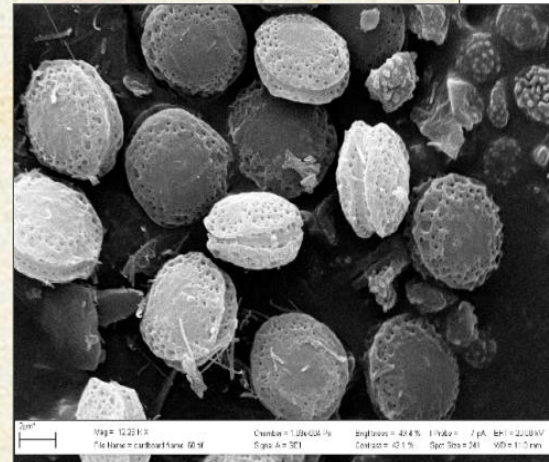
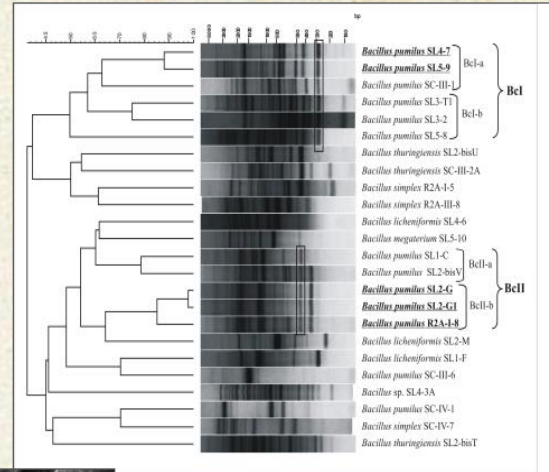


Mikroorganizmy



Like

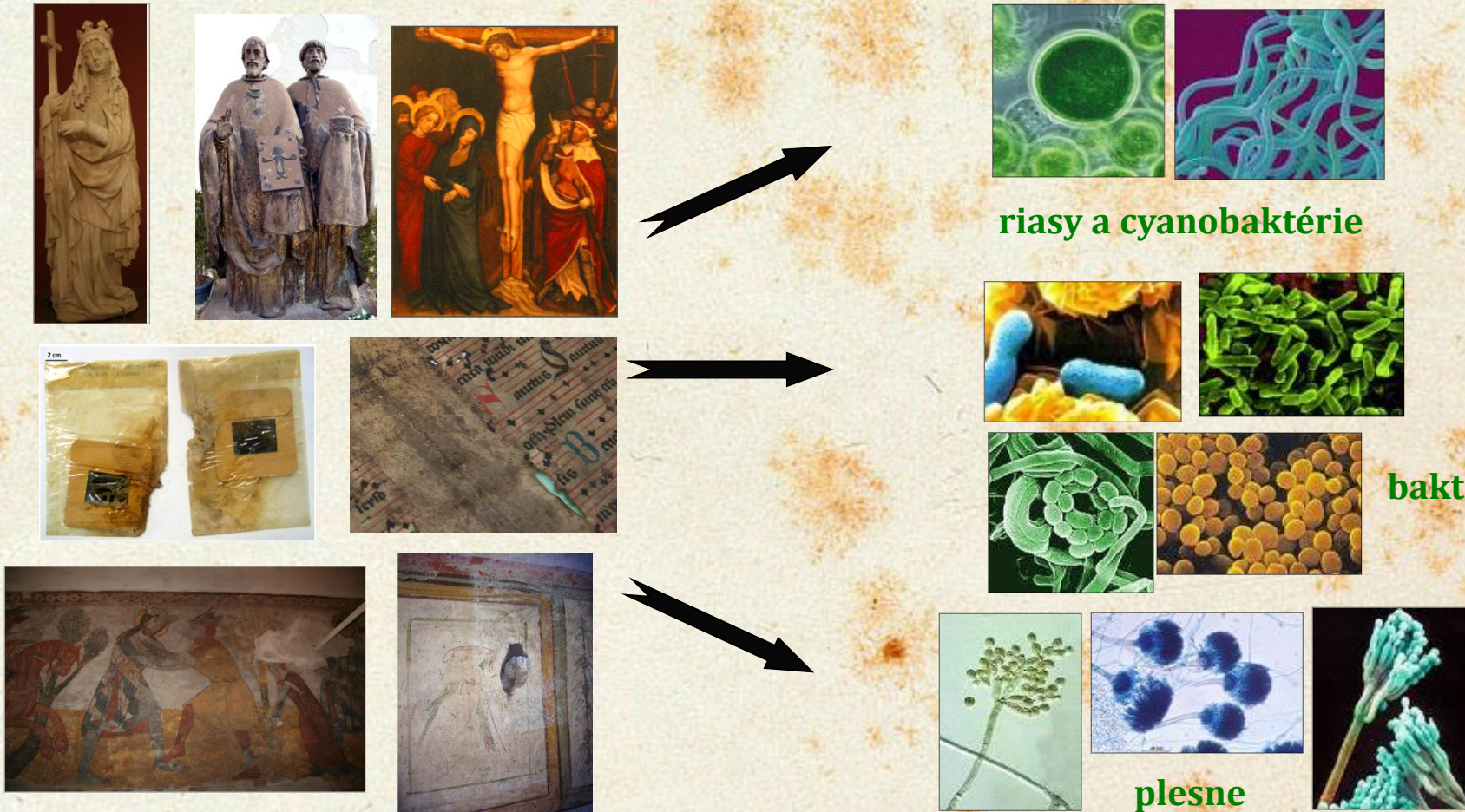
Umenie



Kto je tam a čo robí?

Biodeteriorácia je fyzikálne a chemické poškodenie spôsobené aktivitami živých organizmov.

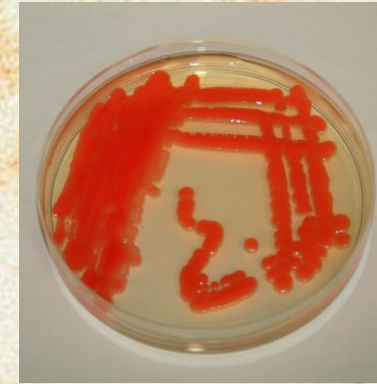
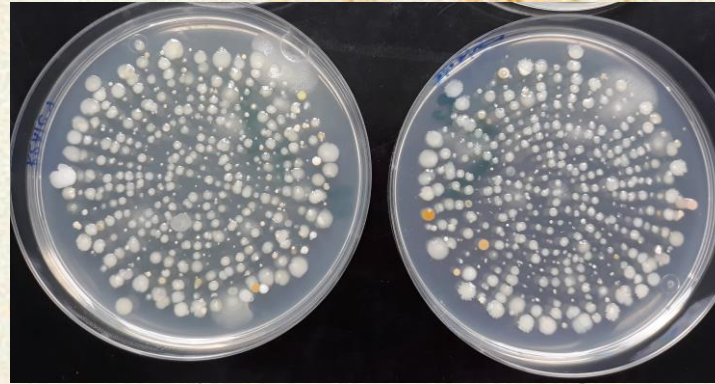
Mikroorganizmy žijú vo viac alebo menej komplexných komunitách a biofilmoch.



Mikrobiálne komunity je možné študovať dvoma rôznymi stratégiami

- kultivačne závislá

dôležité je určenie vhodných podmienok pre izoláciu a kultiváciu čo možno najviac druhov mikroorganizmov

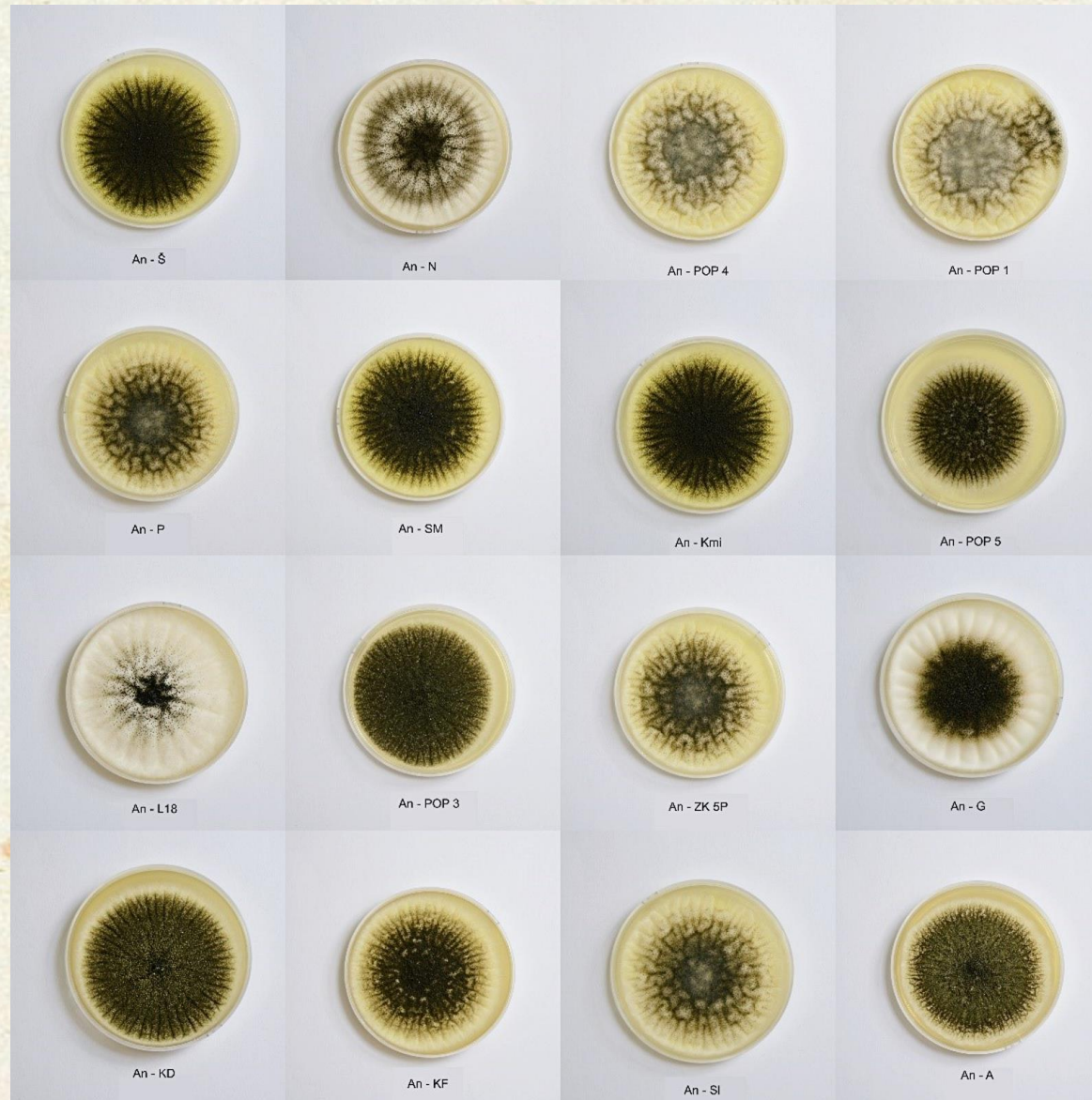


- kultivačne nezávislá

závisí na metódach molekulárnej biológie založenej na analýze nukleových kyselín



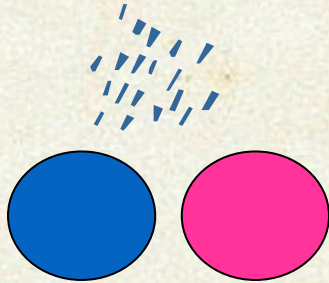
Kultivačně závislé přístupy



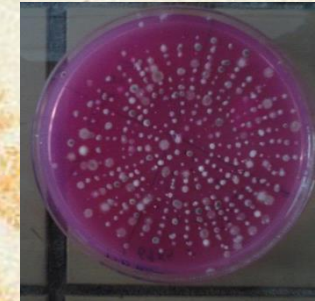
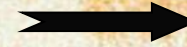
Spôsoby odberu vzoriek na analýzu

Odber vzoriek zo vzduchu

Sedimentačná metóda

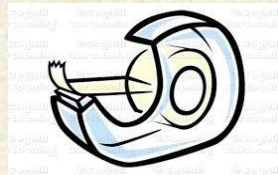


„Air sampler“ metóda



Odber vzoriek z predmetov

Dermatologická lepiaca páska



Sterilný vatový tampón



Sterilná membrána



Skalpel



Mikrobiologické metódy

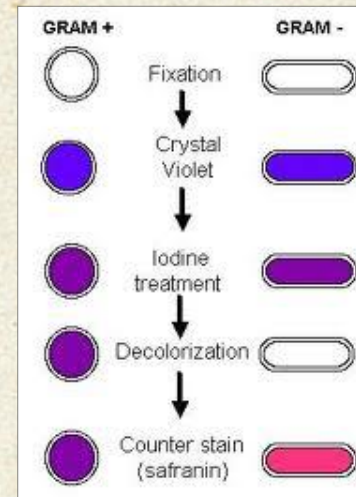
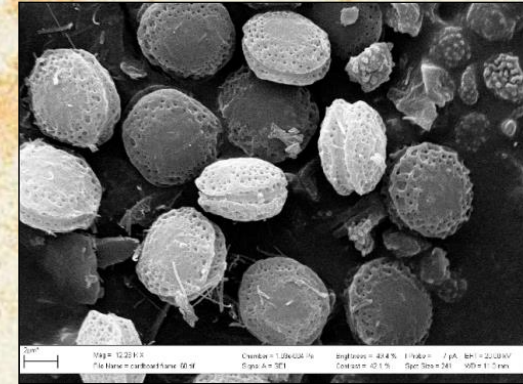
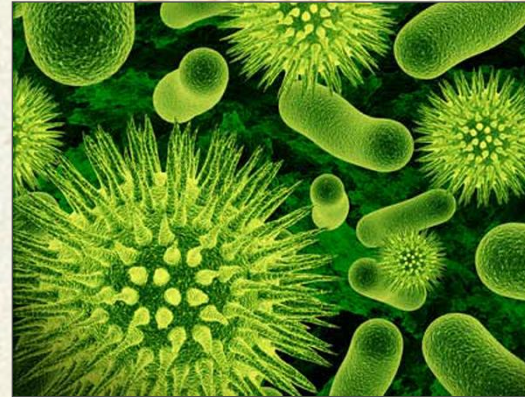
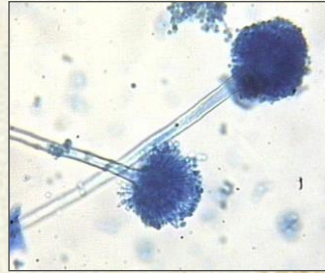
Objekty
záujmu



Odber vzoriek



kultivácia



Gramovo farbenie



API test

Identifikácia pomocou DNA

mikroorganizmy

selekcia mikroorganizmov
pomocou rôznych nástrojov

izolácia DNA

RAMP- PCR

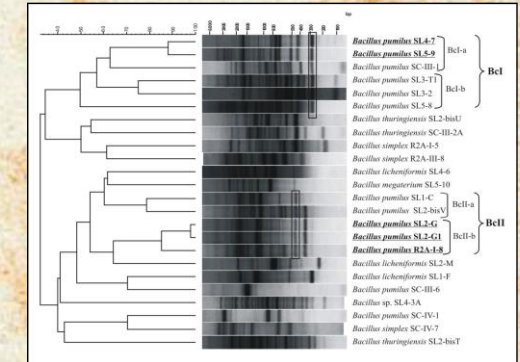
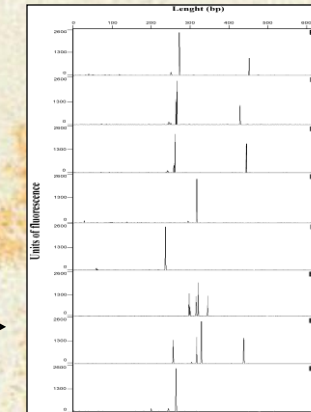
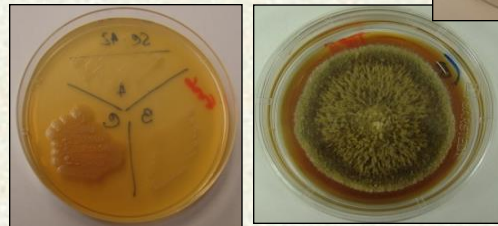
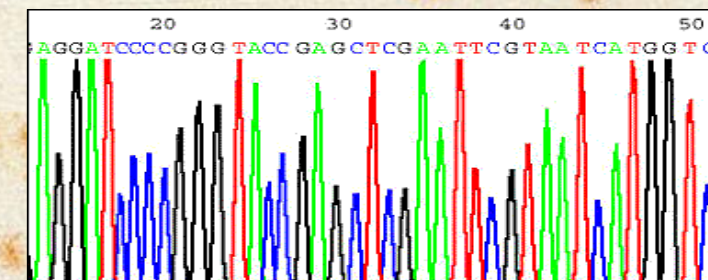
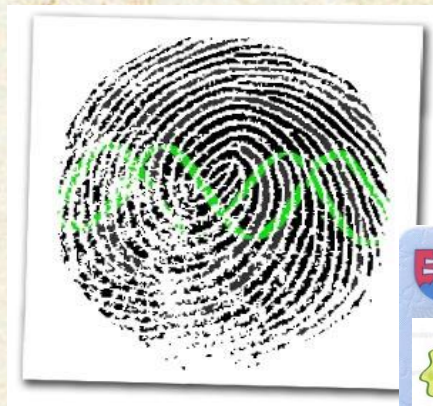
fluorescenčná PCR

Baktérie - 16S rRNA

Plesne - ITS

SANGER sekvenovanie

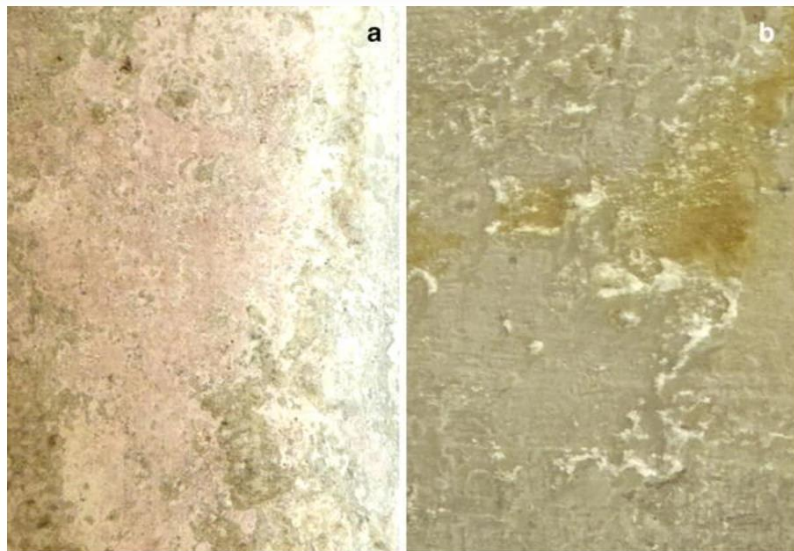
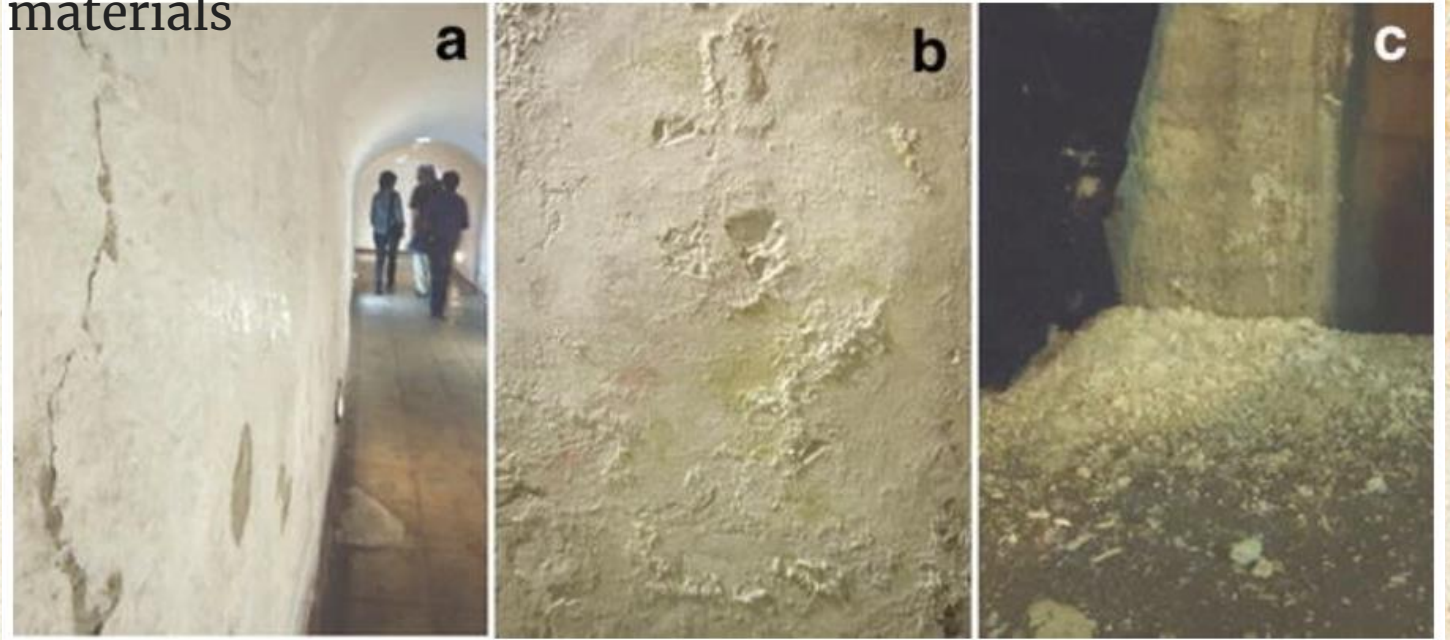
Identifikácia



The Capuchin Catacombs of Palermo



a Water migrating horizontally into the walls of the Catacombs. b Salt crusts of sodium chloride (chlorid sodný) on the walls. c Detached salt contaminating other materials

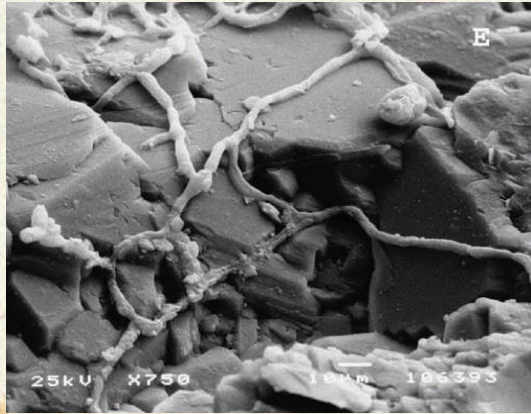


Many of the bacterial isolates were pigmented, ranging from yellow to a strong pink, and were directly related to the discoloration observed in the materials.

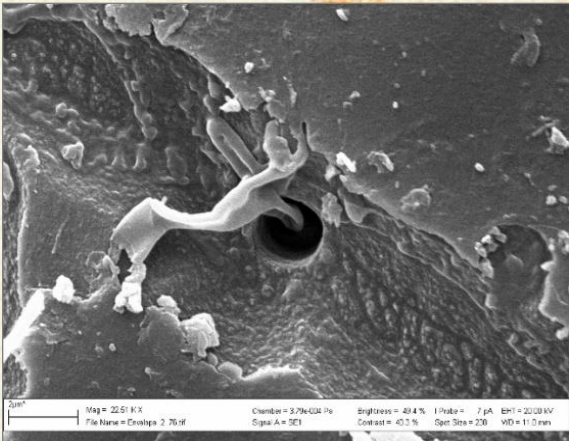
Detected genera: *Oceanobacillus*, *Virgibacillus*, *Halobacillus*, *Idiomarina*, *Bacillus*, *Lysinibacillus*, *Marinococcus* and *Halomonas*

Aktivity mikroorganizmov

1) Rast

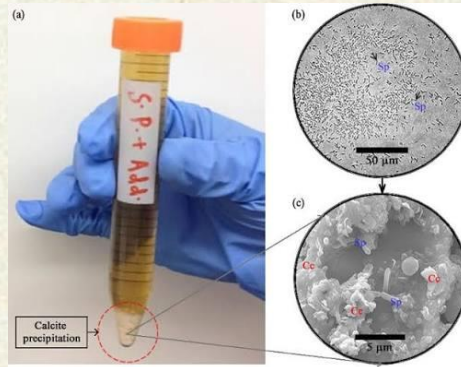


Mikroorganizmy
kolonizujúce sochu

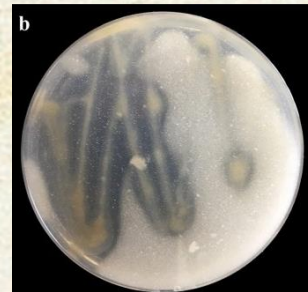


Pleseň vytvárajúca diery
vo fotografii

Zrážanie CaCO_3



Biominingalization



Biosolubilizácia uhličitanu vápenatého

Celulózový Kongo red test



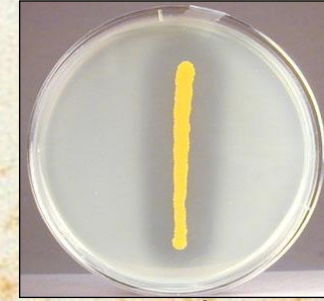
celulóza

Celulolytický test



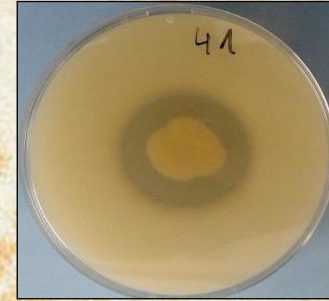
celulóza

Želatínový agar



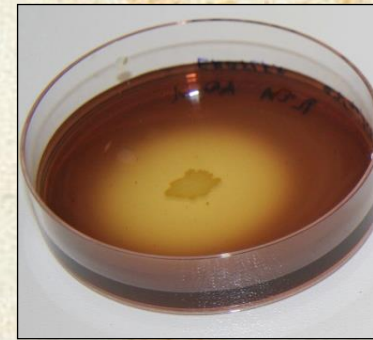
proteíny

Mliečny agar



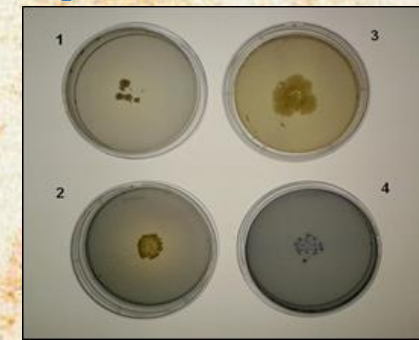
proteíny

Keratinolytický test



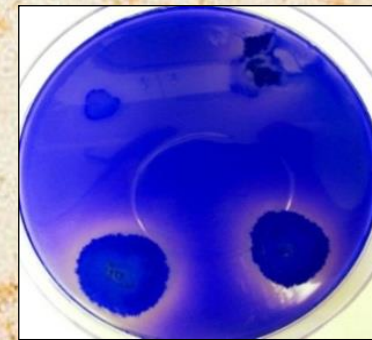
keratín

Spirit Blue médium



tuky

Fibroínový agar



fibroín- hodváb



3) Pigmentácia

Freska Sv. Ladislava - Kostol sv. Kataríny vo Veľkej Lomnici



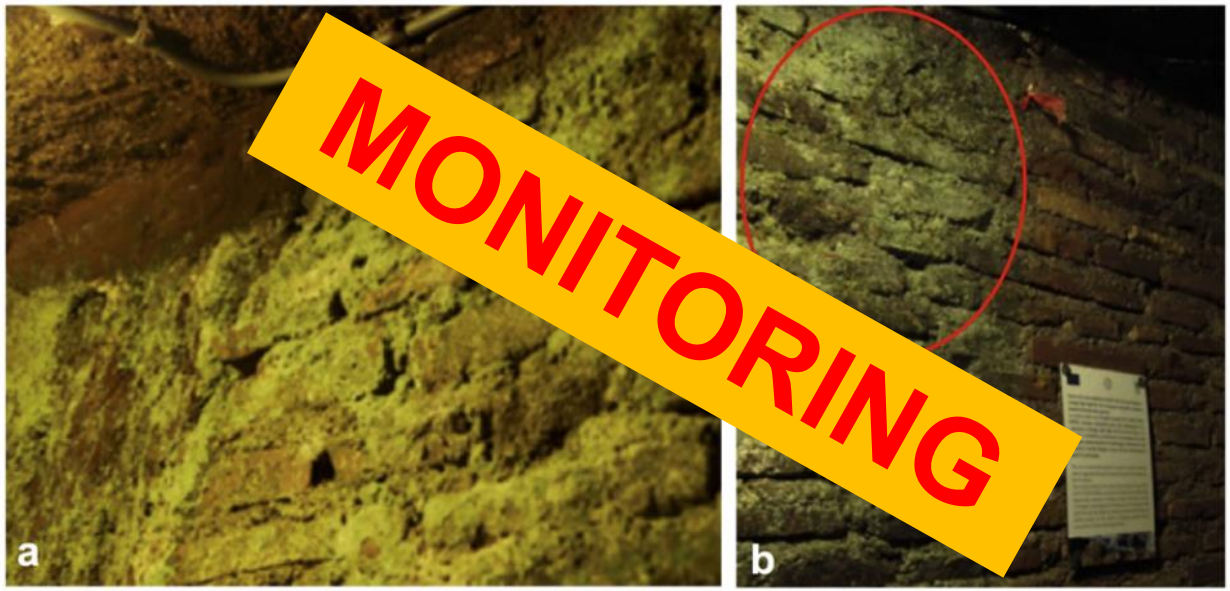
Dvadsať kmeňov húb z viac ako **53 izolátov** bolo schopných **solubilizovať** **CaCO₃** a/alebo degradovať kazeín na agarových platniach.

Bakteriálne kmene prenášané **vzduchom** vykazovali mierne vyššiu proteolytickú aktivitu (58,8 % pozitívnych kmeňov) samostatne alebo v kombinácii so solubilizáciou alebo precipitáciou CaCO₃. Okrem toho bolo **20,6 % baktérií** prenášaných vzduchom schopných produkovať organické kyseliny a **solubilizovať uhličitanové substráty**.

Catacombs Rome - Biocides

The biocide (**Rocima™ 103 - LANXESS**) employed by the restorers consisted of a mixture of chlorides of **alkyl-benzyldimethylammonium (40–60%)** and **N-octyl-2H-isothiazol-3-one (OIT) (7–10%)**, Isopropanol (15–20%), Formic acid (1–2.5%).

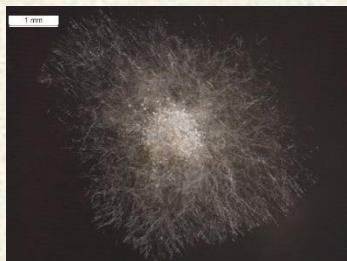
Quaternary ammonium compounds
Kvartérne amóniové zlúčeniny



	CD15	
	Before	After
Bacteria	<i>Streptomyces</i> spp. <i>S. scabrisporus</i> <i>S. paresii</i> <i>S. colombiensis</i> <i>S. lavendulae</i> <i>S. xanthophaeus</i> <i>Nocardia cummidelens</i> <i>Paracoccus yeeii</i>	<i>Chitinophaga soli</i> <i>Lysobacter capsici</i> <i>Pseudomonas marincola</i> <i>Stenotrophomonas maltophilia</i> <i>Inquilinus lanosus</i>
Cyanobacteria	<i>S. julianum</i> <i>Leptolyngbya</i> sp. <i>Symphyonemopsis</i> sp. <i>Chroococcales</i>	<i>S. julianum</i> <i>Symphyonemopsis</i> sp.
Algae	<i>Unicellular coccoid algae</i> <i>Diadasmus</i> sp.	<i>Diadasmus</i> sp.
Fungi ^a	<i>Mycelia sterilia</i> <i>Phoma</i> <i>Penicillium</i> <i>Aspergillus</i> sp.	<i>Aspergillus</i> sp.

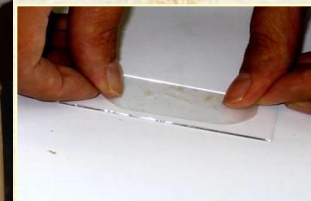
Mikroskopické pozorovanie

Stereo mikroskopia

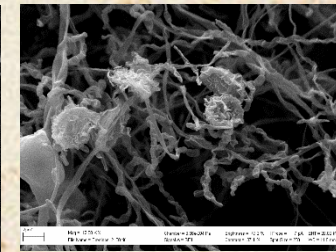
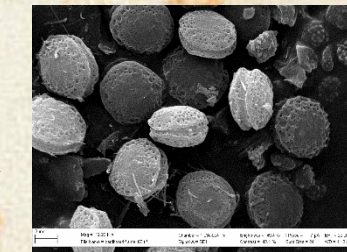
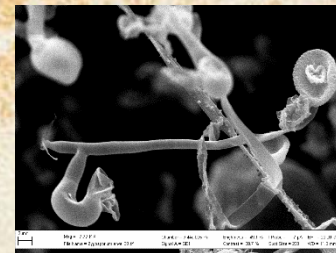
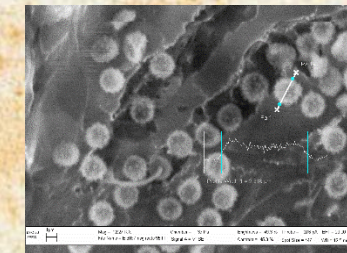
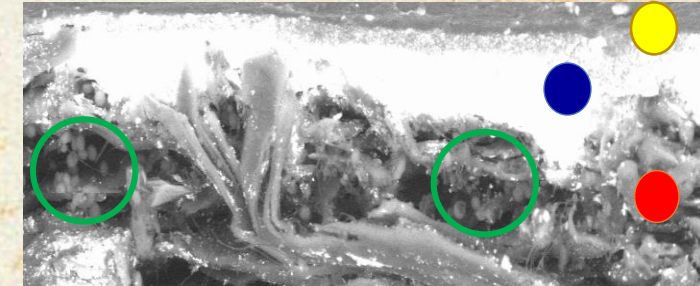


Skenovací elektrónový mikroskop SEM

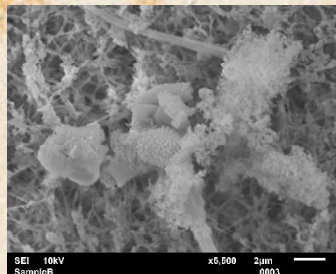
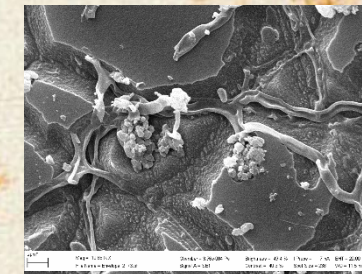
Nedeštruktívny odber vzoriek

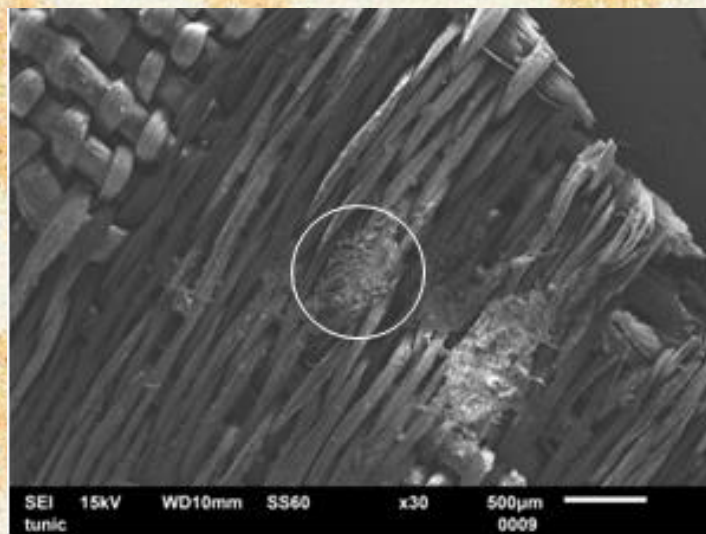


Variable
Pressure
Mode

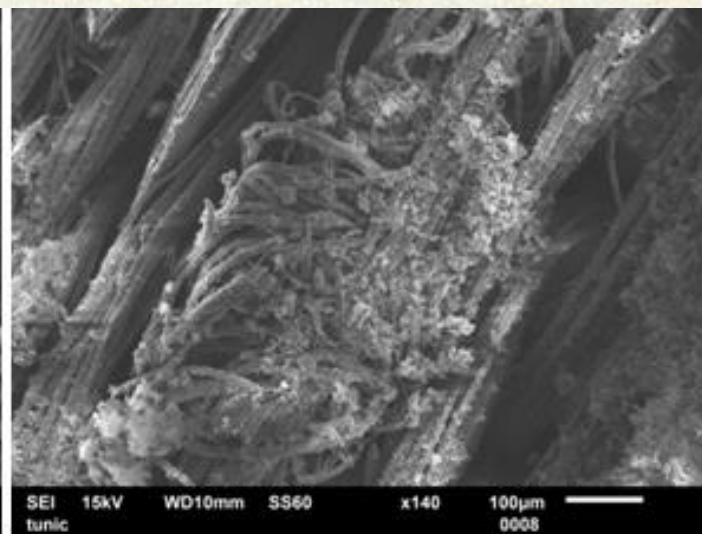


Pozlátenie
vzorky

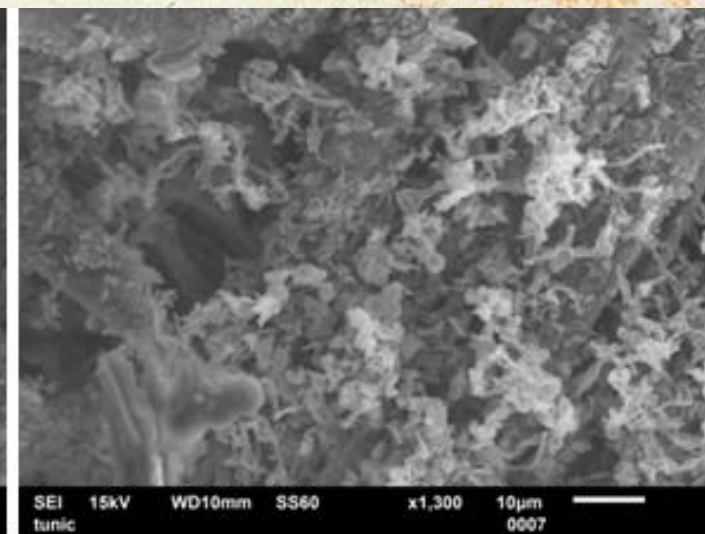




a)

