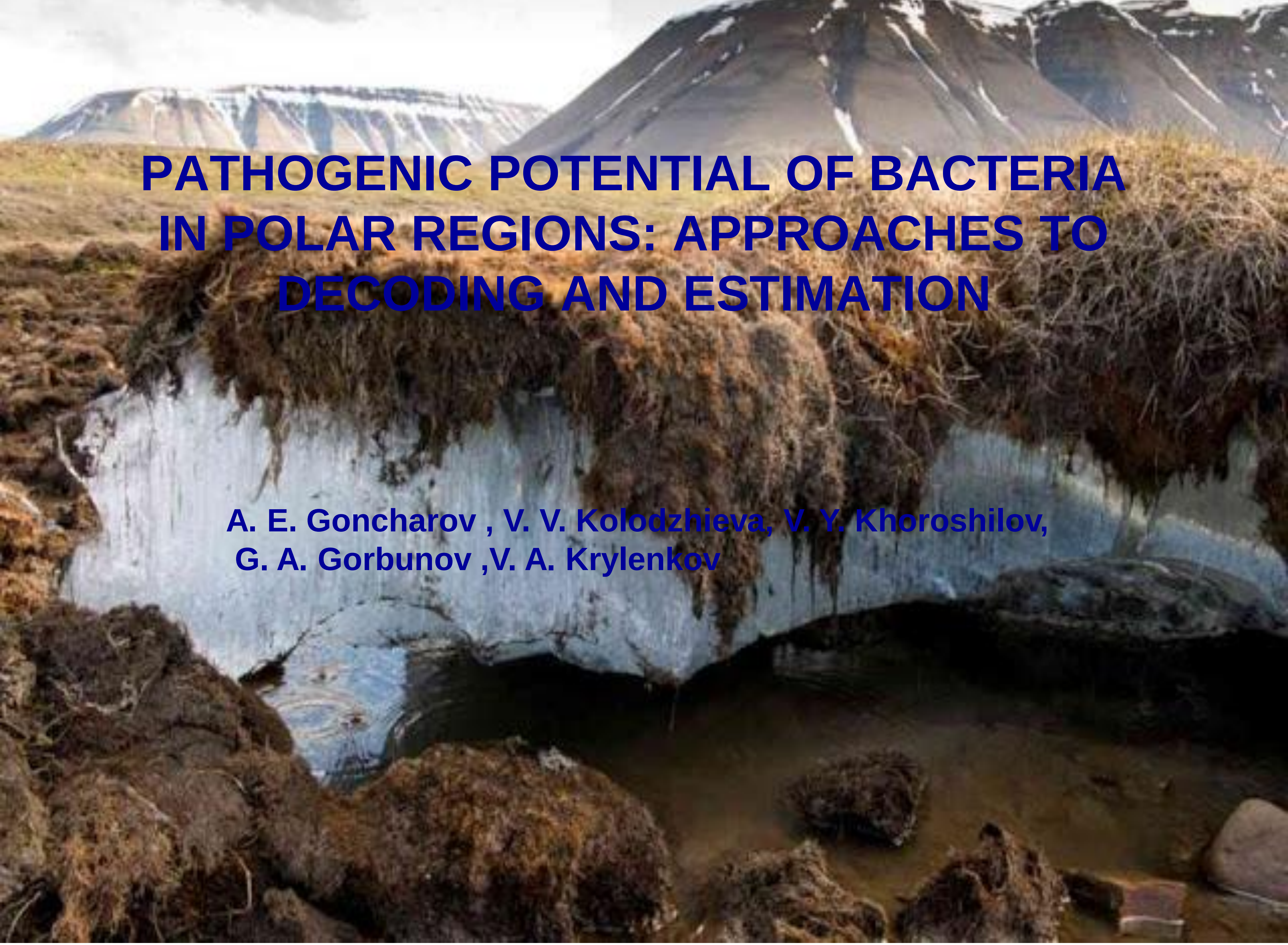
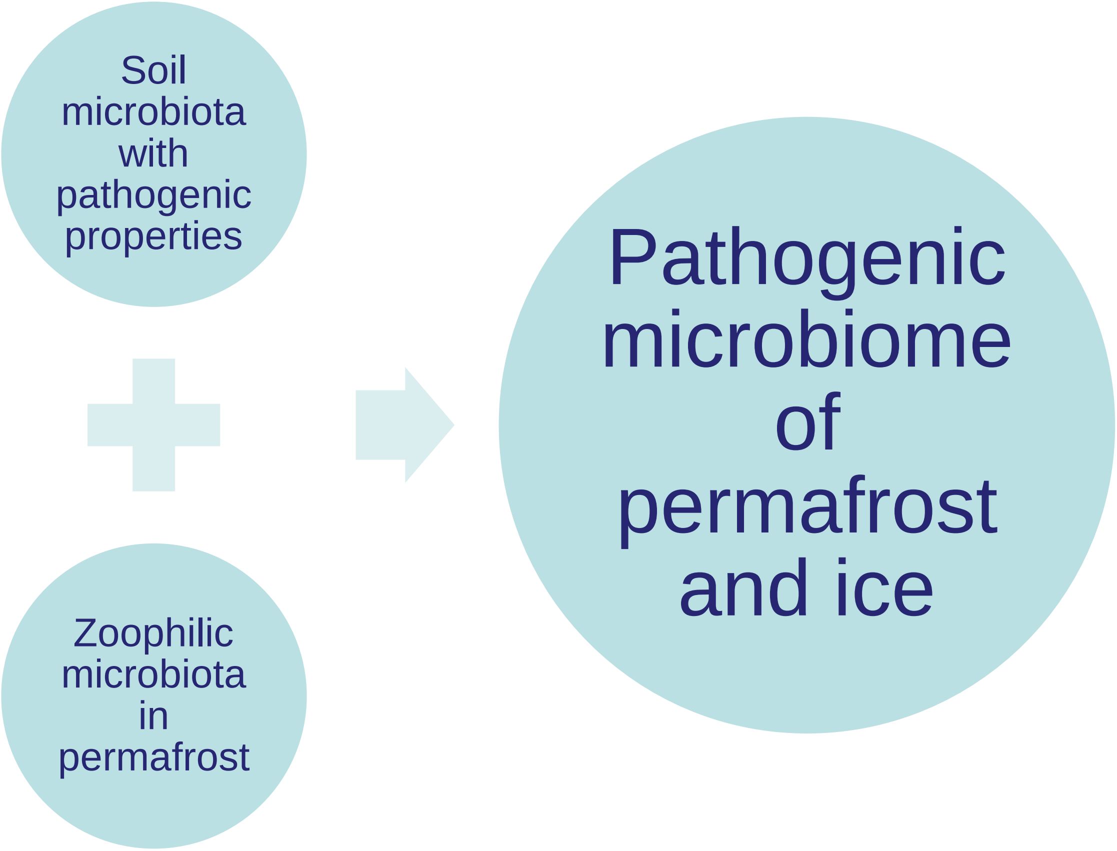


