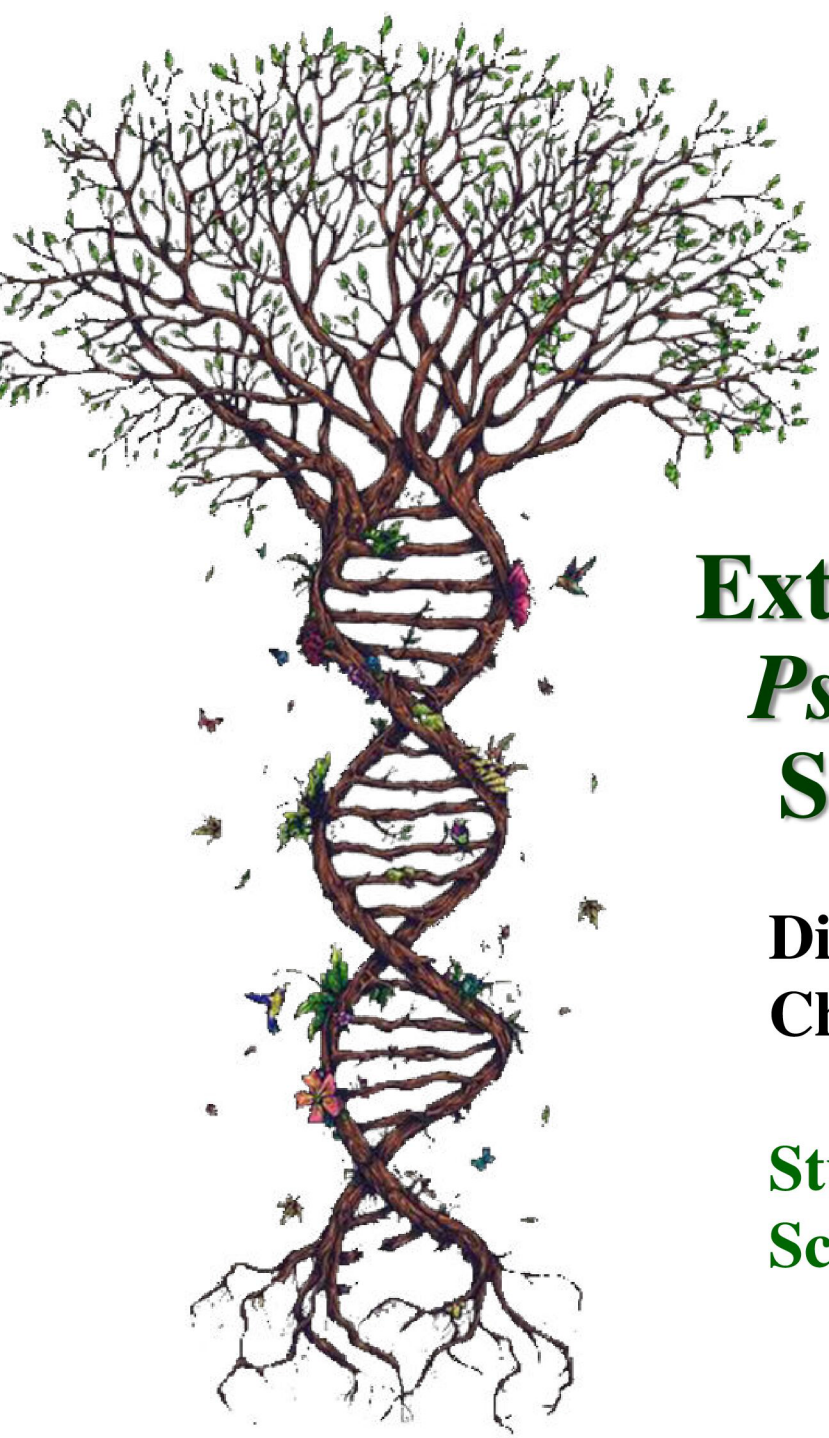


Iceland Liechtenstein Norway grants
"Working together for a green, competitive and inclusive Europe"

GREENCAM for tomorrow

UNIVERSITY OF BUCHAREST

NTNU
Norwegian University of Science and Technology

FACULTY OF CHEMISTRY
DEPARTMENT OF ORGANIC CHEMISTRY, BIOCHEMISTRY AND CATALYSIS

Extracellular cold-active lipases of *Psychrobacter sp. SC65A.3* from Scarisoara Ice Cave, Romania

Dissertation Thesis: Experimental report
Chemistry of Advanced Materials

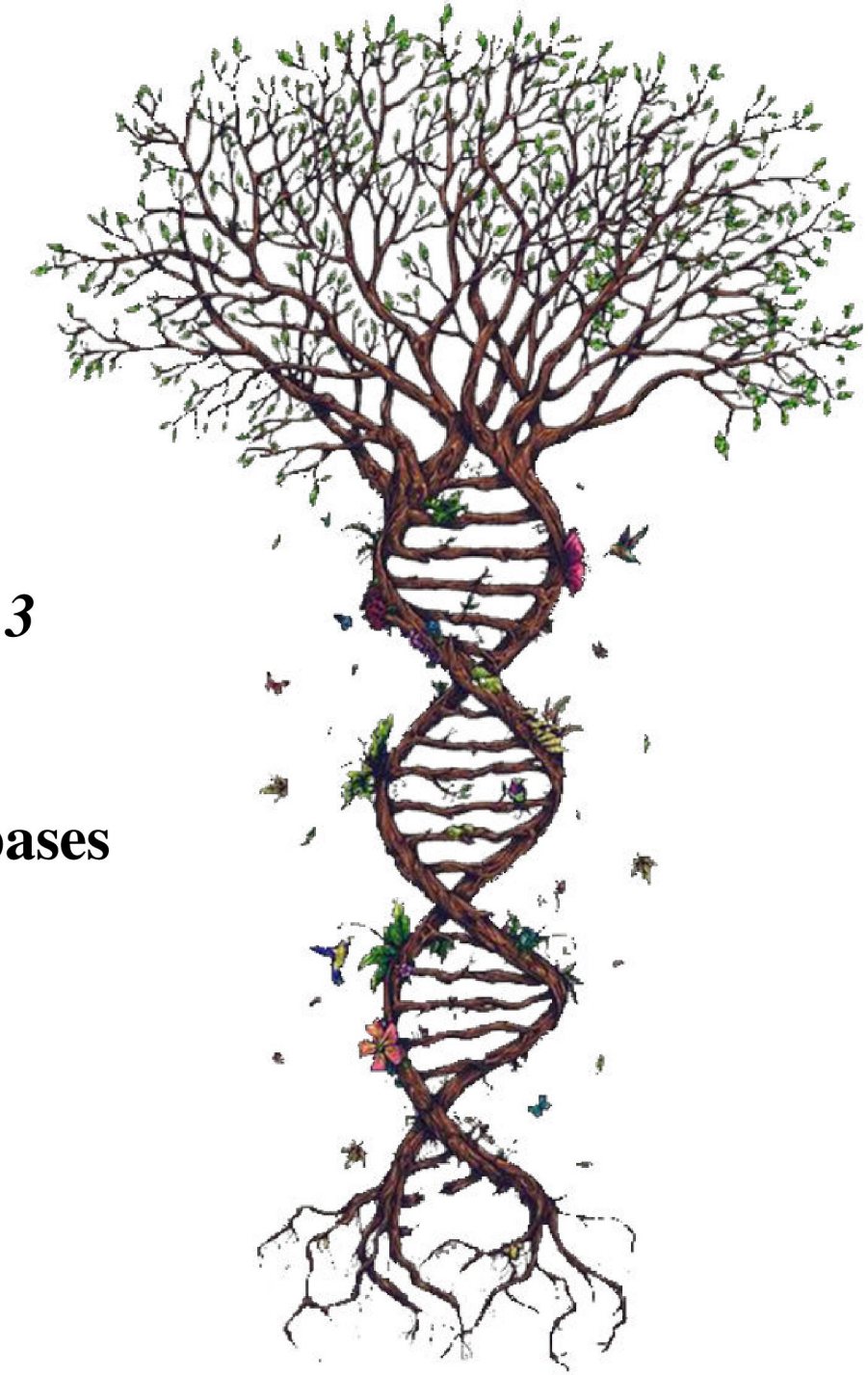
Student: Giulia-Roxana Gheorghiță
Scientific Coordinator: Assoc. Prof. Dr. Mădălina Tudorache

September **2020**, Bucharest

Disclaimer: This was realised with the EEA Financial Mechanism 2014-2021 financial support. Its content (text, photos, videos) does not reflect the official opinion of the Programme Operator, the National Contact Point and the Financial Mechanism Office. Responsibility for the information and views expressed therein lies entirely with the author(s).