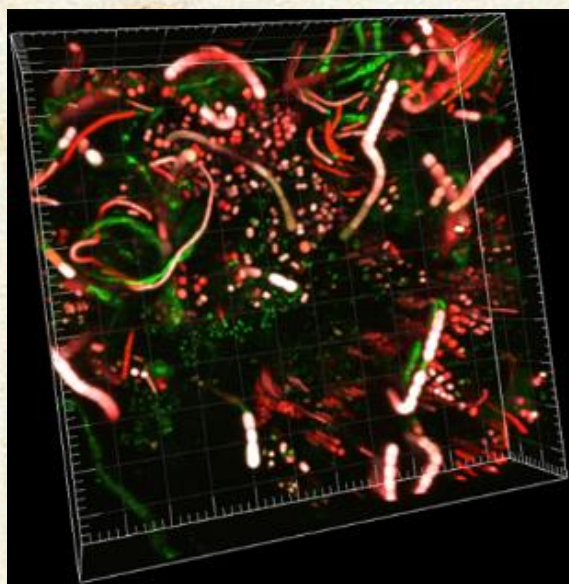
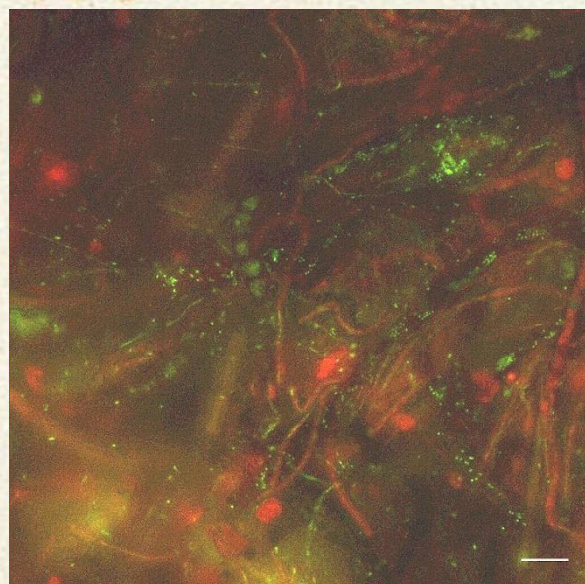


b)



c)

SEM fotografie povrchu tuniky. Oblast' v krúžku je postupne zväčšená na nasledujúcich obrázkoch (b, c)



Rímske katakomby



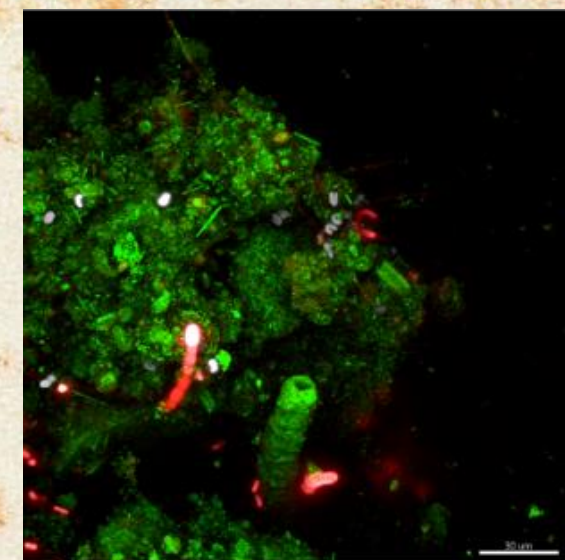
Pred

a

Po

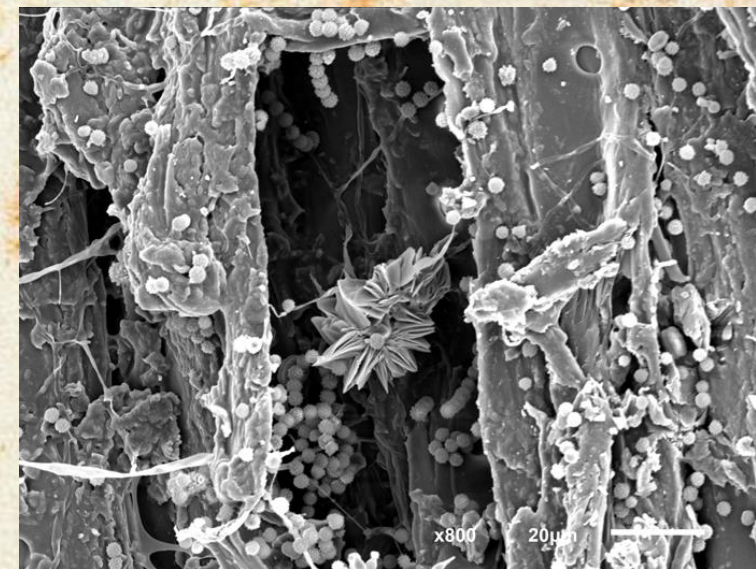
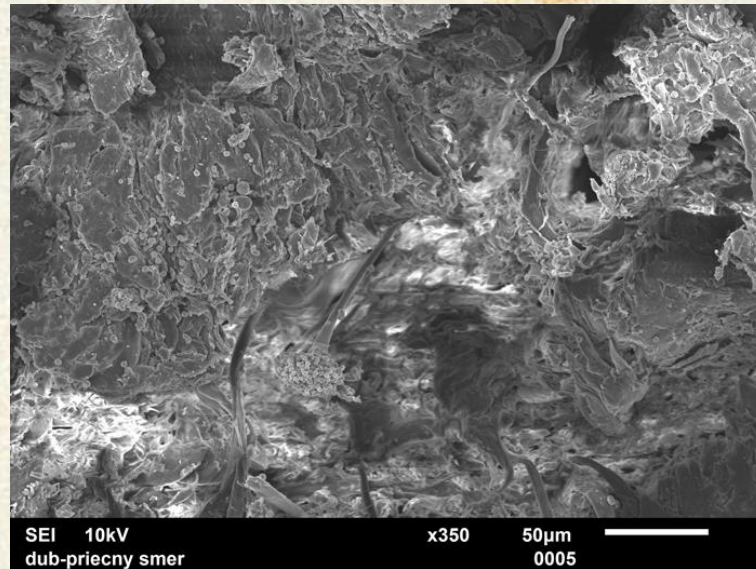
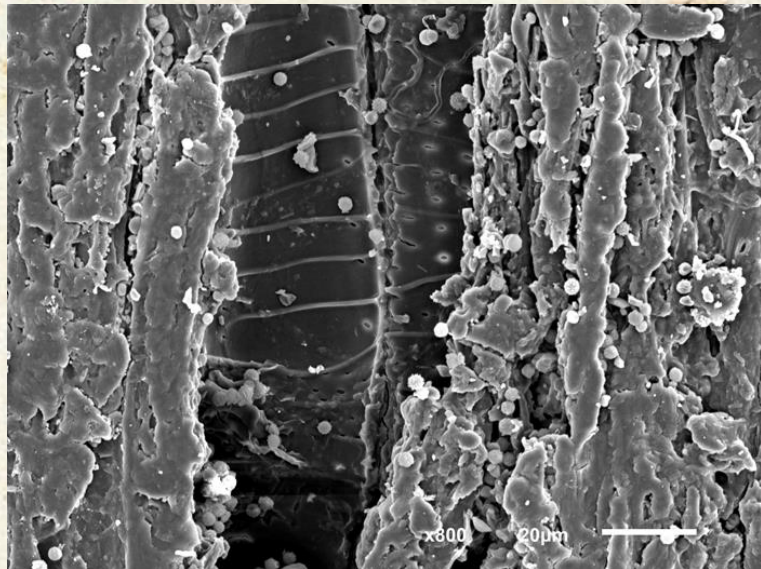


ošetrení biocídmi





Drevené organy

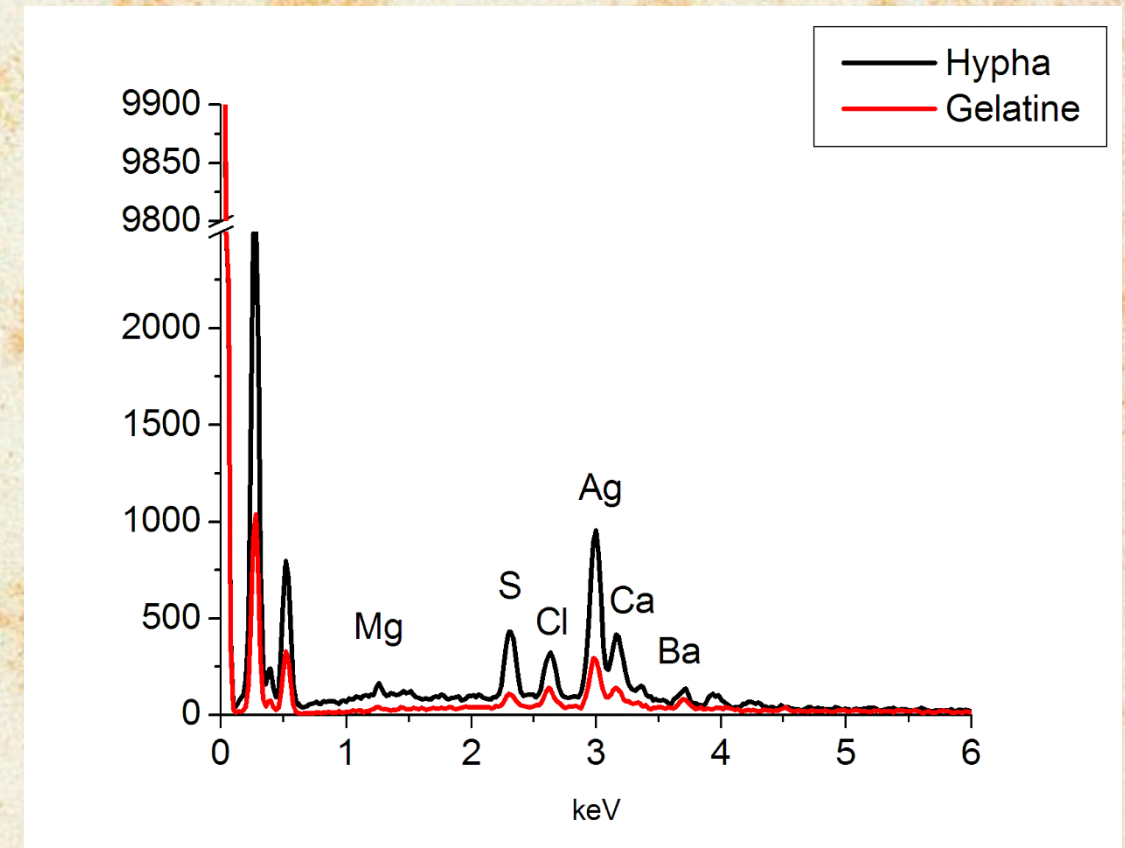
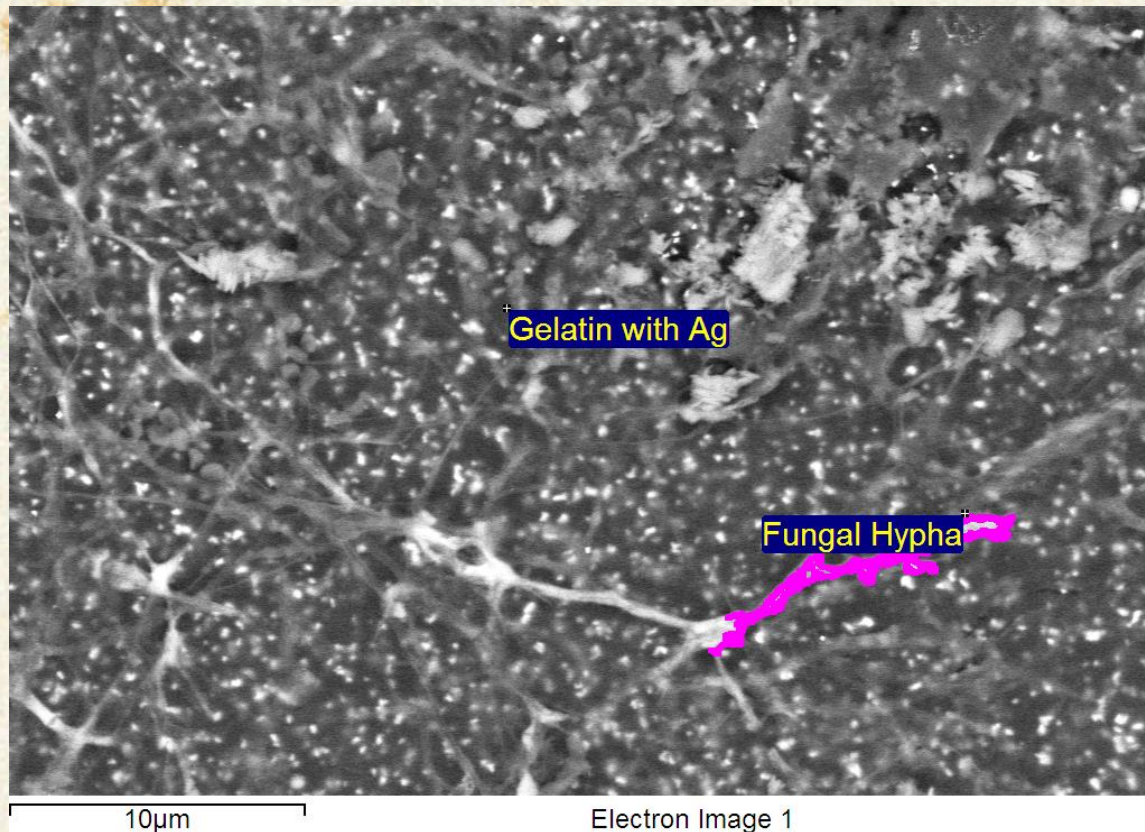


SEM fotografie poškodeného dreva plesňami drevených píštal organu.

Charakterizácia študovaného materiálu

Energy Dispersion Spectroscopy (SEM-EDS)

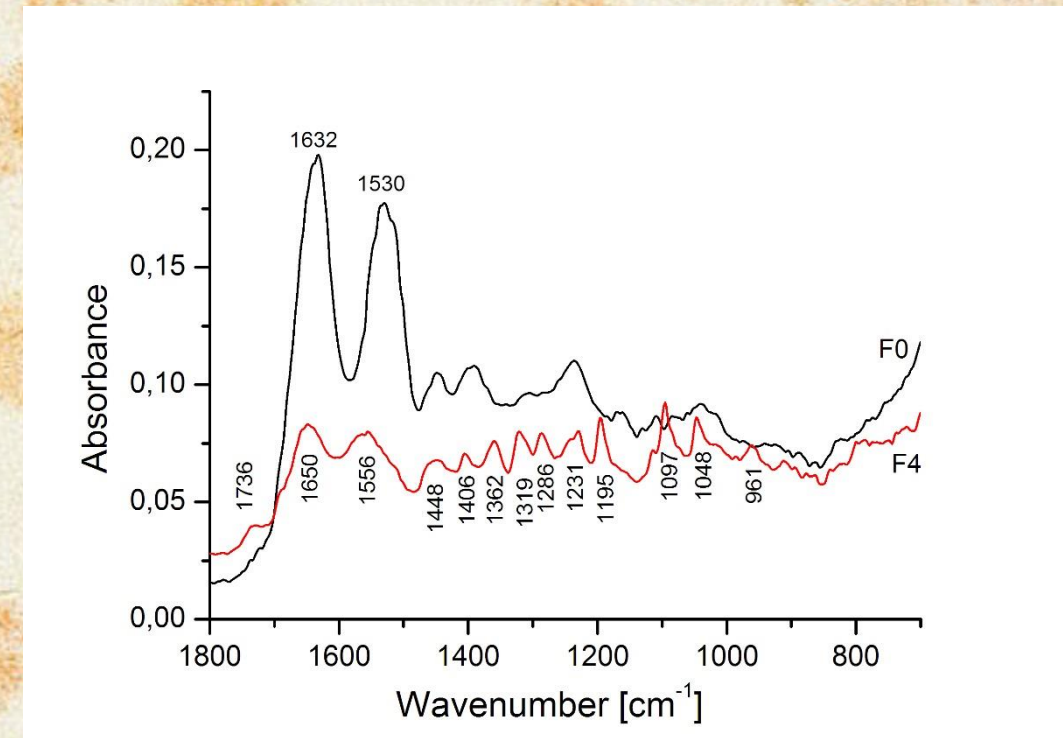
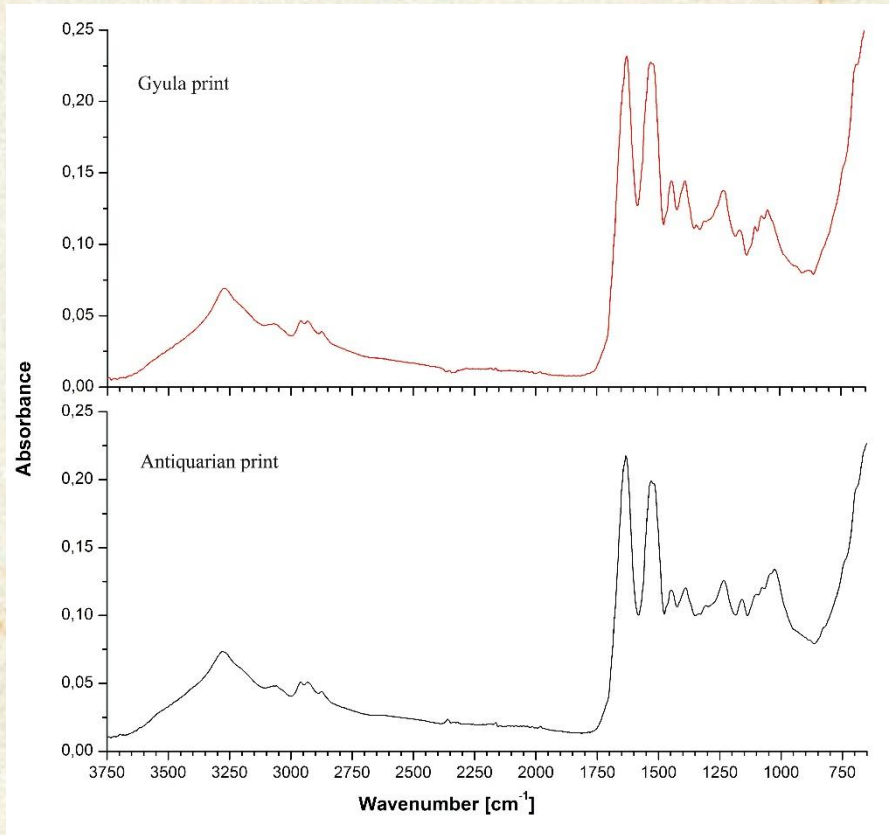
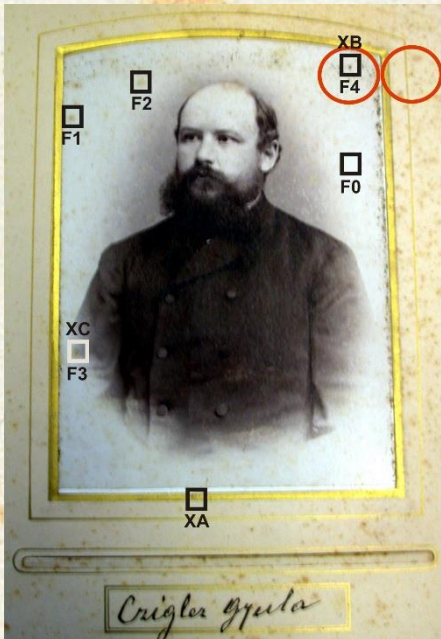
Biosorpcia častíc striebra plesňami



Energy Dispersive X-Ray Spectrometry performed on positive prints affected by moulds, the different kinds of objects pointed with EDS probe: the fungal hypha with Ag deposits; the gelatin without fungal elements.

Charakterizácia študovaného materiálu

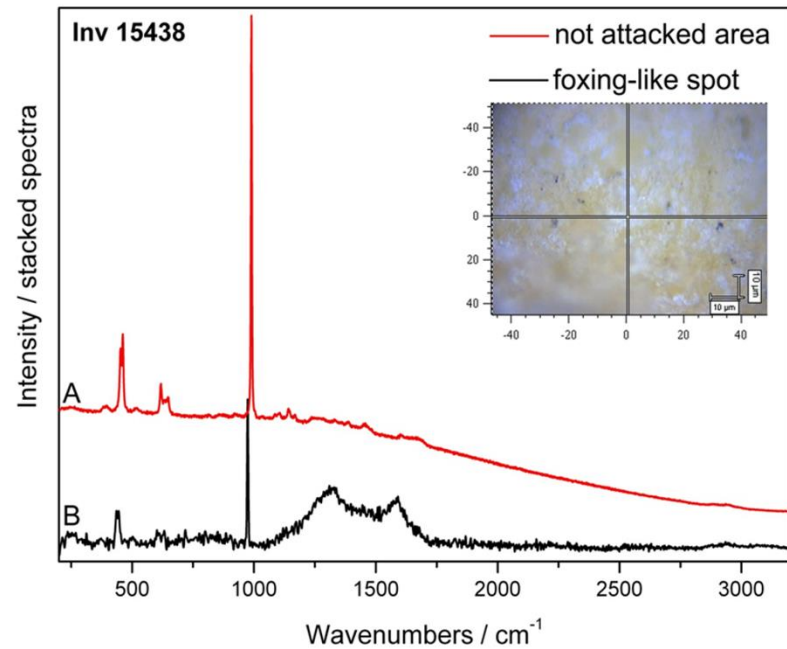
Fourier-transform infrared spectroscopy (FTIR)



FTIR spectroscopy confirmed that these two photographs are albumen prints. The typical absorption peaks for an albumen photograph appear **between 1632 cm⁻¹ and 1161 cm⁻¹**

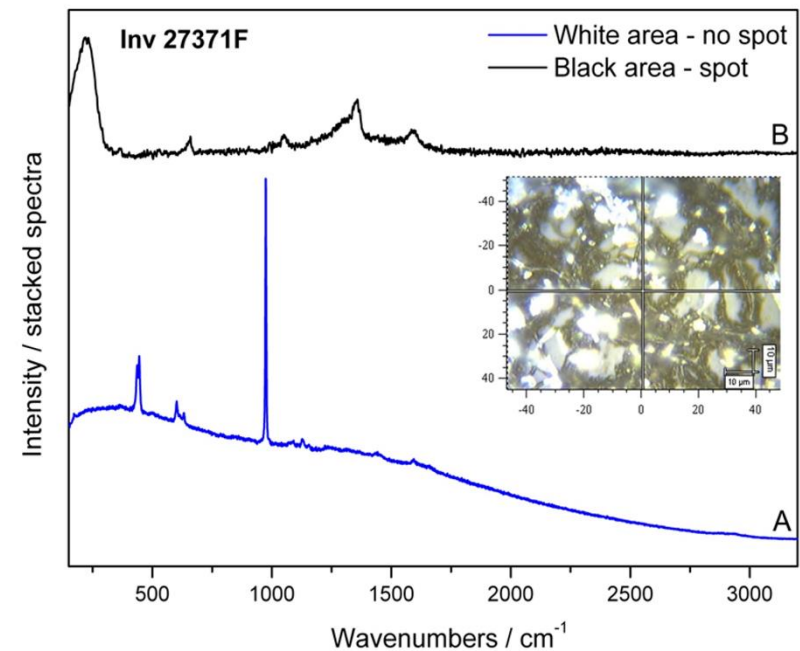
Charakterizácia študovaného materiálu

μ-RAMAN spektroskopia



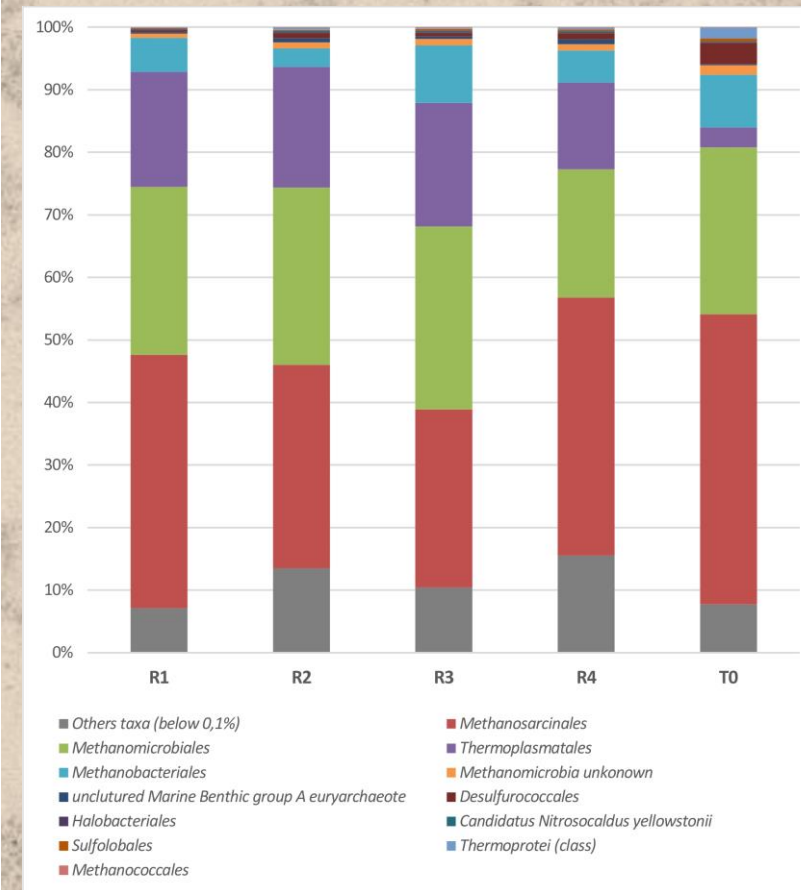
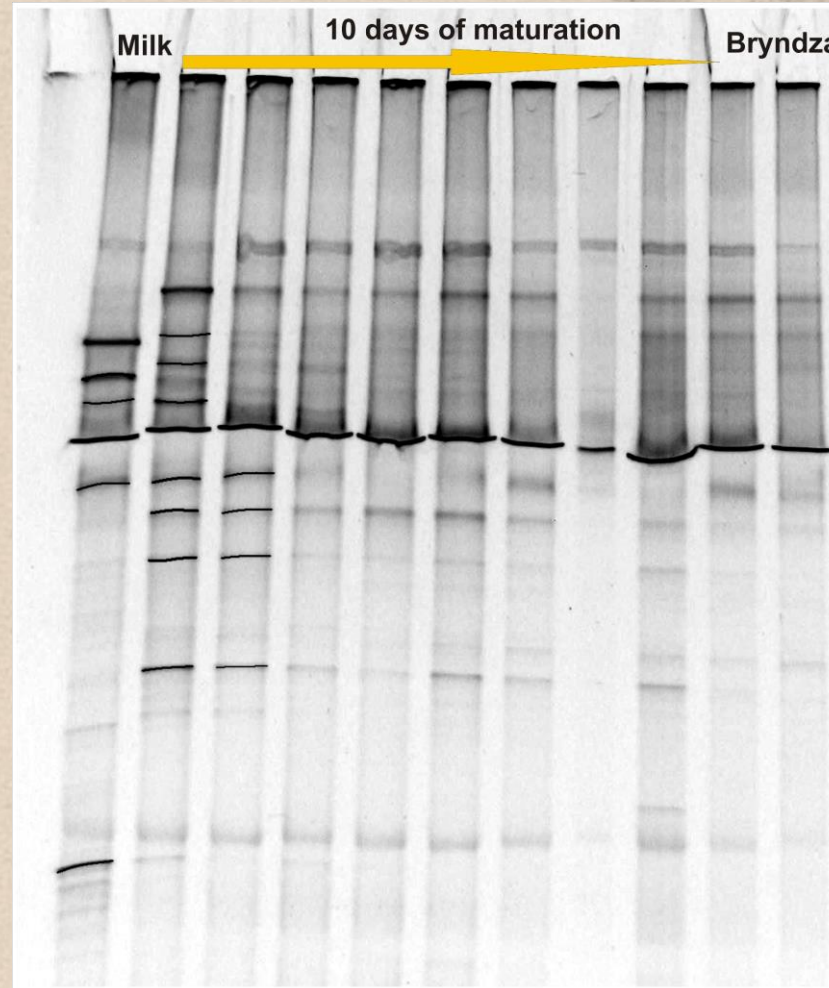
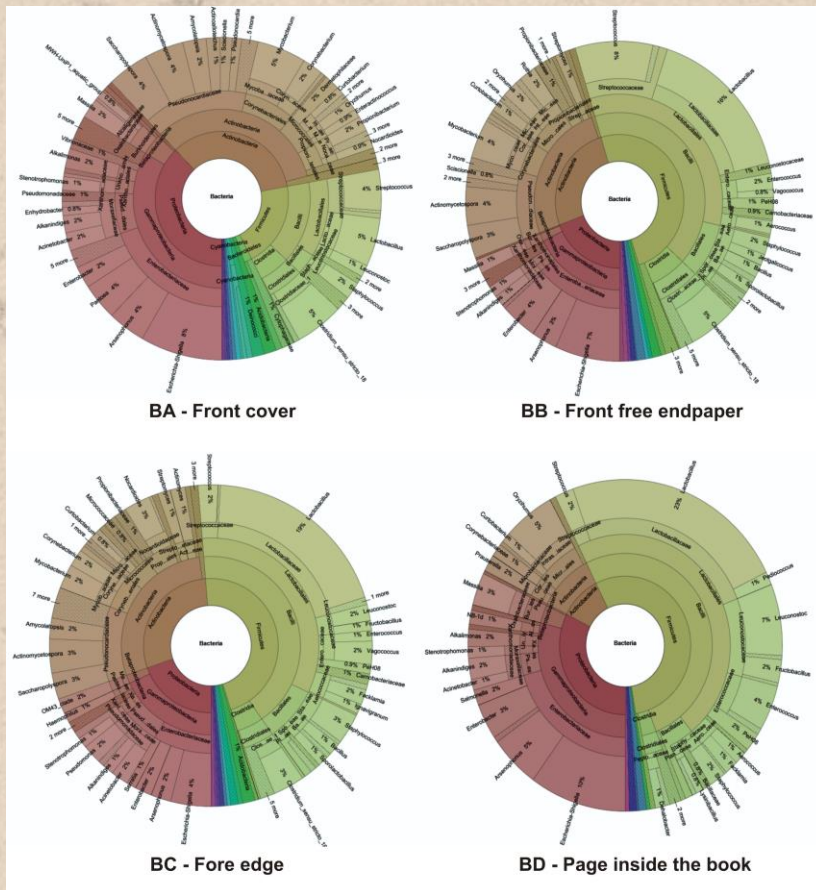
Combination of **μ-RAMAN** and **X-ray fluorescence (XRF)** - In the attacked areas of inv. 15438 it was documented the **formation of a phosphate**, presumably a mixed aluminum iron-potassium phosphate that could be attributable to the hydrolytic activity of bacteria. **The disappearing of barite** in the attacked areas could be attributable to the metabolic activity of microorganisms: **barite** could have been leached and differently re-precipitated in a configuration not detectable by Raman

Analýzy foxingových škvŕn



The spectrum of attacked areas of inv. 27371F is different and it can refer to the formation of a **mixture of oxides**, among them **barium titanate**.

Kultivačne nezávislé prístupy



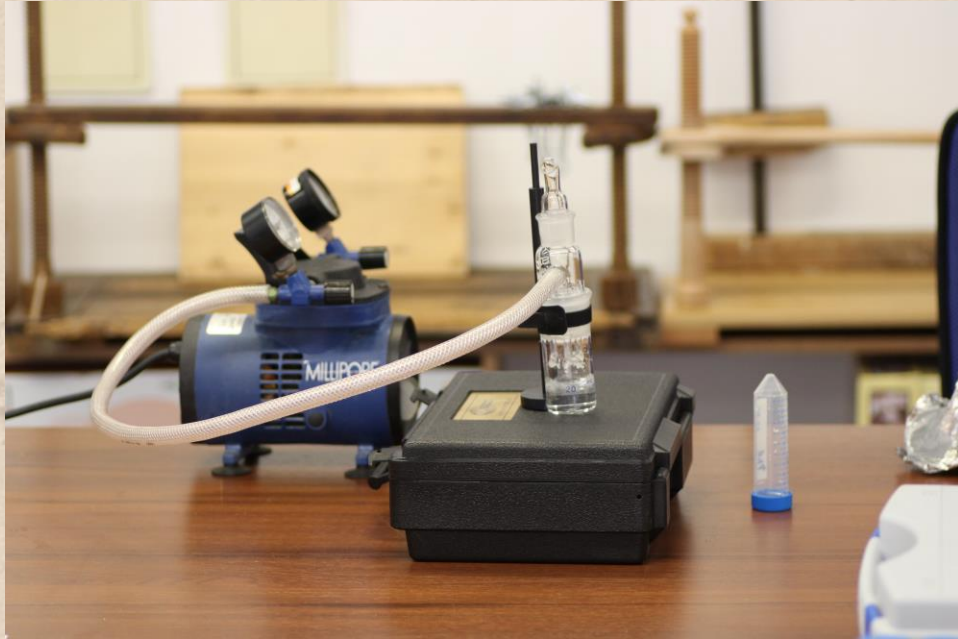
CSI MIAMI



CSI Bratislava



Vzorkovanie

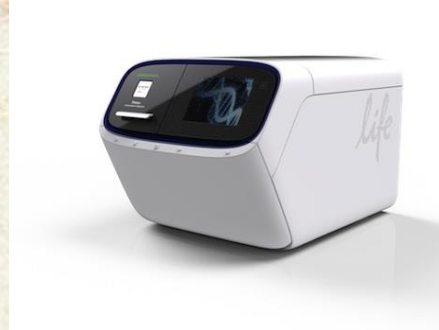


Mikroflóra zo vzduchu

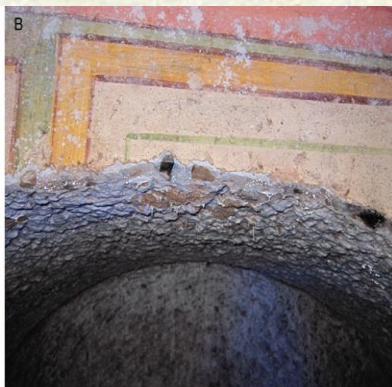


FASHIONABLE TREND

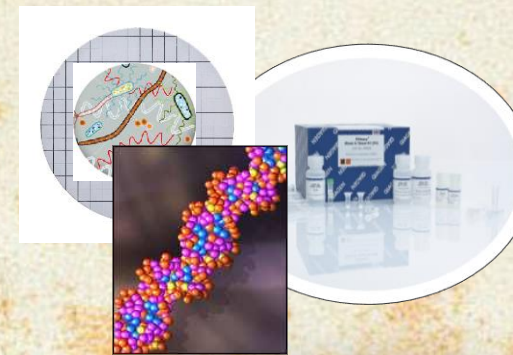
- 2nd Generation Sequencing (Next Generation Sequencing – NGS):
massive parallel sequencing



Typický pracovný postup spoločnosti Illumina



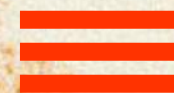
Odber vzoriek
pomocou
nitrocelulózovej
membrány alebo
lepiacej pásky



DNA extrakcia



PCR amplifikácia
~ 700 bp



baktérie 16S rRNA
plesne 28S rRNA



PCR purifikácia



vzorky pripravené
pre NGS analýzu

Úžasná Bioinformatická Práce

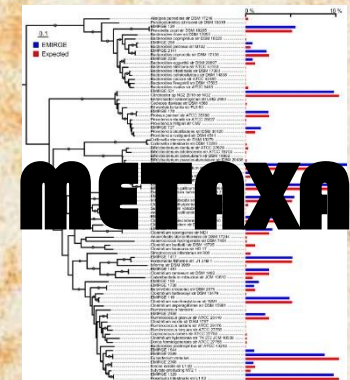
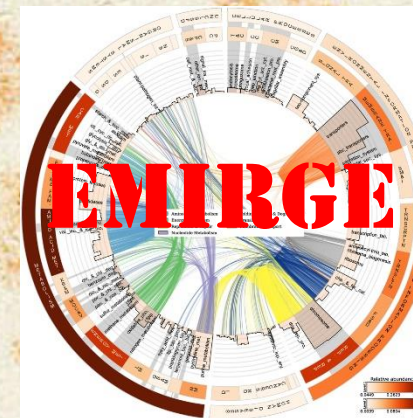
BaseSpace[®]
Genomics Cloud Computing

powered by illumina[®]