PATHOGENIC POTENTIAL OF BACTERIA IN POLAR REGIONS: APPROACHES TO DECODING AND ESTIMATION

A. E. Goncharov , V. V. Kolodzhieva, V. Y. Khoroshilov,
G. A. Gorbunov ,V. A. Krylenkov

Ice and permafrost as a reservoir of pathogenic microorganisms

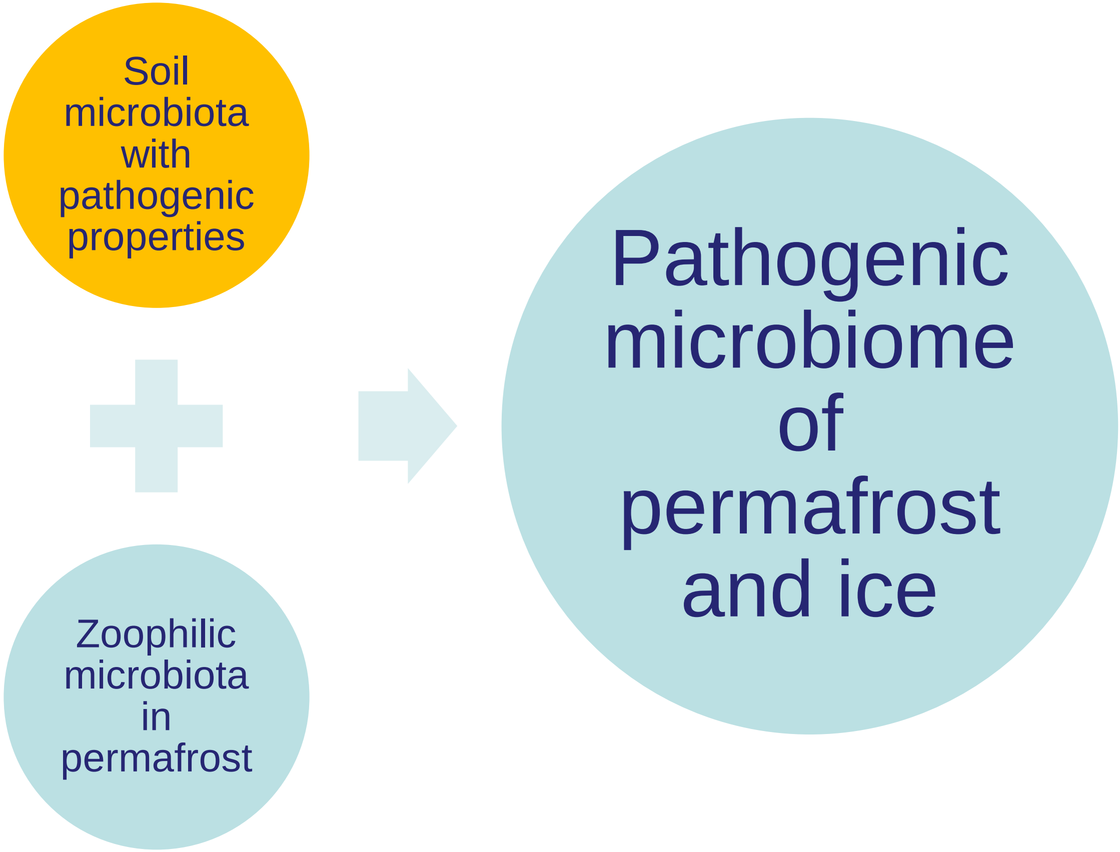
Taxonomic group	Disease	Possibility of long-term viability in permafrost and ice	Ref.
Enterobacteriaceae	Intestinal infections	+	Goodwin, K., Loso, M. G., and Braun, M. (2012).
Enterococcus spp.	Nosocomial infections	+	Goncharov A, Grigorjev S, Karaseva A et al. (2016)
Cryptococcus	Opportunistic infections	+	Turchetti, B., Selbmann, L., Blanchette, R. A., Di Mauro, S., Marchegiani, E., Zucconi, L., et al. (2015).
Bacilus anthracis	Anthrax	+	Nicholson, W.L.; Munakata, N.; Horneck, G.; Melosh, H.J.; Setlow, P. (2000)
Influenza A viruses	Influenza	+	Zhang, G., Shoham, D., Gilichinsky, D., Davydov, S., Castello, J. D., and Rogers, S. O. (2006).
Poxviruses	Smallpox	DNA can be preserved, preservation of functional viral particles is debatable	Biagini, P., Thèves, C., Balaresque, P., Géraut, A., Cannet, C., Keyser, C., et al. (2012).



Soil microbiota with pathogenic properties

Zoophilic microbiota in permafrost

Pathogenic microbiome of permafrost and ice



Soil microbiota with pathogenic properties

Zoophilic microbiota in permafrost

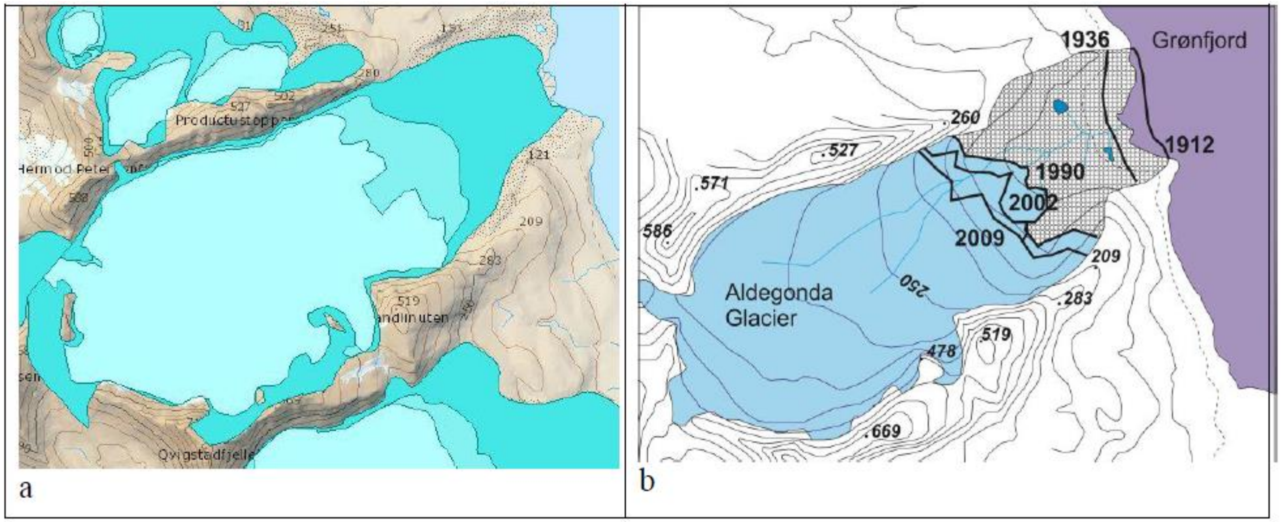
Pathogenic microbiome of permafrost and ice

Outbreak of anthrax on the Yamal Peninsula, 2016

- > 2,300 deer fell on the winter road to the town of Muzhi (160 km south of Salekhard)
- «zombie infection»: recent deaths of animals in the region was in 1911 and 1914.
- 23 confirmed cases, 2 deaths

Photo from https://inews.co.uk/news/world/russia-dealt-anthrax-released-melting-permafrost/

Bacteria from melting Aldegonda Glacier, Svalbard (Spitsbergen), 2018

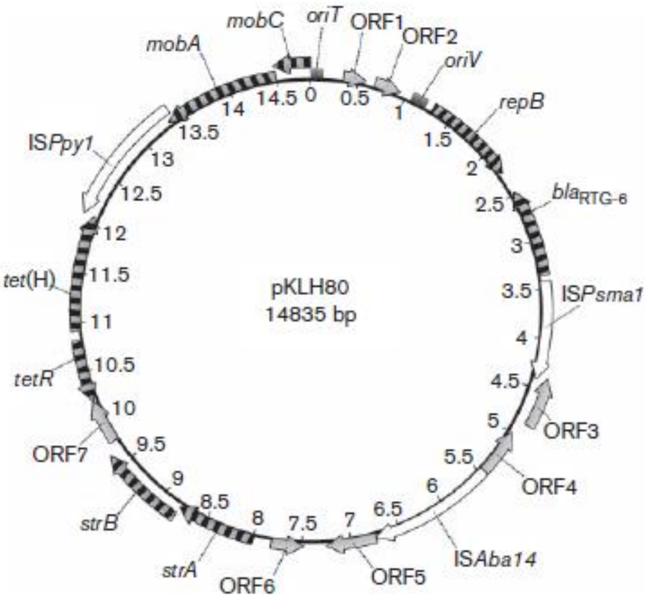


Glacier Aldegonda: a - its boundaries in 1936, 1990 and 2010, b – glacier boundaries in different years

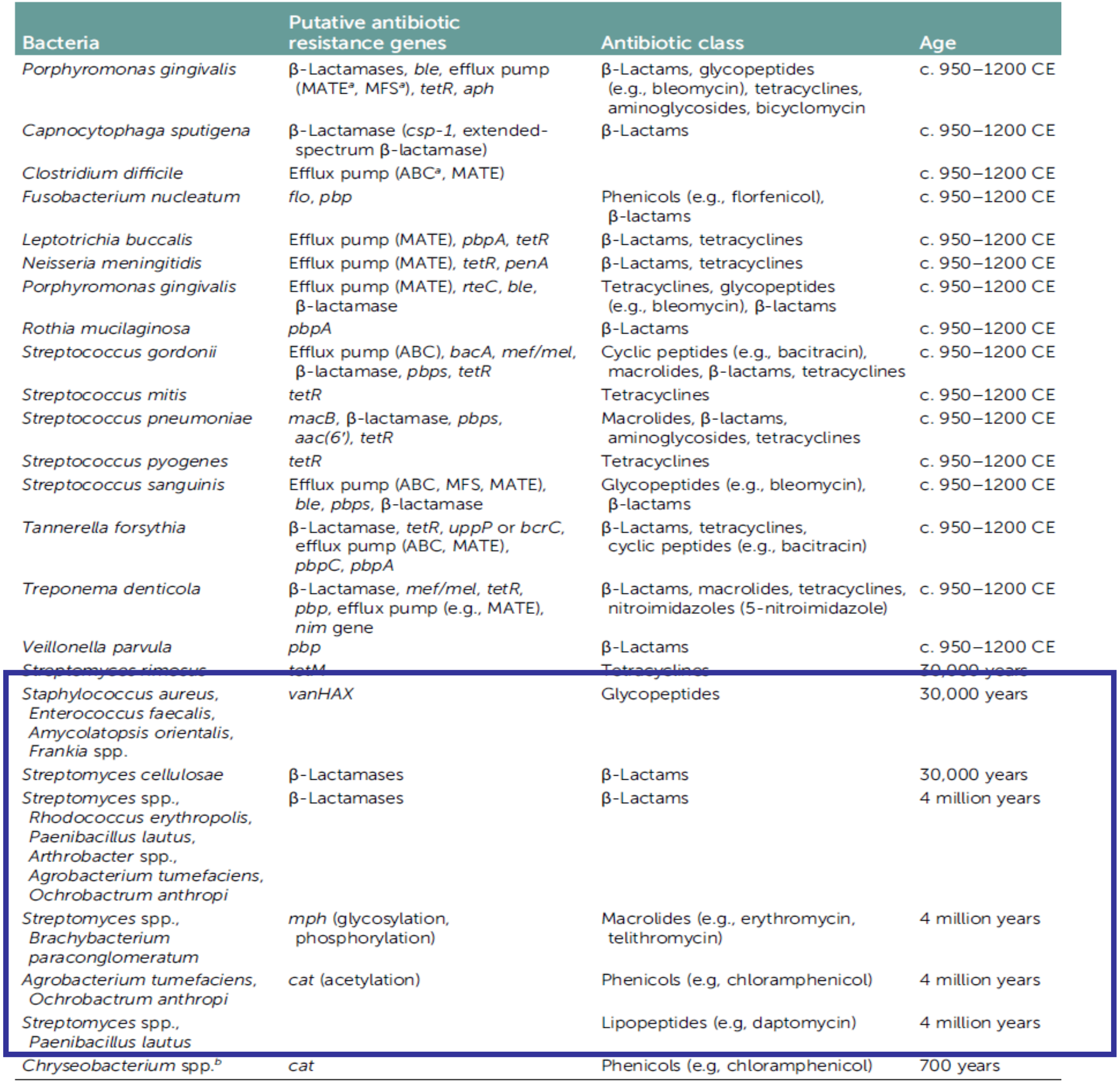
- Acinetobacter pittii
- Acinetobacter radioresistans
- Acinetobacter sp.
- Yersinia intermedia