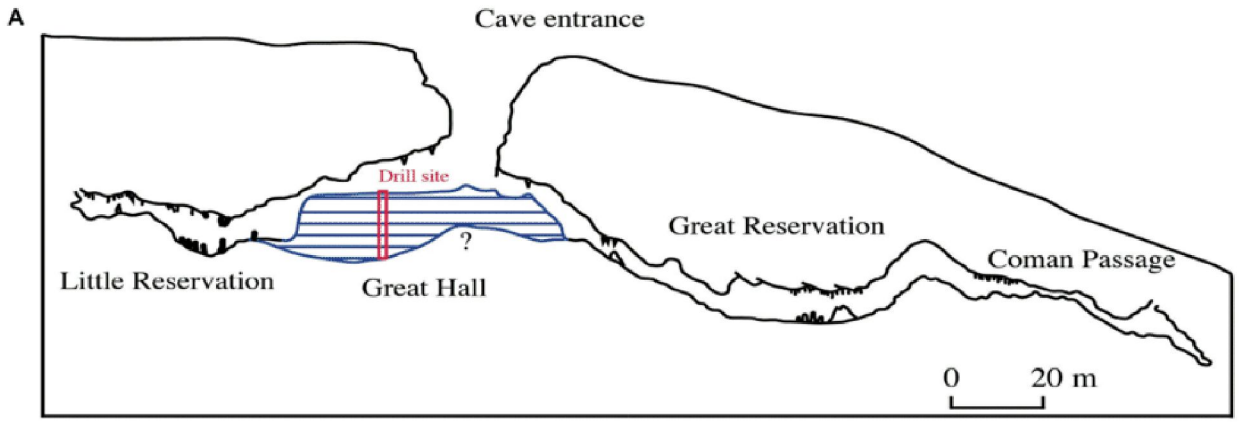
OUTLINE

- Cold-adapted strain of *Psychrobacter sp. SC65A.3*
- Structural analyses of extracellular lipases
- Biochemical characterization of extracellular lipases
- Conclusions



Psychrobacter sp. SC65A.3 from Scarisoara Ice Cave, Romania

The *Psychrobacter sp. SC65A.3* strain was isolated from **perennial ice deposits** of Scarisoara cave and its draft genome sequence was determined in the **Laboratory of Dr. Cristina Purcarea** from the **Institute of Biology, Bucharest**.



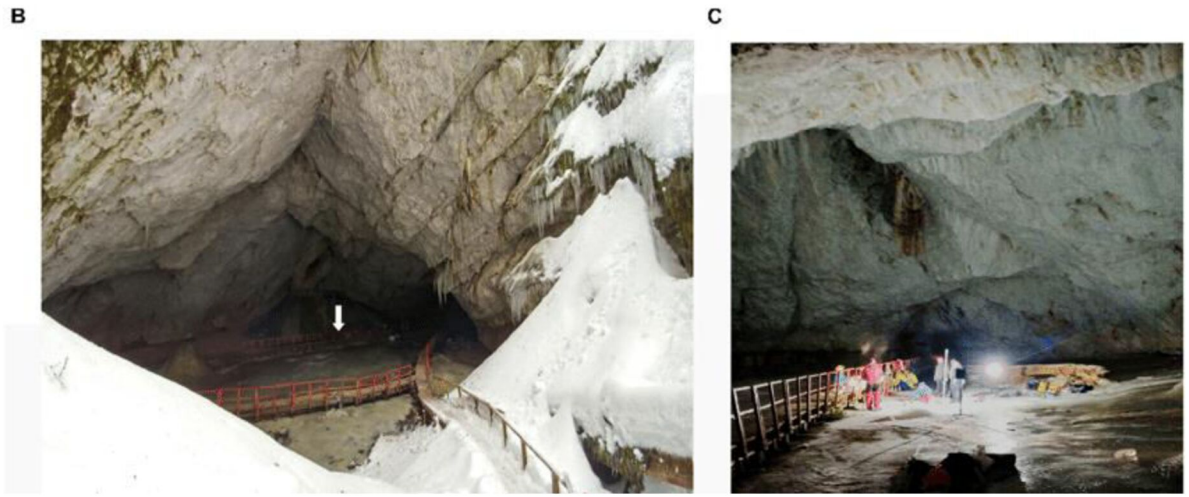


Figure I. Scarisoara Ice Cave presentation

Paun, V.I., Purcarea, C. et al, Frontiers in Microbiology, 2019, 10 (1193):1-19

Strain cultivation of *Psychrobacter sp. SC65A.3*

The *Psychrobacter* strains usually grow well on standard complex media such as **Trypticase Soy Agar** and **Reasoner's** medium at **15°C** for 2-3 days.

Spread plate technique

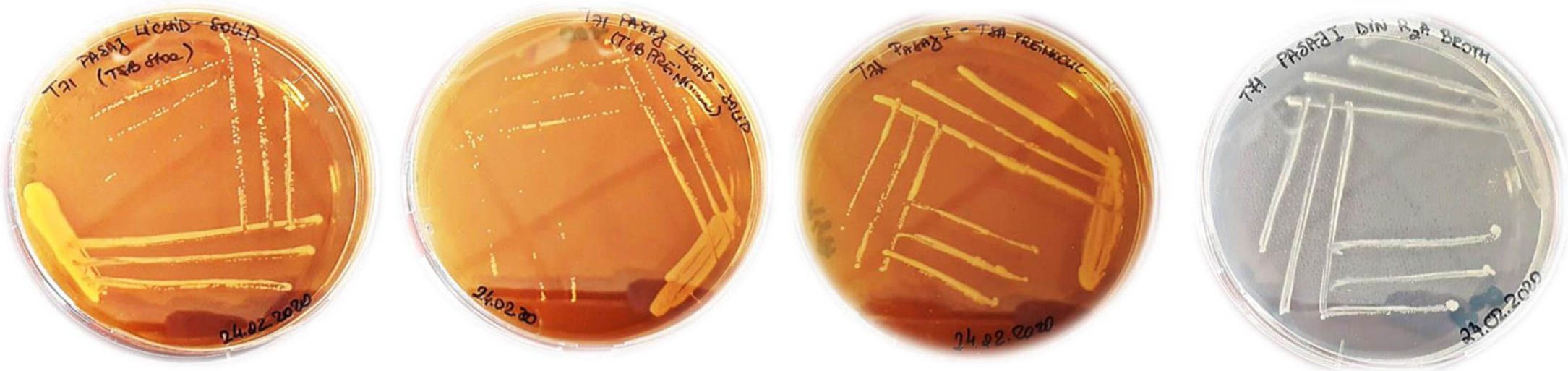


Figure II. Liquid to solid passages on TSA from TSB glycerol stock, TSB and TSA pre-inoculum

Juni, E., The Prokaryotes, Springer Science+Business Media, New York, 1992.