Software



Databases



Analýza múmie Žofie Šerédiovej

Odber vzoriek z prostredia



zo vzduchu z kaplnky

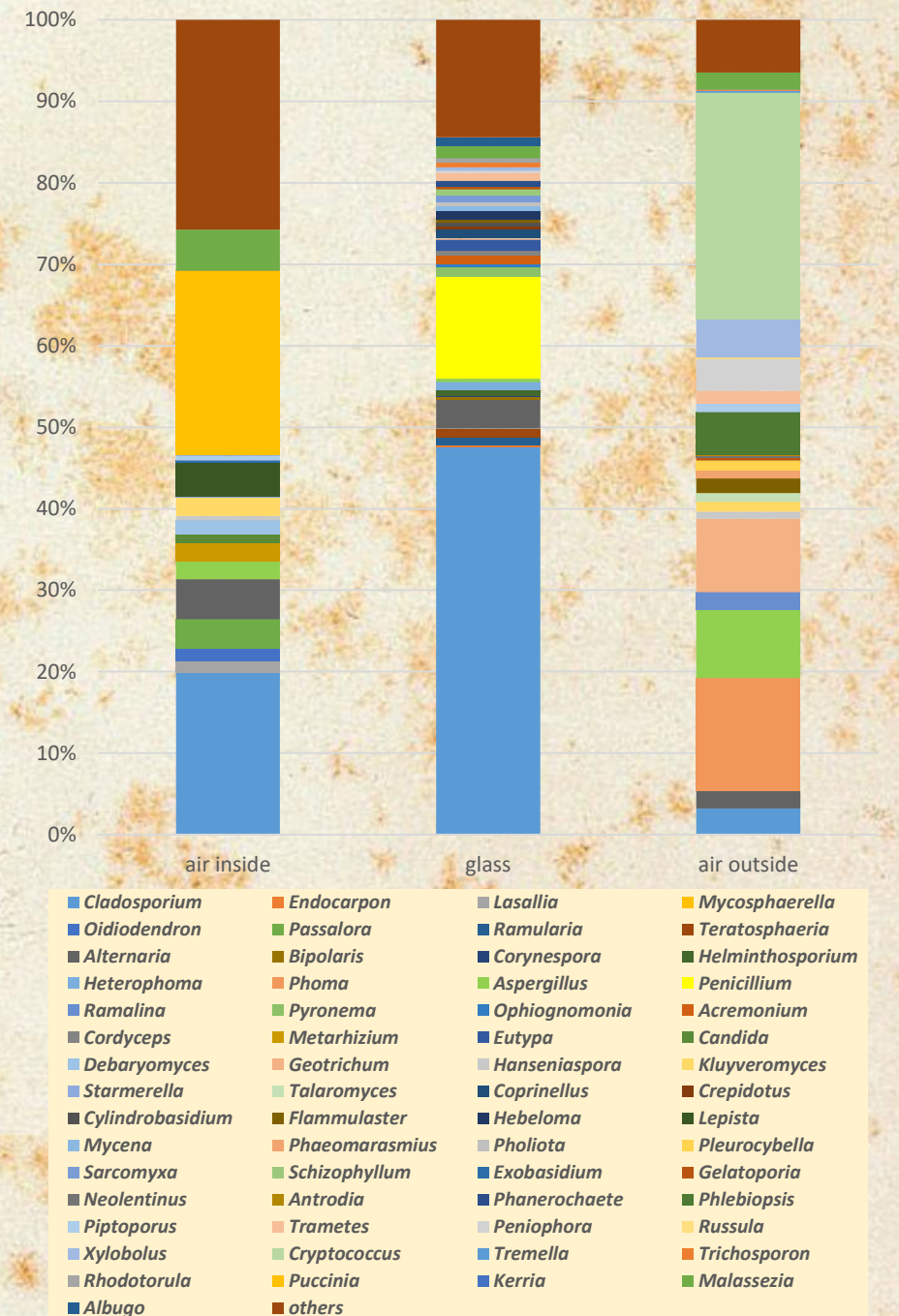
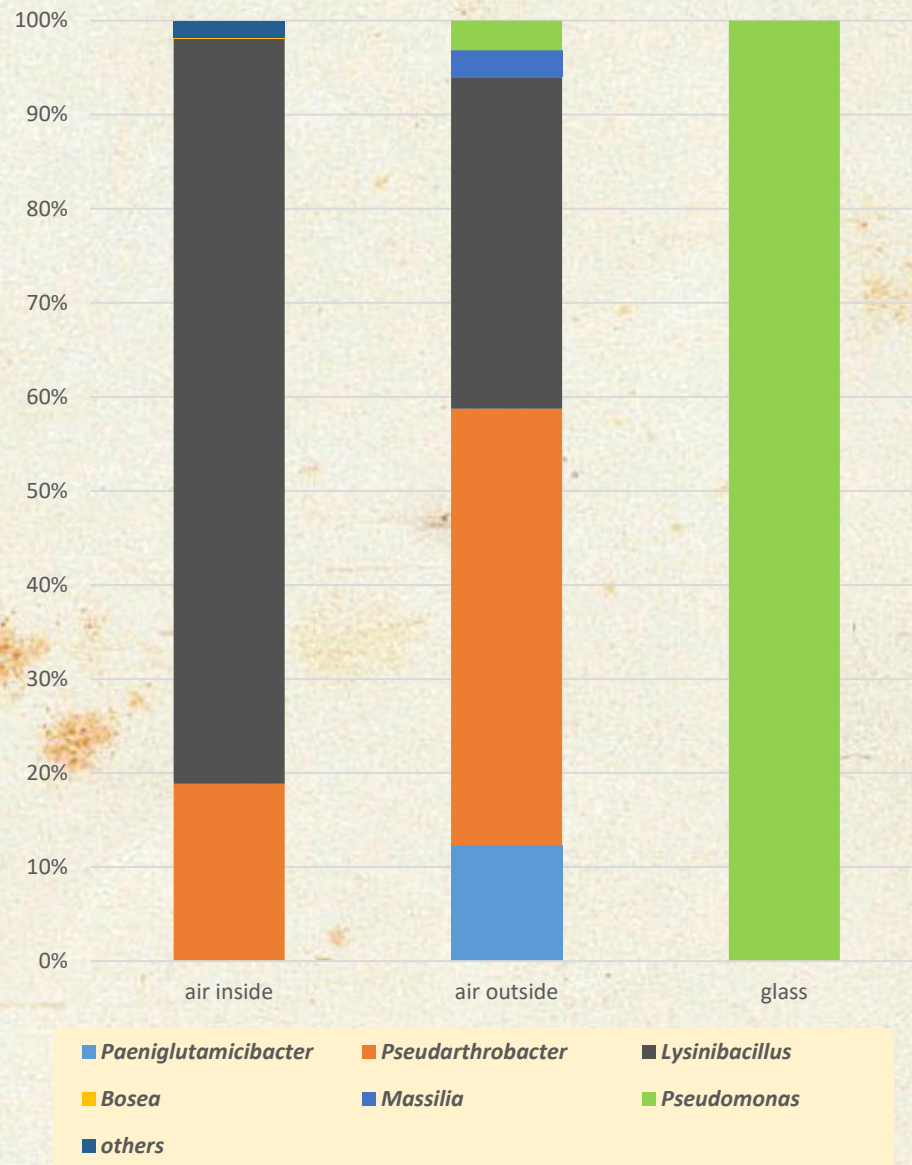


z vnútra skleneného sarkofágu

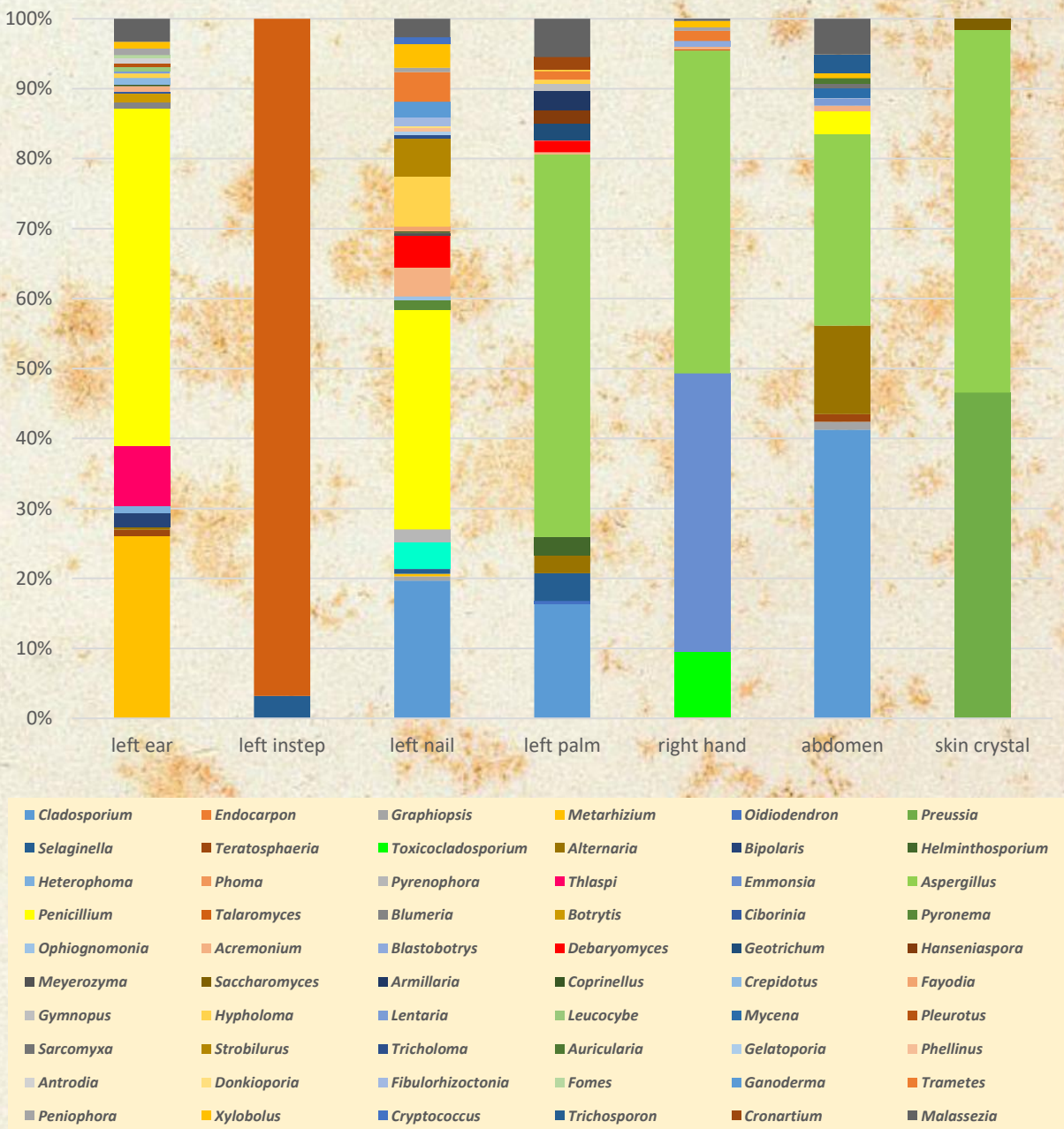
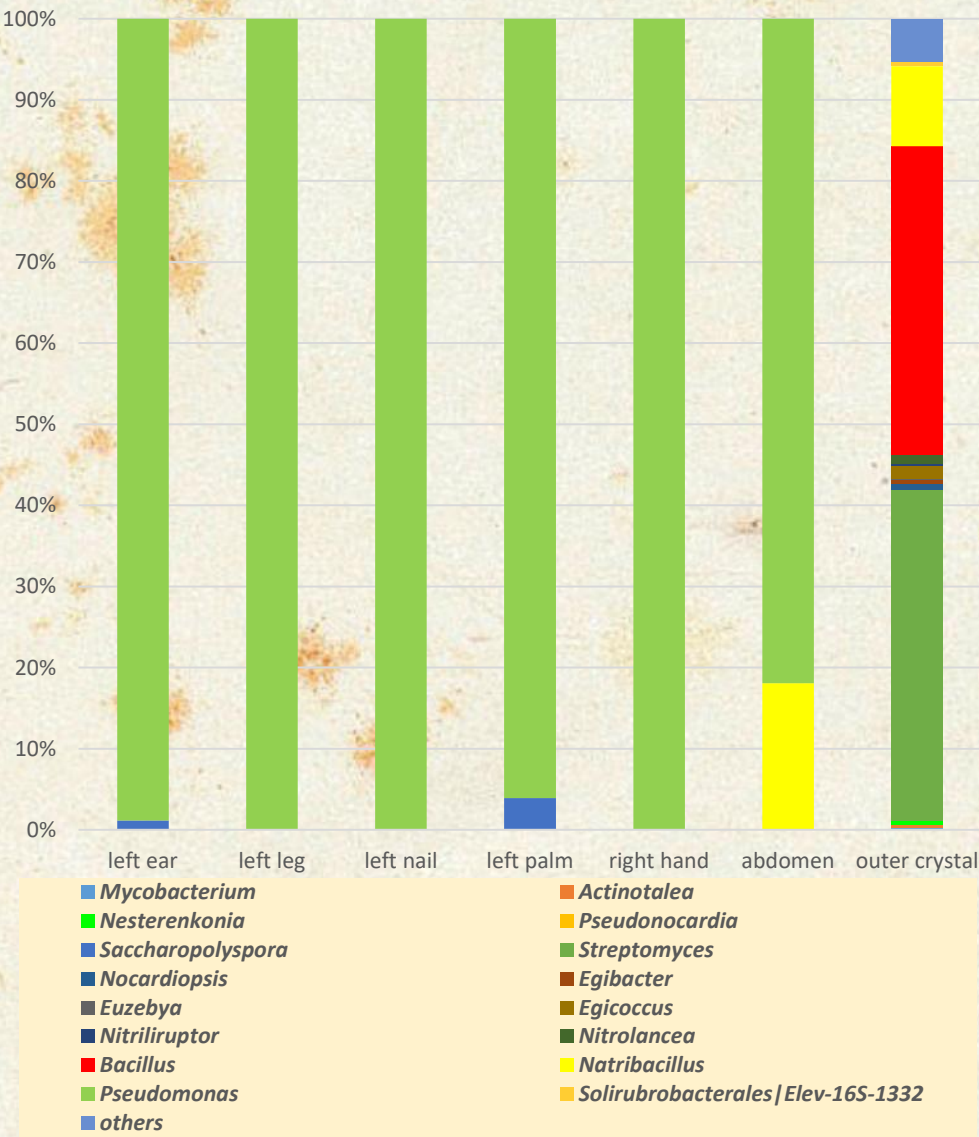
Odber vzoriek z tela múmie



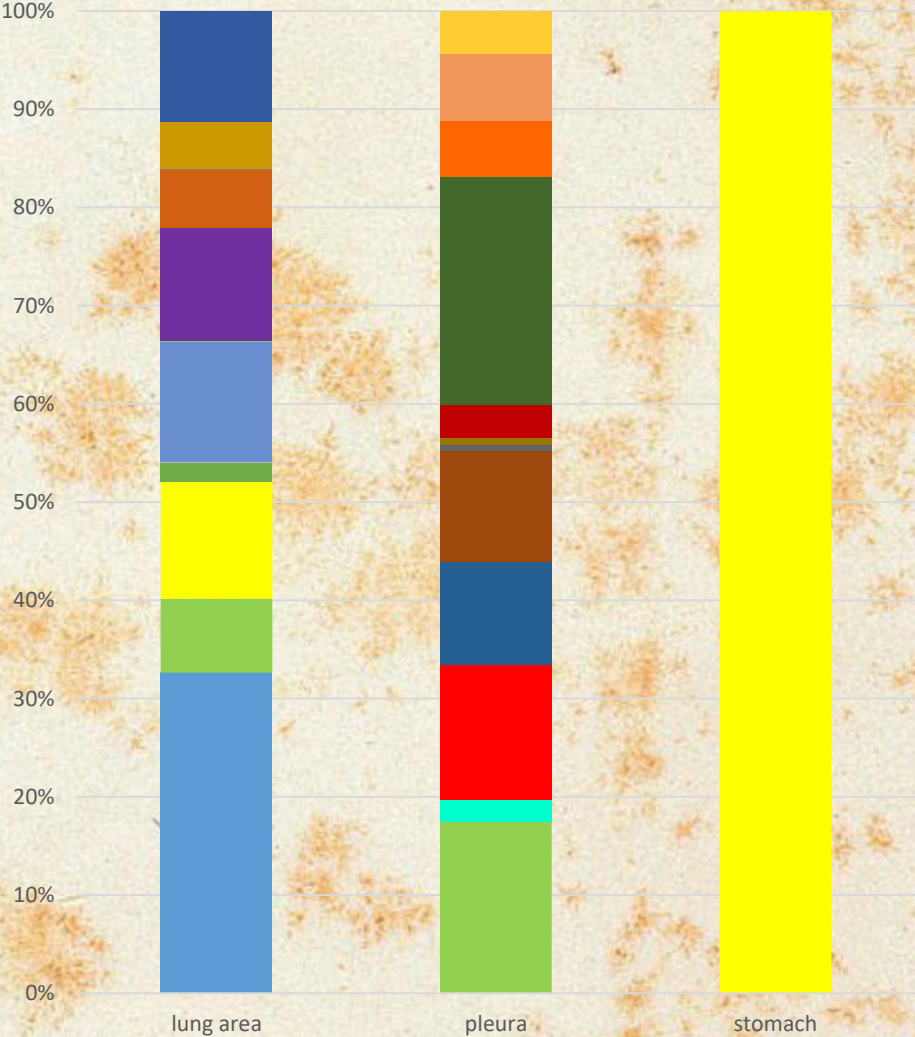
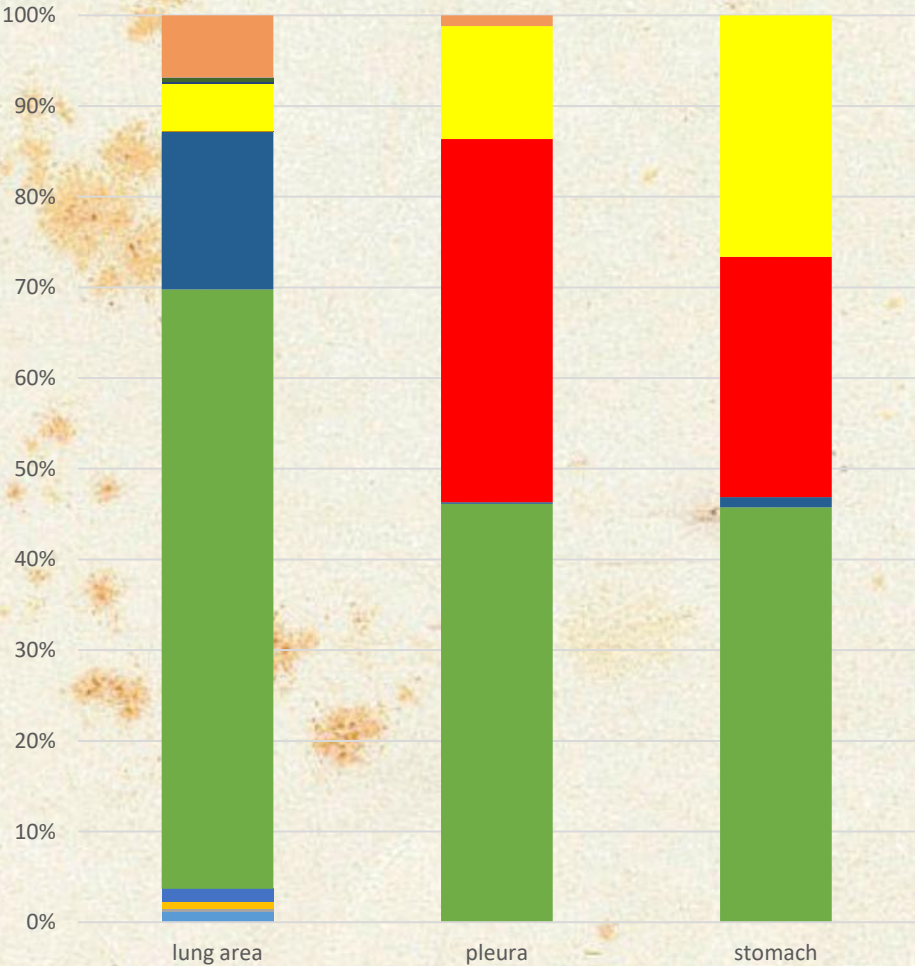
Prostredie – Krásna Hôrka



Múmia – povrch tela



Múmia orgány

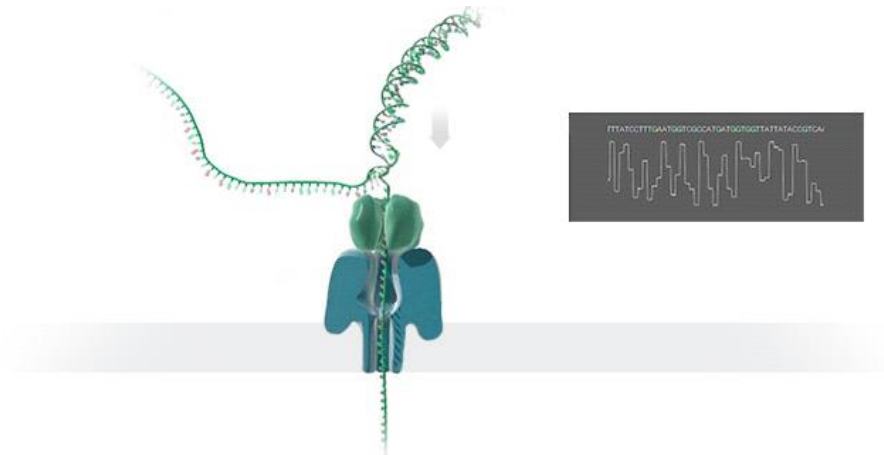
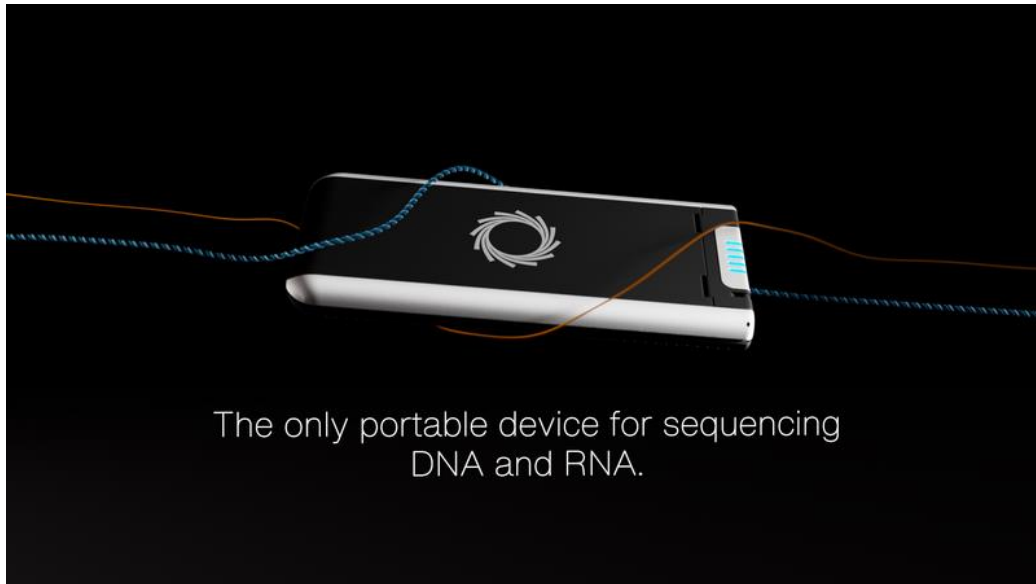


And now, something funny.....

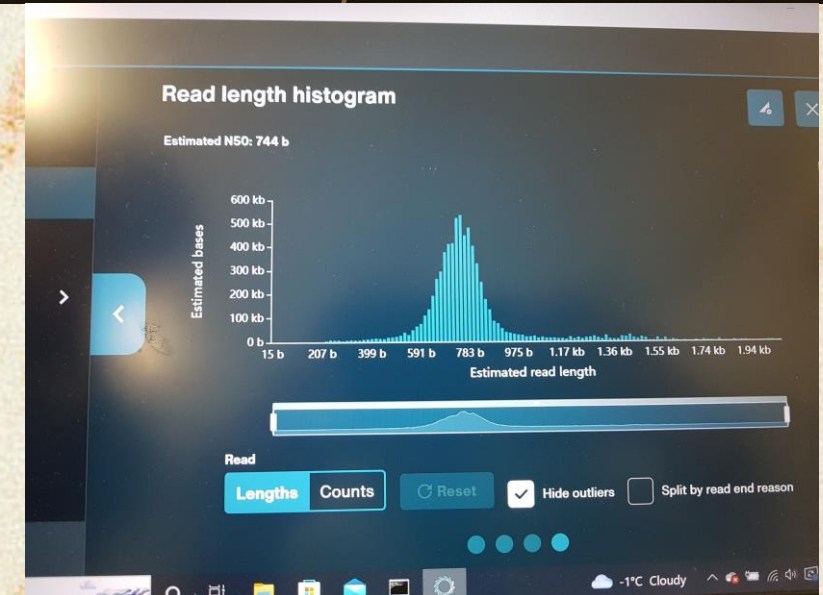
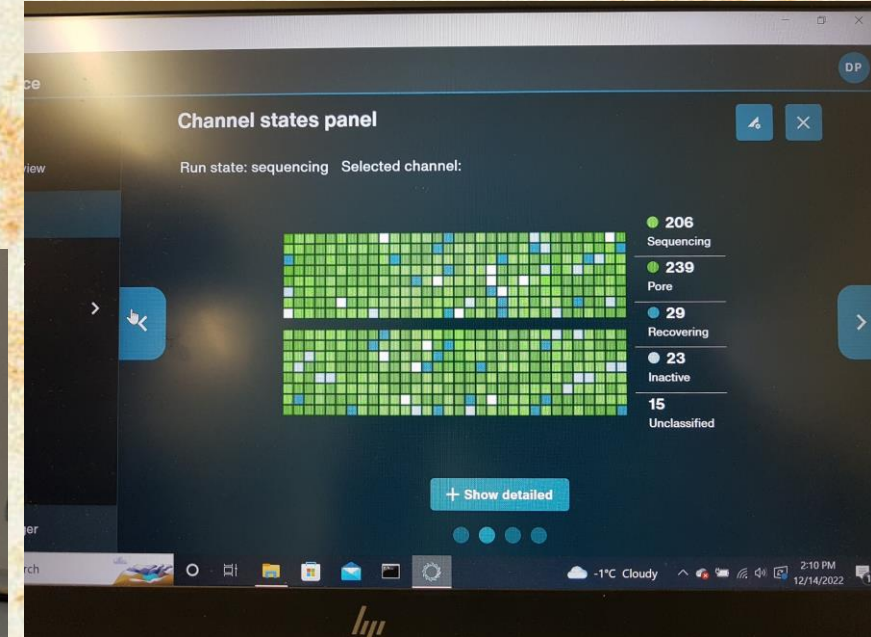


Minions?

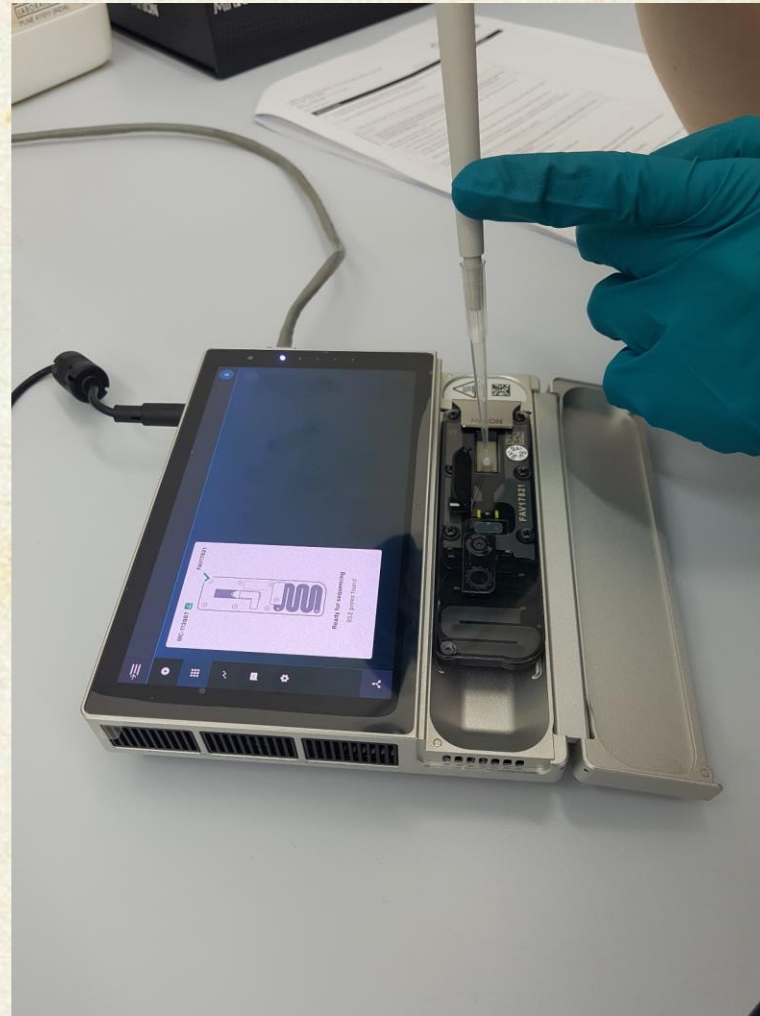
Sekvenovanie DNA a RNA v reálnom čase: Technológia Oxford Nanopore



3rd Generation Sequencing – MinION, Oxford Nanopore Technologies

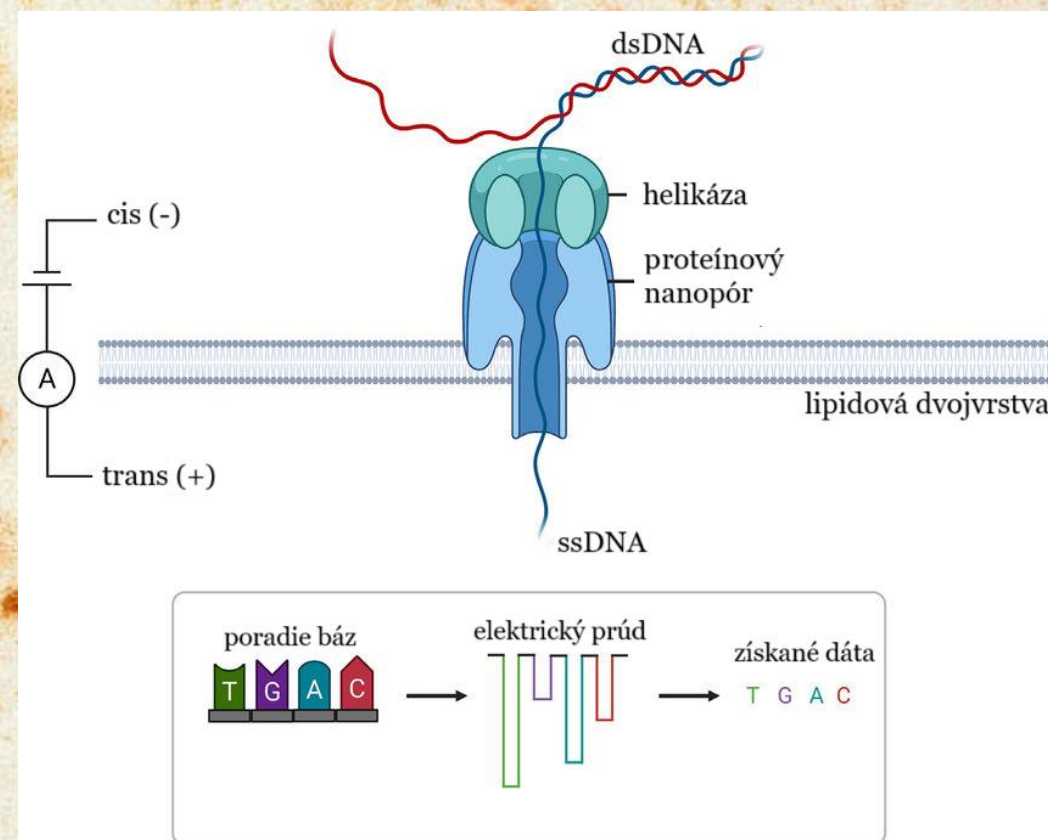
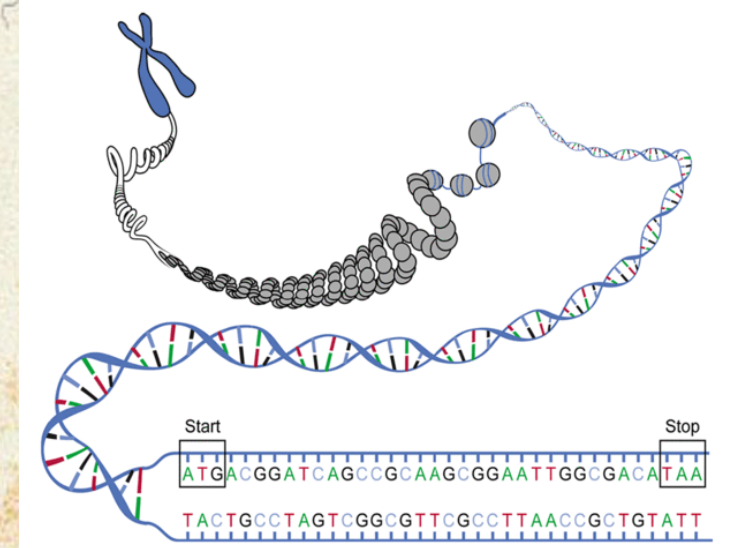


MinION Mk1C



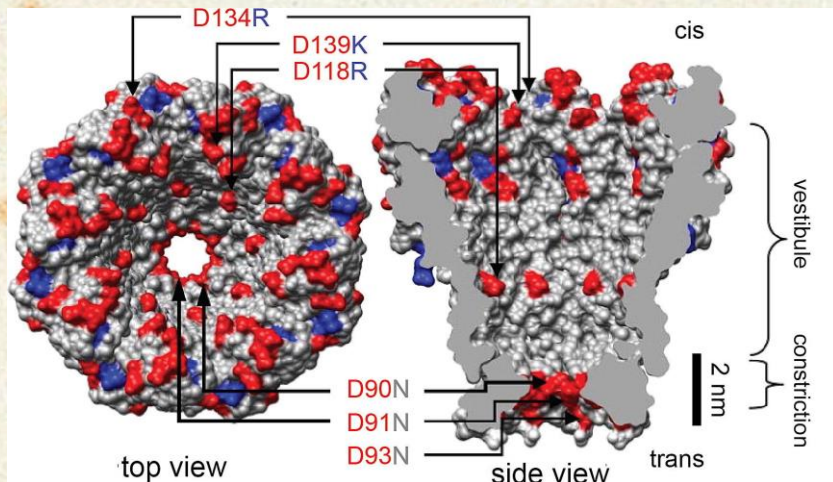
Úvod do technológie Oxford Nanopore (ONT) sekvenovania

- Čo je sekvenovanie DNA?
- Oxford Nanopore Technologies (ONT): sekvenovanie jednej molekuly pomocou nanopórov
- Každý nukleotid má špecifickú štruktúru, pričom počas prechodu cez nanopór každý nukleotid spôsobuje špecifickú zmenu elektrického prúdu. Na základe tejto zmeny vieme identifikovať, poradie nukleotidov v reťazci DNA.
- Výhody ONT sekvenovania



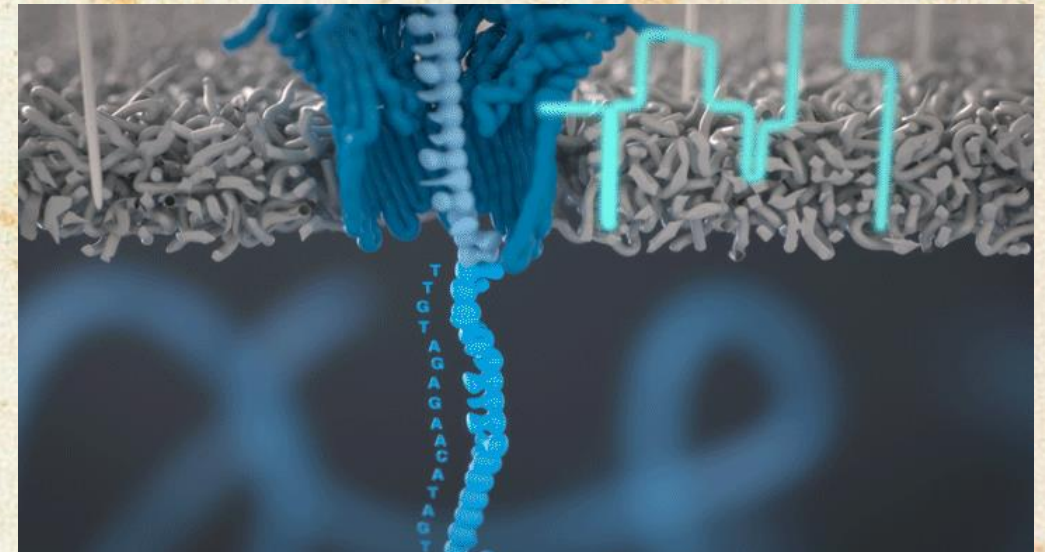
Princípy Nanopore sekvenovania

- NANOPORE-proteín, ktorý slúži ako brána medzi dvoma systémami.
- Starostlivo navrhnuté proteínové nanopóry prostredníctvom mutácie kľúčových zvyškov v bareli póru.



Structure and charge distribution of wild-type MspA- *Mycobacterium smegmatis* porin A (Laszlo *et al.*, 2016)

- Nukleová kyselina prechádza cez proteínový nanopór, zatiaľ čo sa monitorujú zmeny elektrického prúdu.
- Výsledný signál sa dekoduje, aby poskytol špecifickú sekvenciu DNA alebo RNA.