Cryolithosphere is a source of antibiotic resistance genes

- pKLH80 plasmid from Psychrobacter maritimus MR29-12 strain found in permafrost Arctic soil (abs. age >15,000 years)
-contains beta-lactamase blaRTG-6, tetracycline and streptomycin resistance genes,
-Can repilicate in modern strains of acinetobacters
- probable precursor of blaRTG in modern clinical strains



Petrova M, Kurakov A, Shcherbatova N, Mindlin S.Genetic structure and biological properties of the first ancient multiresistance plasmid pKLH80 isolated from a permafrost bacterium. Microbiology. 2014 Oct;160(Pt 10):2253-63.

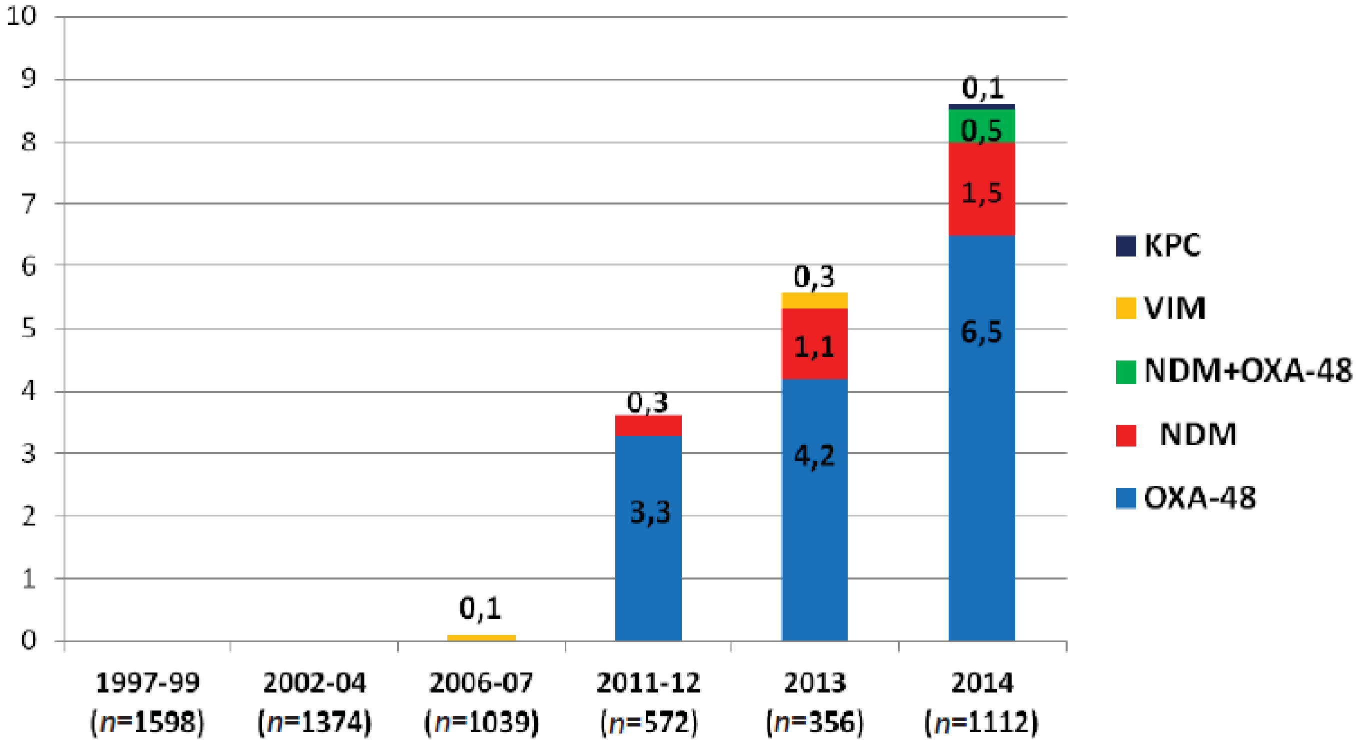


Bacteria	Putative antibiotic resistance genes	Antibiotic class	Age
Porphyromonas gingivalis	β-Lactamases, *ble*, efflux pump (MATE[a], MFS[a]), *tetR*, *aph*	β-Lactams, glycopeptides (e.g., bleomycin), tetracyclines, aminoglycosides, bicyclomycin	c. 950–1200 CE
Capnocytophaga sputigena	β-Lactamase (*csp-1*, extended-spectrum β-lactamase)	β-Lactams	c. 950–1200 CE
Clostridium difficile	Efflux pump (ABC[a], MATE)		c. 950–1200 CE
Fusobacterium nucleatum	*flo*, *pbp*	Phenicols (e.g., florfenicol), β-lactams	c. 950–1200 CE
Leptotrichia buccalis	Efflux pump (MATE), *pbpA*, *tetR*	β-Lactams, tetracyclines	c. 950–1200 CE
Neisseria meningitidis	Efflux pump (MATE), *tetR*, *penA*	β-Lactams, tetracyclines	c. 950–1200 CE
Porphyromonas gingivalis	Efflux pump (MATE), *rteC*, *ble*, β-lactamase	Tetracyclines, glycopeptides (e.g., bleomycin), β-lactams	c. 950–1200 CE
Rothia mucilaginosa	*pbpA*	β-Lactams	c. 950–1200 CE
Streptococcus gordonii	Efflux pump (ABC), *bacA*, *mef/mel*, β-lactamase, *pbps*, *tetR*	Cyclic peptides (e.g., bacitracin), macrolides, β-lactams, tetracyclines	c. 950–1200 CE
Streptococcus mitis	*tetR*	Tetracyclines	c. 950–1200 CE
Streptococcus pneumoniae	*macB*, β-lactamase, *pbps*, *aac(6')*, *tetR*	Macrolides, β-lactams, aminoglycosides, tetracyclines	c. 950–1200 CE
Streptococcus pyogenes	*tetR*	Tetracyclines	c. 950–1200 CE
Streptococcus sanguinis	Efflux pump (ABC, MFS, MATE), *ble*, *pbps*, β-lactamase	Glycopeptides (e.g., bleomycin), β-lactams	c. 950–1200 CE
Tannerella forsythia	β-Lactamase, *tetR*, *uppP* or *bcrC*, efflux pump (ABC, MATE), *pbpC*, *pbpA*	β-Lactams, tetracyclines, cyclic peptides (e.g., bacitracin)	c. 950–1200 CE
Treponema denticola	β-Lactamase, *mef/mel*, *tetR*, *pbp*, efflux pump (e.g., MATE), *nim* gene	β-Lactams, macrolides, tetracyclines, nitroimidazoles (5-nitroimidazole)	c. 950–1200 CE
Veillonella parvula	*pbp*	β-Lactams	c. 950–1200 CE
Streptomyces rimosus	*tetM*	Tetracyclines	30,000 years
Staphylococcus aureus, *Enterococcus faecalis*, *Amycolatopsis orientalis*, *Frankia* spp.	*vanHAX*	Glycopeptides	30,000 years
Streptomyces cellulosae	β-Lactamases	β-Lactams	30,000 years
Streptomyces spp., *Rhodococcus erythropolis*, *Paenibacillus lautus*, *Arthrobacter* spp., *Agrobacterium tumefaciens*, *Ochrobactrum anthropi*	β-Lactamases	β-Lactams	4 million years
Streptomyces spp., *Brachybacterium paraconglomeratum*	*mph* (glycosylation, phosphorylation)	Macrolides (e.g., erythromycin, telithromycin)	4 million years
Agrobacterium tumefaciens, *Ochrobactrum anthropi*	*cat* (acetylation)	Phenicols (e.g, chloramphenicol)	4 million years
Streptomyces spp., *Paenibacillus lautus*		Lipopeptides (e.g, daptomycin)	4 million years
Chryseobacterium spp.[b]	*cat*	Phenicols (e.g, chloramphenicol)	700 years