Lipases of the strain

Screening of the sequence of this cold-adapted bacterium revealed several **genes coding** mainly **four lipase protein sequences**, in addition to **thio-/phospho-lipases and carboxylesterases**.

Tabel I.	Protein sequence
Lipase 2_65A.3	MSNSTVLSVNTLLNKAVKTLNLMSFGQDKNPKSTDINLSDEIIDIEESALQDSREDKGLSIKEKILEHHLMTNYQPHLLHYAIKSFGCLPT PILESLIKCLDGPTSKQYLHVDAHLRLILAVNSKLKTPLQLIEMSELRKRFATDAVAMQAPKVWQQASDNLLSNLKQFHKKGDSAISWQ DRTITNADDGDMTIRCYQNETSDNGFGFKKEQTSNPDETVLLFFHGGGFCIGDLNTHHEFCHAICEQTGWPVISVDYRLAPEHPAPAAV RDCISAYAWLAEHCEEFGALPSRIVLAGDSAGGGLSTLMAQQIITPNKEAWLDLGDEGQKTFDILQGLPHPMAQMPLYPVTDIETDYPS WELYGEGLLLDHADVAIFDAACLENSPLPRQHILTSPMLGDNRQVCPSYVVAAELDVLRDEAFAYANQLKSFGIAVQTHTVLGAPHGFI HFMSVHQRLGQETQHIITGFANFVREIIKTRALLSA
Lipase 3_65A.3	MLLKRLGLATLLSFSVVGCTTAPNTLAINTTQKIIQYERSKSDLTTQSFTLSSGDKIVYAENGNVAGEPLLLIHGFGGNKDNFTRIARQLE NYNLIIPDLLGFGDSSKPMAADYRSEAQATRLHELLQAKGLASNIHVGGNSMGGAISVAYAAKYPKEVKSLWLIDSAGFWSVGVPKSLE SATLENNPLLVDKKEDFYAMYDFVMSKPPYIPKSVKAVFAQERIANKAVESKILAQIVEDNVEQRAKVIAEYNIPTLVVWGEEDKVIKPE TVTLIKEIIPQSQVITMPKIGHVPMIEAVKDTANDYKAFREGLKN

The deduced amino acid sequence of the putative **Lipase 2_65A.3** and **Lipase 3_65A.3** were selected for evaluating the structural elements related to a **cold-adaptation mechanism** that necessities to be adequately elucidated.

STRUCTURAL ANALYSES of EXTRACELLULAR LIPASES

Table II.	Lipase homologs	Lipase 2_65A.3		Lipase 3_65A.3	
		Identity (%)	Similarity (%)	Identity (%)	Similarity (%)
Psychrophiles	*Psychrobacter sp. G*	98.96	99.37	99.68	99.68
	Glaciibacter superstes	28.88	43.10	27.75	43.54
	Moritella sp. PE36	28.24	43.92	27.67	42.76
Mesophiles	*Pseudomonas aeruginosa*	32.78	48.96	36.92	58.06
	Escherichia coli	29.08	43.32	26.33	43.41
Hyperthermophile	*Stenotrophomonas maltoflia*	30.43	41.47	35.11	57.44

https://blast.ncbi.nlm.nih.gov/

STRUCTURAL ANALYSES of EXTRACELLULAR LIPASES

Homology and sequence alignment

Lipase 2 from Scarisoara vs. Lipase from Psychrobacter sp. G

```
CLUSTAL O(1.2.4) multiple sequence alignment

LIP2_65A.3          MSNSTVLSVNTLLNKAVKTLNLMSFGQDKNPKSTDINLSDEIIDIEESALQDSREDKGLS   60
WP_020444543.1      MSNSTVLSVNTLLNKAVKTLNLMSFGQDKNPKSTDINVSAEIIDIEESALQDSREDKGLS   60
                    ************************************:* ********************

LIP2_65A.3          IKEKILEHHLMTNYQPHLLHYAIKSFGCLPTPILESLIKCLDGPTSKQYLHVDAHLRLIL   120
WP_020444543.1      IKEKILEHHLMTNYQPHLLHYAIKSFGCLPTPILESLITCLDGPTSKQYLHVDAHLRLIL   120
                    ************************************.********************

LIP2_65A.3          AVNSKLKTPLQLIEMSELRKRFATDAVAMQAPKVWQQASDNLLSNLKQFHKKGDSAISWQ   180
WP_020444543.1      AVNSKLKTPLQLIEMSELRKRFATDAVAMQAPKVWQQASDNLLSNLKQFHKKGDSAISWQ   180
                    ************************************************************

LIP2_65A.3          DRTITNADDGDMTIRCYQNETSDNGFGFKKEQTSNPDETVLLFFHGGGFCIGDLNTHHEF   240
WP_020444543.1      DRTIANADDGDMTIRCYQNETSDNGFGFKKEQTSNPDETVLLFFHGGGFCIGDLNTHHEF   240
                    ****:********************************************************

LIP2_65A.3          CHAICEQTGWPVISVDYRLAPEHPAPAAVRDCISAYAWLAEHCEEFGALPSRIVLAGDSA   300
WP_020444543.1      CHAICEQTGWPVISVDYRLAPEHPAPAAVRDCISAYAWLAEHCEEFGALPSRIVLAGDSA   300
                    ************************************************************

LIP2_65A.3          GGGLSTLMAQQIITPNKEAWLDLGDEGQKTFDILQGLPHPMAQMPLYPVTDIETDYPSWE   360
WP_020444543.1      GGGLSTLMAQQIITPNKEAWLDLGDEGQKTFDILQGLPHPMAQMPLYPVTDIETDYPSWE   360
                    ************************************************************

LIP2_65A.3          LYGEGLLLDHADVAIFDAACLENSPLPRQHILTSPMLGDNRQVCPSYVVAAELDVLRDEA   420
WP_020444543.1      LYGEGLLLDHADVAIFDAACLENSPLPRQHILTSPMLGDNRQVCPSYVVAAELDVLRDEA   420
                    ************************************************************

LIP2_65A.3          FAYANQLKSFGIAVQTHTVLGAPHGFIHFMSVHQRLGQETQHIITGFANFVREIIKTRAL   480
WP_020444543.1      FAYANQLKSYGIAVQTHTVLGAPHGFIHFMSVHQRLGQETQHIITGFANFVREIIKTRAL   480
                    **********:*************************************************

LIP2_65A.3          LSA 483
WP_020444543.1      LSA 483
                    ***
```