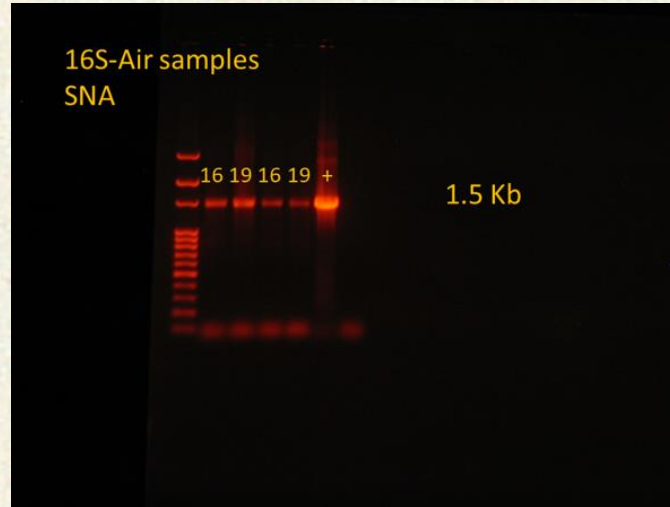




Príprava knižnice – wet lab

Sekvenovanie dlhých amplicónov

pomocou MinION (Nanopore sequencing)



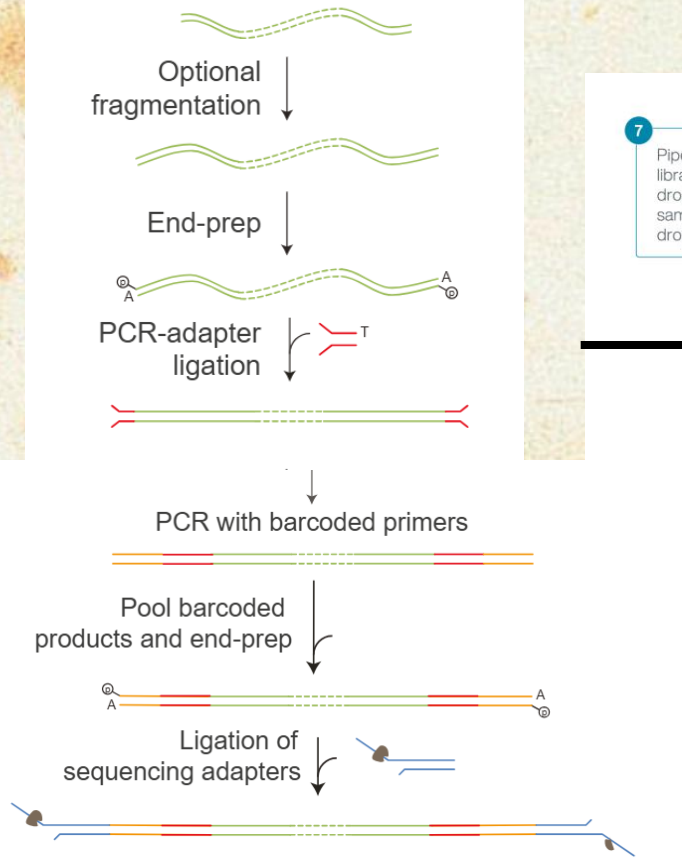
- GENY MARKEROV špecifické pre baktérie alebo huby: 16S rRNA, 28S rRNA, ITS
- Tieto gény musia byť amplifikované pomocou PCR amplifikácie, a preto sa tento prístup nazýva AMPLIKÓNOVÉ SEKVENOVANIE.
- Univerzálne PCR priméry použité na amplifikáciu.



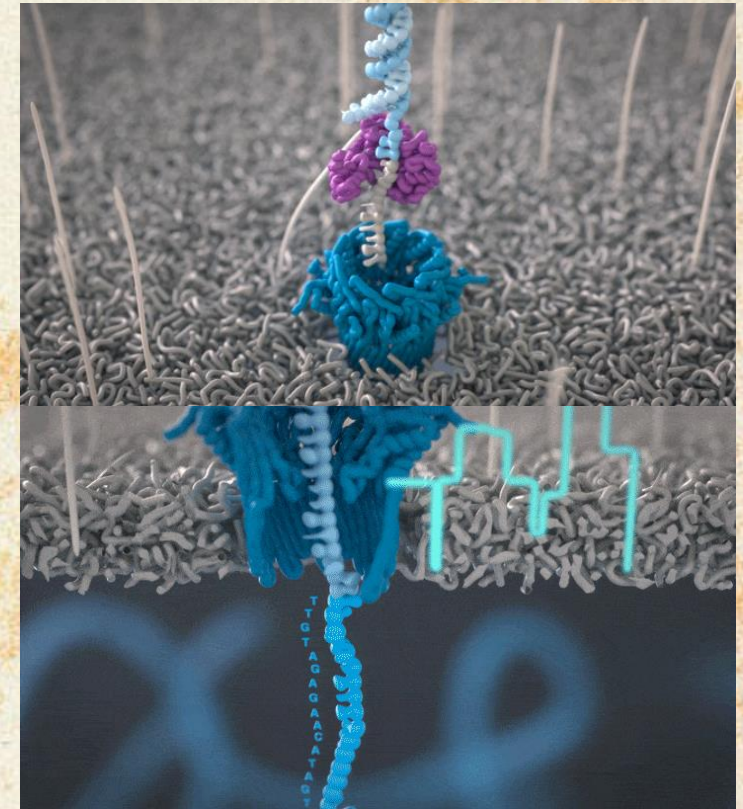
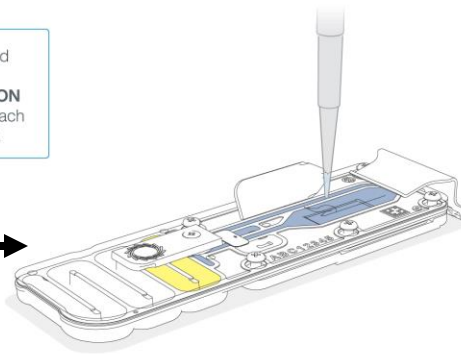
- Rýchla, automatizovaná príprava knižnice
- Dáta dostupné v reálnom čase (pre rýchle poznatky, ak sú potrebné)

Ako funguje technológia ONT sekvenovania

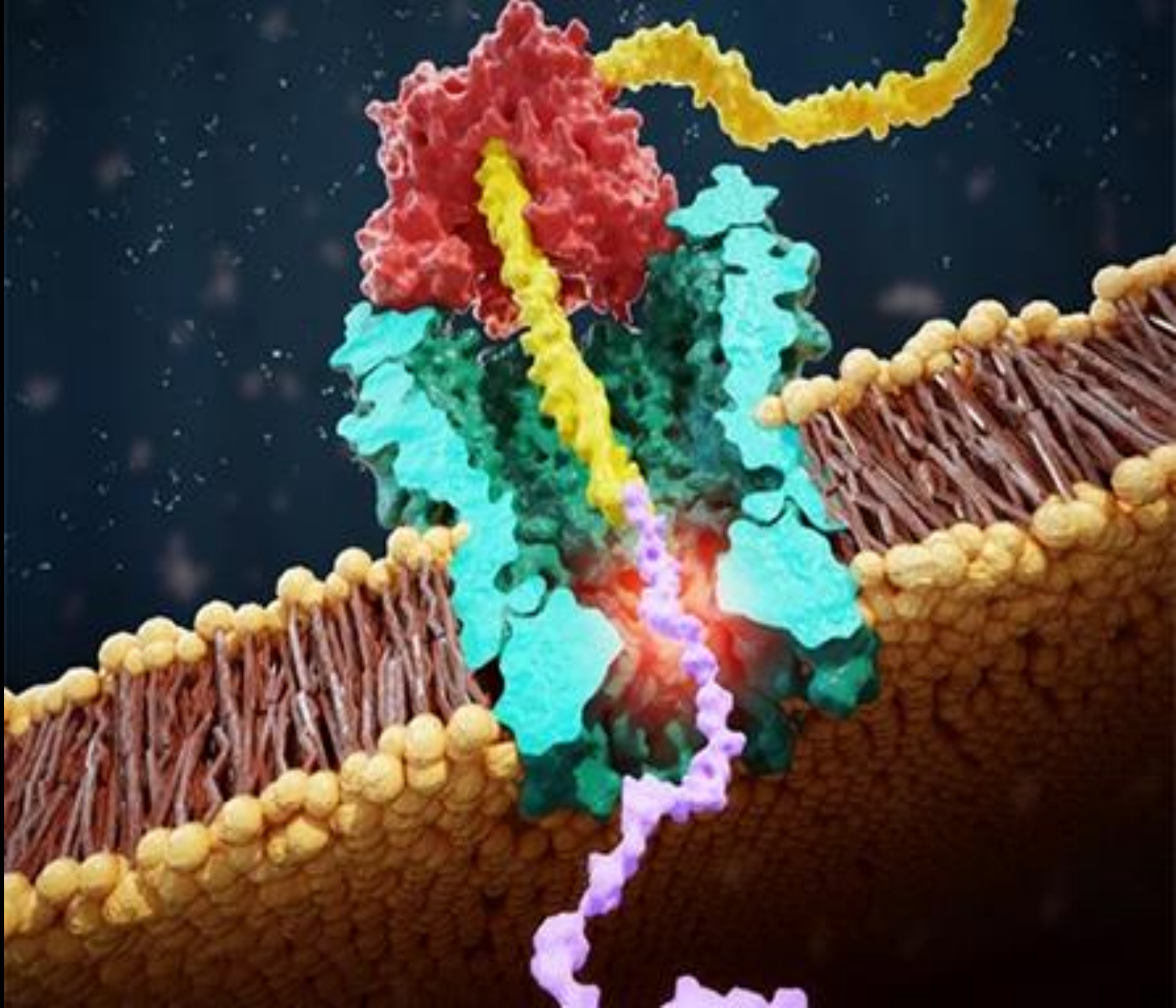
Barcoding protocol for genomic DNA

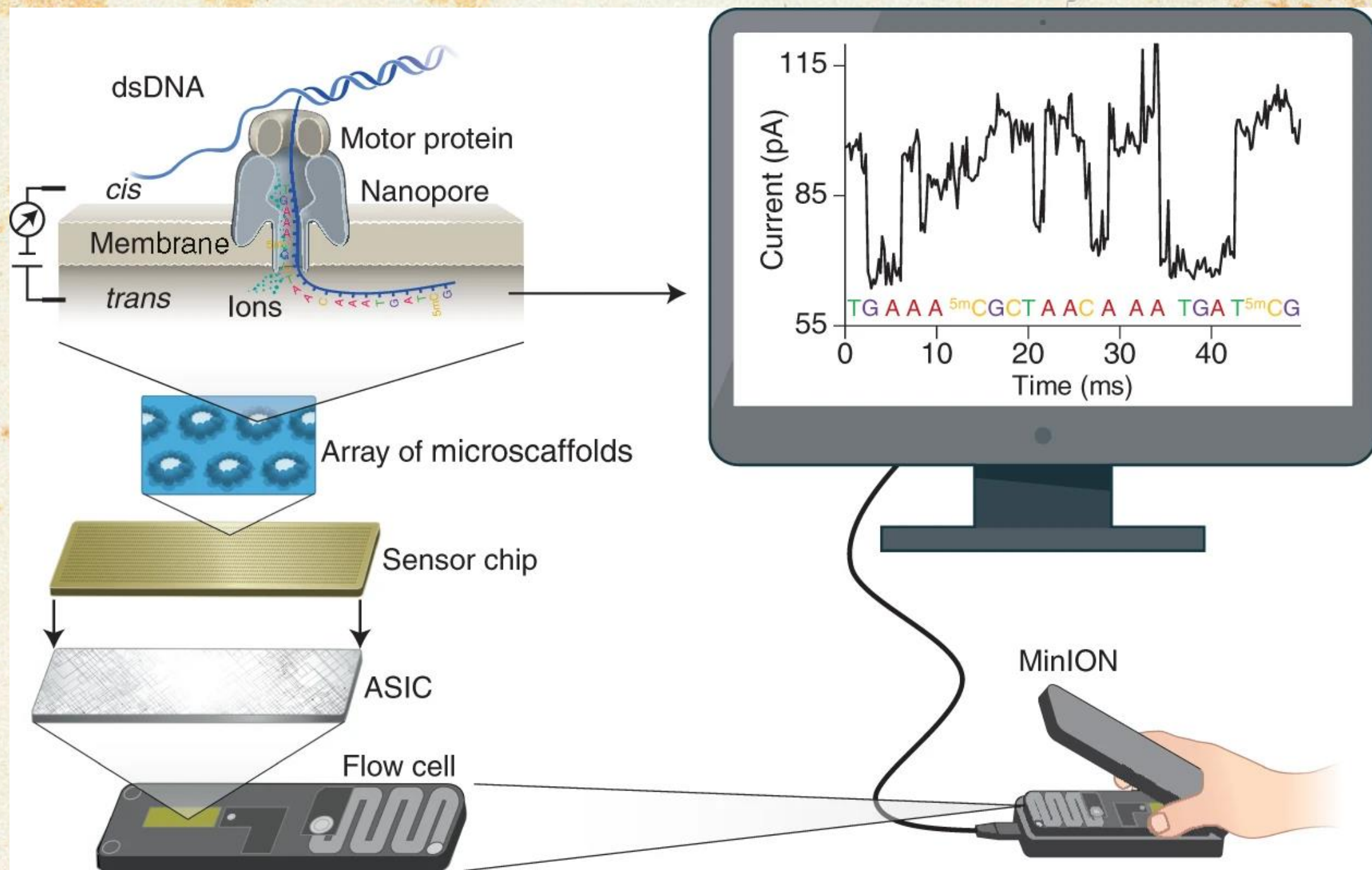


7
Pipette mix the prepared library and load 75 µl dropwise into the SpotON sample port, ensuring each drop flows into the port.



Samotné sekvenovanie



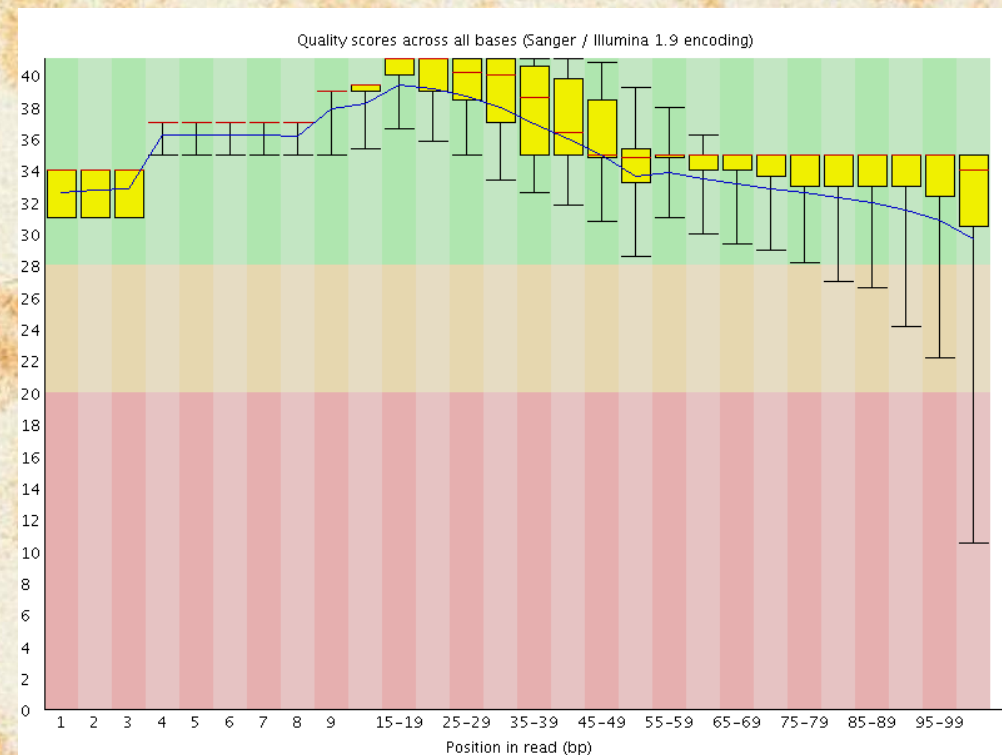
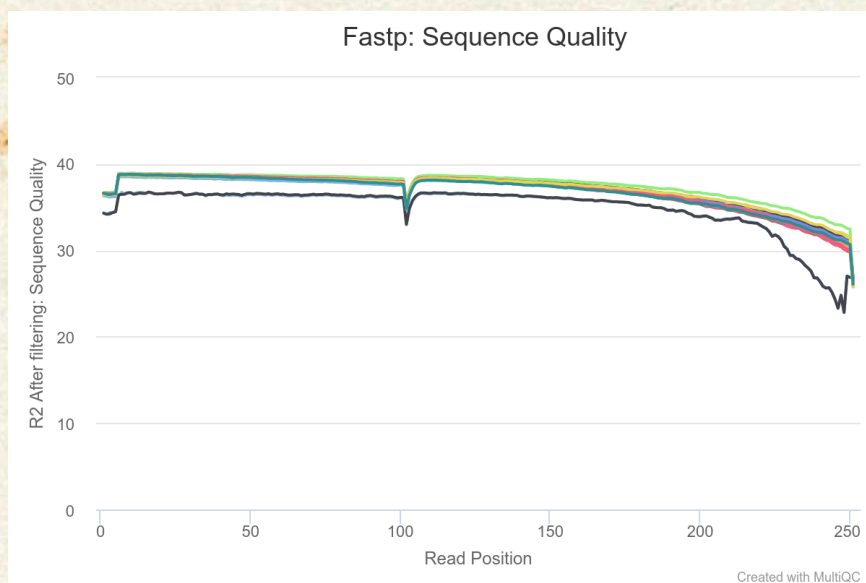


Bioinformatická analýza



Predúprava a úprava dát

- **Dorado** – nástroj na **basecalling**, **demultiplexing** a **trimming**
- **nanoFilt/fastp** – filtrovanie dát na základe parametrov
- **Flye** – nástroj na assembly (zarovnanie/mapovanie)
- **fastQC** – nástroj na vyhodnotenie kvality dát



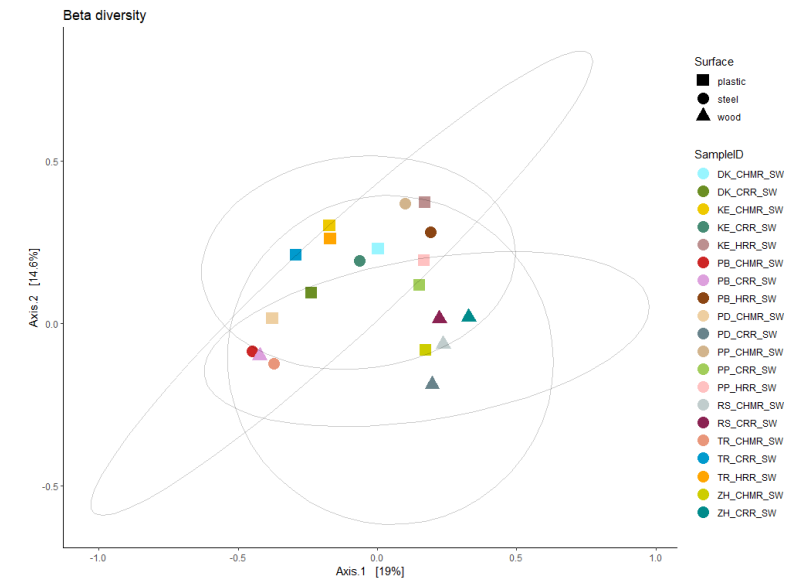
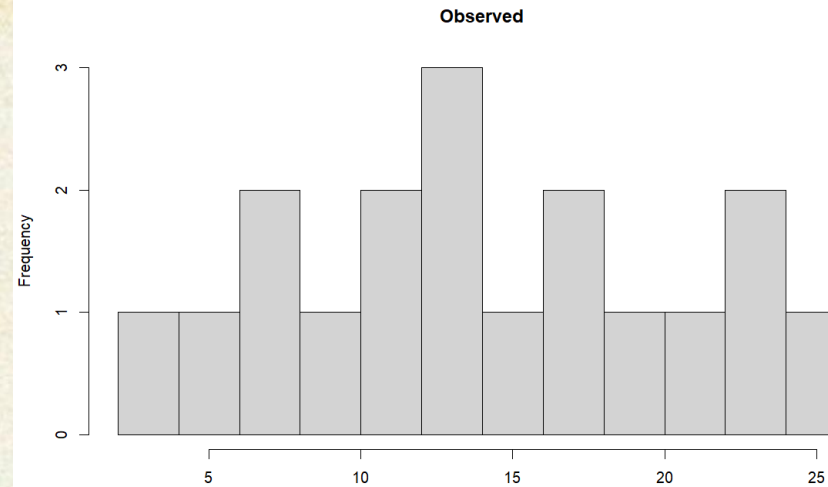
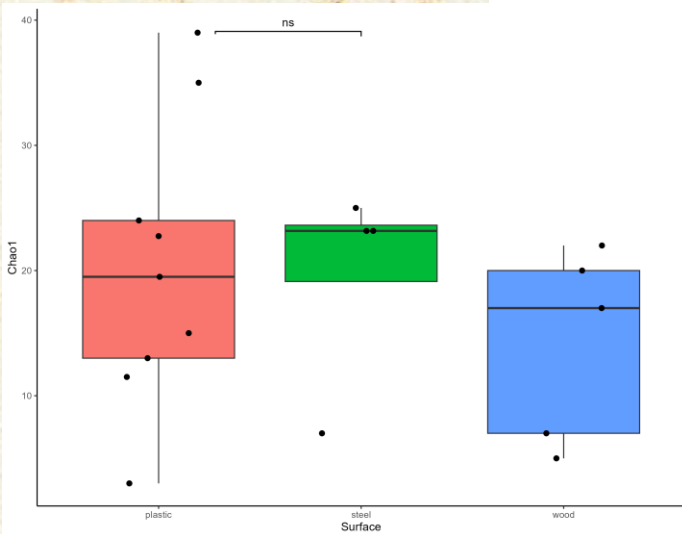
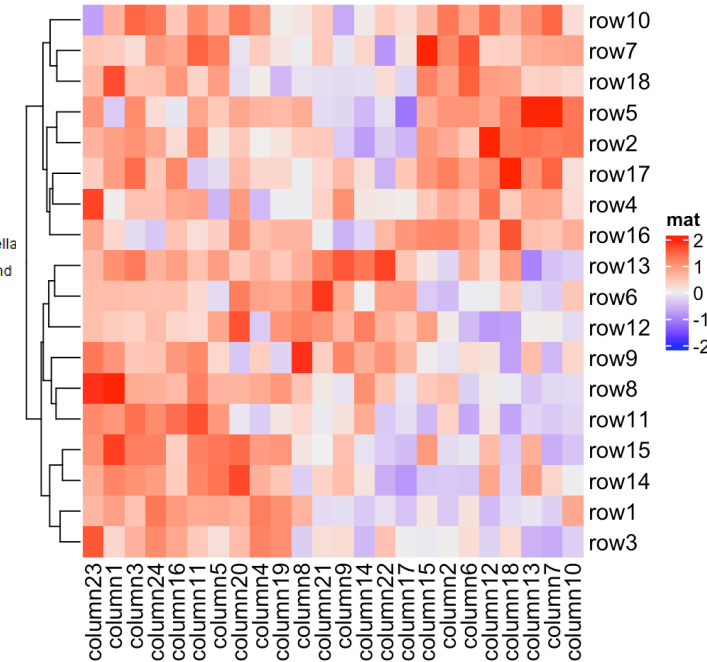
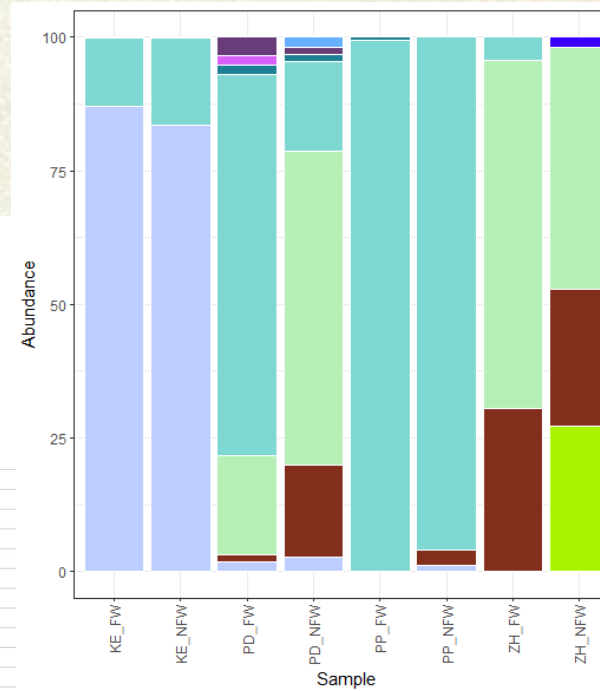
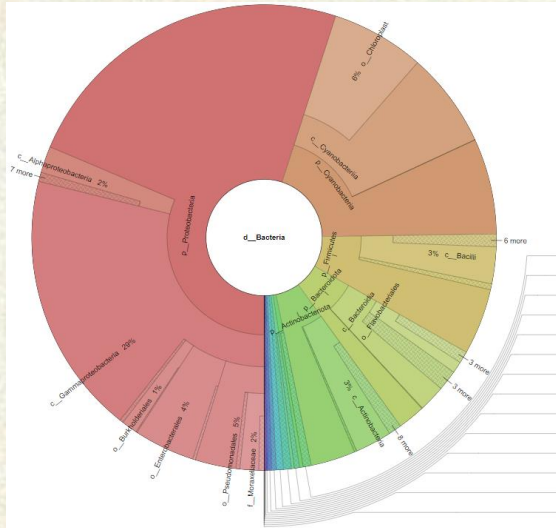
Taxonomická identifikácia



- Rôzne nástroje a databázy – pre baktérie a huby (Silva, Greengenes, Standars, Unipore, PFPlus)
 - Kraken 2, MetONTIME, Emu

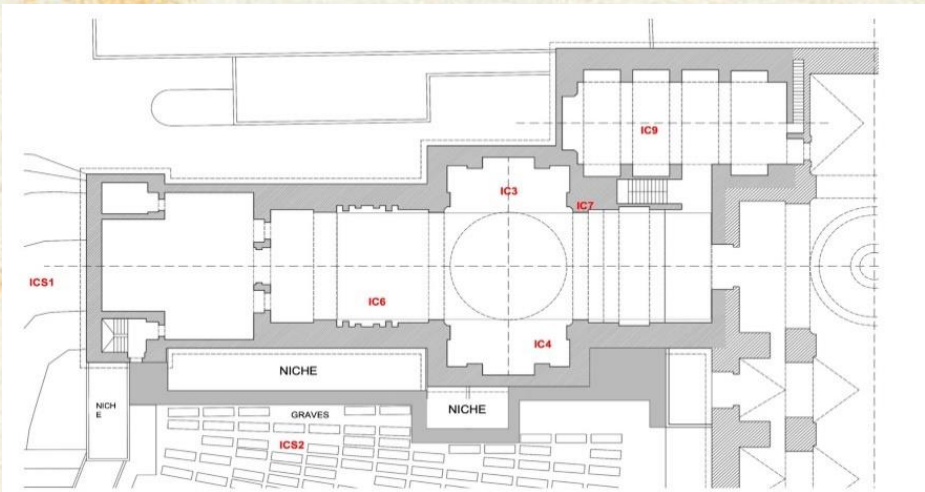
Vizualizácia

- R studio
- Phyton
- Krona



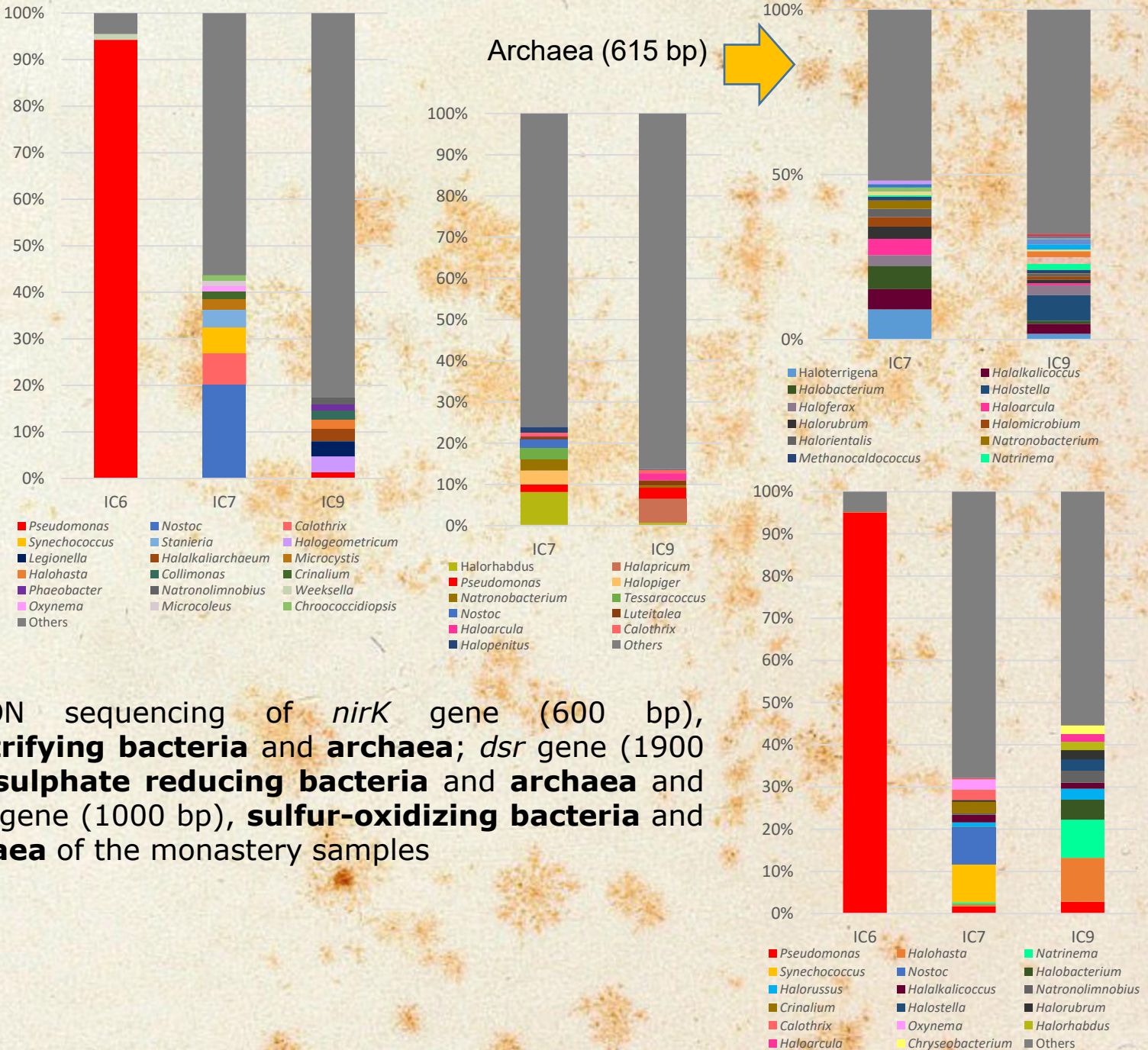
Long-amplicon MinION-based sequencing study in a salt-contaminated twelfth century granite-built chapel

Jelena Pavlović¹ · Pilar Bosch-Roig² · Magdalena Rusková¹ · Matej Planý¹ · Domenico Pangallo^{1,3} · Patricia Sanmartín^{4,5}



Code	Description, Orientation	Picture
IC3	Heterogenous damp to touch dark staining and darker*, bio-desalinated slab from the chapel paving slabs. South	
IC4	Homogeneous damp to touch dark staining and lighter*, bio-desalinated slab from the chapel paving. North	
IC6	Heterogenous damp to touch dark staining and darker*, partially bio-desalinated chapel paving slab. North	
IC7	No damp dark staining, untreated slab from the inner wall of the chapel. South	
IC9	Homogeneous damp dark staining and darker*, untreated church paving slab. South-west	
ICS1	Soil surrounding the chapel and adjoining the cemetery. North	
ICS2	Soil surrounding the chapel and not adjoining the cemetery. East	

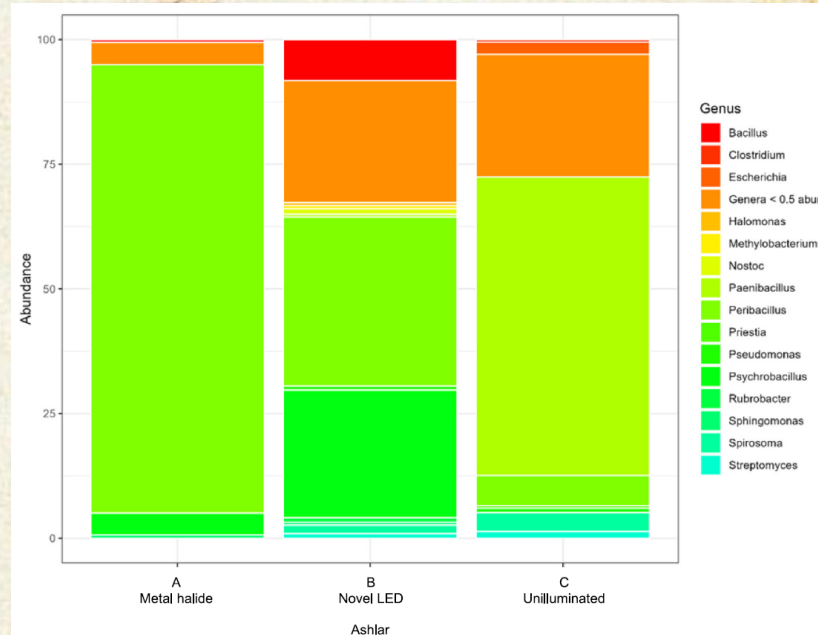
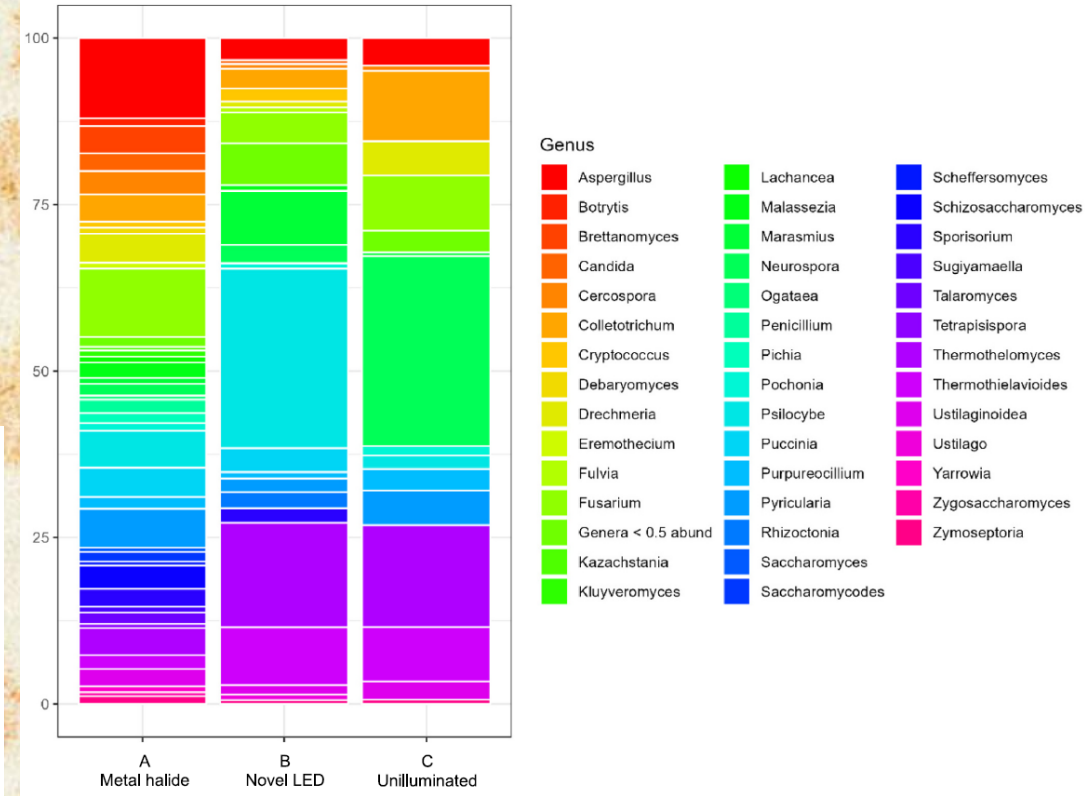
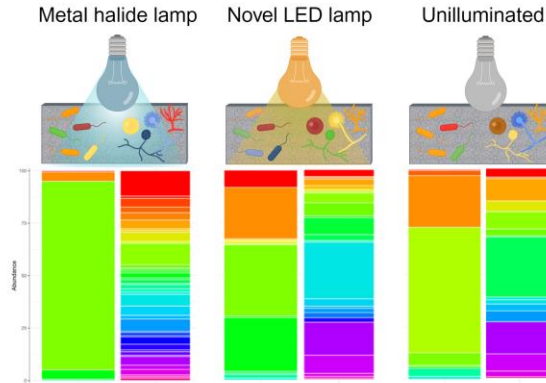
MinION sequencing of *nirK* gene (600 bp), denitrifying bacteria and archaea; *dsr* gene (1900 bp), sulphate reducing bacteria and archaea and *soxB* gene (1000 bp), sulfur-oxidizing bacteria and archaea of the monastery samples



TAXONOMIC PROFILING – Kraken2

- Taxonomic classification of amplified ribosomal genes of **bacterial and fungal DNA from stone samples** and shotgun metagenomic Sequencing.
- Database used: PlusPF Standard plus Refeq protozoa & fungi

(1) Relative abundance of **fungal ribosomal gene** at the genus level detected in the stone samples