permafrost

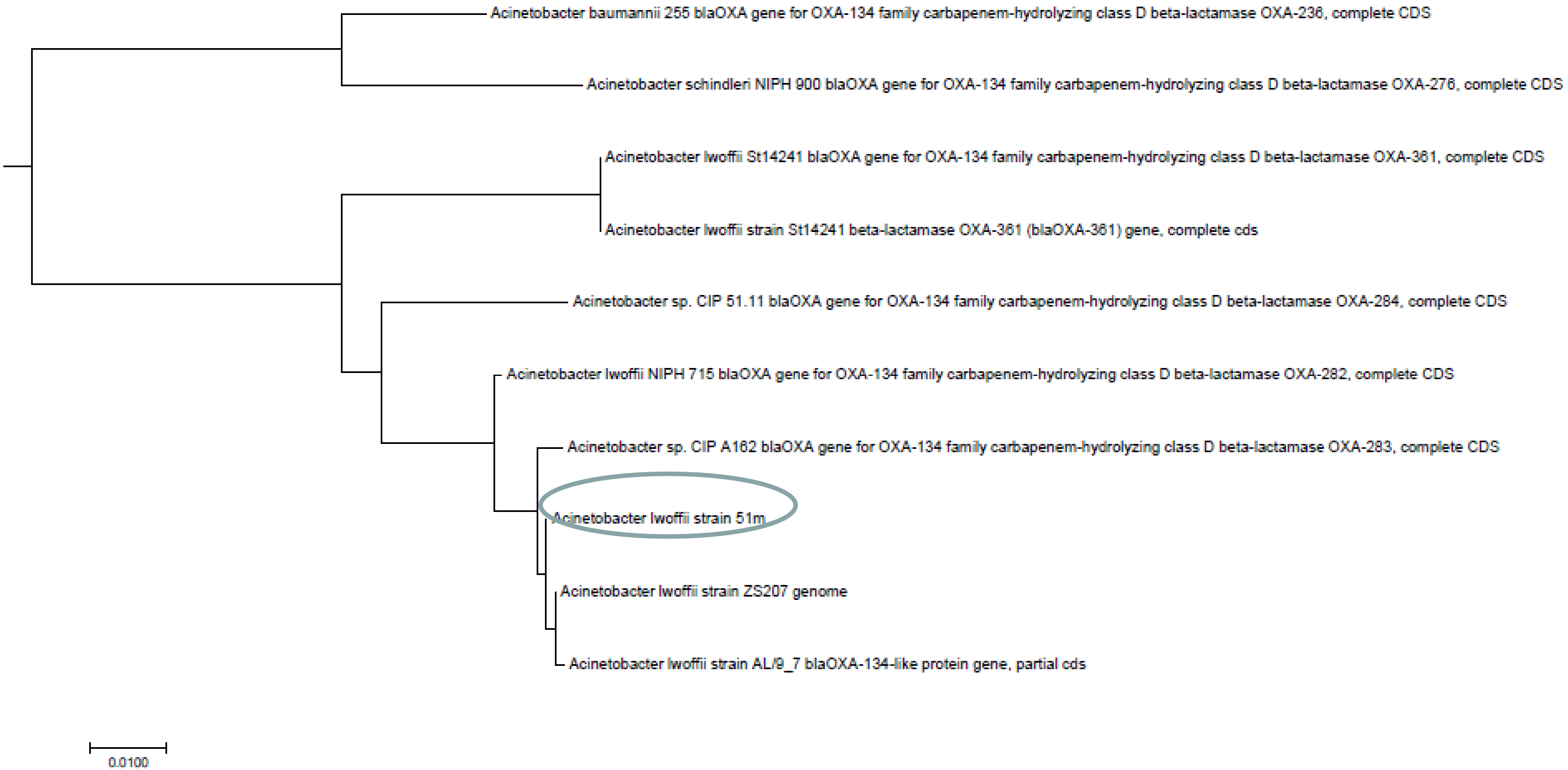
Olaitan AO, Rolain JM. 2016. Ancient resistome. Microbiol Spectrum 4(4):PoH-0008-2015. doi:10.1128/microbiolspec.PoH -0008-2015.

Epidemic of nosocomial *Enterobacteriaceae* that have carbapenemases in Russia



Edelstein M. V., Zhuravlev V. S., Shek E. A. (2017)

Carbapenemase class D in the genome of the ancient strain of Acinetobacter lwoffii from “Malolyakhovskiy mammoth»



The global spread of vancomycin-resistant Enterococcus faecium CC17

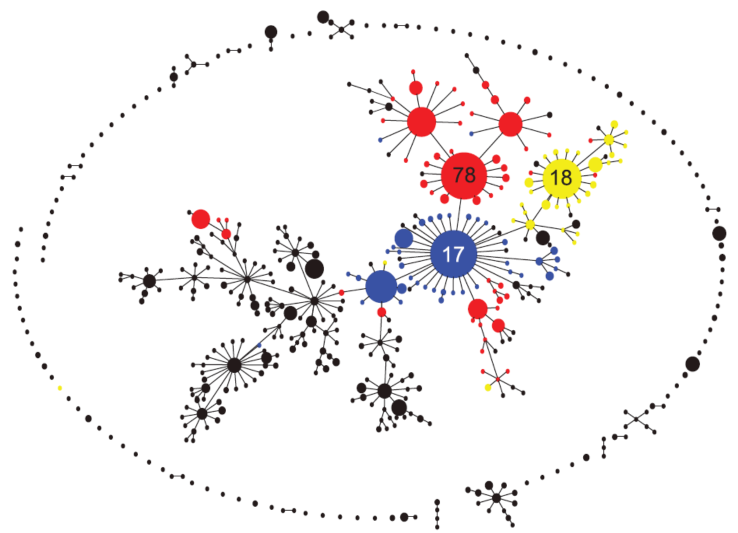
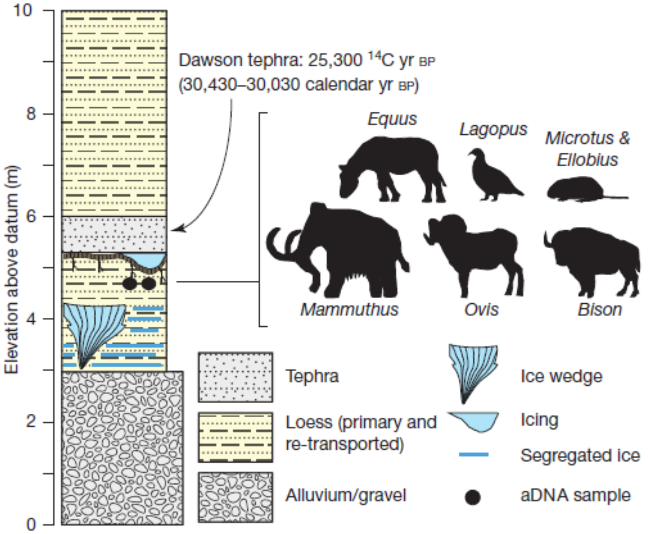
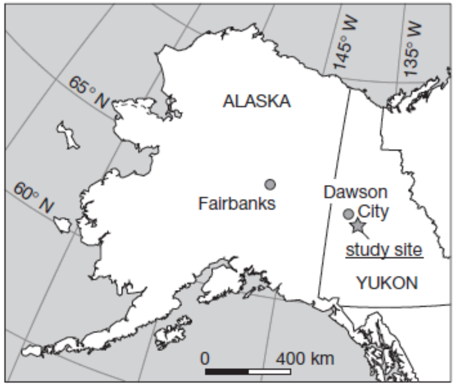


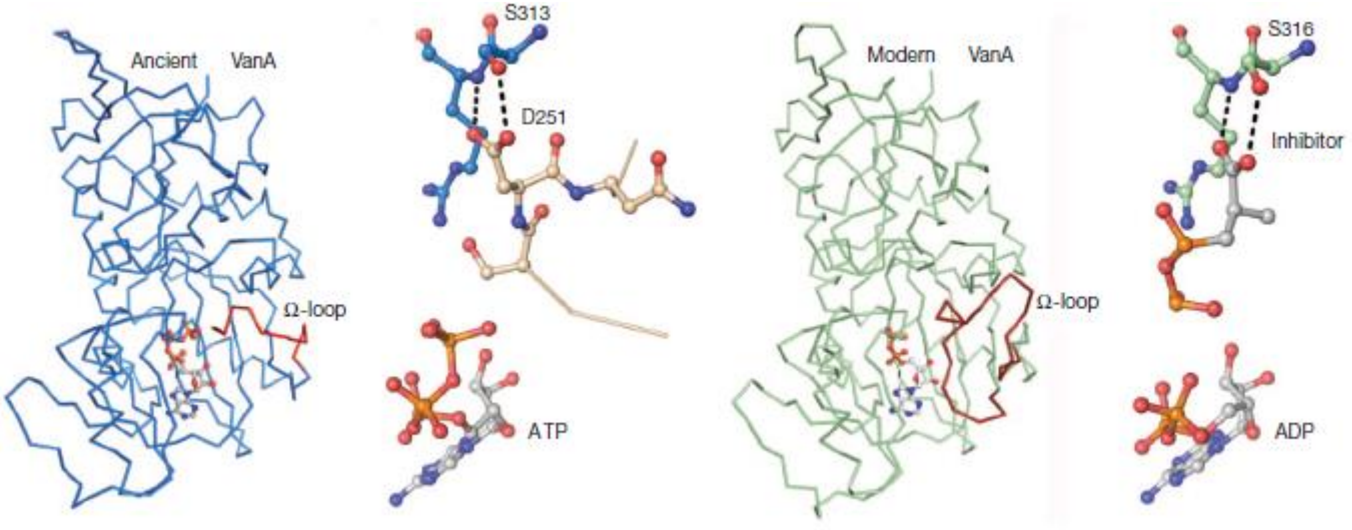
FIG 1 eBURST-based population snapshot of *E. faecium* based on 519 STs representing 1,749 isolates contained in the online *E. faecium* MLST database (http://efaecium.mlst.net). STs belonging to lineages 17, 18, and 78 are color coded in blue, red, and yellow, respectively.