Lipase 3 from Scarisoara vs. Lipase from Psychrobacter sp. G

```
CLUSTAL O(1.2.4) multiple sequence alignment

LIP3_65A.3          MLLKRLGLATLLSFSVVGCTTAPNTLAINTTQKIIQYERSKSDLTTQSFTLSSGDKIVYA   60
WP_020442424.1      MLLKRLSLATLLSFSVVGCTTAPNTLAINTTQKIIQYERSKSDLTTQSFTLSSGDKIVYA   60
                    ******.*****************************************************

LIP3_65A.3          ENGNVAGEPLLLIHGFGGNKDNFTRIARQLENYNLIIPDLLGFGDSSKPMAADYRSEAQA   120
WP_020442424.1      ENGNVAGEPLLLIHGFGGNKDNFTRIARQLENYNLIIPDLLGFGDSSKPMAADYRSEAQA   120
                    ************************************************************

LIP3_65A.3          TRLHELLQAKGLASNIHVGGNSMGGAISVAYAAKYPKEVKSLWLIDSAGFWSVGVPKSLE   180
WP_020442424.1      TRLHELLQAKGLASNIHVGGNSMGGAISVAYAAKYPKEVKSLWLIDSAGFWSVGVPKSLE   180
                    ************************************************************

LIP3_65A.3          SATLENNPLLVDKKEDFYAMYDFVMSKPPYIPKSVKAVFAQERIANKAVESKILAQIVED   240
WP_020442424.1      SATLENNPLLVDKKEDFYAMYDFVMSKPPYIPKSVKAVFAQERIANKAVESKILAQIVED   240
                    ************************************************************

LIP3_65A.3          NVEQRAKVIAEYNIPTLVVWGEEDKVIKPETVTLIKEIIPQSQVITMPKIGHVPMIEAVK   300
WP_020442424.1      NVEQRAKVIAEYNIPTLVVWGEEDKVIKPETVTLIKEIIPQSQVITMPKIGHVPMIEAVK   300
                    ************************************************************

LIP3_65A.3          DTANDYKAFREGLKN   315
WP_020442424.1      DTANDYKAFREGLKK   315
                    **************:
```

Homolog	Lipase 2_65A.3		Lipase 3_65A.3	
	Identity (%)	Similarity (%)	Identity (%)	Similarity (%)
Psychrobacter sp. G	98.96	99.37	99.68	99.68

: partially conserved residues
. conserved residues
*** identical residues**

Sievers, F. et al, Mol. Syst. Biol., 2011, 7:539

STRUCTURAL ANALYSES of EXTRACELLULAR LIPASES

Primary structure analysis

Protein size and pI

- shortening the enzyme sequence at increasing environmental temperatures;

Table III. Homologous lipases	No. of amino acids	Molecular weight (Da)	Theoretical pI
Psychrobacter sp. SIC Lipase 2_65A.3	483	53626.97	5.33
Psychrobacter sp. G	483	53527.84	5.33
Glaciibacter superstes	321	33908.18	4.75
Moritella sp. PE36	305	34088.98	6.13
Pseudomonas aeruginosa	321	34743.32	4.72
Escherichia coli	322	35255.95	5.19
Stenotrophomonas maltoflia	308	32737.20	4.78

Table III. Homologous lipases	No. of amino acids	Molecular weight (Da)	Theoretical pI
Psychrobacter sp. SIC Lipase 3_65A.3	315	34606.86	6.93
Psychrobacter sp. G	315	34650.96	7.72
Glaciibacter superstes	209	21844.94	4.74
Moritella sp. PE36	334	36512.78	4.96
Pseudomonas aeruginosa	315	34820.18	6.09
Escherichia coli	301	34348.64	8.54
Stenotrophomonas maltoflia	311	34898.25	6.00

- a more acidic pI suggests structural mechanisms for low-temperature adaptation of the surface of the enzyme in psychrophilic bacteria.

Santiago, M. et al, Frontiers in Microbiology, 2016.

Amino acid composition

• lowered amount of **bulky aromatic residues** as many **flexible nonpolar amino acids** are responsible for the structural flexibility of the enzyme in cold environments:

Lipase 2_65A.3: Phe + Tyr + Trp = **7.4 %** < Gly + Ala + Val + Leu + Ile + Met + Pro = **44.2%**

Lipase 3_65A.3: Phe + Tyr + Trp = **7.7 %** < Gly + Ala + Val + Leu + Ile + Met + Pro = **47.2%**

- a slight decrease of **Glu** content along with **Asp** proved the reduction of ionic interactions;
- **cysteines** cause the formation of interchain disulfide bonds intended for a lower protein flexibility providing higher stability;
- decreasing **Gln** residues along with increasing **Lys** residues and lowering **Pro** content stand for thermal lability of the enzyme;
- high value of **His** and **Met** residues are established for structure stability at low temperatures.

Mondini, A. et al, Rom. J. Biol.-Plant Biol., 2019, 64: 19-30.

STRUCTURAL ANALYSES of EXTRACELLULAR LIPASES

Secondary structure analysis

Table V. Homologous proteins	Secondary structure composition (%)			
	Helix	Sheet	Coil	Turn
Psychrobacter sp. SIC Lipase 2	50.1	31.9	11.4	6.6
Glaciibacter superstes	51.4	28.7	13.4	6.5
Moritella sp. PE36	40	37.4	15.7	6.8
P. aeruginosa	46.7	31.8	14.3	7.2
Escherichia coli	51.2	35.4	7.8	5.6
Stenotrophomonas maltophilia	47.1	34.4	12	6.5

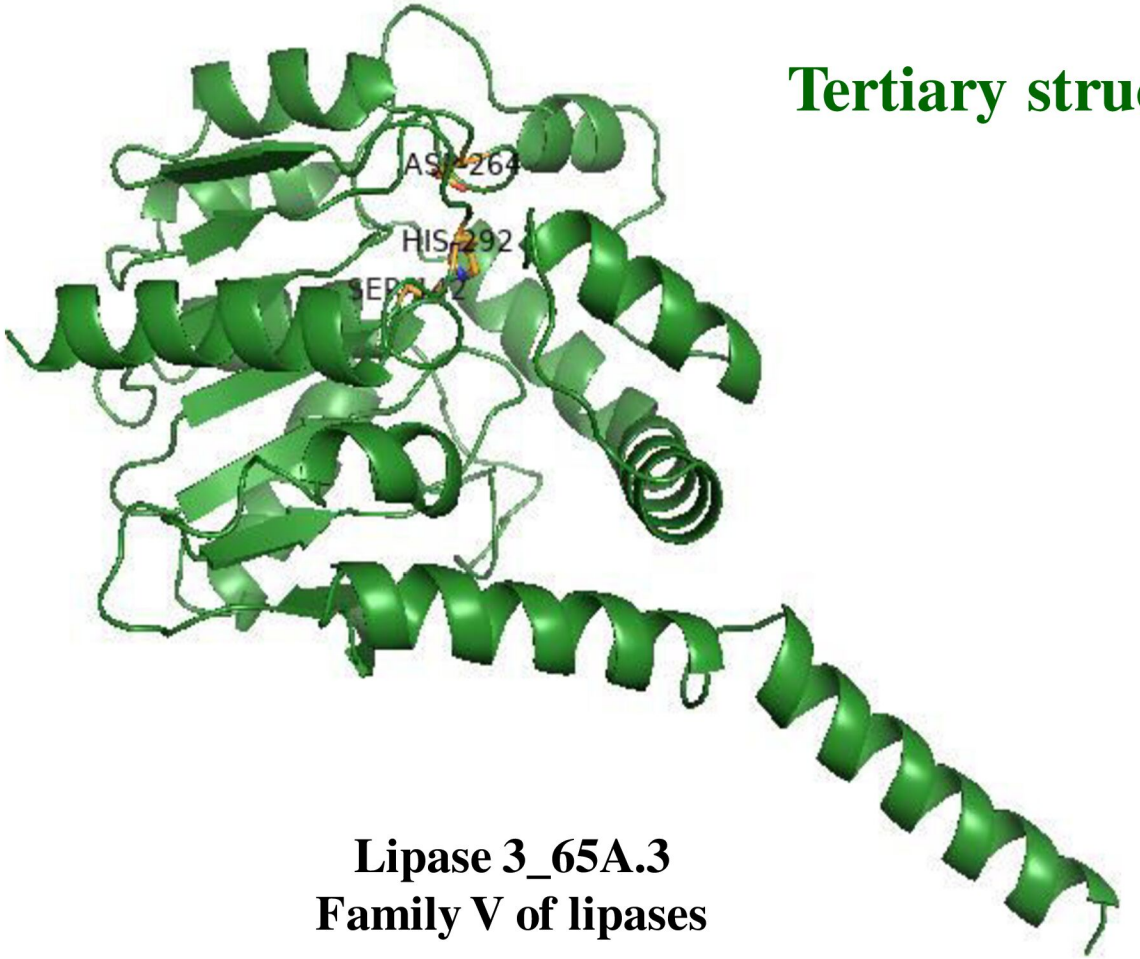
Table V. Homologous proteins	Secondary structure composition (%)			
	Helix	Sheet	Coil	Turn
Psychrobacter sp. SIC Lipase 3	57.8	25.4	10.1	6.7
Glaciibacter superstes	34.5	37.3	19.1	9.1
Moritella sp. PE36	39.5	45.2	10.2	5.1
P. aeruginosa	64.8	21	7.9	6.3
Escherichia coli	39.5	37.2	17.3	6
Stenotrophomonas maltophilia	49.8	28.6	14.5	7.1