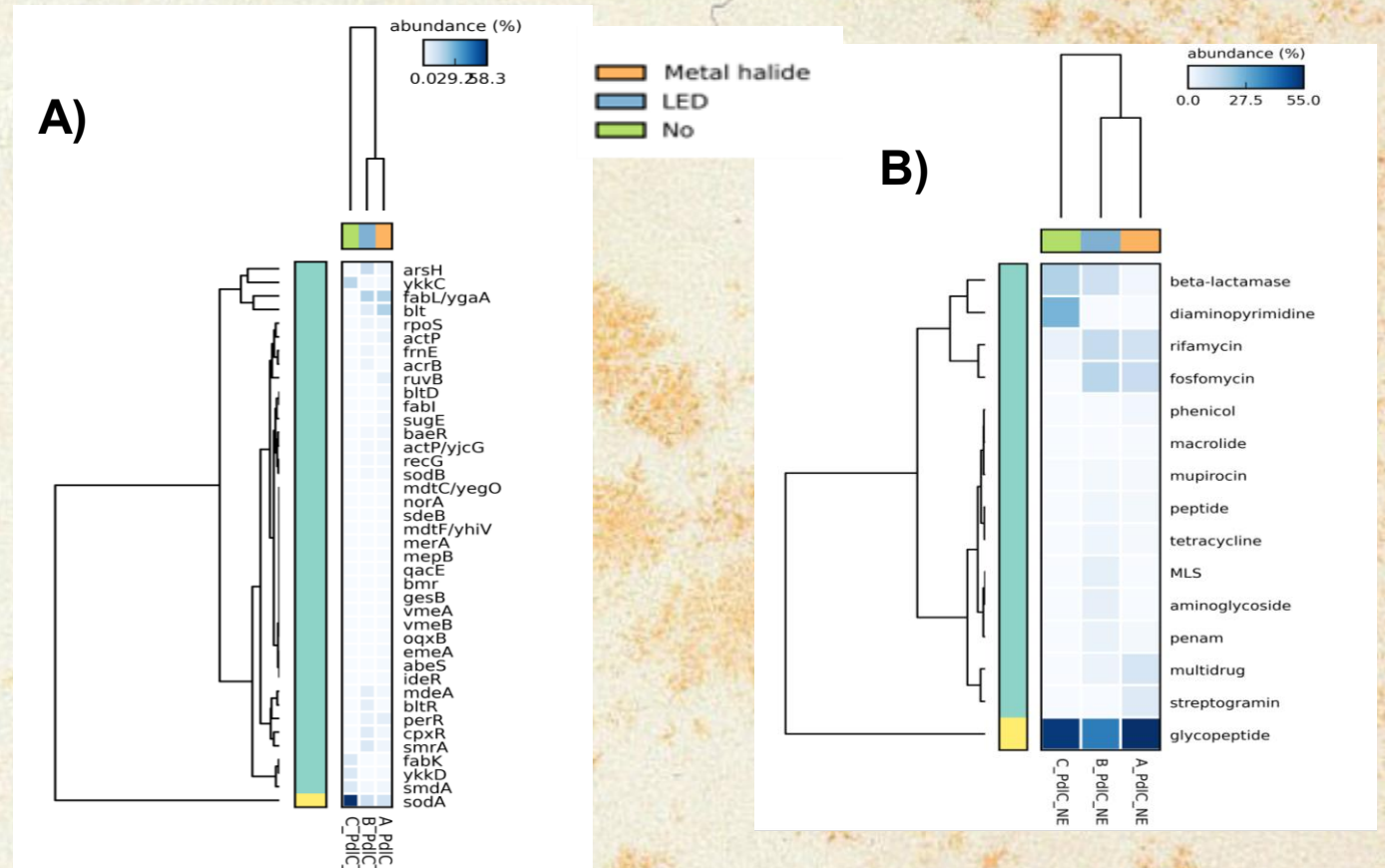
(2) Relative abundance of **bacterial genera** detected in the stone samples

FUNCTIONAL GENES PROFILING

- DIAMOND Blast - high-throughput program for aligning DNA reads or protein sequences against a protein reference database, at up to 20,000 times the speed of BLAST, with high sensitivity
- Blast+ command line - a suite of command-line tools used for sequence similarity searching (blastn, blastx, blastp)
- EPI2ME – ARMA (The Antimicrobial Resistance Mapping Application)
- ANTIBIOTIC RESISTANCE GENES – CARD database (<https://card.mcmaster.ca/>)
- BIOCIDES RESISTANCE GENES – BacMet database (<http://bacmet.biomedicine.gu.se/>)

$$\text{ARG}_i \text{ abundance (gc/Gb)} = \frac{\sum_1^m \frac{\text{Alignment}_{\text{end}} - \text{Alignment}_{\text{start}}}{\text{Length}_{\text{reference}} \text{ (bp)}}}{\frac{\sum_1^n \text{Length}_{\text{read}} \text{ (bp)}}{10^9 \text{ bp/Gb}}}$$

Dai et al., 2022



Relative abundance of Biocide (A) and Antibiotic Resistance genes (B)

- Metagenomic fastq reads -> ARG annotation against CARD using Diamond Blastx and biocide annotation against BacMet using blastn
- Filtering criteria included an 80% similarity and an E-value of 1e-5
- Normalization of the data: ARG abundance is expressed as the equivalent full length of gene copies per Giga base pair of sequences (gc/Gb) in a sample or
- copy number of ARGs is normalized to the total gigabase pairs (Gbp) of the sample

$$A_i = \frac{C_i}{C_g}(1)$$

Arango_Argoty et al., 2019

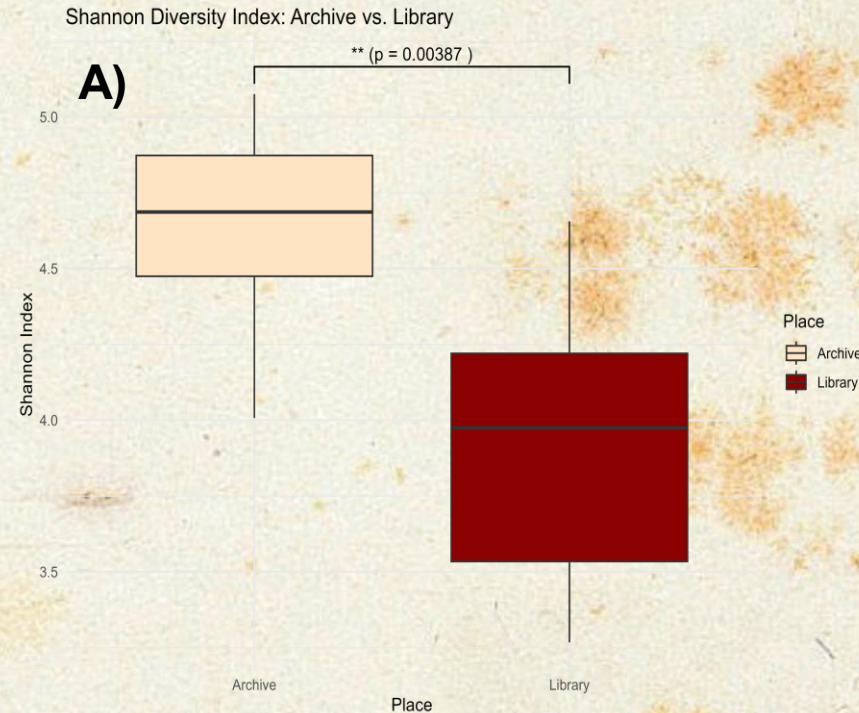
DIVERSITY ANALYSIS

α diversity: Measures the diversity within a single ecological community or sample, capturing both species richness and evenness.

Shannon Diversity Index: A metric that quantifies the entropy (diversity) in a community by considering both the abundance and evenness of species present.

β diversity: Measures the difference in diversity between ecological communities or samples, often assessing species turnover or dissimilarity across environments.

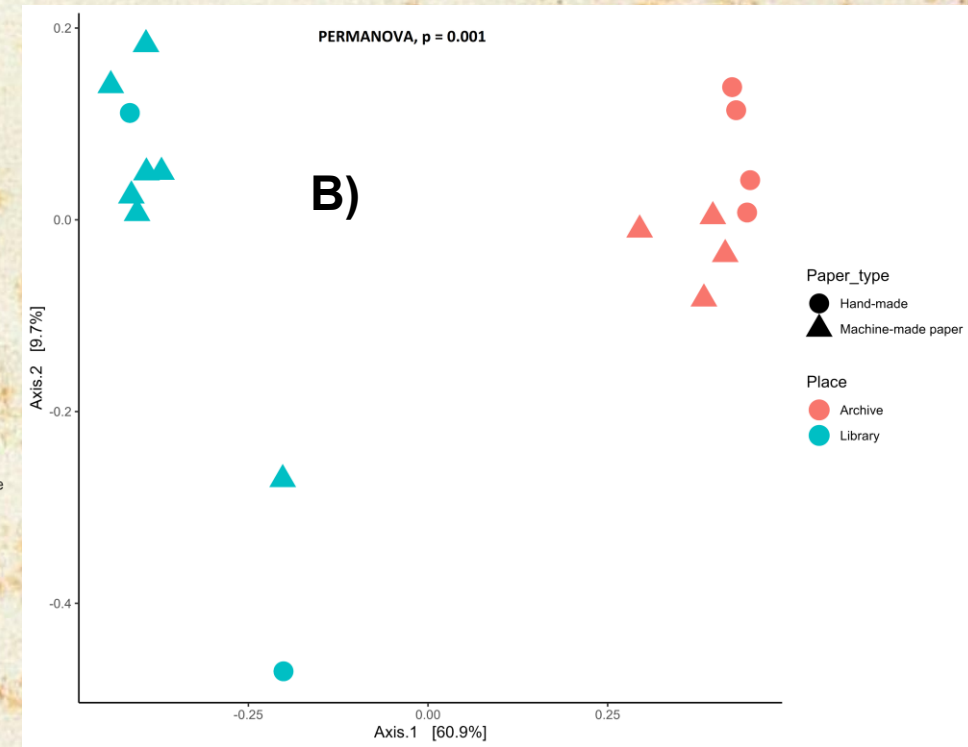
Bray-Curtis dissimilarity matrices can be used to quantify differences between samples, and these matrices can be visualized using ordination methods such as NMDS (Non-metric Multidimensional Scaling) or PCoA (Principal Coordinates Analysis) to explore patterns of community composition across samples



A) Differences in Shannon diversity index between Library and Archive group. Statistical significance was assessed using an unpaired t-test.

$$H = -\sum_{j=1}^S p_i \ln p_i$$

Eq 1. Shannon diversity index



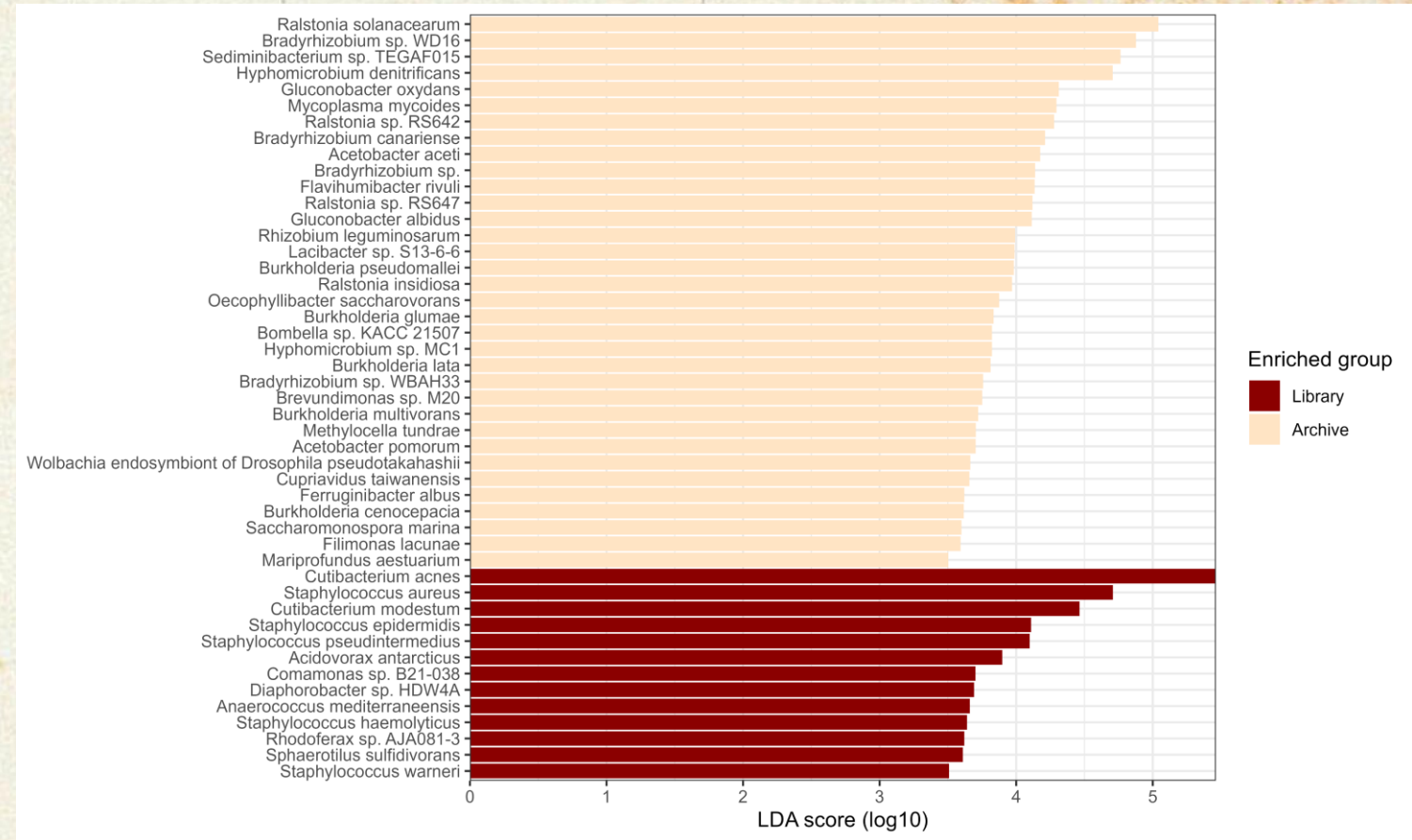
B) PCoA plots of beta diversity in Library and Archive microbial community using Bray-Curtis distance. Statistical significance was assessed using permutational multivariate analysis of variance (PERMANOVA) Permanova

$$BC_d = \frac{\sum |x_i - x_j|}{\sum (x_i + x_j)}$$

Eq. 2. Bray-Curtis dissimilarity

DIFFERENTIAL ABUNDANCE ANALYSIS

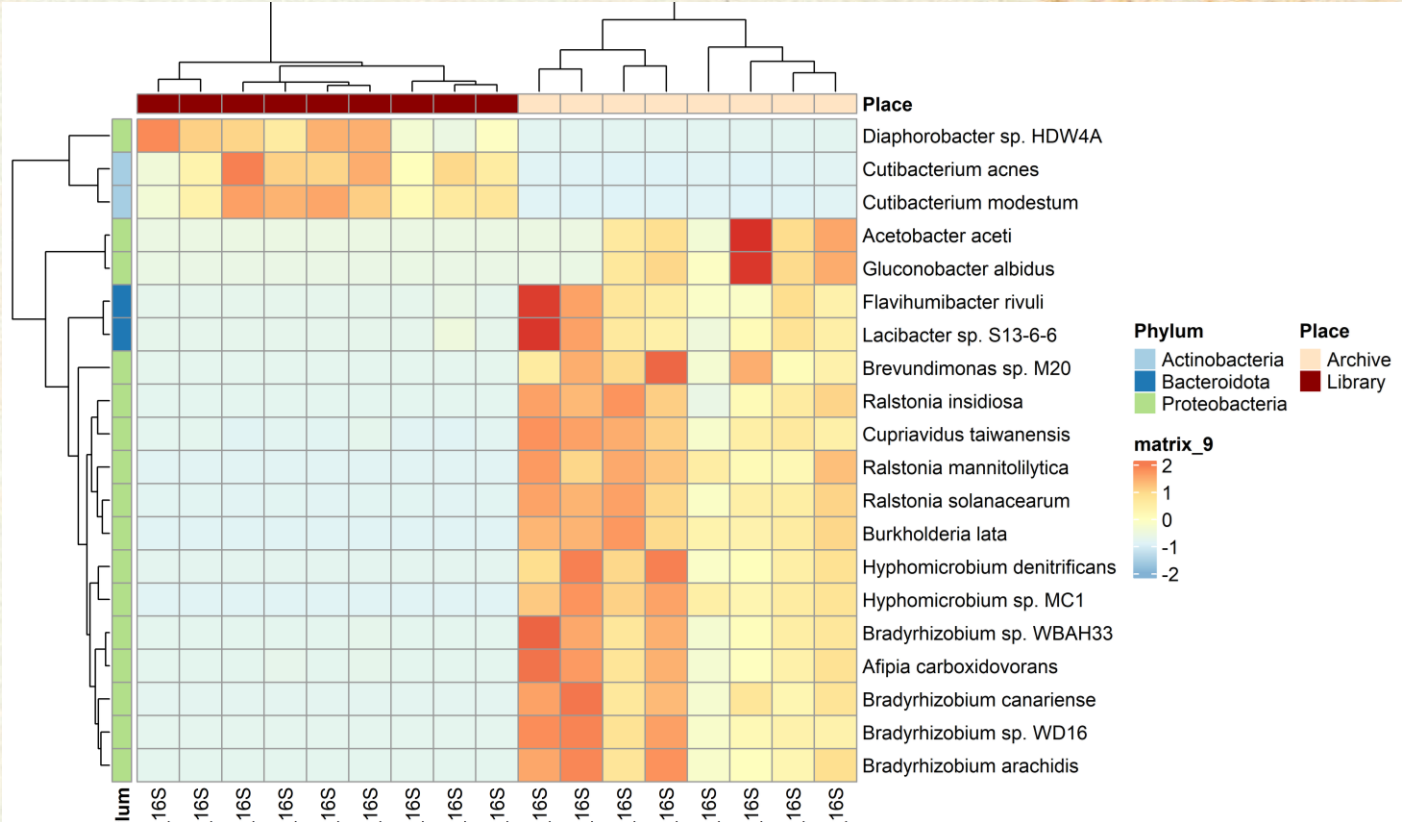
- **LefSE(Linear discriminant analysis Effect Size)** – statistical tool used for biomarker discovery identifying features (like organisms or genes) that differ significantly in abundance across biological groups
- <http://galaxy.biobakery.org/>
- R package “microbiomeMarker”
- Statistical Tests in LefSe:
Kruskal-Wallis rank-sum test: non-parametric test is used to detect features with significant differences
Wilcoxon rank-sum test: for pairwise comparisons between subgroups
- **Linear Discriminant Analysis (LDA)** – estimate the effect size of each feature.



feature	enrich_group	ef_lda	pvalue	padj
Cutibacterium acnes	Library	5.46234105	0.00053201	0.00053201
Diaphorobacter sp. HDW4A	Library	3.69199783	0.00049117	0.00049117
Cutibacterium modestum	Library	4.46407876	0.00053201	0.00053201
Ralstonia solanacearum	Archive	5.04060869	0.00053201	0.00053201
Bradyrhizobium sp. WD16	Archive	4.87623439	0.00033137	0.00033137
Acetobacter aceti	Archive	4.17508337	0.00033137	0.00033137

DIFFERENTIAL ABUNDANCE ANALYSIS

- **DeSeq2** – Statistical tool originally designed for RNA-seq data, adapted for microbiome count data.
- Similar results as LefSe
- **ANCOMBC** (analysis of compositions of microbiomes with bias correction) – is a package containing differential abundance (DA) and correlation analyses for microbiome data.



OTU	Phylum	Species	log2Fold Change	lfcSE	stat	pvalue	padj	Enrichment Direction
2714924	Proteobacteria	<i>Diaphorobacter</i> sp. HDW4A	8.30996	1.02534	8.104553	5.29E-16	4.04459E-14	Library
1747	Actinobacteria	<i>Cutibacterium acnes</i>	5.95306	0.66797	8.91215	5.01E-19	5.736E-17	Library
2559073	Actinobacteria	<i>Cutibacterium modestum</i>	6.22314	0.66695	9.330619	1.05E-20	1.34024E-18	Library
435	Proteobacteria	<i>Acetobacter aceti</i>	-9.68626	1.20223	-8.05691	7.82E-16	5.60441E-14	Archive

Table showing DeSeq2 results with log2 fold change in significantly different bacterial species

Heatmap representing DeSeq2 results showing log2 fold change in significantly different bacterial species

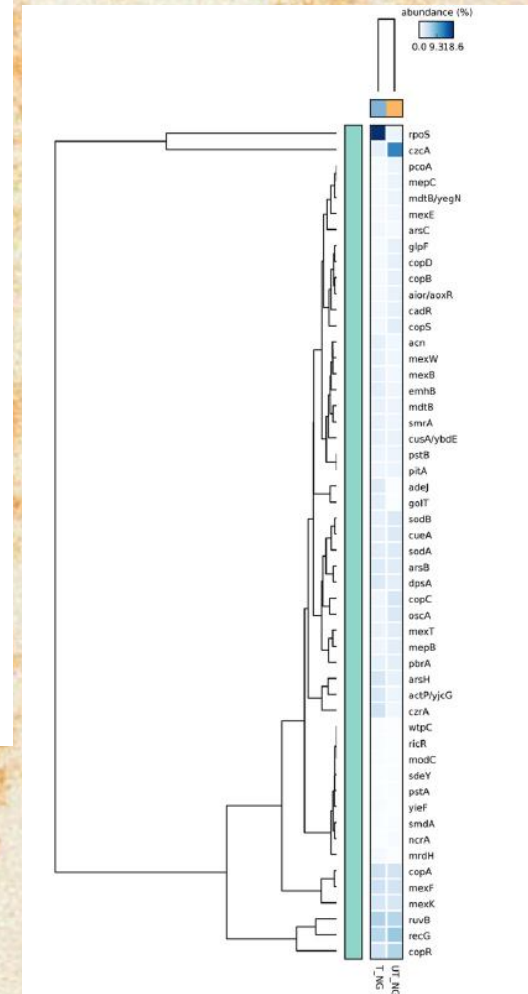
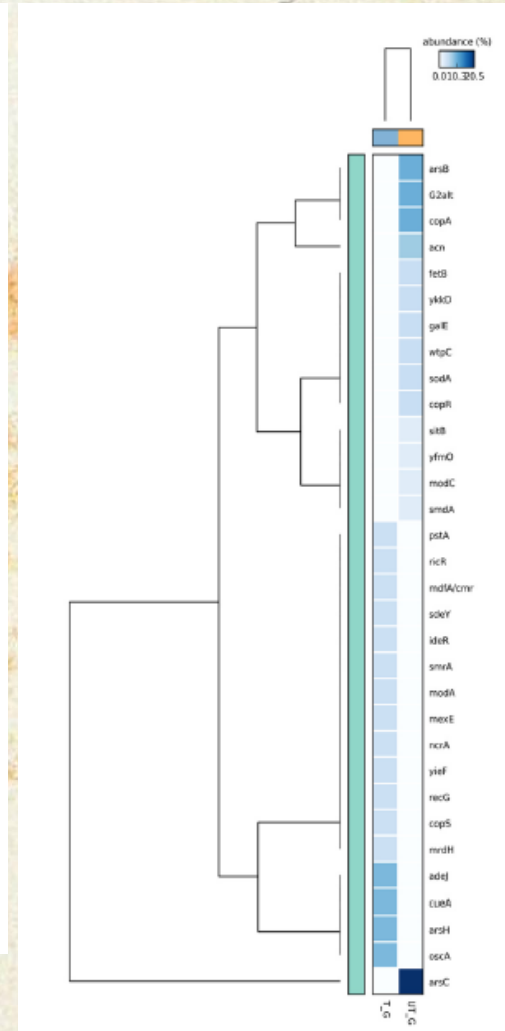
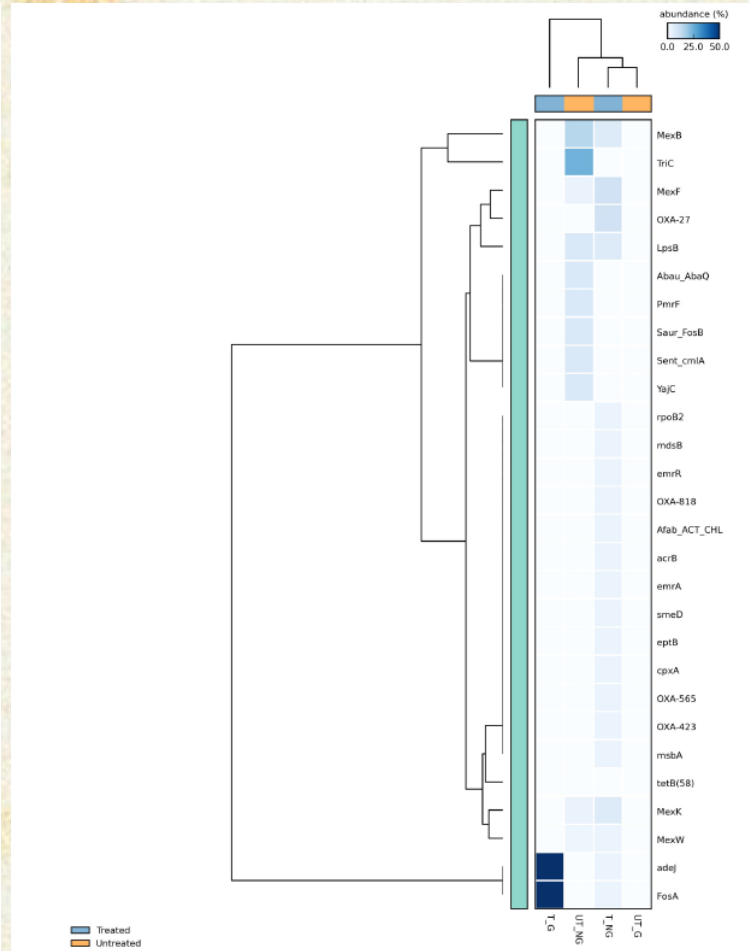
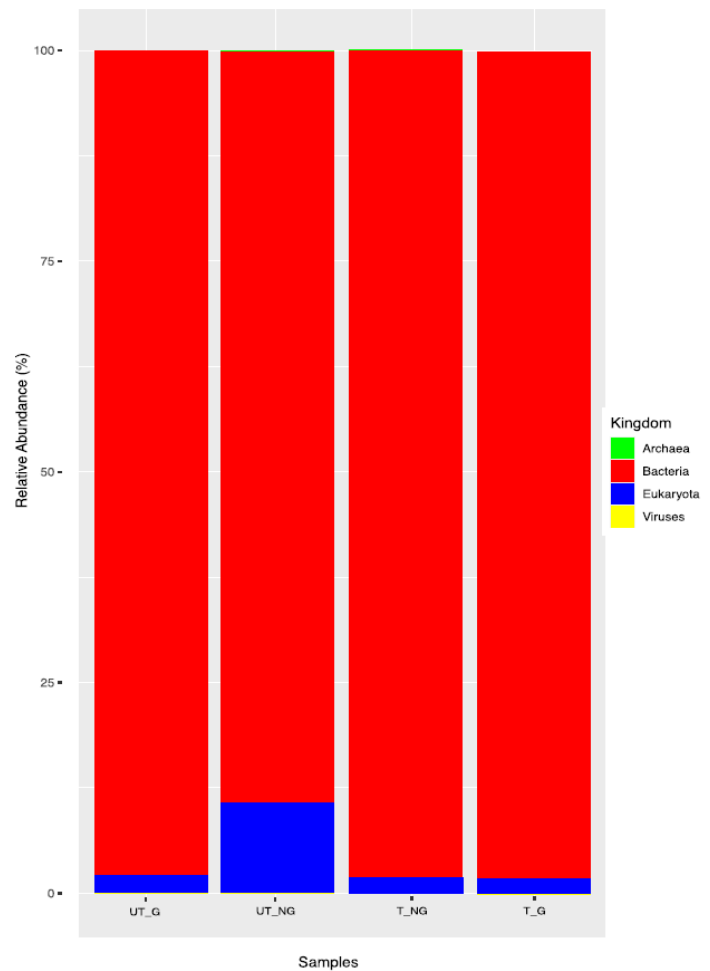
untreated with
greening (**UT_G**) and
untreated with no-
greening (**UT_NG**) at
Casa do Deán.



treated with no-greening
(**T_NG**) and treated with
greening (**T_G**) at **Casa
do Cabildo**.



Biocides based on quaternary
ammonium salts (**Biotin R[®]**
and **New Des[®] 50** - CTS srl)
and **ammonium bicarbonate**.



Baktérie a prítomnosť génov rezistencie na antibiotiká a biocídy

Zdá sa, že vplyv čistenia ulíc na bakteriálny mikrobióm bol väčší ako vplyv chemickej aplikácie. Bakteriálna aj hubová diverzita bola vyššia v oblastiach odberu vzoriek postihnutých čistením ulíc, zatiaľ čo ARG a BRG boli rozšírenejšie v oblastiach odberu vzoriek, ktoré prešli reštauračnou úpravou pred 15 rokmi.

SICK BUILDING SYNDROME

SBS

„**Syndróm chorých budov**“ označuje situáciu, keď ľudia, ktorí trávajú čas v konkrétnej budove, pociťujú súbor symptómov, ktoré sa zdajú byť spojené s časom stráveným v nej.

Medzi bežné symptómy patria **bolesti hlavy, podráždenie očí, nosa a hrdla, suchá alebo svrbiaca pokožka, únava**, ťažkosti so sústredením, závraty a nevoľnosť. Tieto symptómy bývajú zvyčajne mierne, no môžu výrazne ovplyvniť pohodlie a produktivitu postihnutých osôb.

Medzi podozrivé prispievajúce faktory často patrí **nedostatočné vetranie**, ktoré umožňuje koncentráciu znečisťujúcich látok v interiéri, **chemické kontaminanty** zo zdrojov ako koberce, nábytok, čistiace prostriedky alebo kopírovacie stroje, ktoré uvoľňujú **prchavé organické zlúčeniny**, a **biologické kontaminanty** ako **plesne**, prach, **peľ** alebo **baktérie** zo stojatej vody v potrubiach či zvlhčovačoch vzduchu.

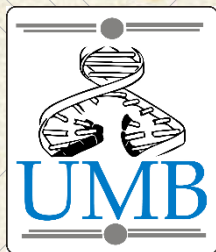


SUSTAINABLE ARCHIVES
& GREENER APPROACHES

Microbial Risks Within Archival Settings

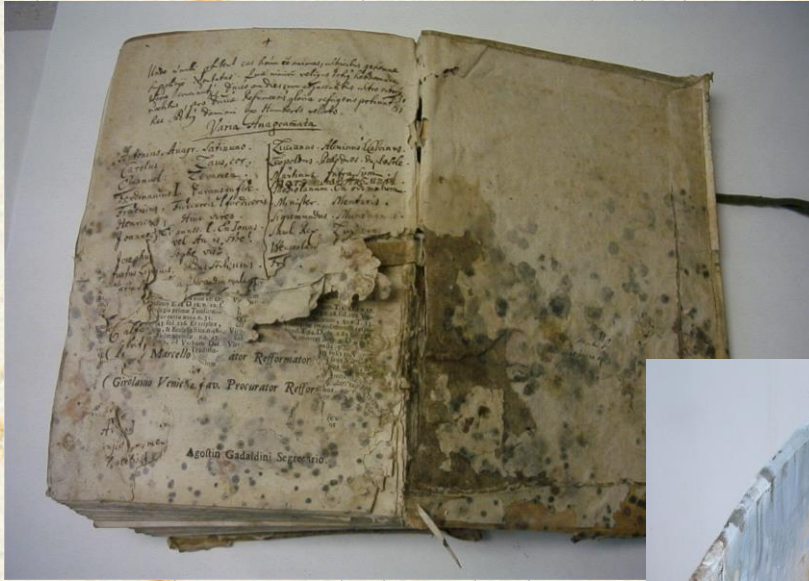
Domenico Pangallo & the Bratislava's Group

Bratislava's Group



Co-funded by
the European Union

Targets of the Microbial Analysis



Archival objects



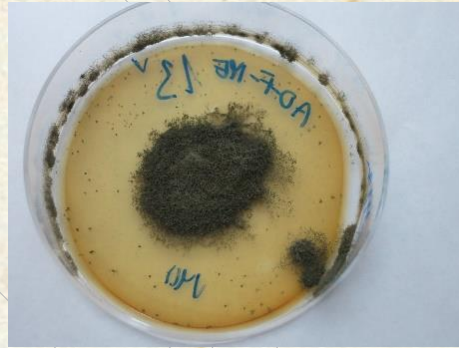
Surrounding environment



Approaches for the identification of Microbial Communities

- Culture-dependent (CD) - kultivačne závislá

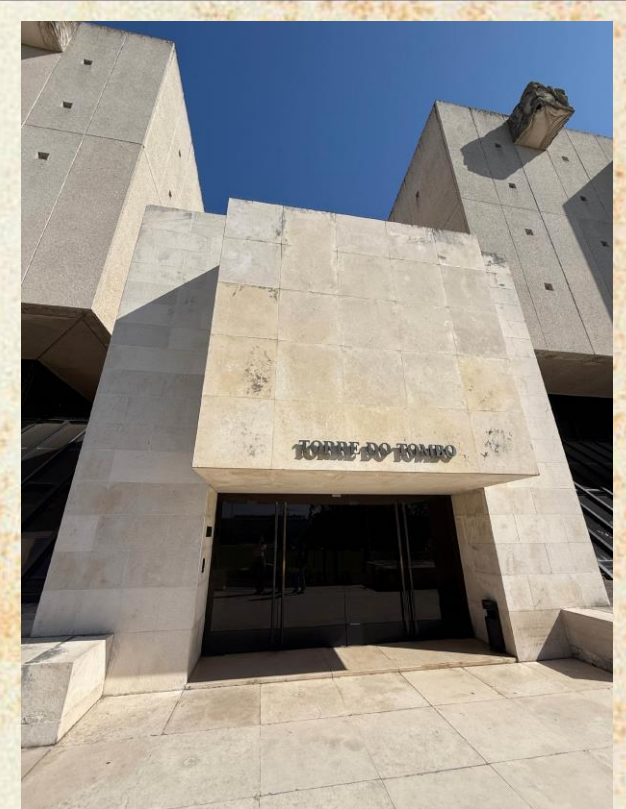
Cultivation, Isolation and Characterization of Microorganisms by the use of Culture Media



- Culture independent (CI) - kultivačne nezávislá

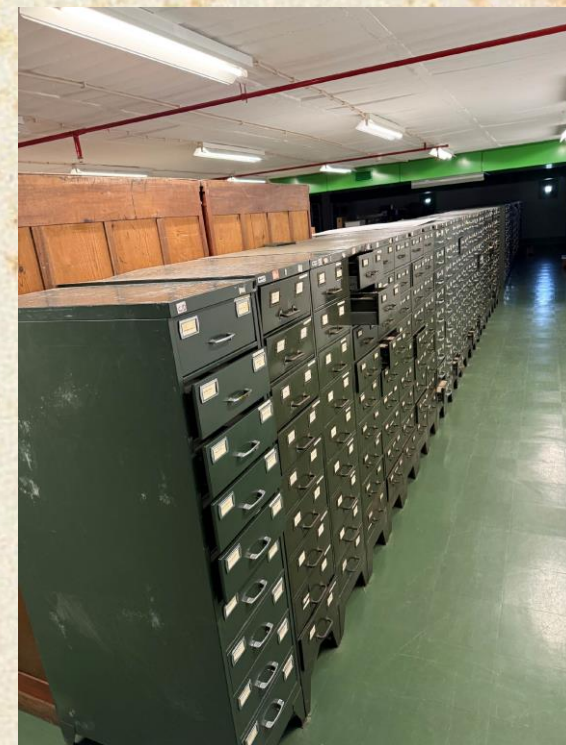
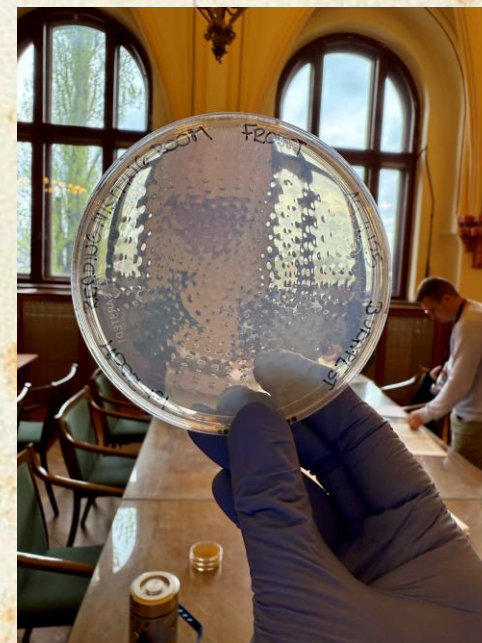
Identification of the microorganisms by the extraction of total DNA and using high-throughput sequencing approach



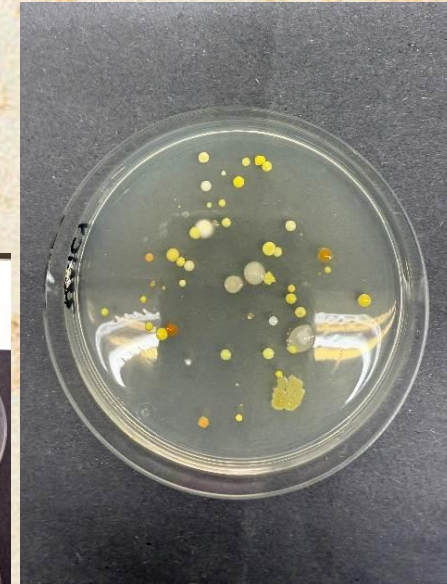
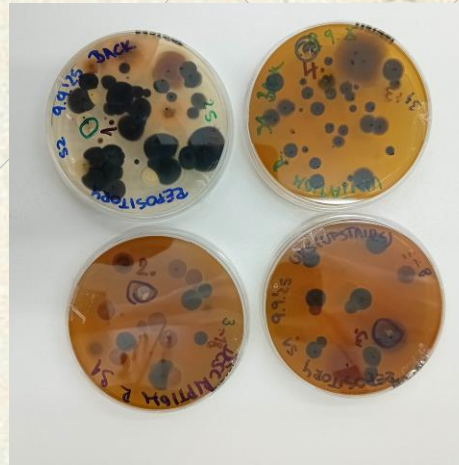
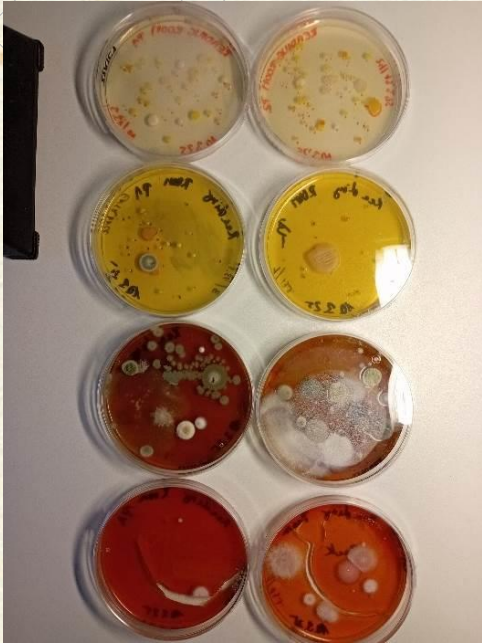
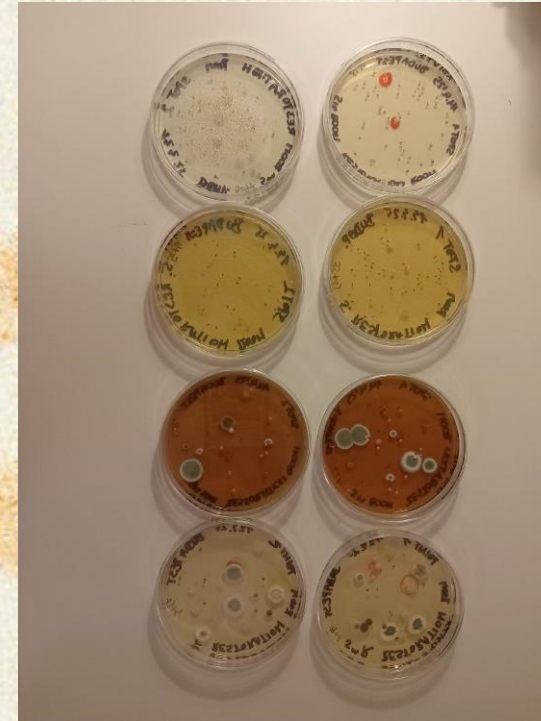
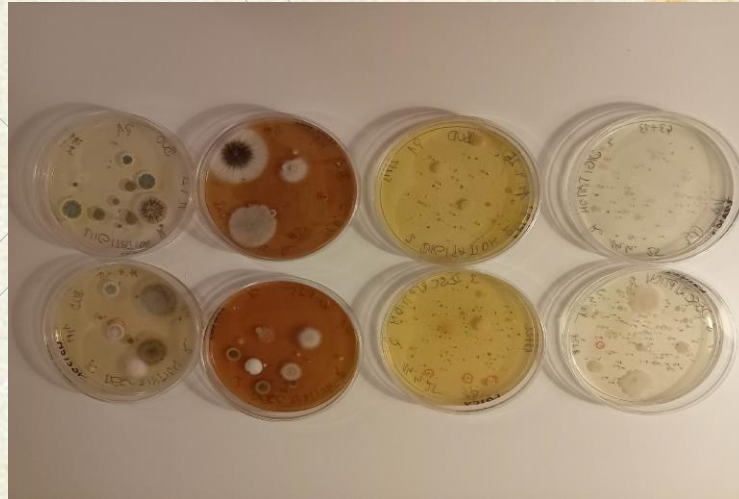


Five Historical Archives:

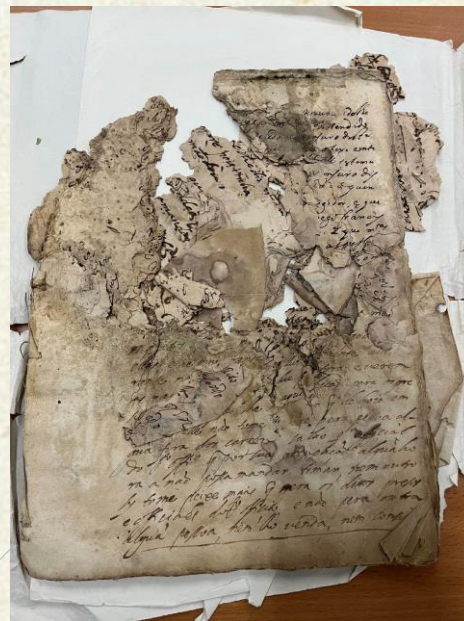
Florence – Italy
Budapest – Hungary
Lisbon – Portugal
Malta
Madrid - Spain



Kultivačne závislá



Historické dokumenty - Kultivačne závislá



Guidelines about **CFU/m³** in INDOOR AIR



**World Health
Organization**

WHO Guidelines for Indoor Air Quality: Dampness and Mould (2009)

Total bacterial count:

- **<500 CFU/m³** is considered good air quality.
- **500–1000 CFU/m³** is acceptable.
- **>1000 CFU/m³** may indicate poor ventilation or contamination.