Willems RJ et. al, 2005

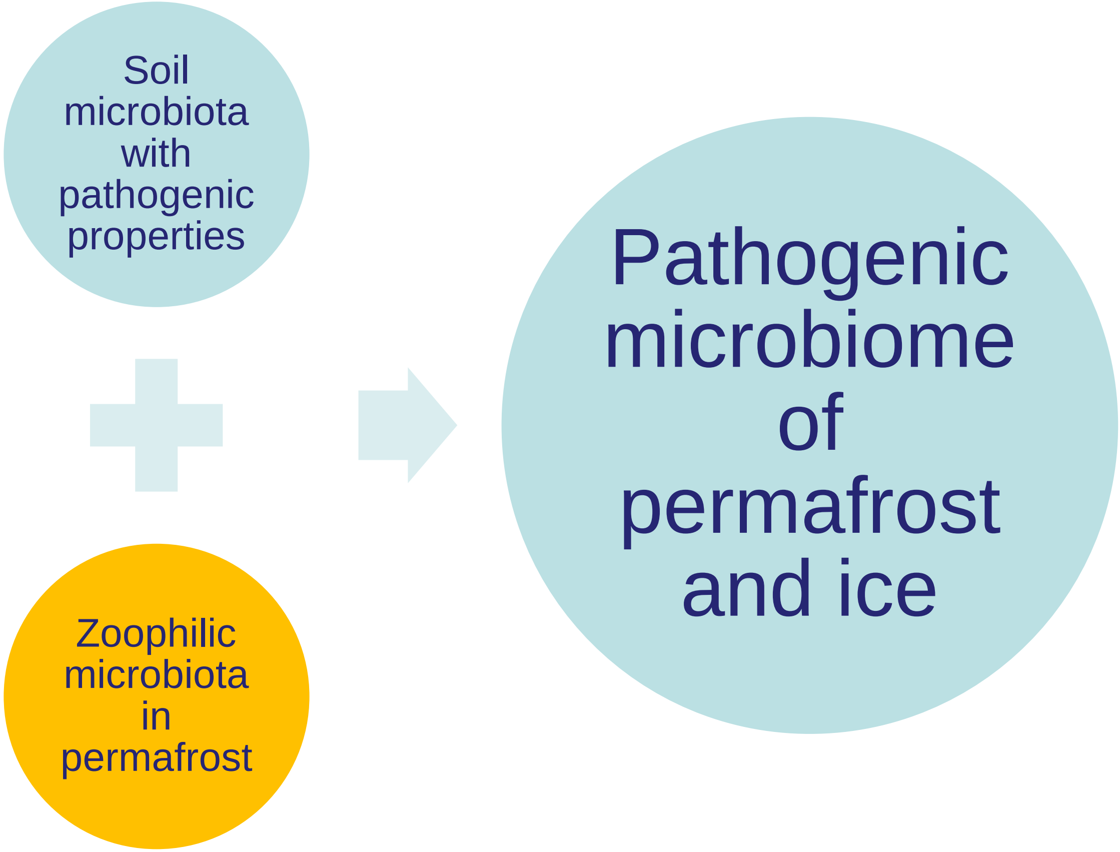




Comparison of modern and ancient VanA monomer structures. The sigma-loop is coloured red and detailed in the ball-and stick figures. Ligands are shown in grey. Dashed lines represent hydrogen bonds.



D'Costa, Vanessa M., et al. "Antibiotic resistance is ancient." *Nature* 477.7365 (2011): 457.



Soil microbiota with pathogenic properties

Zoophilic microbiota in permafrost

Pathogenic microbiome of permafrost and ice

Antarctic migratory birds as potential vectors for intercontinental transfer of pathogenes

Species (in russian)	Migrations	Bacterial isolates (+ pathogenic species)*	
Южнополярный поморник (Catharacta maccormicki)	migration to the Northern hemisphere (to the coasts of Alaska and Greenland).	Salmonella spp. Acinetobacter guillouiae, Citrobacter braakii, Enterococcus faecalis	
Серебристо-серый буревестник (Fulmarus glacialoides)	temperate and sometimes tropical latitudes of the southern hemisphere	Enterobacteriaceae Enterococcus spp.	

* 2018, 63 Russian antarctic expedition, Krylenkov VA, Goncharov AE, Khoroshilov VV