- relatively high content of random coils in the amino acid sequence of the psychrophilic enzymes;
- a decreased content of turns for lipases;
- a lower sheet content reduced ionic interactions.

Ashok Kumar T., Wide Spectrum: Research Journal, 2013, 1(9):15-19

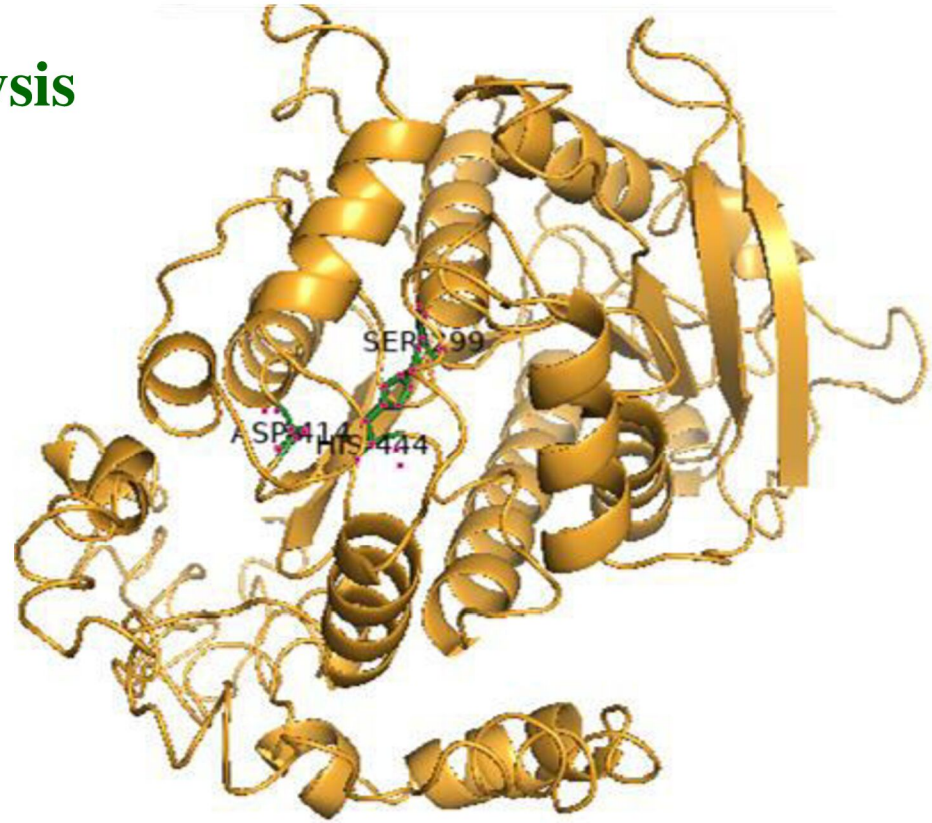
STRUCTURAL ANALYSES of EXTRACELLULAR LIPASES

Tertiary structure analysis



Lipase 3_65A.3
Family V of lipases

Conserved motifs HGGGF and GNSMG
Catalytic triad: Ser142-Asp264-His292
Signal peptide: Met1-Ala27



Lipase 2_65A.3
Family IV of lipases

Conserved motifs HGGGF and GDSAG with Gly297
Catalytic triad: Ser299-Asp414-His444
Consensus sequence LDXL with Asp414
His444 within PHG

Yang, J. , Yan, R. et al, Nature Methods, 2015, 12(1):7-18.

STRUCTURAL ANALYSES of EXTRACELLULAR LIPASES

Multiple sequence alignment and conserved regions

Lipase 2 and its homologous proteins

```
LIP2            AVNSKLKTPLQLIEMSELRKRFATDAVAMQAPKV------WQQASDNLLSNLKQFHKKGD 174
P.sp.G          AVNSKLKTPLQLIEMSELRKRFATDAVAMQAPKV------WQQASDNLLSNLKQFHKKGD 174
Moritella       QIELGIR---ELVEGFVE----AGCPCPSKQ-SVIQRREGYIDSV-----VL------AG 44
G.superstes     PYDPELTASLAAM--GDL----AYISVTLETLPAARA----SRPTQSVDELL------AG 52
S.maltophilia   -LEPALQQFVDAVAAHPL----PEELRELRA---------ISESALPQLQGA------PQ 42
P.aeruginosa    -LNPDIAAYLELVGNGRS----SGKSLPMHQLTVQQAREQFDQSSALMDPGL------DE 51
E.coli          -LHPDLAAFLELVEFGRL----TGRSLPMHAMDVAQARAEFEGSSQVLDPSP------PG 51

LIP2            SAISWQDRTITNADDG-DMTIRCYQNETSDNGFGFKKEQTSNPDETVLLFFHGGGFCIGD 233
P.sp.G          SAISWQDRTIANADDG-DMTIRCYQNETSDNGFGFKKEQTSNPDETVLLFFHGGGFCIGD 233
Moritella       PSPKMHEEFEDEFD---GIRIKIFKPTS----------E---KLLPLTIYFHGGCFVSGG 88
G.superstes     RAVDHVEHTVPGQGGEPDVVLSVFTPRG----------LAA--PVPVVYHAHGGGMIMGD 100
S.maltophilia   PVAHVIEHTVIARDGQ-ALDVRLYTPEG----------LPDGP-APALLFAHGGGWFQCS 90
P.aeruginosa    PLARVETLFVPARDGT-SLPARLYSPQG----------LSASPSLPGVLYLHGGGYVVGS 100
E.coli          NVT-ASELQITARDGT-RLAARLYRQG----------DAGAALQPVILYLHGGGYVVGS 98

LIP2            LNTHHEFCHAICEQTGWPVISVDYRLAPEHPAPAAVRDCISAYAWLAEHCEEFGALPSRI 293
P.sp.G          LNTHHEFCHAICEQTGWPVISVDYRLAPEHPAPAAVRDCISAYAWLAEHCEEFGALPSRI 293
Moritella       FATHEQQMRQLAKLSNTIVVCIRYRLAPEYHYPAAHDDVYKASIHIHDHGYEYGGDPKRI 148
G.superstes     RFANIGRVLDWVENLGIVAVSVEYRLAPEAPYPAAVEDCYAGLLWTVEHAADLGIDPERV 160
S.maltophilia   LAVYDGPCRALANASGCVIVAVGYRLAPEHPFPVPLHDVADAWSWLQDNAERLGLDPQRL 150
P.aeruginosa    LDSHDALCASLAERAGCVVLSLAYRLAPEWRFPTAAEDAEDAWCWLAAEAERLGIDPQRL 160
E.coli          LDSHDSVCRRLAALGEFAVLAADYRLAPEQQFPKALHDVLDAANWLAEQAASLGLDNRRV 158

LIP2            VLAGDSAGGGLSTLMAQQIITPNKEAWLDLGDEGQKTFDILQGLPHPMAQMPLYPVTDIE 353
P.sp.G          VLAGDSAGGGLSTLMAQQIITPNKEAWLDLGDEGQKTFDILQGLPHPMAQMPLYPVTDIE 353
Moritella       SFVGDSAGAHLALVTSLRLK--------------------AKSNWLPRKQVLIYPMLDPQ 188
G.superstes     IIGGGSSGGGITAGLALLLR--------------------DRGGPKVAGQWLSSPMLDDR 200
S.maltophilia   AIGGDSAGGNLAAACLLLR--------------------DLGLPQPCHQLLLYPALDAG 190
P.aeruginosa    AVAGDSVGGSLCAVLSRQLALR-------------------GDASQPRLQVLIYPVTDAS 201
E.coli          VLAGDSVGASLAAVLAITSVQQ-----------------PEALAFKPLAQLLFYPVTDIS 201

LIP2            TDYPSWELYGEGLLLDHADVAIFDAACLENSPLP-RQHILTSPMLGDN-RQVCPSYVVAA 411
P.sp.G          TDYPSWELYGEGLLLDHADVAIFDAACLENSPLP-RQHILTSPMLGDN-RQVCPSYVVAA 411
Moritella       GKSDSYSQNGKDFIITGGMLLSGFEMYLEGSNV-SNKHPEISLLLRNDFSGLPPTYIVTA 247
G.superstes     DVTVSSKQYVDGAIWSGRSNDTAWRALLGDDFQTENVSIYAAPARATDLSNLPPAFIDVG 260
S.maltophilia   MGSDSYRDYATGYYLSAELMQRCWQAYLGNLDQPS---SLASPAQATDLQGLAPASVLSC 247
P.aeruginosa    RTRQSIERYAVGHLLEKDSLEWFYQHYQRSPEDRQ--DPRFSPLLGAVPAELAPTLLLVA 259
E.coli          CQRESHREHAEGYLLETPTLEWFYQHYAPQREQRL--DWRVSPLLSTLRQPLPPAYLSVA 259

LIP2            ELDVLRDEAFAYANQLKSFGIAVQTHTVLGAPHGFIHFMSVHQRLGQETQHIITGFANFV 471
P.sp.G          ELDVLRDEAFAYANQLKSYGIAVQTHTVLGAPHGFIHFMSVHQRLGQETQHIITGFANFV 471
Moritella       ELDPLRDEGEELYKKLLSSGVDAYCDRYLGVIHGFFQLSAVSKSAVRCIE----NVARQI 303
```