Total fungal count (molds & yeasts):

- **<500 CFU/m³** is considered normal.
- **500–1000 CFU/m³** is acceptable but requires monitoring.
- **>1000 CFU/m³** may indicate fungal contamination.



**MINISTERO
DELLA
CULTURA**

Good air quality in archives, libraries, and museums **should not exceed**
750 CFU/m³ for bacteria and **150 CFU/m³** for fungi.

ROOM – Florence	BACTERIA		FUNGI		T / RH%
	R2A	LB	PDA	DRBC	
Repository 2 P1	330	230	20	0	16.2 °C / 57%
Repository 2 P2	340	30	20	20	15.8 °C / 61%
Repository 11	580	300	10	10	17.5 °C / 52%
Digitation 19	370	160	20	0	18.8 °C / 52%
Visitors room P1	420	270	70	10	20.7 °C / 50%
Visitors room P2	520	120	260	70	21 °C / 49%
Office 43	300	140	120	20	19.8 °C / 55%

ROOM - Budapest	BACTERIA		FUNGI		T / RH%
	R2A	LB	PDA	YGC	
Repository P1 (49)	710	340	40	120	20.6 °C / 31%
Repository P2 (38)	260	250	50	60	19.4 °C / 32%
Repository P3 (22)	280	150	50	80	19.2 °C / 32%
Digitation	600	380	150	190	23.6 °C / 36%
Visitors room P1	1560	760	200	160	23.3 °C / 35%
Visitors room P2	1010	310	170	130	23.5 °C / 35%
Office	1150	770	180	150	22.3 °C / 37%
Restoration Lab P1	450	510	290	260	22.5 °C / 45%
Restoration Lab P2	610	300	230	170	23.2 °C / 42%

ROOM - Lisbon	BACTERIA		FUNGI		T / RH%
	R2A	LB	PDA	YGC	
Repository corpo 18	80	20	20	20	20.8 °C / 59%
Repository corpo 51 (AC)	30	60	20	40	22 °C / 56%
Reproduction room, office	120	80	50	10	23.6 °C / 54%
Digitation P1	110	150	50	30	25.5 °C / 51%
Digitation P2	200	120	40	60	24.1 °C / 51%
Visitors room P1	430	490	50	40	22.5 °C / 58%
Visitors room P2	160	20	20	60	22.8 °C / 55%
Description room, office	290	140	140	120	23.3 °C / 55%
Restoration Lab P1	100	40	30	20	23.9 °C / 54%
Restoration Lab P2	130	100	20	30	24 °C / 54%

500 CFU/m³ for both bacteria and fungi

750 CFU/m³ for bacteria - 150 CFU/m³ for fungi

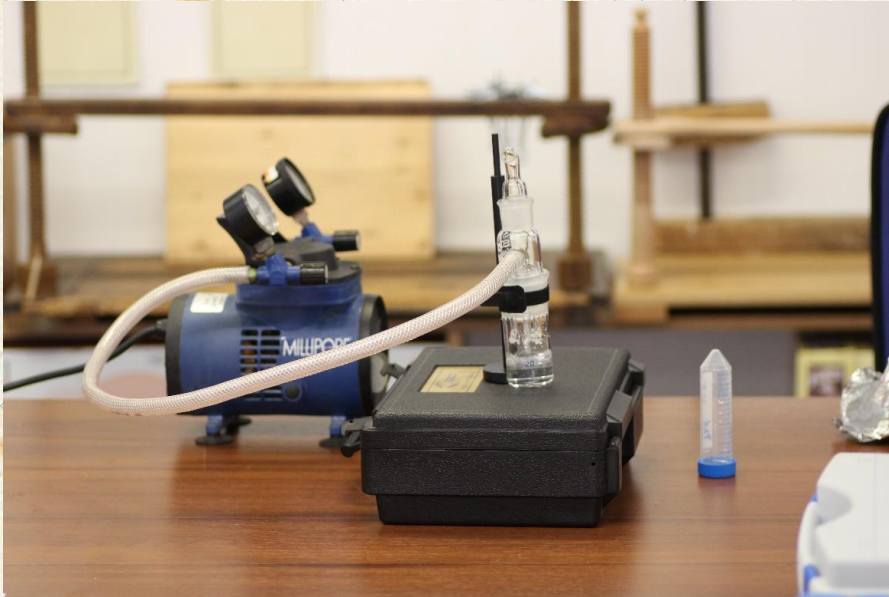
ROOM - Malta	BACTERIA		FUNGI		T / RH%
	R2A	LB	PDA	YGC	
Repository Up S1	520	280	150	160	29 °C / 70%
Repository back S2	350	400	380	410	28 °C / 66%
Repository front S3	500	470	490	430	28.4 °C / 66%
Digitation S1 (AC)	390	230	430	280	25.9 °C / 70%
Visitors room S1 back	140	110	350	390	28.1 °C / 64%
Visitors room S2 front	170	260	490	350	28.2 °C / 64%
Description room S1 (AC)	720	250	320	240	25.5 °C / 42%
Restoration Lab S1 (AC)	1120	600	700	450	25.9 °C / 73%

500 CFU/m³ for both bacteria and fungi

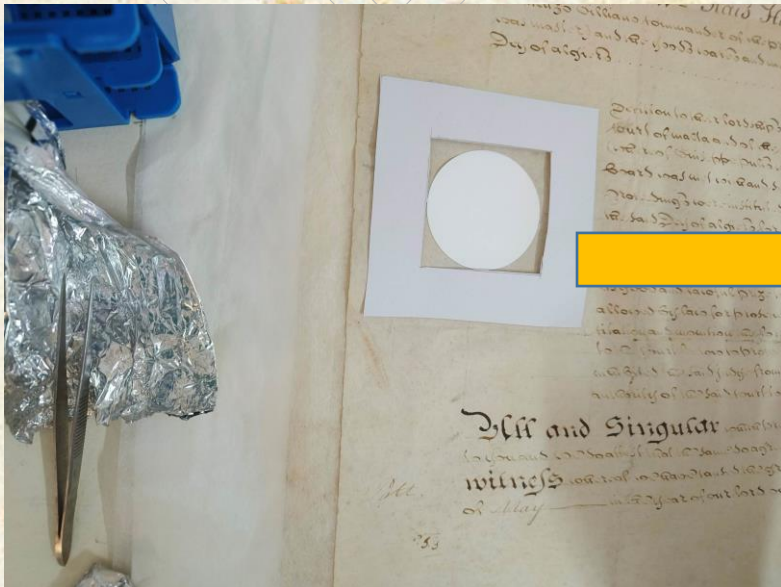
750 CFU/m³ for bacteria
150 CFU/m³ for fungi

ROOM - Madrid	BACTERIA		FUNGI		T / RH%
	R2A	LB	PDA	YGC	
Repository; SP 1	180	140	130	120	19.4 °C / 44%
Repository; SP 2	130	90	80	80	20 °C / 44%
Digitization room	90	60	80	150	21.8 °C / 41%
Visitors' room; SP 1	600	430	290	260	22.1 °C / 40%
Visitors' room; SP 2	940	710	290	410	22.8 °C / 42%
Restoration laboratory; SP 1	310	240	170	260	21.2 °C / 41%
Restoration laboratory; SP 2	150	610	180	180	20.5 °C / 41%
Office 1 (description)	720	250	320	240	21.6 °C / 40%

High-Throughput Sequencing (**DNA extraction**) - kultivačne nezávislá

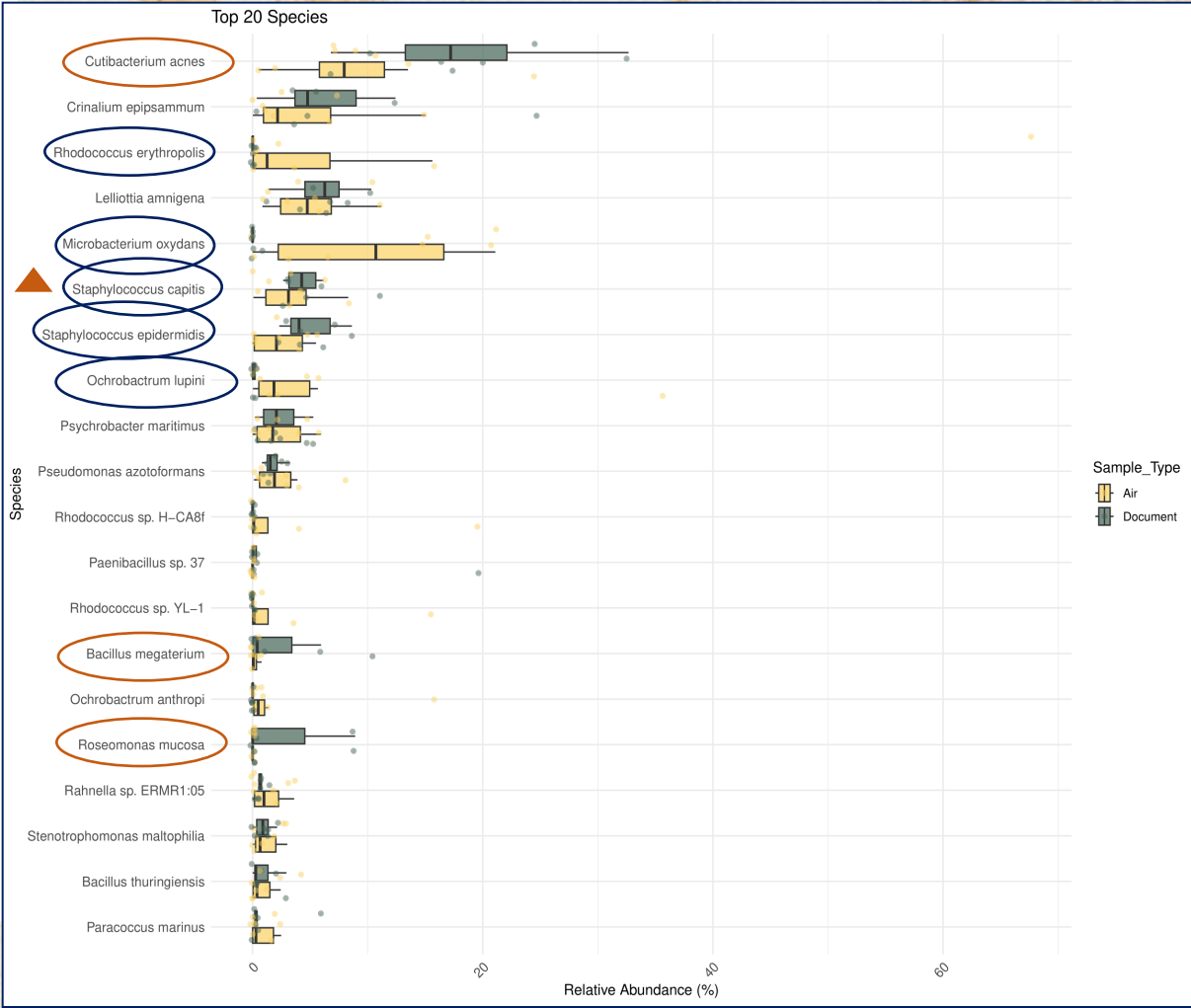
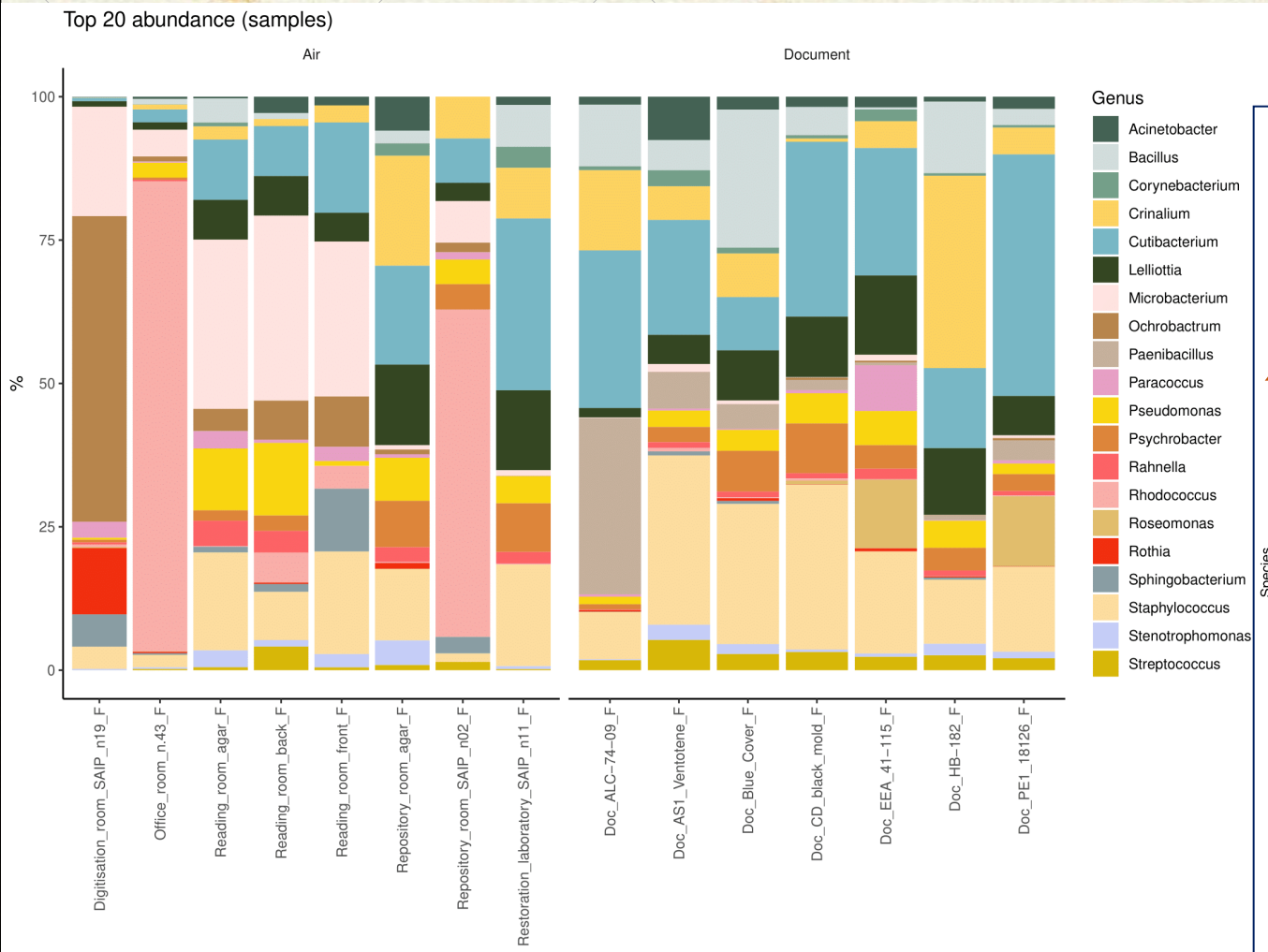


The solution containing airborne microorganisms was **filtered** through 0.2 µm nitrocellulose membranes.



The microbial samples are recovered by 0.2 µm nitrocellulose membranes.

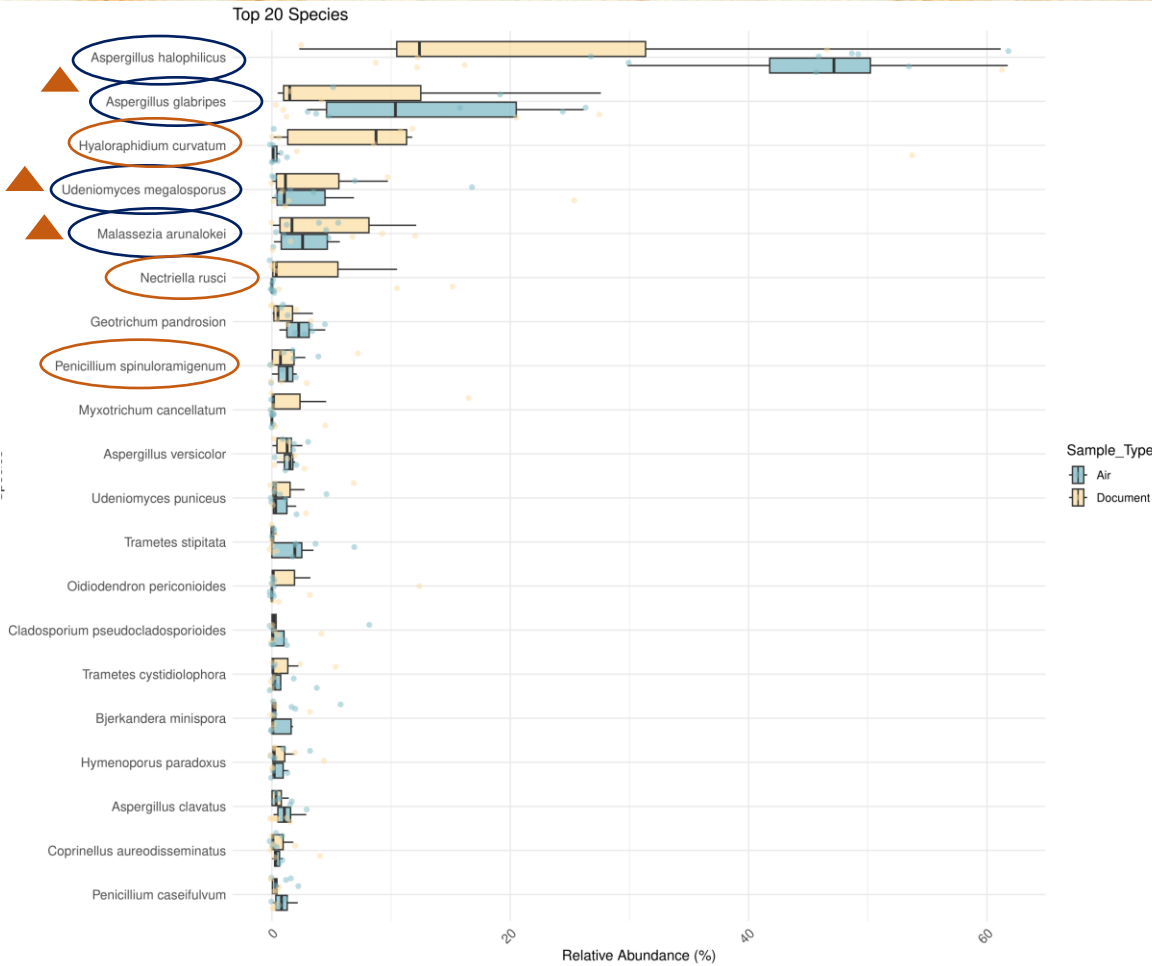
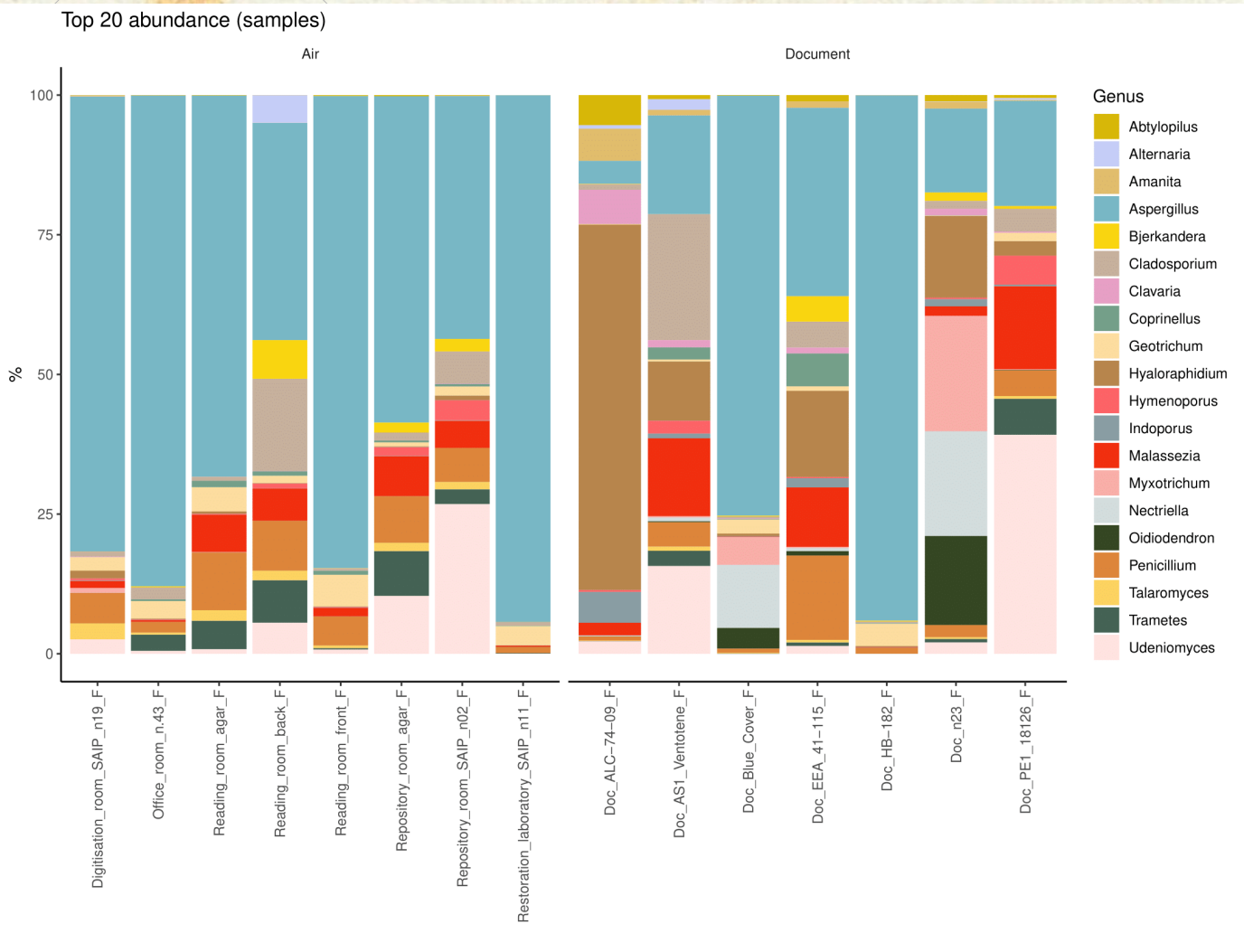
Bacterial 16S rRNA gene (Historical Archives of the European Union)



Air: Microbacterium, Rhodococcus, Ochrobactrum, Staphylococcus

Documents: Cutibacterium, Roseomonas, Bacillus,

Fungal 28S rRNA gene (Historical Archives of the European Union)

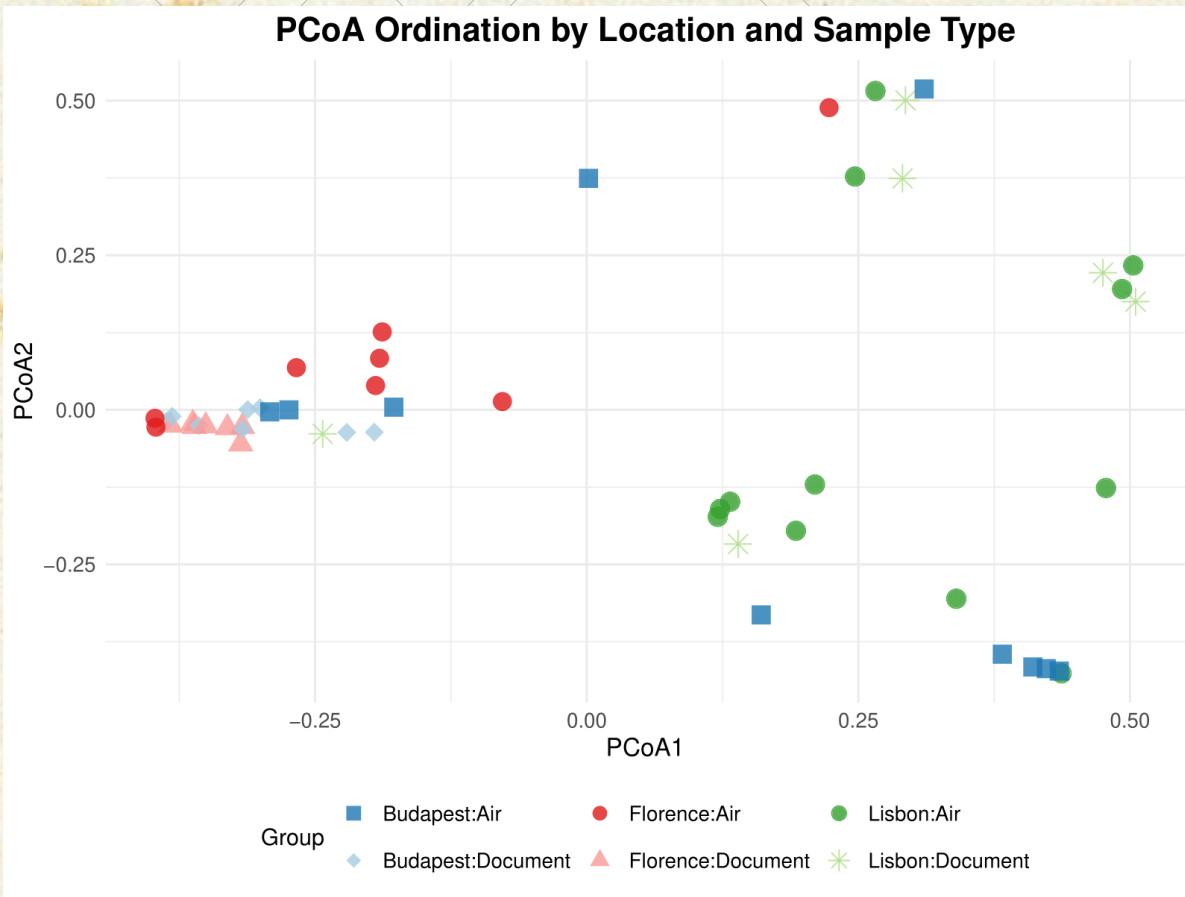


Air: *Aspergillus*, *Udeniomyces*, *Malassezia*

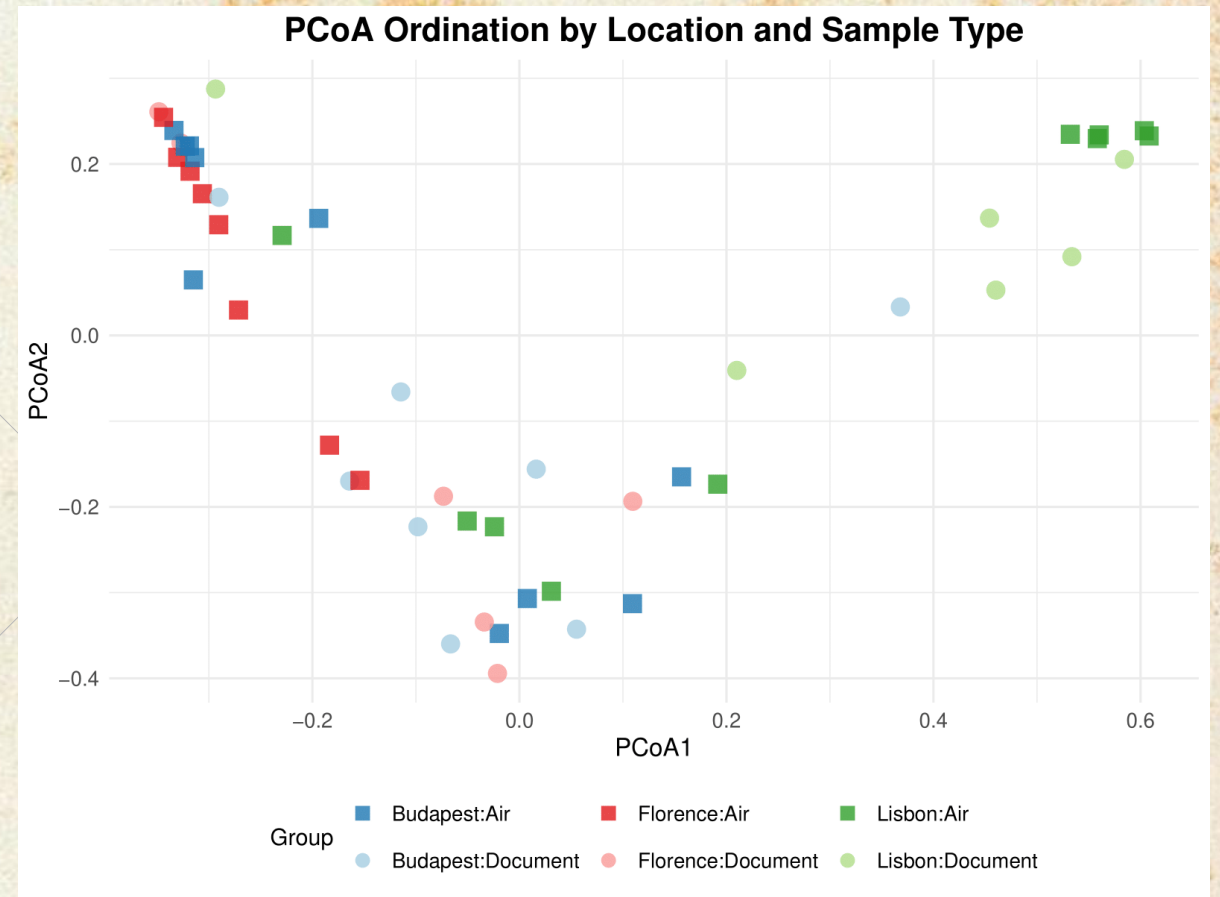
Documents: *Hyaloraphidium*, *Aspergillus*, *Udeniomyces*,
Nectriella, *Malassezia*, *Penicillium*

COMPARISON: Florence, Budapest, and Lisbon

16S rRNA



28S rRNA



COMPARISON: Florence, Budapest, and Lisbon

Comparison	p-value	Signif
Budapest:Document vs Lisbon:Document	0.0005	***
Florence:Air vs Lisbon:Air	0.0002	***
Florence:Document vs Lisbon:Document	0.0008	***
Budapest:Air vs Budapest:Document	0.0017	**
Florence:Air vs Florence:Document	0.0061	**
Budapest:Air vs Florence:Air	0.0022	**
Budapest:Document vs Florence:Document	0.0026	**
Budapest:Air vs Lisbon:Air	0.0291	*
Lisbon:Air vs Lisbon:Document	0.1195	ns

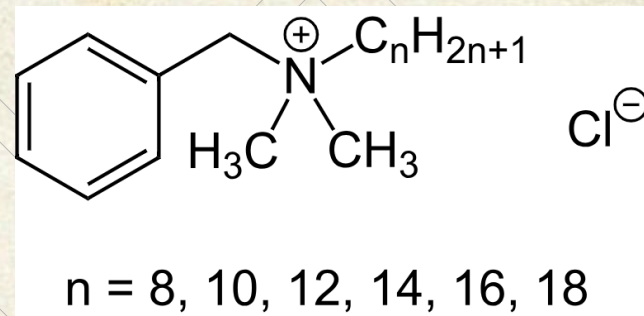
16S rRNA sequencing (Bacteria)

28S rRNA sequencing (Fungi)

Comparison	p-value	Signif
Florence:Air vs Lisbon:Air	0.0004	***
Budapest:Air vs Lisbon:Air	0.0018	**
Budapest:Document vs Lisbon:Document	0.0083	**
Florence:Document vs Lisbon:Document	0.0096	**
Budapest:Air vs Budapest:Document	0.017	*
Budapest:Air vs Florence:Air	0.0176	*
Budapest:Document vs Florence:Document	0.0927	.
Florence:Air vs Florence:Document	0.0604	.
Lisbon:Air vs Lisbon:Document	0.5295	ns

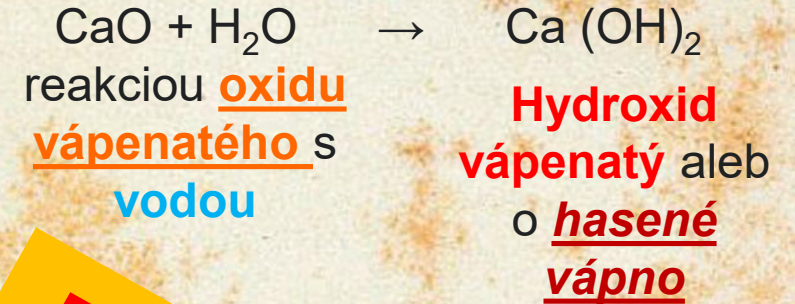
Biocides and Lime plaster

Several long-chain aliphatic benzylic QACs (including **benzalkonium chloride** relatives such as **cetalkonium chloride**, **benzododecinium chloride**, etc.) are used in cultural heritage preservation and have been **flagged by ECHA for possible future RESTRICTION**.