photo: Todd FC, 2004 http://www.birdlife.org/datazone

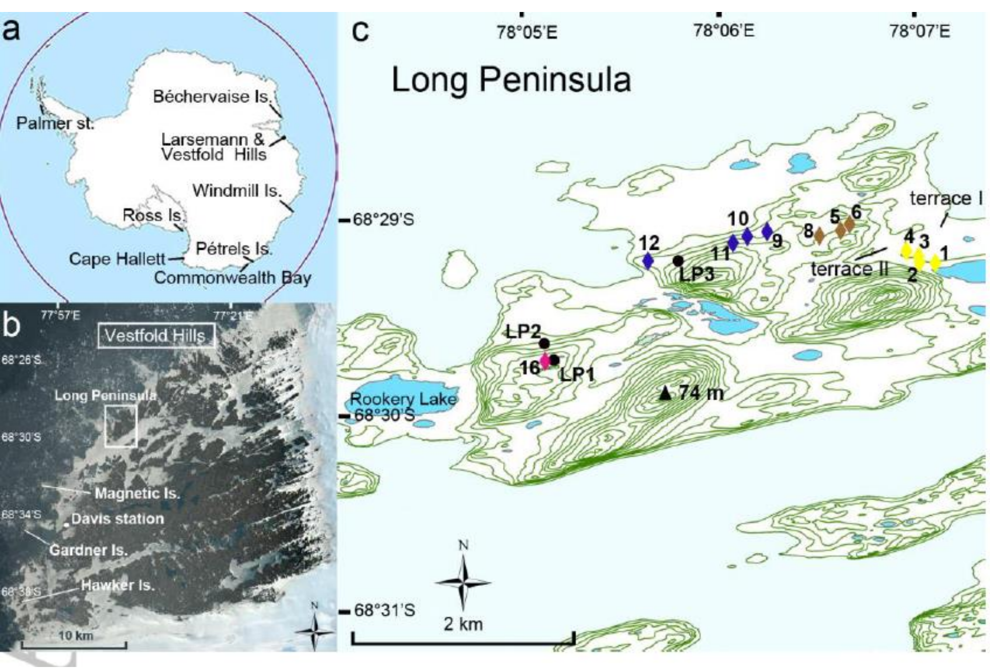

2018/1/5 14:14

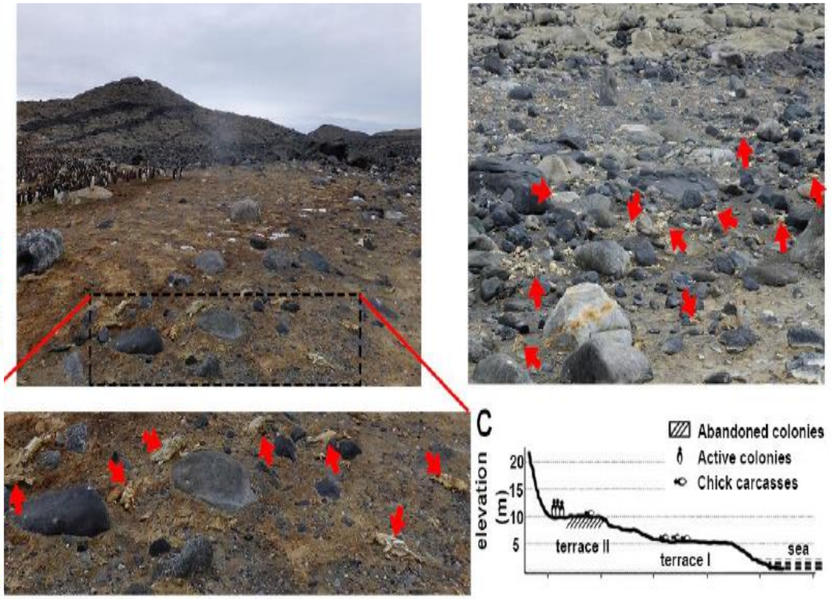

2018/1/5 14:32

Determinants of pathogenicity of ancient bacteria

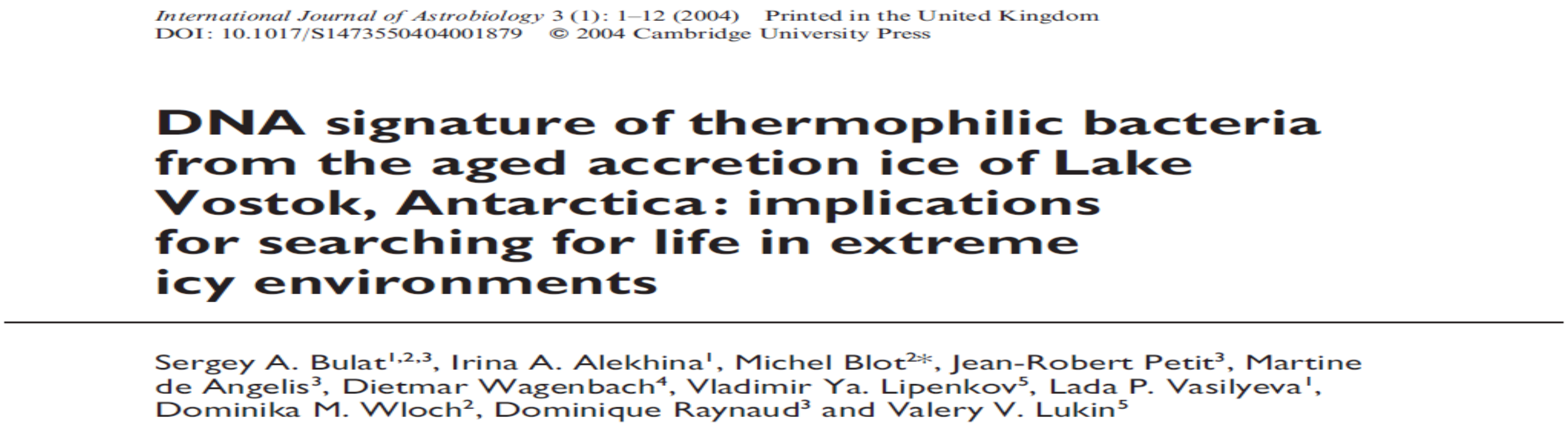
Determinants of pathogenicity found in the genome	genome	Animal (fossil)	Calibrated age (according to radiocarbon dating)
Siderophores: aerobactin and enterobactin operons	*Serratia fonticola 5l* *Serratia plymutica str. tumat*	Alces alces Canis lupus	12 460 ± 50
Pathogenicity island SDI-1 Salmonella spp.	*Serratia fonticola 5l*	Alces alces	8980± 50
Perfringolysin O, hemolysins, collagenase	*Clostridium perfringens BzA*	Bison priscus	48000-50000
hemolysin	*Serratia fonticola 5l* *Serratia plymutica str. tumat*	Alces alces Canis lupus	12 460 ± 50
hemolysin, IS-16 (marker of epidemic clones of enterococci)	*Enterococcus faecium str. 58*	Mammuthus primigenius	28800± 150

Mass mortality of penguins ~750 and ~200 years BP





Gao, Y., Yang, L., Xie, Z., Emmerson, L., Southwell, C., Wang, Y., & Sun, L. (2018). Last Millennium Adélie Penguin Mortality and Colony Abandonment Events on Long Peninsula, East Antarctica.
Journal of Geophysical Research: Biogeosciences. doi:10.1029/2018jg004550



International Journal of Astrobiology 3 (1): 1–12 (2004) Printed in the United Kingdom
DOI: 10.1017/S1473550404001879

DNA signature of thermophilic bacteria from the aged accretion ice of Lake Vostok, Antarctica: implications for searching for life in extreme icy environments

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Sergey A. Bulat[1,2,3], Irina A. Alekhina[1], Michel Blot[2*], Jean-Robert Petit[3], Martine de Angelis[3], Dietmar Wagenbach[4], Vladimir Ya. Lipenkov[5], Lada P. Vasilyeva[1], Dominika M. Wloch[2], Dominique Raynaud[3] and Valery V. Lukin[5]

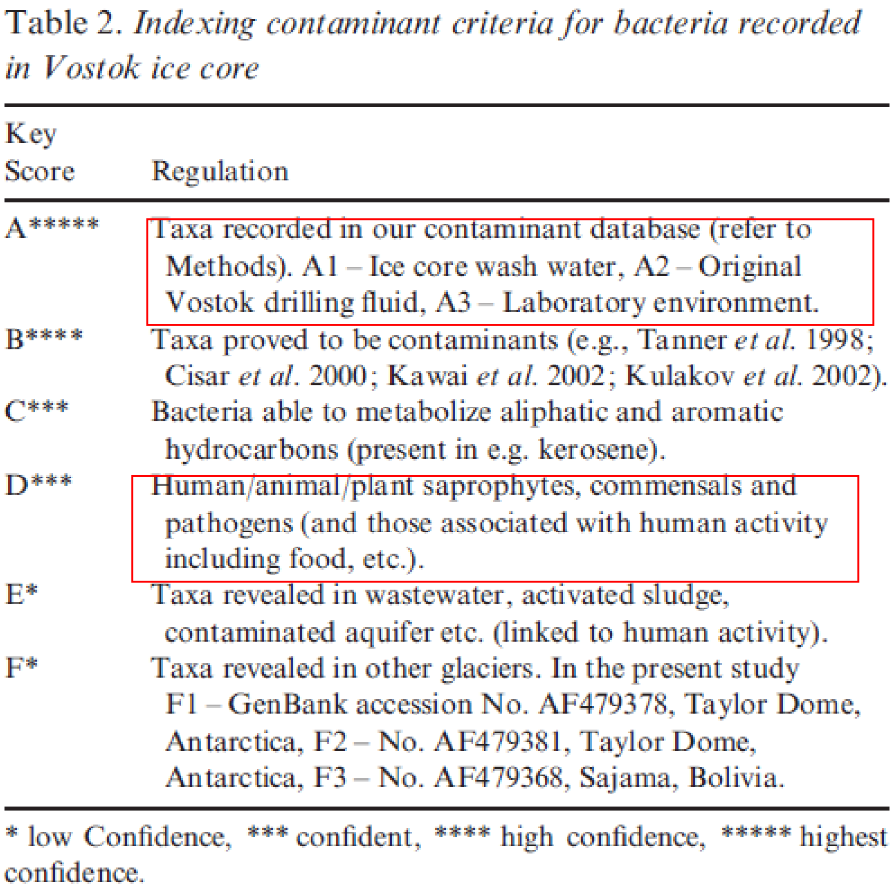


Table 2. *Indexing contaminant criteria for bacteria recorded in Vostok ice core*

Key Score	Regulation
A*****	Taxa recorded in our contaminant database (refer to Methods). A1 – Ice core wash water, A2 – Original Vostok drilling fluid, A3 – Laboratory environment.
B****	Taxa proved to be contaminants (e.g., Tanner *et al.* 1998; Cisar *et al.* 2000; Kawai *et al.* 2002; Kulakov *et al.* 2002).
C***	Bacteria able to metabolize aliphatic and aromatic hydrocarbons (present in e.g. kerosene).
D***	Human/animal/plant saprophytes, commensals and pathogens (and those associated with human activity including food, etc.).
E*	Taxa revealed in wastewater, activated sludge, contaminated aquifer etc. (linked to human activity).
F*	Taxa revealed in other glaciers. In the present study F1 – GenBank accession No. AF479378, Taylor Dome, Antarctica, F2 – No. AF479381, Taylor Dome, Antarctica, F3 – No. AF479368, Sajama, Bolivia.

* low Confidence, *** confident, **** high confidence, ***** highest confidence.

Table 3. *Bacterial 16S rDNA clones recovered from Vostok ice core and presumed to be contaminants*

Number of allelic clones[a] in rDNA clone library							
3607	3607b	3607c	3551	3001	Closest neighbours in GenBank	Contamination criterion	Total score[b]
Alpha-*Proteobacteria*							
3			1		100 % *Sphingomonas paucimobilis* (U37337)	A3, C, F1	9
	3				98–100 % *Sphingobium yanoikuyae* (AY047219)	A3, C	8
1	8				99–100 % *Sphingomonas natatoria* (AB024288)	A2, F2	6
3				4	99–100 % Uncultured bacterium D from beetle larva intestine (AJ459874)	D	3
	2				99.8 % Uncultured bacterium clone E6 from shower curtain biofilms (AY268237)	D	3
Beta-*Proteobacteria*							
	2				97 % rDNA clone pACH94 (AY297809)		
					96 % *Rhodocyclus sp.* R6 (AJ224937), rDNA clones (AF204245), (AF502232), (AF204244)	Linked to E Likely contaminant (AF532058)	NA[c]
Gamma-*Proteobacteria*							
1	4		1		98–99 % *Acinetobacter johnsonii* (X89775)	A3	5
		2			99 % *Pseudomonas rhodesiae* (AF064459)	B	4
		6			98 % *Haemophilus parainfluenzae* (M75081)	D	3

The study of the Malolyakhovskiy mammoth

The mammoth remains have been found in the summer of 2012 on the Malyi Lyakhovsky island between the Laptev Sea and East Siberian Sea
Dating age is ~ 28,000 years

The study of «Malolyakhovskiy mammoth”