Lipase 3 and its homologous proteins

```
E.coli          TRGQSFSFRGQSIRYWTAG--QGEPLLLLHGFPTASWDWRYLWGPLAQRFRLIACDMLGF 70
G.superstes     ------------------------------------------------MIVPVHPGF 9
Moritella       EESQWMDINGMRIHYRDEGNPNGQPIVLVHGILSSLHTWDEWHKGLTADYRIISLDVPGF 120
LIP3            TTQSFTLSSGDKIVYAENGNVAGEPLLLIHGFGGNKDNFTRIARQL-ENYNLIIPDLLGF 103
P.sp.G          TTQSFTLSSGDKIVYAENGNVAGEPLLLIHGFGGNKDNFTRIARQL-ENYNLIIPDLLGF 103
P.aeruginosa    SEHSVQV-DNLEIAYLEGGSEKNPTLLLIHGFGADKDNWLRFARPLTERYHVVALDLPGF 99
S.maltophilia   TEKTLRV-DDLDIRYYEGGPQDAETILLVHGFGADKDNWPRFARFLTSHYHVLAVDLPGF 99

E.coli          GDSAKPVDHLYSLMEQ-ADLQQALLAELKVDQPVHLLAHDYGGSVAQELLARHHEQRANI 129
G.superstes     NGTPRPESLR--TPRGLAELYVRMLDALGLE-DVTVIGNSIGGWIAAEIAALGSSRVSGV 66
Moritella       GLTGGPENPDDYSETLLHSSFEQFVAQLQLD-DFILVGNSLGGYISAQYAANNPGKIKKL 179
LIP3            GDSSKPMAADYRSEAQ-ATRLHELLQAKGLASNIHVGGNSMGGAISVAYAAKYPKEVKSL 162
P.sp.G          GDSSKPMAADYRSEAQ-ATRLHELLQAKGLASNIHVGGNSMGGAISVAYAAKYPKEVKSL 162
P.aeruginosa    GDSSKPQQASYDVGTQ-AERVANFAAAIGVR-RLHLAGNSMGGHIAALYAARHPEQVLSL 157
S.maltophilia   GDSSQPPSISYDVGTQ-AERLVDFTQALGIG-RLHLVGNSMGGHIVALFAARHPQQVFSL 157

E.coli          ASCVFLNSGLFPESCRMLLIQKLLLSRLG-WLVGRSFGRDDLVRS-----VIQVYGSCTQ 183
G.superstes     V--LVDAVGLVVPGHPYVD-FYSLTP----AEVAA---RS----YYDP---ERFGVDPSK 109
Moritella       I--LIDPAGAPQEL-PFLL-SFASMPGINSLAA------NVFPPFIVAMGVKDVYGDPER 229
LIP3            W--LIDSAGFWSVGVPKSL-ESA-TLENNPLLVDK---KEDFYAMYD-----FVMSKPPY 210
P.sp.G          W--LIDSAGFWSVGVPKSL-ESA-TLENNPLLVDK---KEDFYAMYD-----FVMSKPPY 210
P.aeruginosa    A--LIDNAGVMPARKSELF-EDLERG-ENPLVVRQ---PEDFQKLLD-----FVFVQPPP 205
S.maltophilia   A--LFDNAGVEAPQRSVFF-QRLYAGQANPLVVSR---PEDFPPLLD-----LVFHTRPP 206

E.coli          PSESVLDDFWSLIAAN-RGTRILHKLVGYMPERRVHRERWVGAMQHEGVPLRFINGVVDP 242
G.superstes     LPS-EVRAAMA---------GNRTALAVYGGDMTDPT--LASRLPGVDVPVLVVWGAADR 157
Moritella       ITKANMDRYIHLSLRPGAKQA-YANTIAMLAEKNDRH--APLNFSSITAPTLLMWGEKDI 286
LIP3            IPK-SVKAVFA--QERIANKAVESKILAQIVEDNVEQ--RAKVIAEYNIPTLVVWGEEDK 265
P.sp.G          IPK-SVKAVFA--QERIANKAVESKILAQIVEDNVEQ--RAKVIAEYNIPTLVVWGEEDK 265
P.aeruginosa    LPA-PLKRYLG--ERAVAASAFNAQIFEQLRQRYIPL---EPELPKIEAPTLLLWGDRDR 259
S.maltophilia   LPA-RLRDYLS--ERAVERSGLNAAIFEQLRDRYIPL---EPELPRITAPTLLLWGDQDQ 260

E.coli          LSGAHMVERYRQLVPEPDTVQLQGIGHYPHTEAPVQVLRHYLAFREQPLSCFQKKVAWS  301
G.superstes     IGDLEVGKAYAELVPGARLEVIPDAGHLPQIETPSRLIELVGSFTSTSV----SGS---  209
Moritella       WVPATLSEQWLANISGSTLITYPKAGHVPMEEIPQQTLQDALTFIDLK-----------  334
LIP3            VIKPETVTLIKEIIPQSQVITMPKIGHVPMIEAVKDTANDYKAFREGLKN---------  315
P.sp.G          VIKPETVTLIKEIIPQSQVITMPKIGHVPMIEAVKDTANDYKAFREGLKK---------  315
P.aeruginosa    VLDVSSIEVMRPLLKRPSVVIMENCGHVPMVERPEETAQHYQAFLDGVRNAQVAGR---  315
S.maltophilia   ILDRSSIEVMKPLLRQPSVVVMEDCGHVPMIERPEETARHYLAFLAGLPRR--------  311
```


Sievers, F. et al, Mol. Syst. Biol., 2011, 7:539

BIOCHEMICAL CHARACTERIZATION of EXTRACELLULAR LIPASES

Evidence of lipolytic activity using different substrates

Assay with 1% tributyrin ► Positive reaction: a transparent halo around the bacterial growth zone as tributyrin hydrolysis took place.