Some commercial Formulations: Preventol RI50, BAC-50

Hasenie (hydratácia)



Karbonát (vzniká pri vytvrdzovaní/zrení)



pH ~12 – 13
Antimicrobial effect

pH klesne na hodnoty blízke neutrálnym, typicky okolo **8 – 9**.
Pripravené na mikrobiálnu kontamináciu

Dezinfekcia prírodnými zlúčeninami

Microorganisms and Essential oils tested by *in vitro* experiment

Bacteria

Escherichia coli
Salmonella typhimurium
Yersinia enterocolitica
Staphylococcus aureus
Listeria monocytogenes
Enterococcus faecalis

Fungi

Alternaria alternata
Aspergillus fumigatus
Chaetomium globosum
Cladosporium cladosporioides
Penicillium chrysogenum

Essential Oils



Oregano



Thyme



Clove



Arborvitae

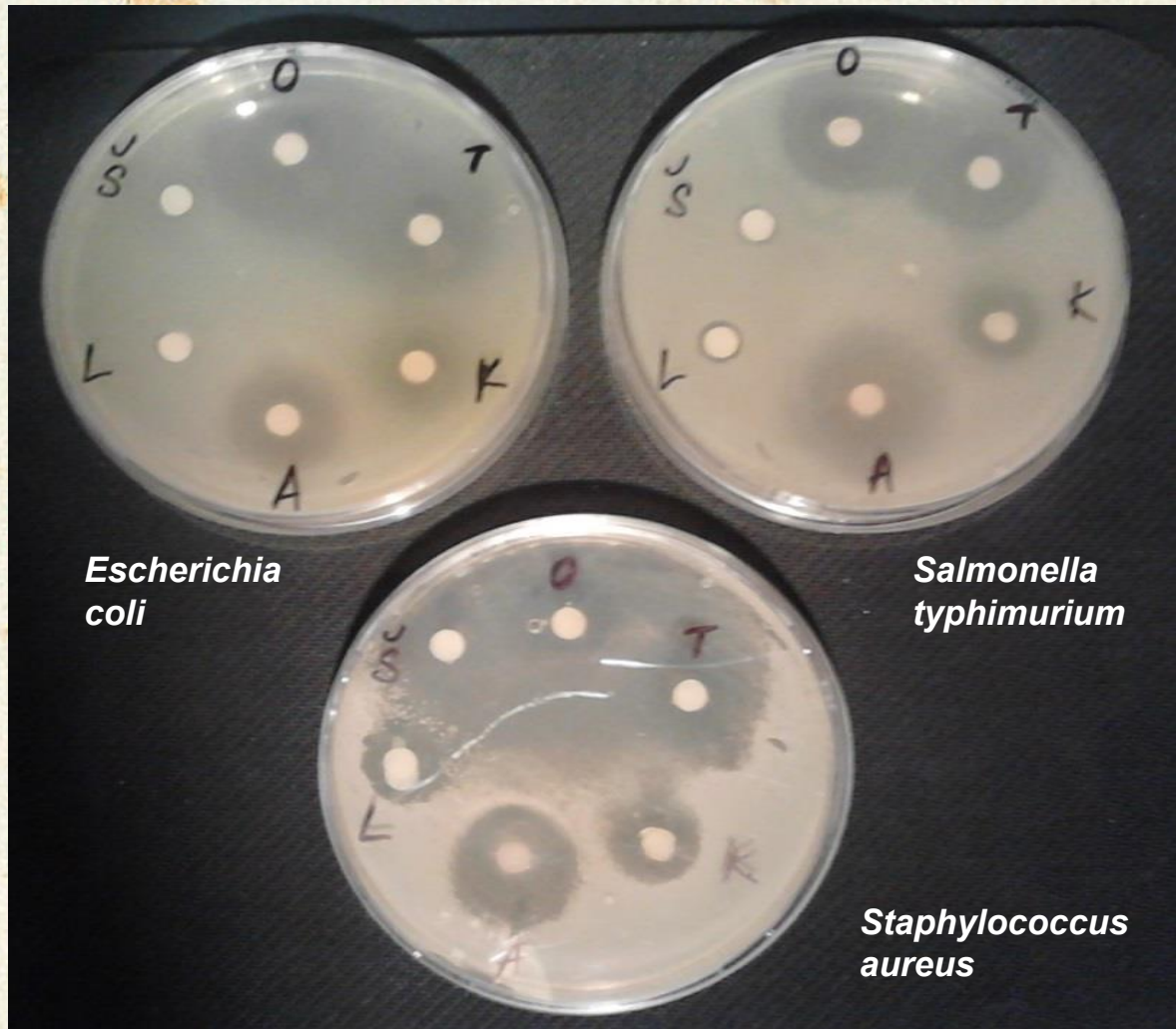


Lavender

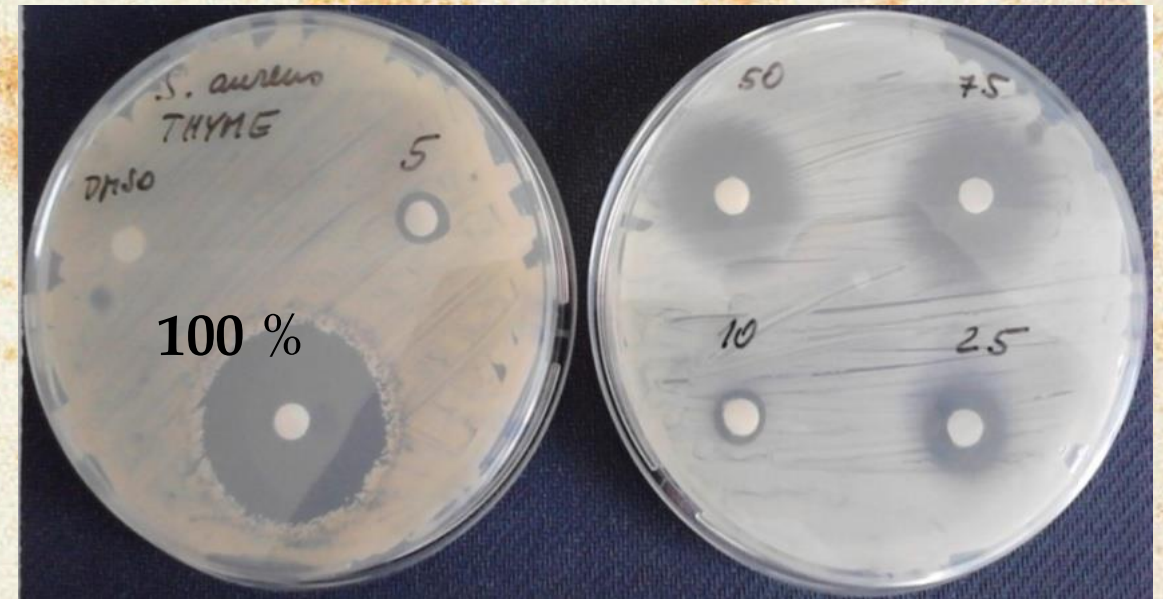


Clary sage

Antibakteriálne účinky esenciálnych olejov



O - Oregáno, T - Tymián, K - Klinček, A - Tuja,
L - Levanduľa, Š - Šalvia



Účinok riedeného **tymiánového** esenciálneho oleja na *S. aureus*.

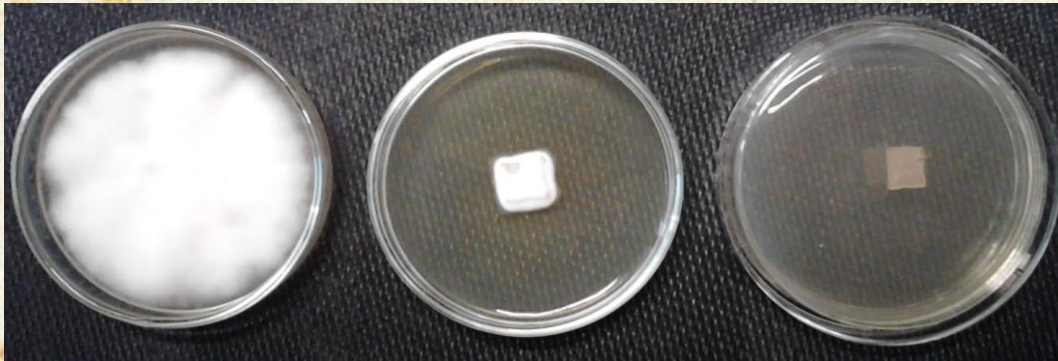
Použitie koncentrácie (**100%, 75%, 50%, 25%, 10% a 5%**) (% w/v).

Inhibitory effect of EOs on mycelial growth of fungi “in vitro”

Control

MIC

MFC



Chaetomium globosum

MIC - minimal inhibitory concentration (the lowest concentration of EO that completely prevent the visible fungal growth) - **Agar dilution methods / mycelium squares**

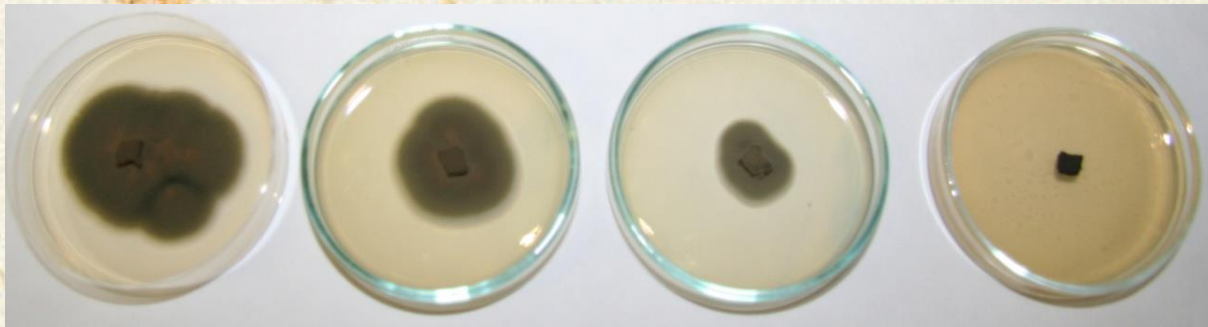
MFC - minimal fungicidal concentration (the lowest concentration at which no colony growth was observed after subculturing into fresh medium) - **Agar dilution methods / mycelium squares**

Control

1% EO

10% EO

50% EO



Cladosporium cladosporioides

Control

1% EO

10% EO

50% EO



Alternaria alternata

antifungal activity of thyme EO

Dezinfekcia použitím esenciálních olejov



Klinček



Arborvitae



Fotografia pred

Fotografia po



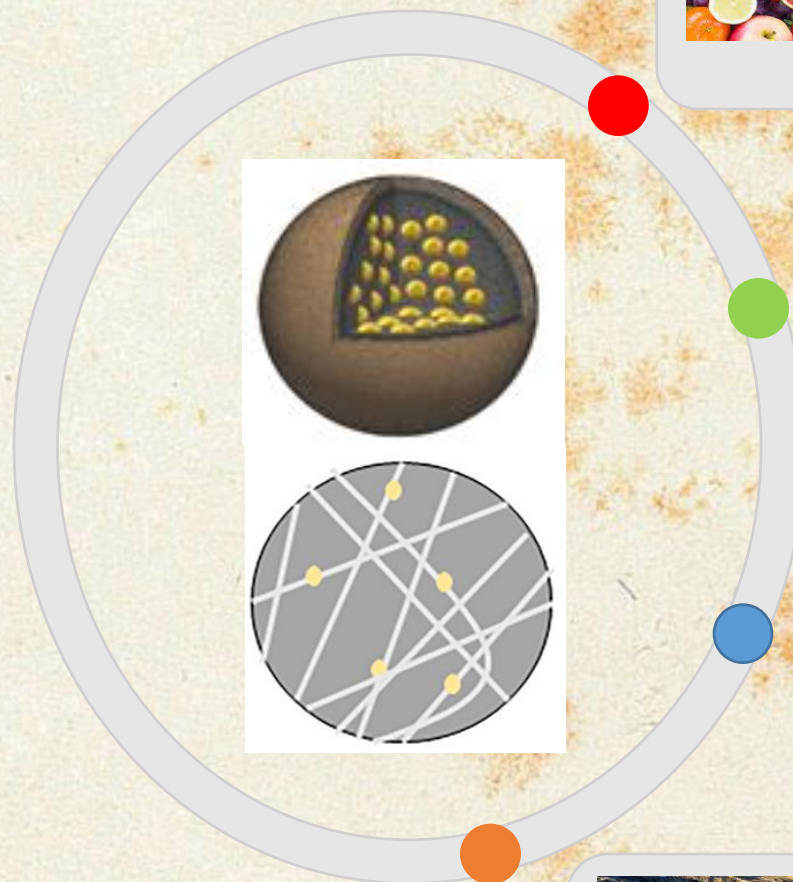
Oregano



Tymián



Využitie antimikrobiálnych účinkov nanomateriálov s EO



Potravinárstvo



Poľnohospodárstvo

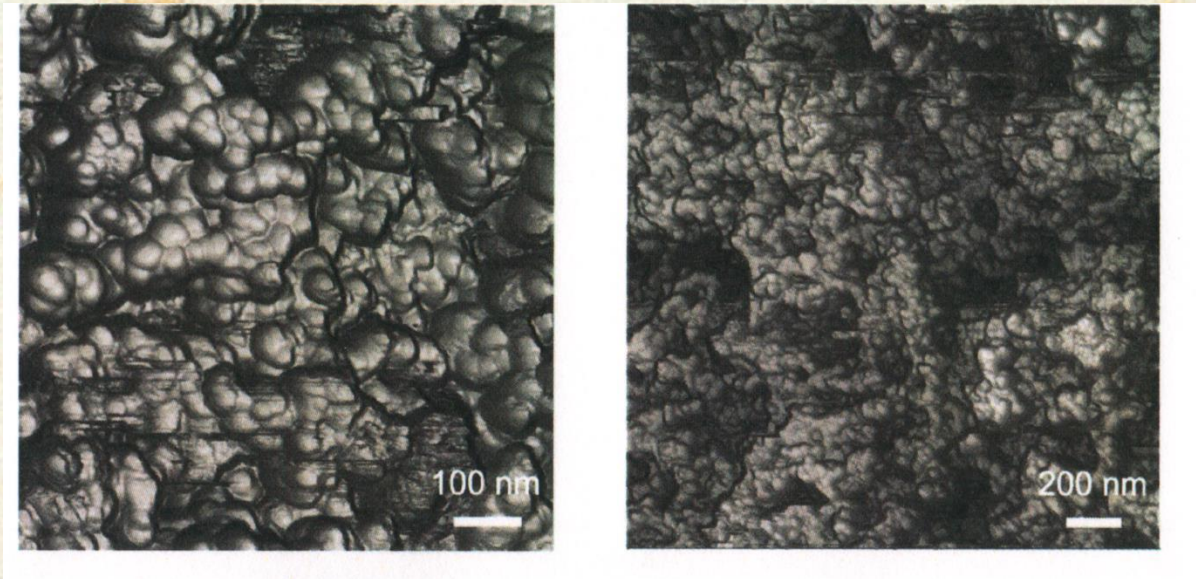
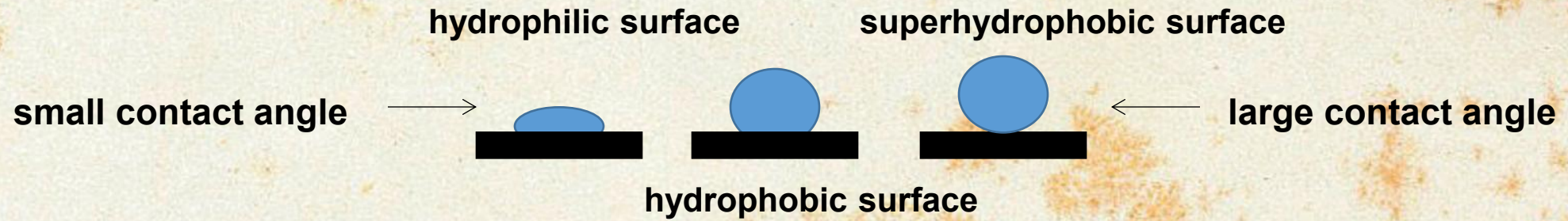


Medicína a farmácia



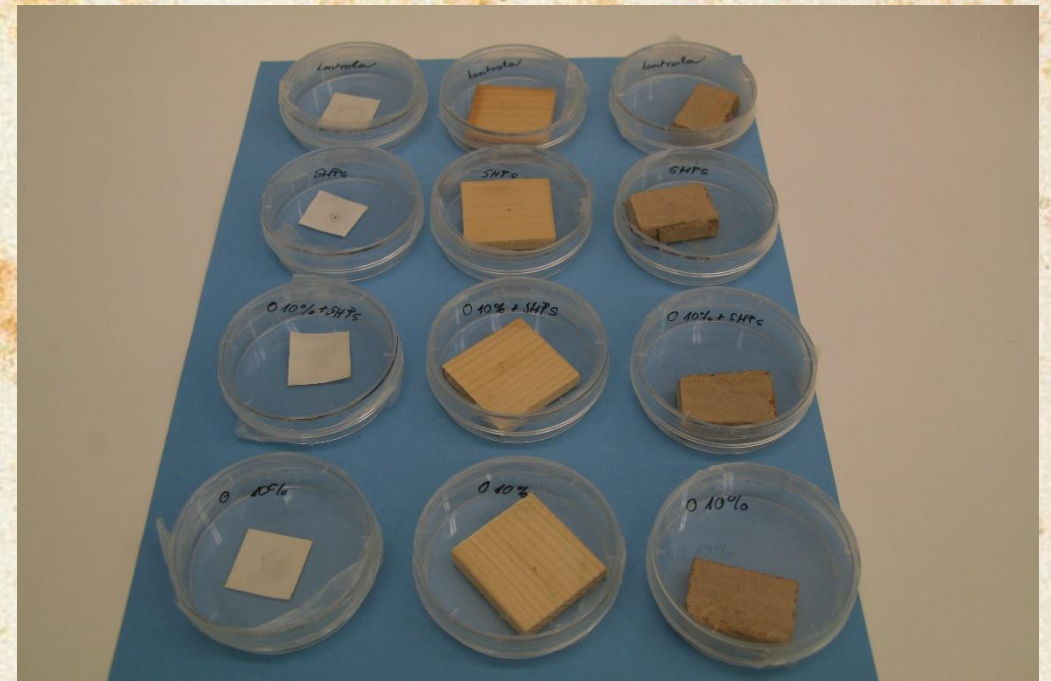
Stavebníctvo, kultúrne dedičstvo

NanoTechnologies



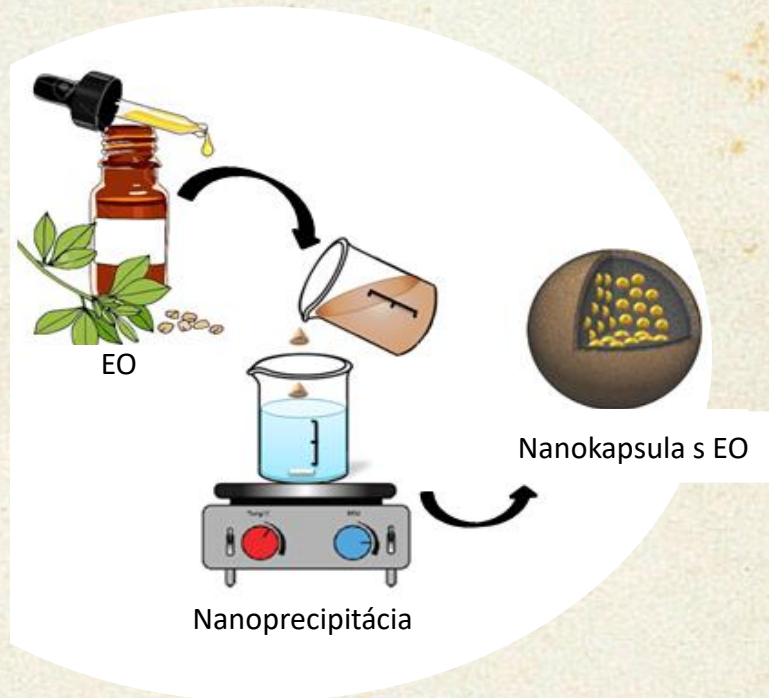
AFM images of the superhydrophobic particles monolayer on silicon substrate scanned in the tapping mode over various areas

- for increase the antifungal effect of essential oils
- prevents from the penetration of water into the substrate



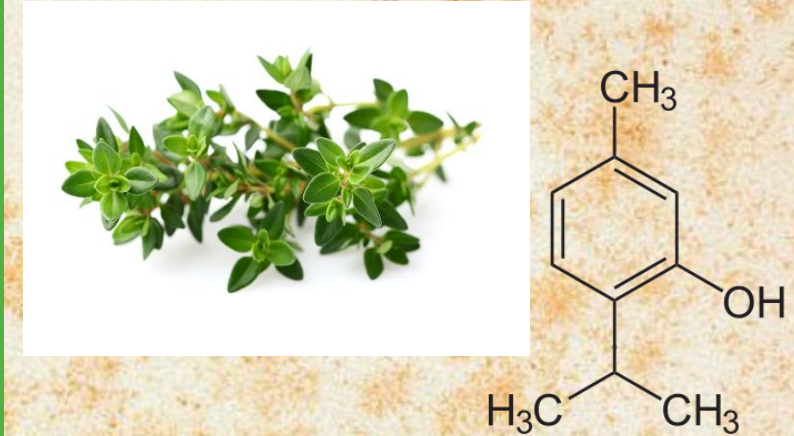
Spojenie EO a nanočastíc

- EO – antimikrobiálny účinok
- Nanočastica – transport a riadené uvoľňovanie bioaktívnej zložky
 - Poly (ϵ -kaprolaktón) – biodegradovateľný a biokompatibilný



Obrázok 5: Schématické zobrazenie nanokapsulácie (Rivas *et al.*, 2017-upravené)

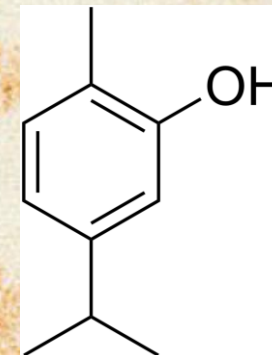
Thymus capitatus - thymol



Th-NC → 175 nm

Or-NC → 171 nm

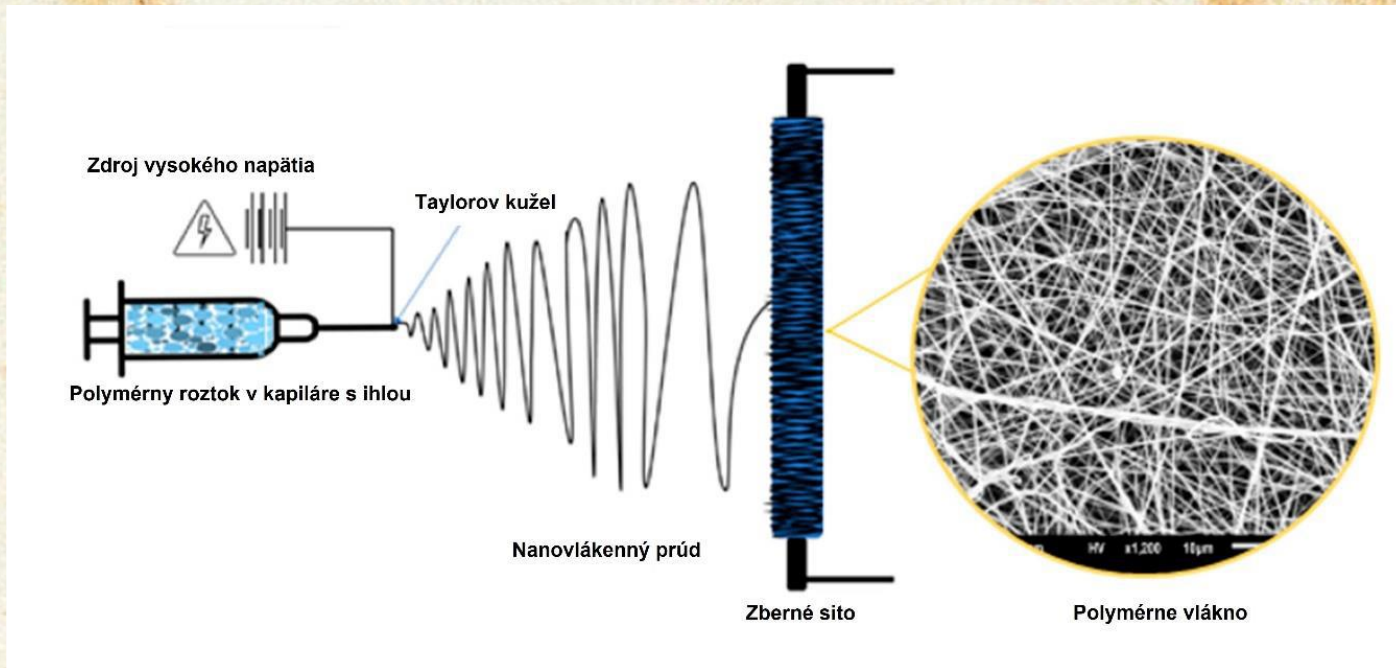
4 °C – 40 °C, 30 dní



Origanum vulgare - karvakrol

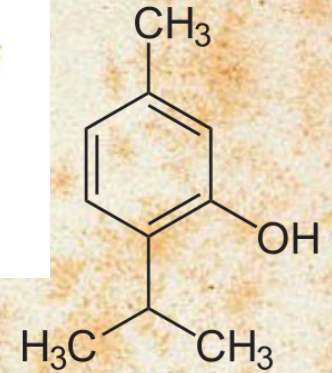
Spojenie EO a nanovlákién

- EO – antimikrobiálny účinok
- Nanovlákná – riadené uvoľňovanie bioaktívnej zložky
 - PLA/PHB/ATBC – biodegradovateľný a kompostovateľný

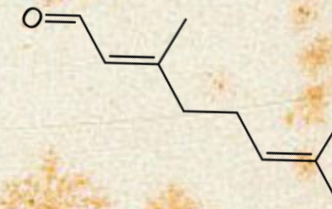


Obrázok 6: Schématické zobrazenie elektrostatického zvlákňovania
(Nirwan *et al.*, 2022 – upravené)

Origanum vulgare - karvakrol

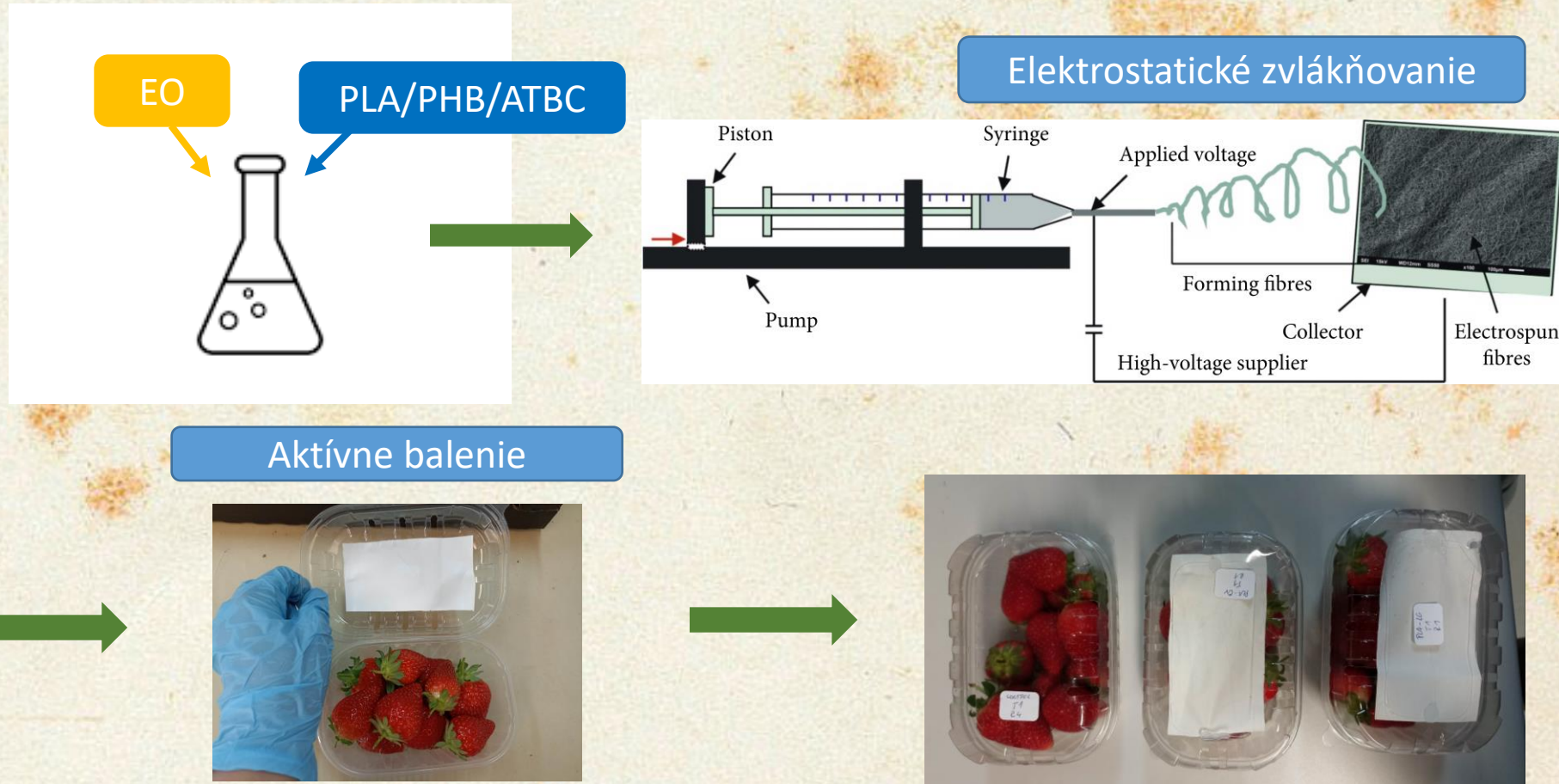


PLA/PHB/ATBC + 5% oregano EO
PLA/PHB/ATBC + 5% citrónová
tráva EO



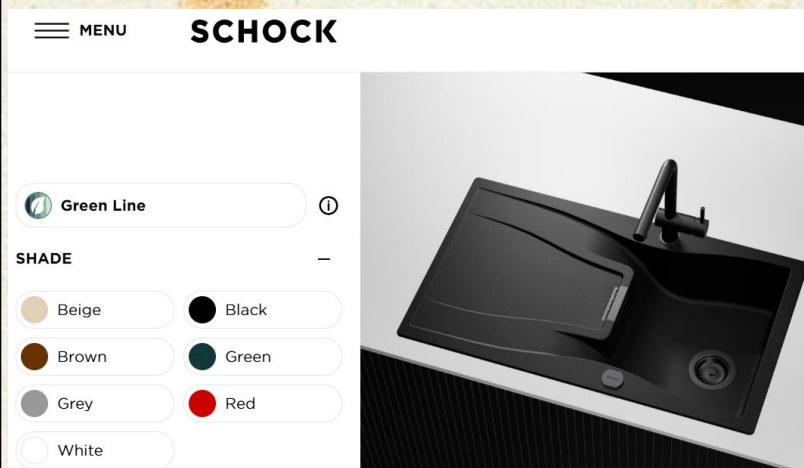
Cymbopogon flexuosus - citral

Aplikácia biologicky rozložiteľných aktívnych obalov na skladovanie jahôd



Aktívne balenie je systém balenia určený na zachovanie biologických, organoleptických vlastností a kvalitu baleného potravinárskeho výrobku, čím sa predlžuje jeho trvanlivosť

Praktické využitie esenciálnych olejov



Pietrzak, K., Otlewska, A., Danielewicz, D., Dybka, K., **Pangallo, D., Kraková, L., Puškárová, A., Bučková, M.**, Scholtz, V., Ďurovič, M. and Surma-Ślusarska, B., 2017. Disinfection of archival documents using thyme essential oil, silver nanoparticles misting and low temperature plasma. *Journal of Cultural Heritage*, 24, pp.69-77.

Benkovičová, M., **Kisová, Z., Bučková, M.**, Majková, E., Šiffalovič, P. and **Pangallo, D.**, 2019. The antifungal properties of super-hydrophobic nanoparticles and essential oils on different material surfaces. *Coatings*, 9(3), p.176.

Kapustová, M., Granata, G., Napoli, E., **Puškárová, A., Bučková, M., Pangallo, D.** and Geraci, C., 2021. Nanoencapsulated essential oils with enhanced antifungal activity for potential application on agri-food, material and environmental fields. *Antibiotics*, 10(1), p.31.

Hofbauerová, M., **Rusková, M., Puškárová, A., Bučková, M.**, Annušová, A., Majková, E., Šiffalovič, P., Granata, G., Napoli, E., Geraci, C. and **Pangallo, D.**, 2023. Protection and disinfection activities of oregano and thyme essential oils encapsulated in poly (ϵ -caprolactone) nanocapsules. *Molecules*, 28(3), p.1018.



Review

Essential Oils and Cultural Heritage Conservation: Are They Safe, Environmentally Friendly, Sustainable, and Negligibly Toxic?

Daniela Pinna  Gels; 2025, 11(12), 978; <https://doi.org/10.3390/gels11120978>

<http://www.bmst.co.kr/>



BIO-CLEANING

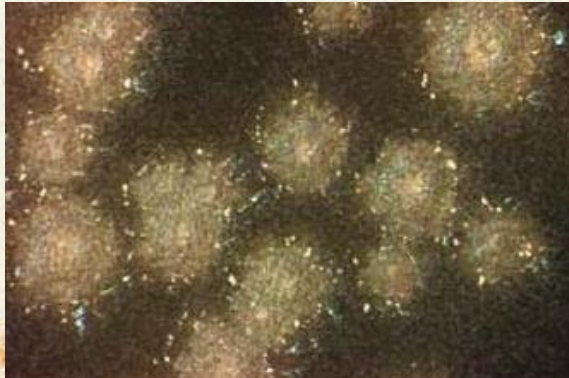
**by Microbial Enzymatic
Extract**

Bioreštaurovanie

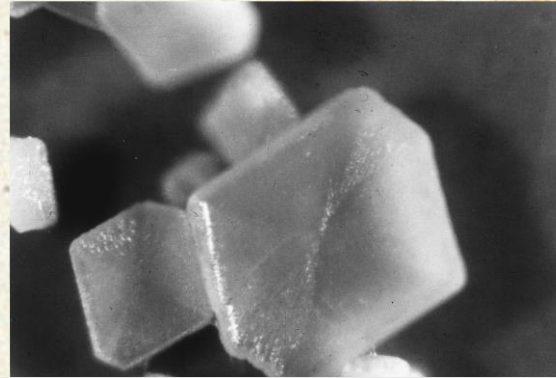
Biokonsolidácia kameňa

Biomineralizácia uhličitanu vápenatého

Bacillus pasteurii, *B.cereus*, *B. subtilis*, *B. sphaericus*, *Micrococcus* sp., *Mytilus californianus*



Baktérie *Bacillus* obalené
uhličitanom vápenatým



Kryštály produkované baktériou *Micrococcus* sp.