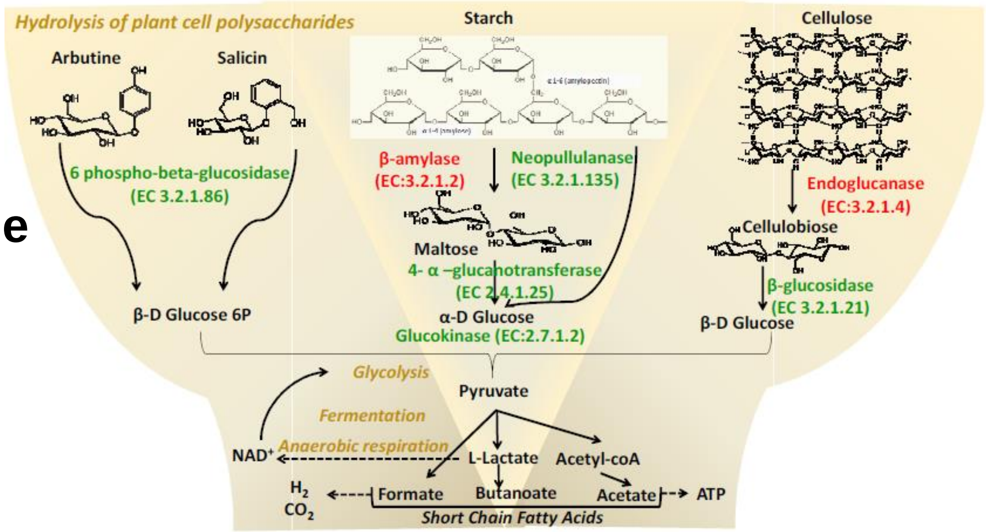
Taxonomic position of isolated bacteria by 16S ribosomal DNA (rDNA) sequencing *

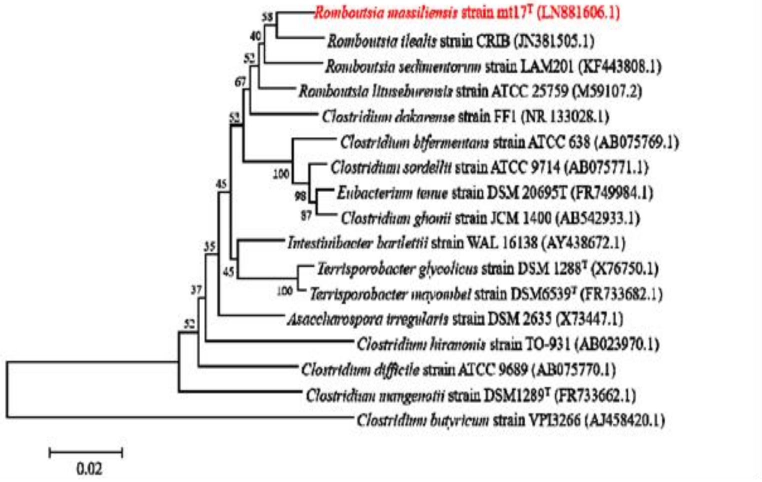
Isolate	Origin	Closest relative (16S rRNA)	% 16S rRNA similarity
1	intestinal contents	Carnobacterium maltaromaticum	96
2	intestinal contents	Enterococcus faecium	98
3	intestinal contents	Romboutsia massiliensis, sp. nov*	98.48*
4	intestinal contents	Acinetobacter lwoffii	99
5	intestinal contents	Enterococcus hirae	99
6	gingival pocket	Enterococcus faecium	98
7	bone marrow	Arthrobacter sp.	99

*fD1 (5'-AGAGTTTGATCCTGGCTCAG- 3') and rP2 (5'-ACGGCTACCTTGTTACGACTT- 3') primer (Weisburg et al., 1991) have been used.

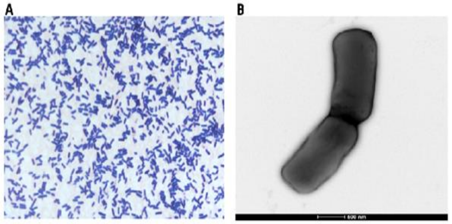
The biology of the isolated bacteria confirms that it is ancient

Genomic and functional analysis of *Romboutsia massiliensis*, sp. nov., reveals adaptation to the small intestine of the mammoth.





Phylogeny of Romboutsia massiliensis, sp. nov mt17



Microscopy of Romboutsia massiliensis, sp. nov mt17A-light microscopy for gram staining, B-transmission electron microscopy using Tecnai G20. The scale size corresponds to 500 nm.

Microbial dark matter: how to study?

only 0.1%–1% of the microbial taxa are cultivable*

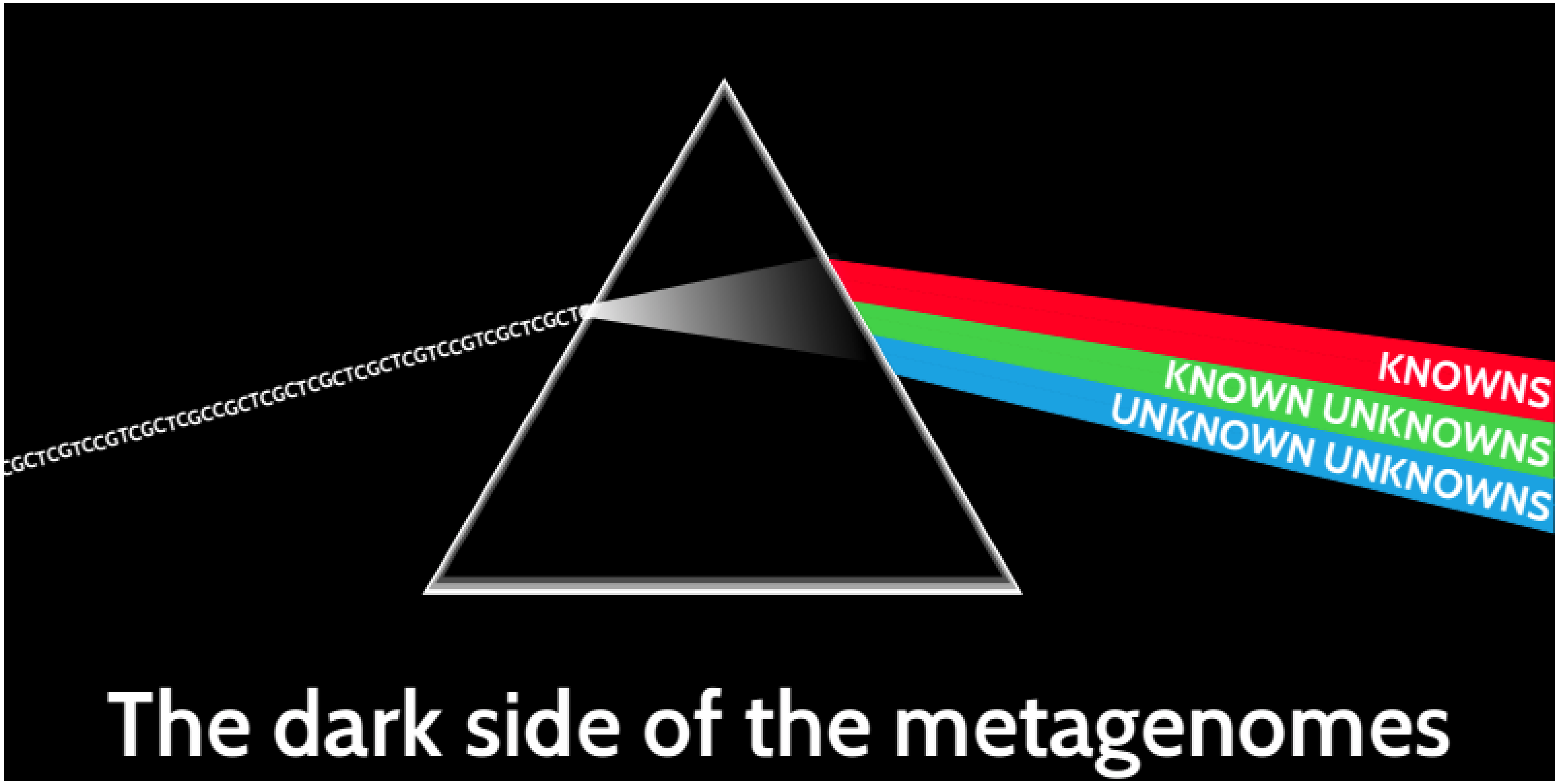
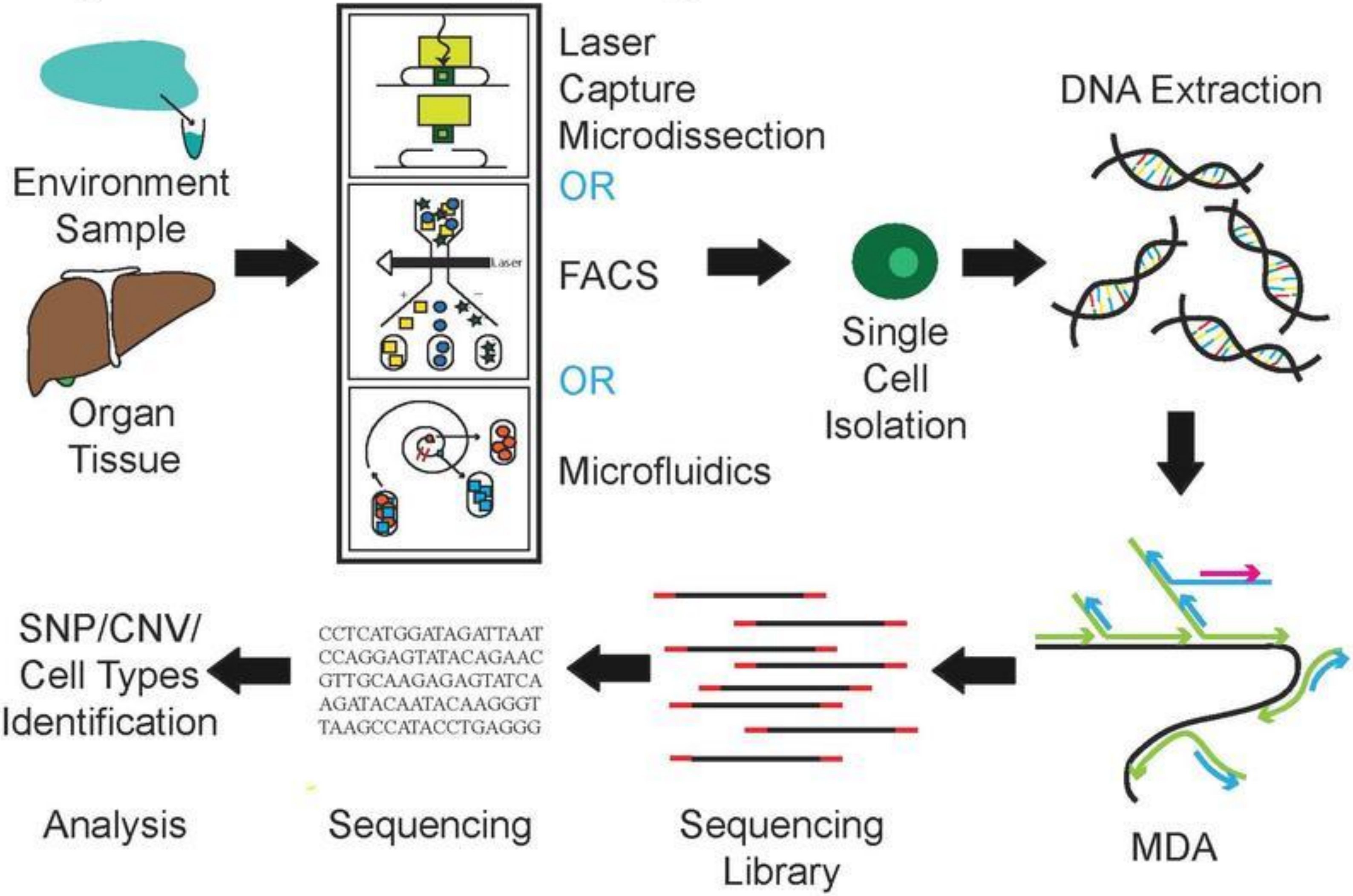