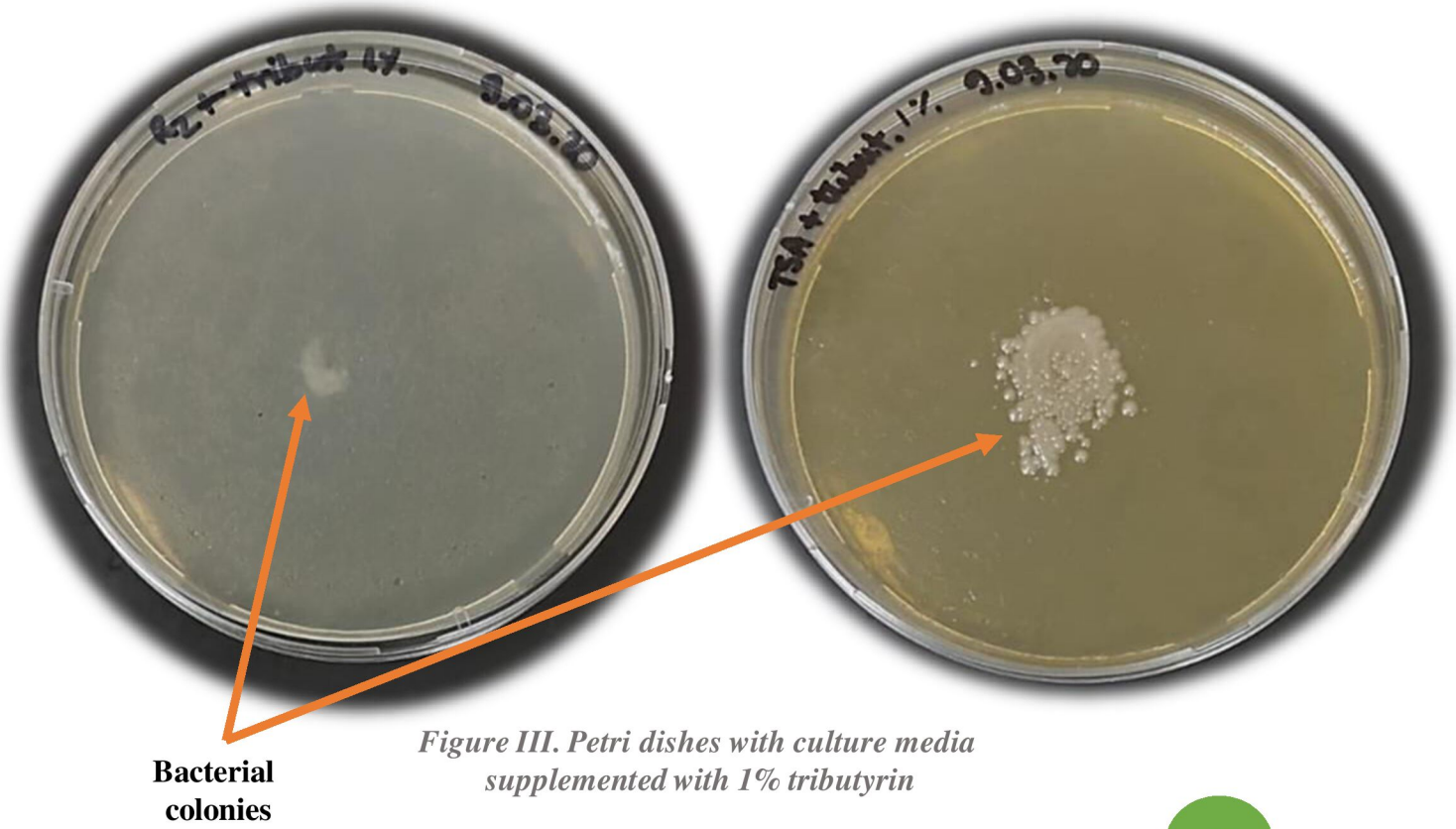


Figure III. Petri dishes with culture media supplemented with 1% tributyrin

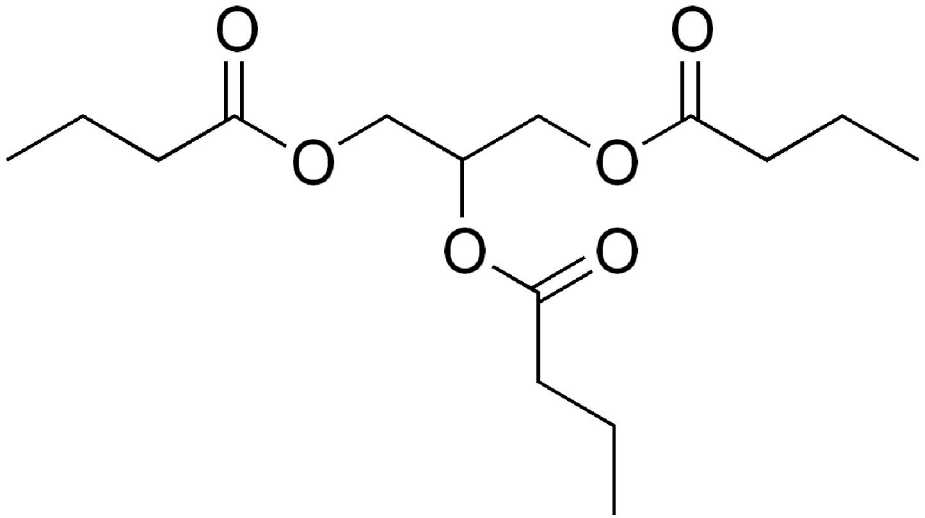


Figure IV. Tributyrin chemical structure

Ramnath, L. et al, Biotechnology Reports , 2017, 15:114-124.

BIOCHEMICAL CHARACTERIZATION of EXTRACELLULAR LIPASES

Evidence of lipolytic activity using different substrates

Assay with 1% Tween 80 + 0.01% $CaCl_2 \cdot 2H_2O$ ►
Positive reaction: white precipitate around the culture spot due to the calcium oleate crystals that formed between Ca^{2+} and the released fatty acids.