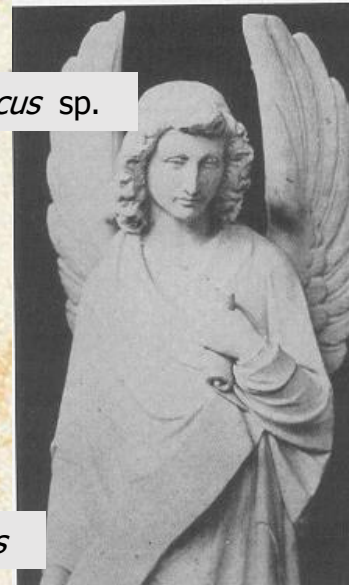


Kryštály produkované baktériou *B. subtilis*

Praktické využitie:

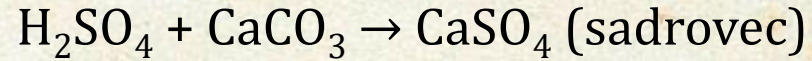
Zníženie pórovitosti kameňa

Zvýšenie pevnosti kameňa



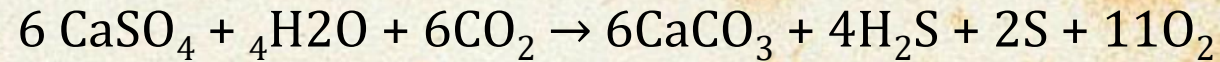
Odstraňovanie čiernych povlakov na sochách

Spaľovanie fosílnych palív → SO_2 rozpustené vo vode → H_2SO_4 (kyslé dažde)



Čierne krusty- kryštály sadrovca zmiešané s nečistotami v atmosfére

Sulfát redukujúce baktérie (*Desulfovibrio desulfuricans*, *Desulfovibrio vulgaris*)



Čierna krusta pred (vľavo) a po (vpravo) aplikácií baktérie *Desulfovibrio vulgaris*

Praktické využitie:

Odstraňovanie neestetických čiernych krúst na sochách

Odstraňovanie organických zvyškov

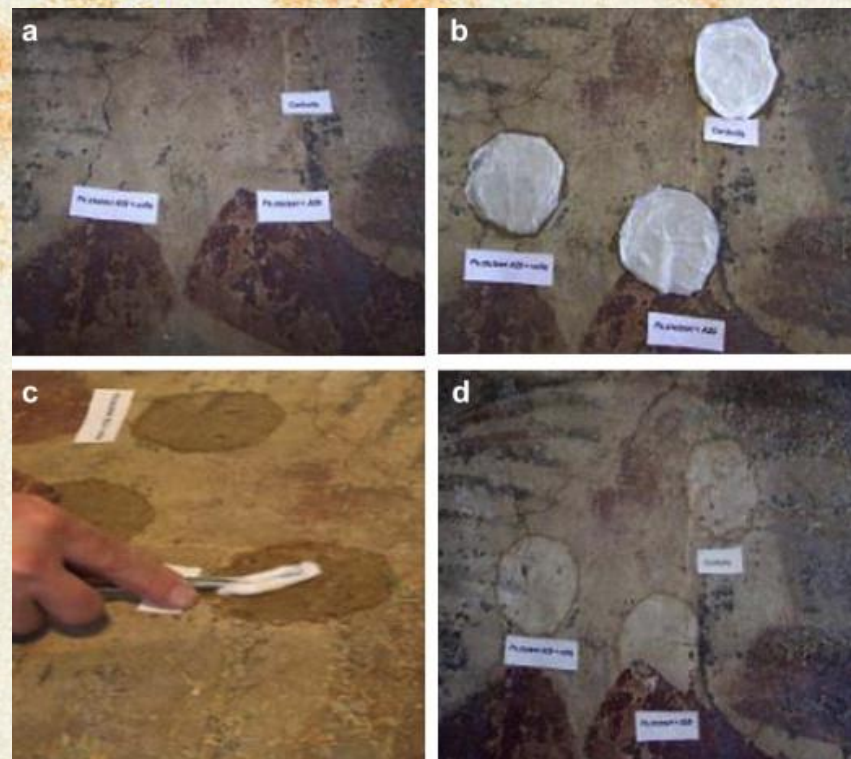
Praktické využitie:

Odstraňovanie zvyškov organických materiálov (napr. bielok, kolagén obsahujúce materiály ako je živočíšny glej) za pomoci hydrolytických enzýmov

Baktéria Pseudomonas stutzeri



Biočistenie a bioreštaurovanie fresky (14. storočie) pomocou baktérie *Pseudomonas stutzeri* (hore pred ošetrováním, dolu po ošetrovaní)

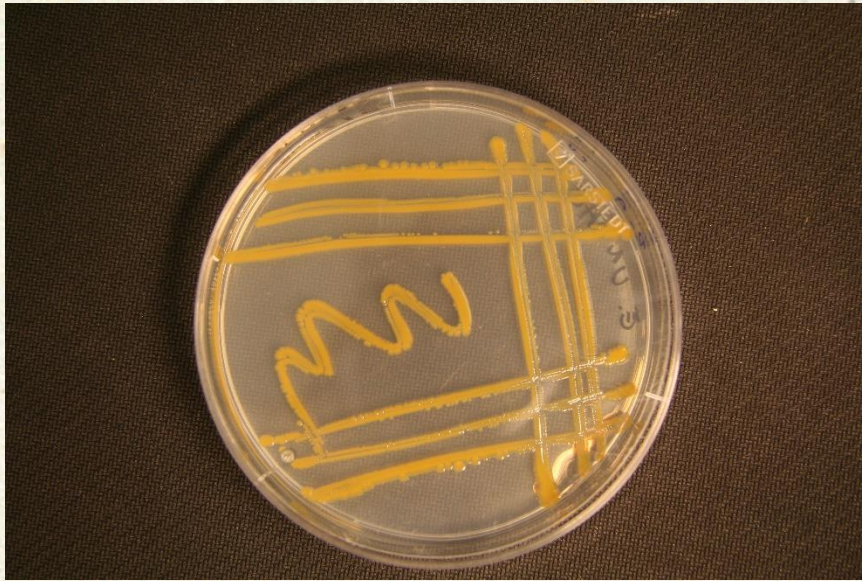


Biočistenie a bioreštaurovanie fresky pomocou baktérie *Pseudomonas stutzeri* a) pred ošetrováním; b) miesto pokryté bavlnenou vrstvou s baktériou; c) odstránenie bavlnenej vrstvy po 2 hodinách; d) výsledok po ošetrovaní.



Airborne bacteria in the chapel of the castle of Krásna Hôrka

Proteolytic assays



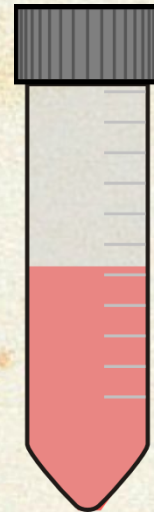
Exiguobacterium undae



Production of extracellular enzymes secretion

- 1. inoculation of *E. undae* in to animal glue broth ($\text{KH}_2\text{PO}_4 = 0.5 \text{ g/l}$; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O} = 0.5 \text{ g/l}$; **animal glue** = 4 g/l)
- 2. Cultivation at 23 °C, 180 rpm, 24 h
- 3. OD measurement at $\lambda = 600 \text{ nm}$; with the $\text{OD} \geq 1.0$ centrifugation for 30 min at 30000 rpm (6 °C)
- 4. The supernatant was filtered through a 0.2 μm filters

**Extracellular
proteolytic enzymatic
extract**



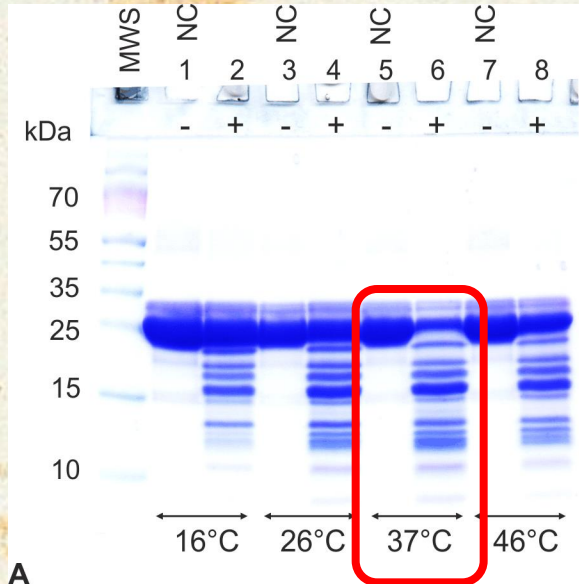
Characterization
of the proteome



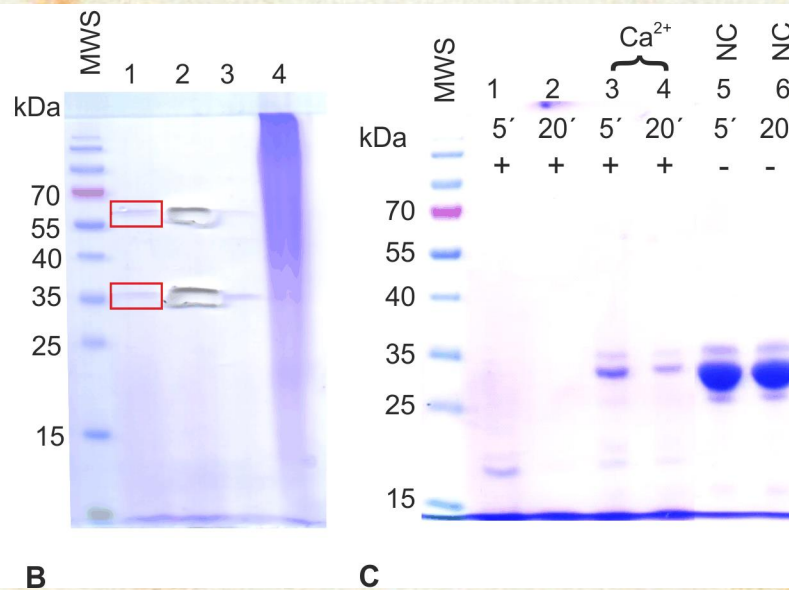
Proteolytic activity

- SDS-PAGE proteolytic degradation, inhibitors and activators
- N-terminal sequencing (Edman degradation)
- Liquid chromatography-mass spectrometry (LC-MS)

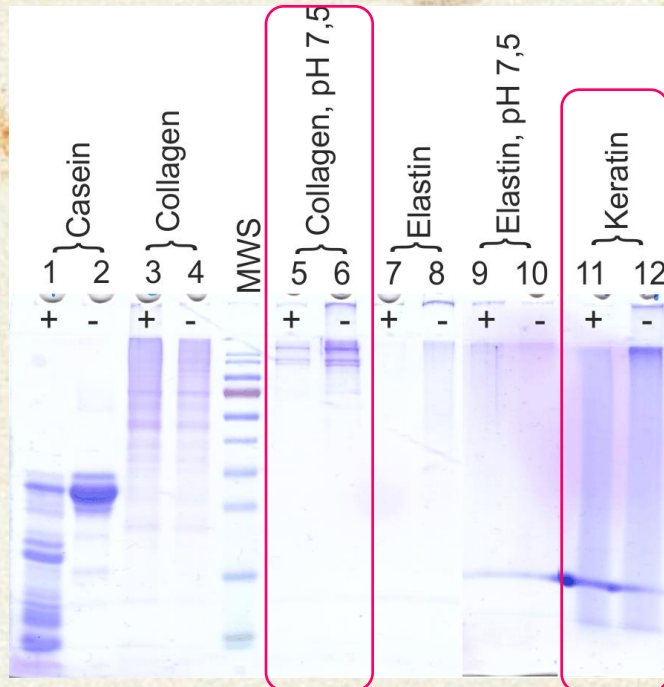
Proteolytic activity against bovine β -casein



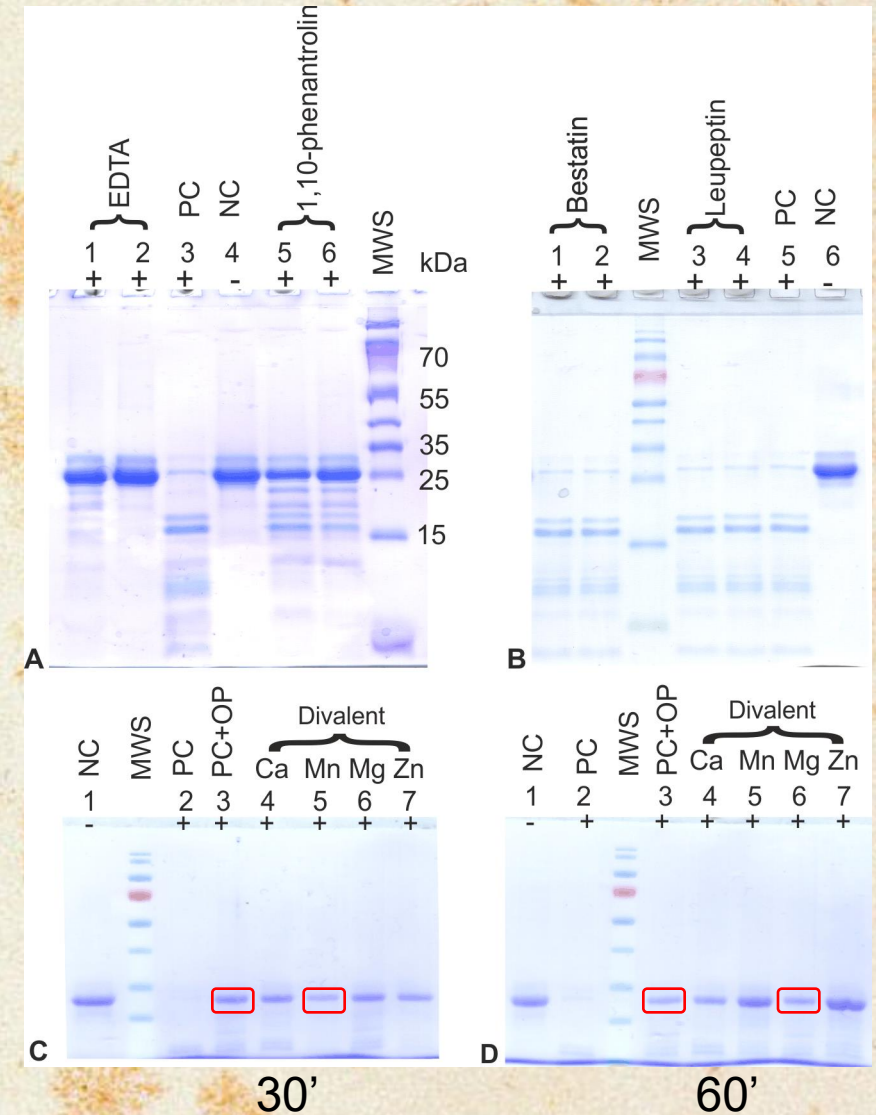
Extracellular proteins identification



The substrate specificity of the *E. undae* enzymatic extract



β -casein degradation in the presence of 25 mM CaCl_2



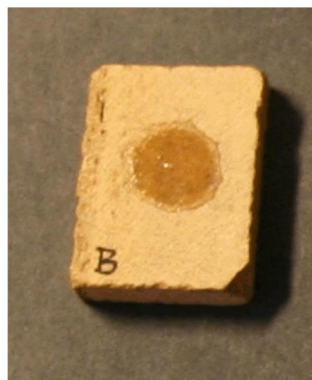
Inhibitors and activators of the *E. undae* enzymatic extract

Skin animal glue



A

Bone animal glue



B



B



B

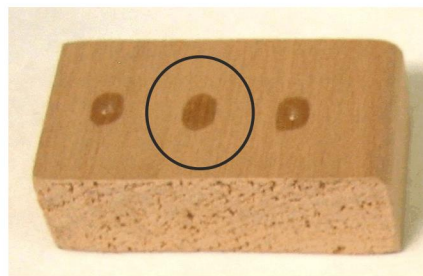


C



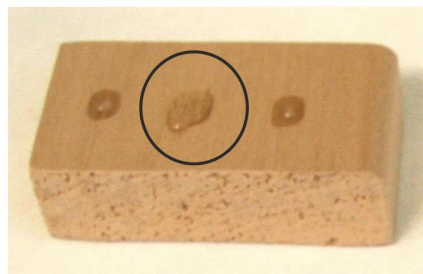
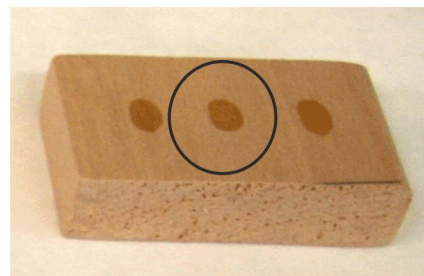
B

Skin animal glue

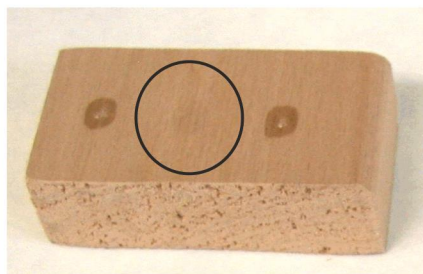
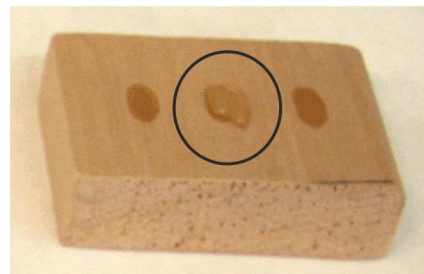


A

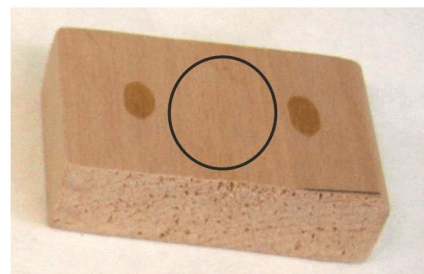
Bone animal glue



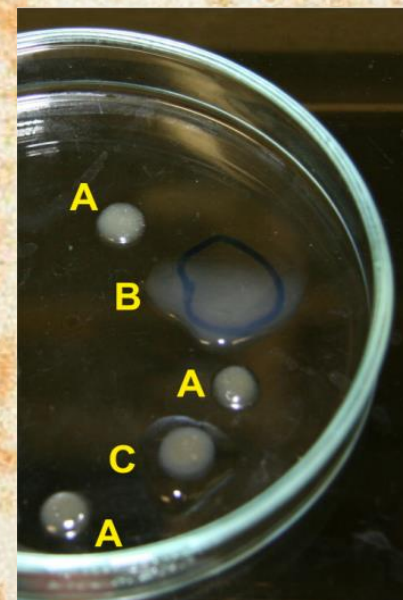
B



C



Fagus sylvatica





Usually, the restores use **hot steam** in order to open the pipe

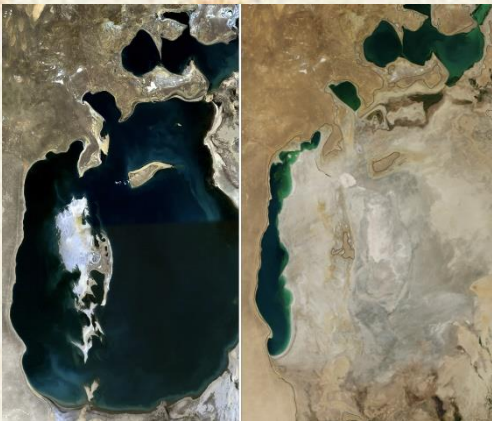
After the application of ***Exiguobacterium* enzymatic extract** (4 h of incubation) the pipe was easily open



Other animal glue degraders



Bacillus flexus



1989

2014

Aral "Sea"
Uzbekistan

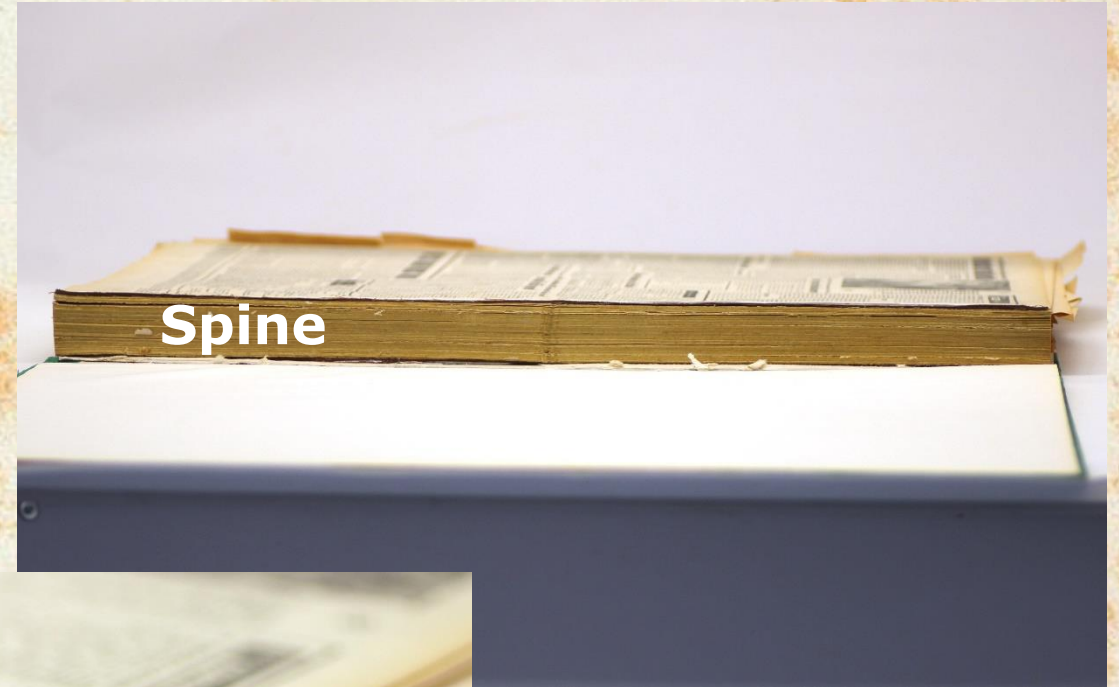


Paracoccus sp.

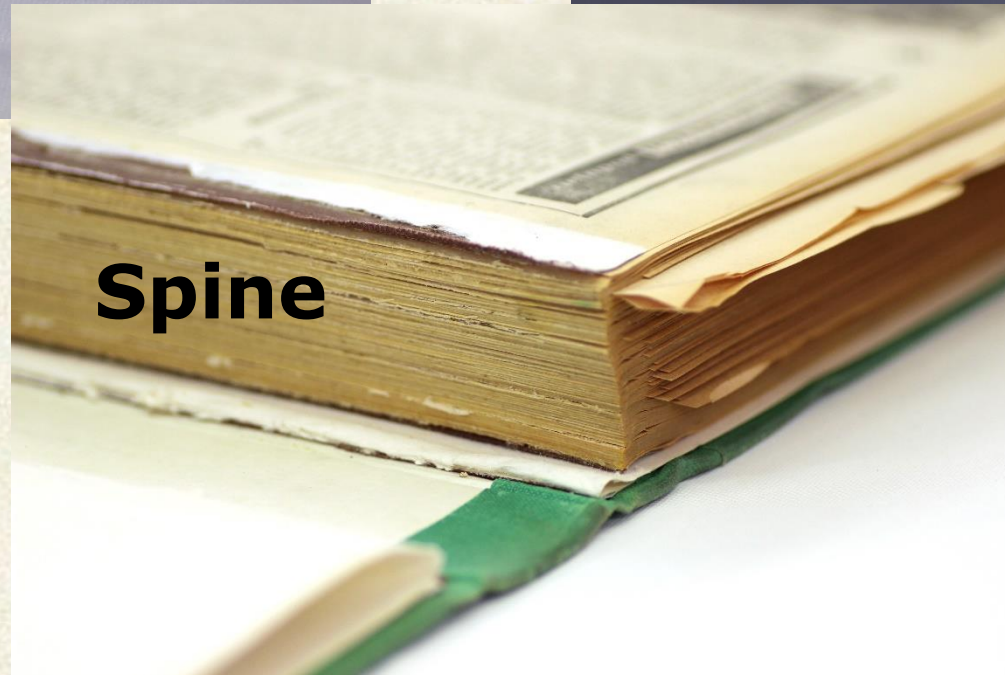


Anadem
found in a
tomb

Newspapers books



Application of a **Tylose solution for 10-15 min and mechanical removal** with a blunt scalpel. The operation is repeated several times



Steam scalpel



**With tylose
and enzymatic
extract**

**Only the
enzymatic
extract**

E. undae



B. flexus

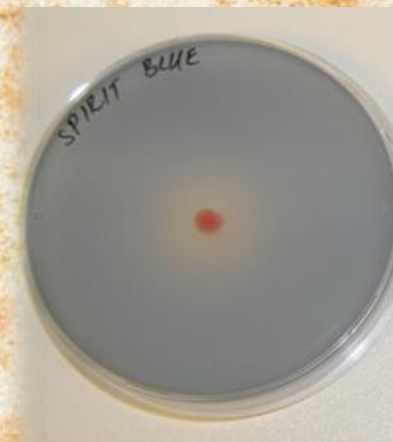




SKRÍNING LIPOLYTICKEJ AKTIVITY KVASINKY *Sporidiobolus metaroseus*

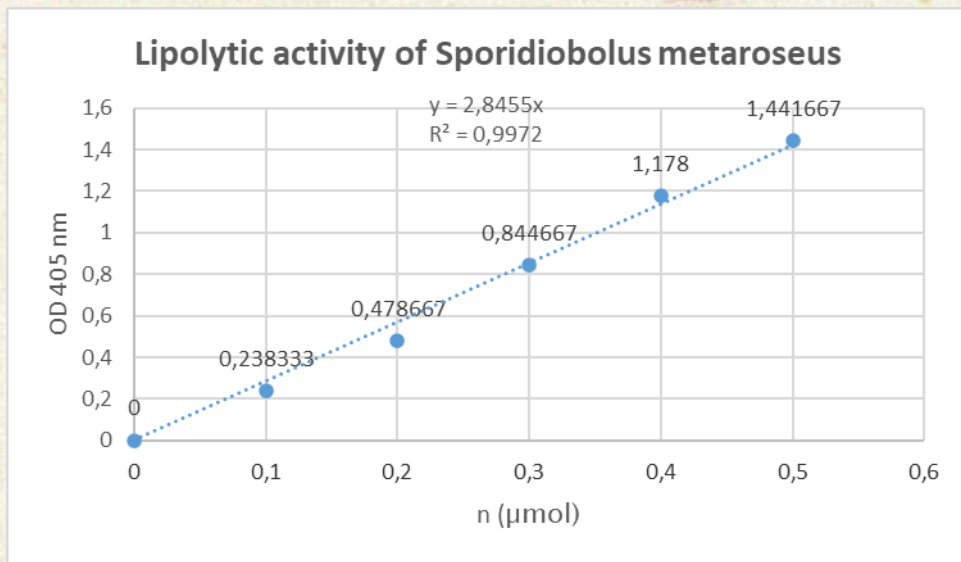
Lipolytická aktivita:

- ❑ Spirit Blue agar + lipidická zmes (Tween 80, voda, ľanový olej (LO))



Kolorimetrická detekčná metóda – hydrolýza p-NPP

- Lipolytická aktivita vypočítaná na základe lineárnej regresnej rovnice

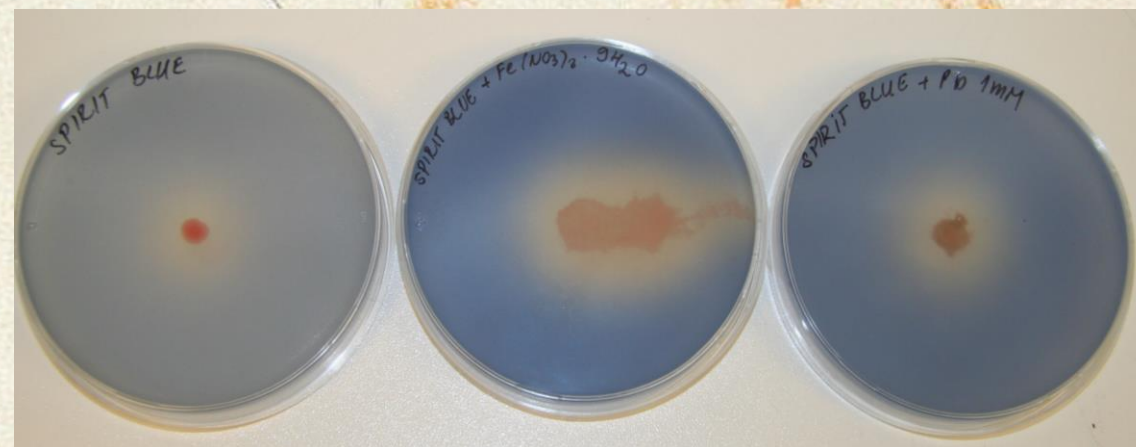


$$x = A_{405} + b/a \cdot V \cdot t$$

↑ Zvýšenie o 60 %

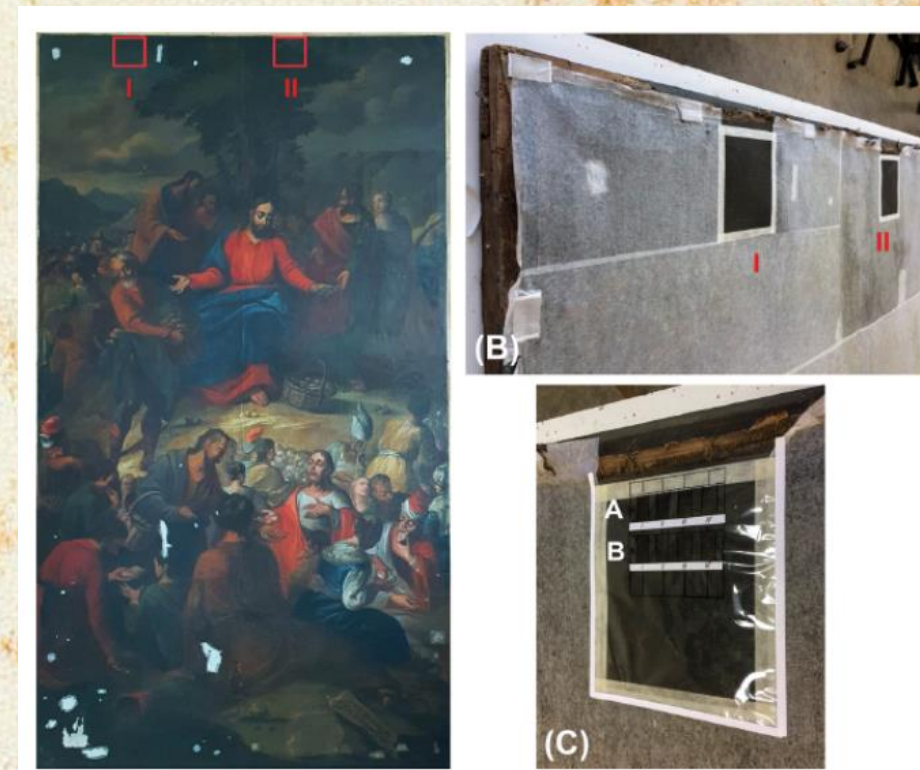
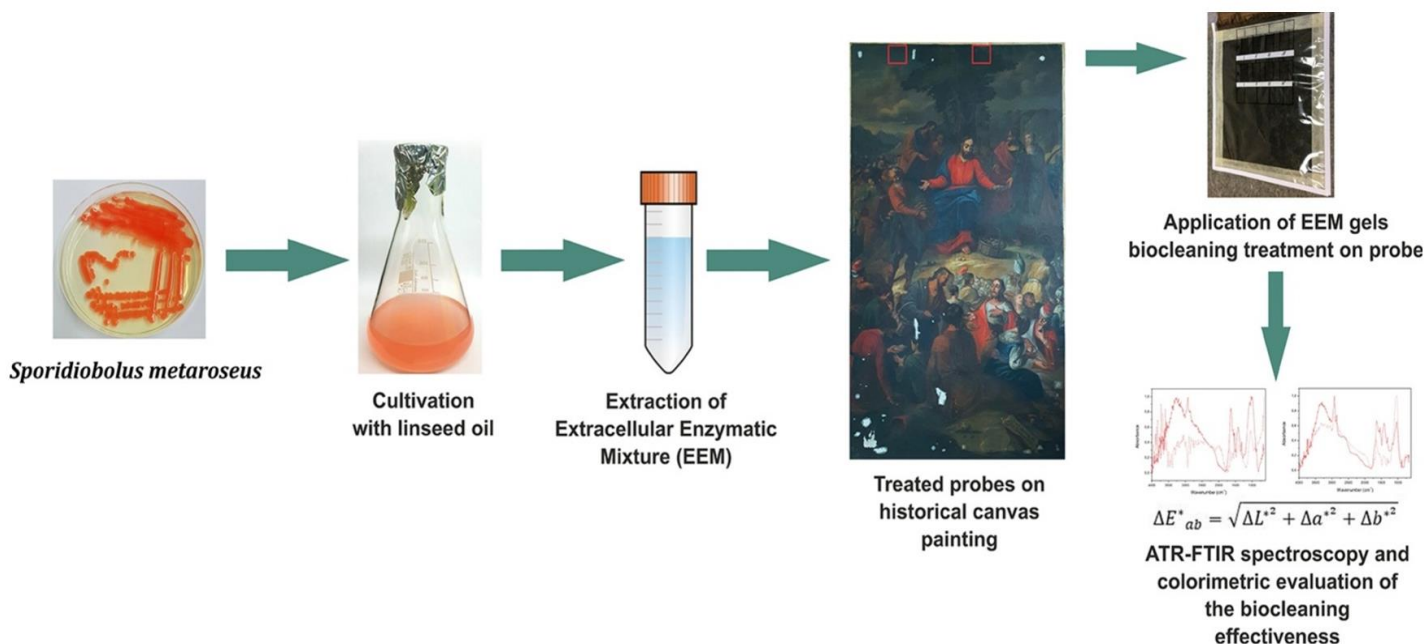
Aktivita:

- bez LO 0.356 U. ml⁻¹
- s LO 0.957 U. ml⁻¹



Determinácia lipol. aktivity kvasinky v prítomnosti kovov železa a olova

BIOLOGICKÉ ČISTENIE OLEJOMAĽBY „MIRACLES OF THE LOAVES AND FISHES“



Aplikácia EEM+nosič na odstránenie nežiadúcej vrchnej vrstvy olejomal'by

- FT-IR analýza → najvhodnejšia kombinácia tylóza-EEM a klučel G-EEM

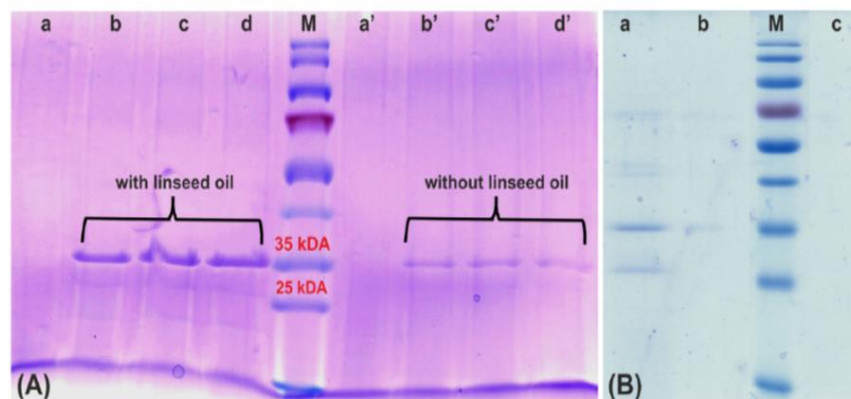


Fig. 3. SDS PAGE gels with EEM. (A) Gel showing the difference of the EEM produced by the adding of linseed oil in R2A broth and without linseed oil; a – a': R2A broth without yeast; b – b': 30 µl of EEM; c – c': 35 µl of EEM; d – d': 40 µl of EEM; M: protein ladder. (B) Gel showing the differences of EEM production in R2A broth with linseed oil (a), and amended with 1 mM of $\text{Fe}(\text{NO}_3)_3 \cdot 9\text{H}_2\text{O}$ (b) and $\text{Pb}(\text{NO}_3)_2$ (c); M: protein ladder.

SDS-PAGE – porovnanie produkcie enzýmov pred a po pridaní ľanového oleja do kultivačného média + v prítomnosti kovov

Závery

Komplexná problematika

Multidisciplinárna kooperácia

**Vyžaduje kombináciu
rôznych techník a metód**

Precízna diagnostika = Najlepšie ošetrovanie



Cenu za vedecko-výskumnú činnosť získal kolektív pracovníkov Laboratória environmentálnej a potravinovej mikrobiológie pod vedením Dr. **Domenica Pangalla**, PhD., z Ústavu molekulárnej biológie SAV v zložení RNDr. **Mária Bučková**, PhD., Mgr. **Lucia Kraková**, PhD., a Mgr. **Andrea Puškárová**, PhD. za výsledky vedecko výskumnej práce

Deteriorácia kultúrneho dedičstva a kvalita tradičných slovenských potravín: štúdium ich mikrobiálnych komunit.

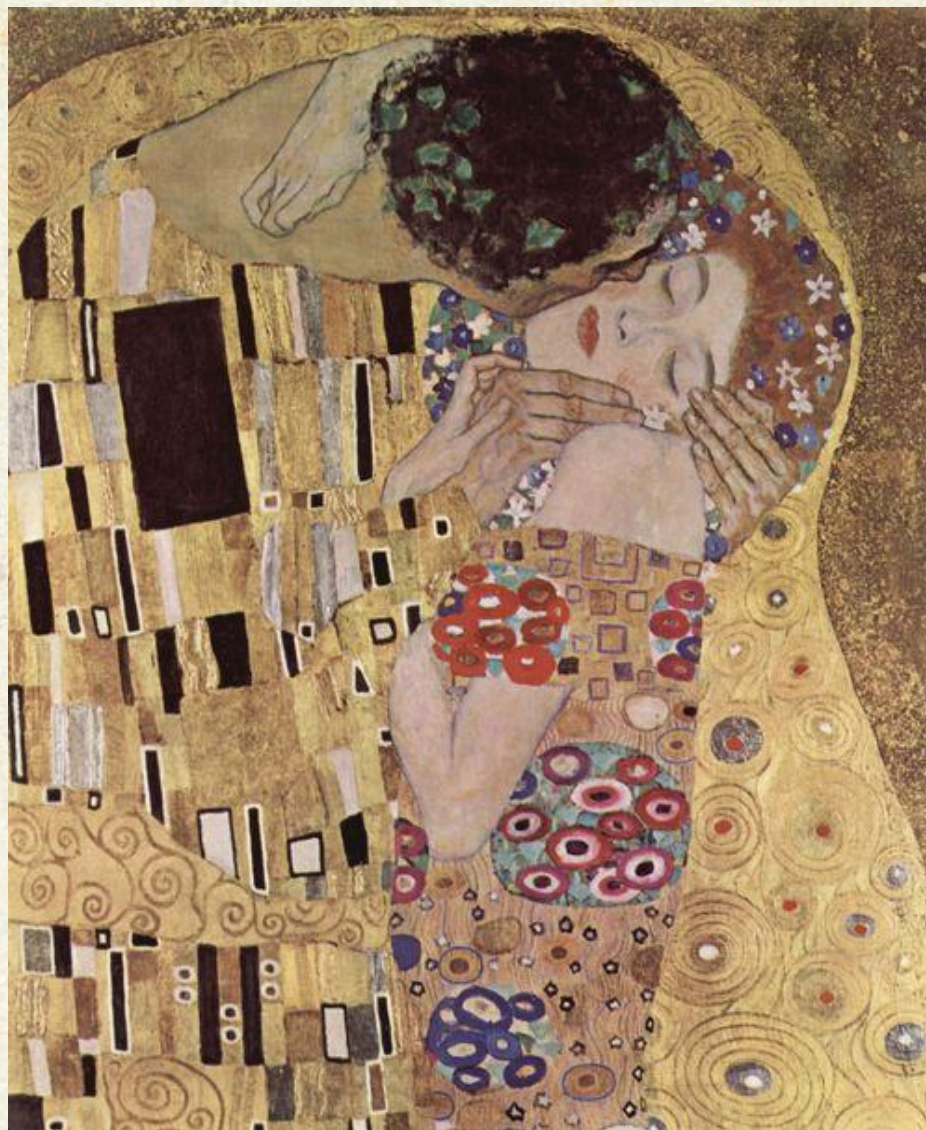




...be careful to results!!!



**Ďakujem
za
pozornosť**



a pekný večer...