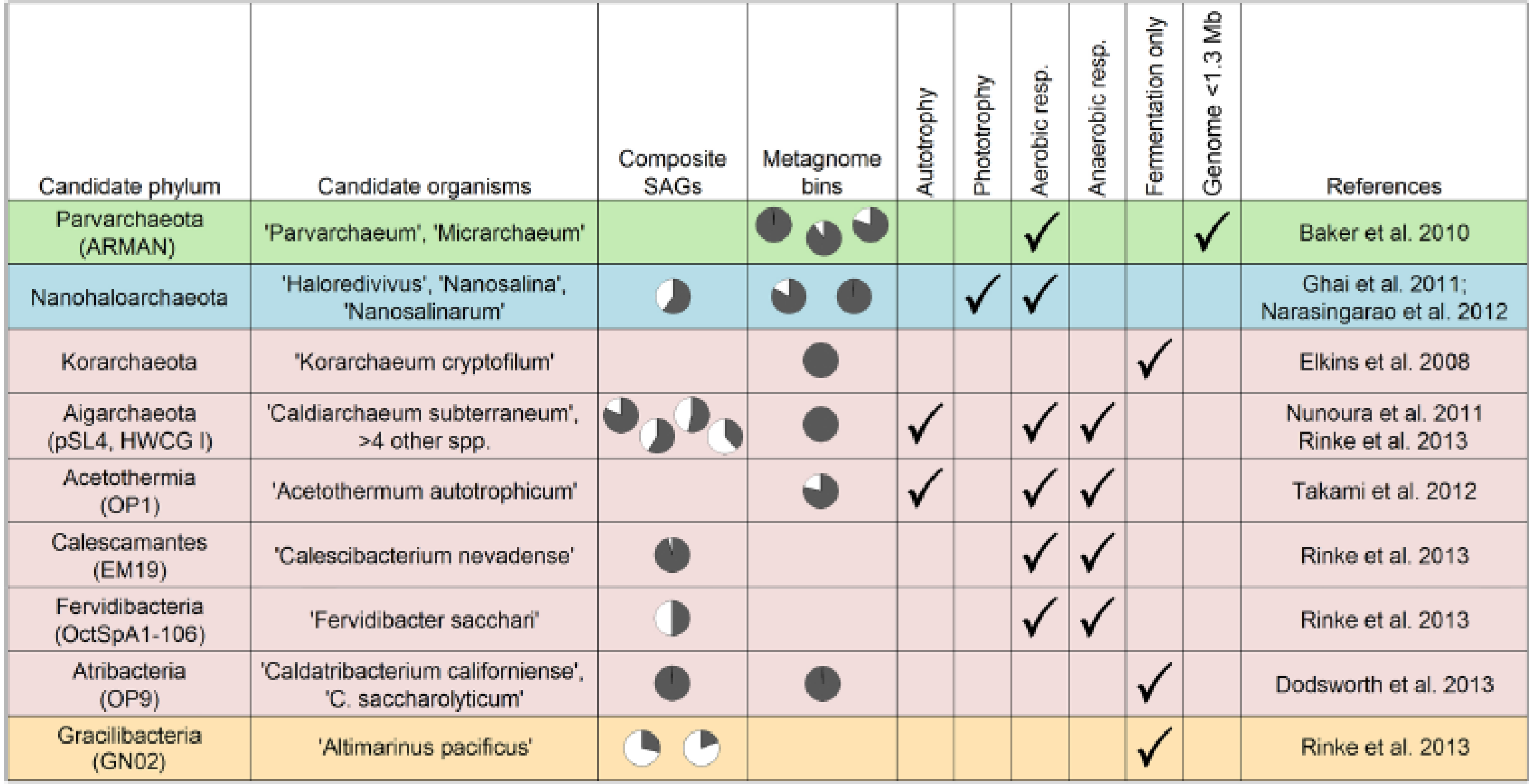
Figure from http://merenlab.org/images/miscellaneous/2017-06-22-microbial-dark-matter/dark-side-of-metagenomes.png

Solden L., Lloyd K. and Wrighton K. (2016)

Single Cell Genome Sequencing Workflow



Summary of genomic coverage and predicted features of extremophilic “microbial dark matter” (MDM) based on metagenome and/or single-cell genome approaches.



Candidate phylum	Candidate organisms	Composite SAGs	Metagnome bins	Autotrophy	Phototrophy	Aerobic resp.	Anaerobic resp.	Fermentation only	Genome <1.3 Mb	References
Parvarchaeota (ARMAN)	'Parvarchaeum', 'Micrarchaeum'		● ● ◕			✓			✓	Baker et al. 2010
Nanohaloarchaeota	'Haloredivivus', 'Nanosalina', 'Nanosalinarum'	◕	◕ ●		✓	✓				Ghai et al. 2011; Narasingarao et al. 2012
Korarchaeota	'Korarchaeum cryptofilum'		●					✓		Elkins et al. 2008
Aigarchaeota (pSL4, HWCG I)	'Caldiarchaeum subterraneum', >4 other spp.	◕ ◕ ◑ ◑	●	✓		✓	✓			Nunoura et al. 2011 Rinke et al. 2013
Acetothermia (OP1)	'Acetothermum autotrophicum'		◕	✓		✓	✓			Takami et al. 2012
Calescamantes (EM19)	'Calescibacterium nevadense'	●				✓	✓			Rinke et al. 2013
Fervidibacteria (OctSpA1-106)	'Fervidibacter sacchari'	◑				✓	✓			Rinke et al. 2013
Atribacteria (OP9)	'Caldatribacterium californiense', 'C. saccharolyticum'	●	●					✓		Dodsworth et al. 2013
Gracilibacteria (GN02)	'Altimarinus pacificus'	◔ ◔						✓		Rinke et al. 2013

Hedlund, B. P., Dodsworth, J. A., Murugapiran, S. K., Rinke, C., & Woyke, T. (2014). Impact of single-cell genomics and metagenomics on the emerging view of extremophile “microbial dark matter.” Extremophiles, 18(5), 865–875. doi:10.1007/s00792-014-0664-7

Conclusion

- The removal of paleomicroorganisms or their genetic material by degradation of permafrost due the global climatic changes is associated with the risk of emergence of new pathogens or activation of forgotten infectious diseases.
- One of the most promising approaches to the study of the pathogenic characteristics of bacteria found in permafrost is the whole genome sequencing.
- an effective monitoring of the pathogenic potential of the polar microbiota should be implemented by using of NGS technologies including genomic (including single-cell genome approaches)and metagenomic sequencing.

Дзякуй за ўвагу, дарагія сябры!