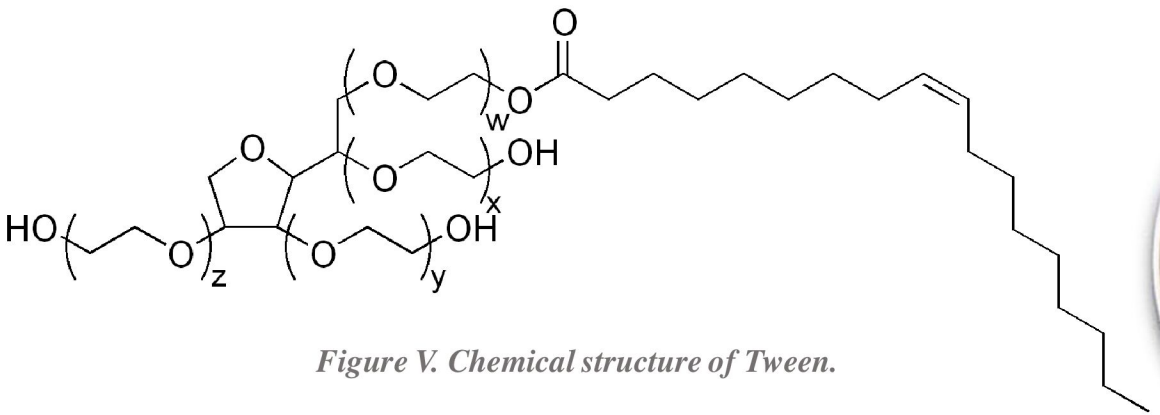


Figure V. Chemical structure of Tween.

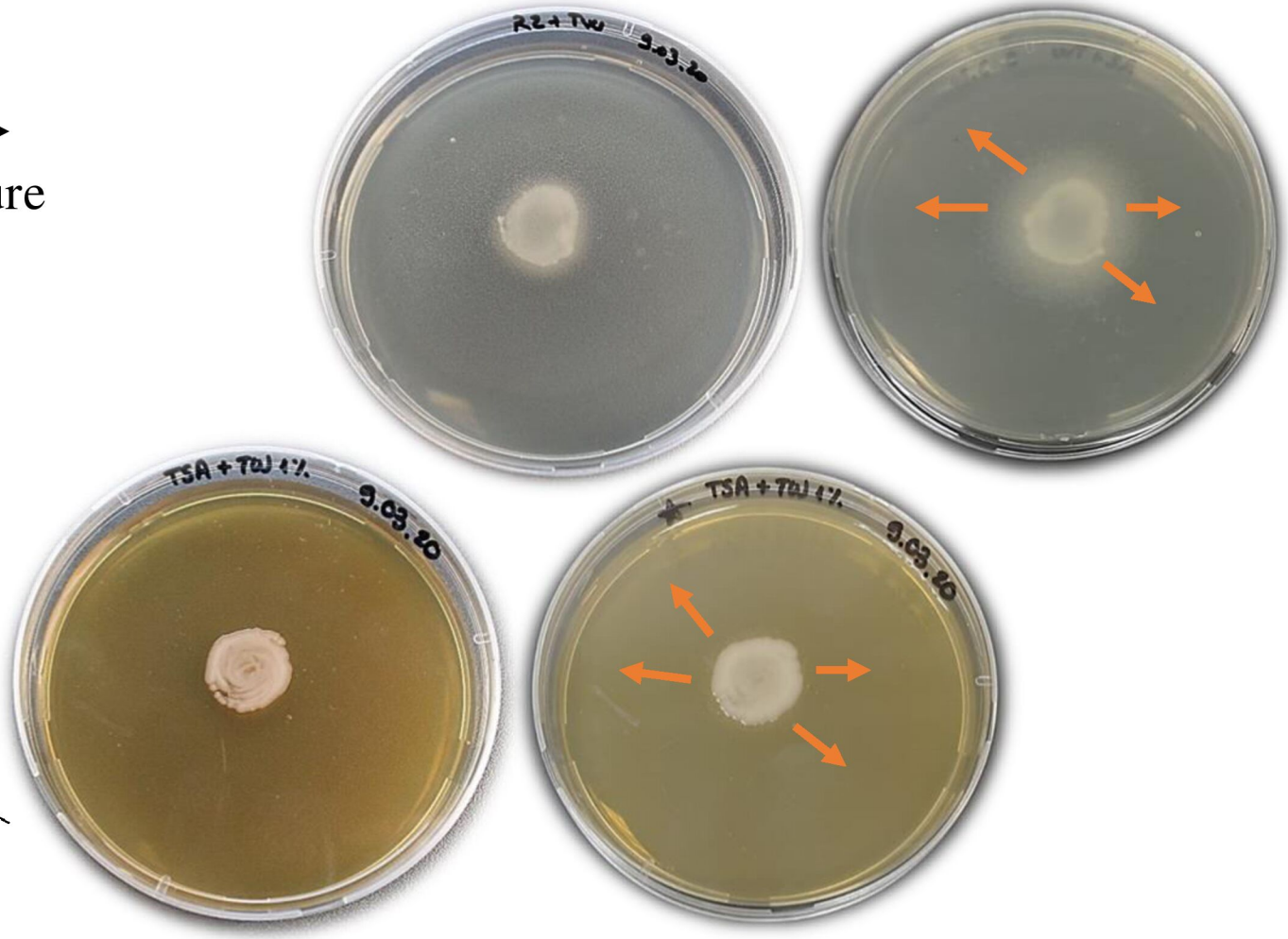


Figure VI. Petri dishes with culture media supplemented with 1% Tween 80
Left: 3 days of hydrolysis; Right: 7 days of hydrolysis

Ramnath, L. et al, Biotechnology Reports , 2017, 15:114-124.

Evidence of lipolytic activity using different substrates

Assay with 0.5%, 1%, 1.5% sun flower oil +0.001% rhodamine B ► Positive reaction: a red-orange fluorescent halo, around the growth area of the bacterial strain visible at 350nm.

Sun flower oil fatty acids profile	Palmitic acid	**5%**
	Stearic acid	**6%**
	Oleic acid	**30%**
	Linoleic acid	**59%**

Figure VII. Petri dishes with culture media supplemented with 0.5%, 1% and 1.5% sun flower oil.

Ramnath, L. et al, Biotechnology Reports , 2017, 15:114-124.

BIOCHEMICAL CHARACTERIZATION of EXTRACELLULAR LIPASES

Evidence of lipolytic activity using different substrates

Assay with 0.5%, 1%, 1.5% olive oil +0.001% rhodamine B ►Positive reaction: a red-orange fluorescent halo, around the growth area of the bacterial strain visible at 350nm.

Olive Oil Fatty acids profile	**Oleic acid**	**55-83%**
	Linoleic acid	3.5-21%
	Palmitic acid	7.5-20%
	Stearic acid	0.5-5%
	α-Linolenic acid	0-1.5%

Figure VIII. Petri dishes with culture media supplemented with 0.5%, 1% and 1.5% olive oil.

Ramnath, L. et al, Biotechnology Reports , 2017, 15:114-124.

CONCLUSIONS

- The *Psychrobacter sp.* SC65A.3 strain extracted from the perennial ice of Scarisoara Ice Cave, Romania is found to contain genes encoding lipases in its draft genome.
- Two extracellular lipases secreted by the psychrobacter strain were identified and subsequently analyzed both structurally and biochemically.
- Visible changes were observed in the protein structure due to the mechanism of adaptation to low temperatures.
- The extracellular lipases excreted by the strain of *Psychrobacter sp.* of SIC could be considered as true lipases. Thus it could be stated that the esters derived from the C4 chains, smaller or slightly larger, cannot be hydrolyzed at the level of the bond between the carboxylic acid and the corresponding hydroxyl.

Perspectives

- Having thus the cold-active enzyme type biocatalyst, it is finally desired to create a reaction system that will capitalize on the biocatalyst in the transesterification of silybin with different fatty acids and fatty acid esters.
- Moreover, it is desired to stabilize the enzymes through specific immobilization systems.

Thank you for your attention!