

*Article*

# **Antifungal effect of Penicillamine due to the selective targeting of L-homoserine O-acetyltransferase**

Aleksandra Kuplińska, Kamila Rząd, Marek Wojciechowski, Sławomir Milewski and Iwona Gabriel\*

Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, Gdańsk, Poland; aleksandra.kuplinska@pg.edu.pl (A.K.); kamila.rzad@pg.edu.pl (K.R.); marek.wojciechowski@pg.edu.pl (M.W.); slawomir.milewski@pg.edu.pl (S.M.); iwona.gabriel@pg.edu.pl (I.G.) \* Correspondence: iwona.gabriel@pg.edu.pl (I.G.); Tel.: +48-58-348-6078 ; Fax: +48-58-347-1144

## **Table of contents:**

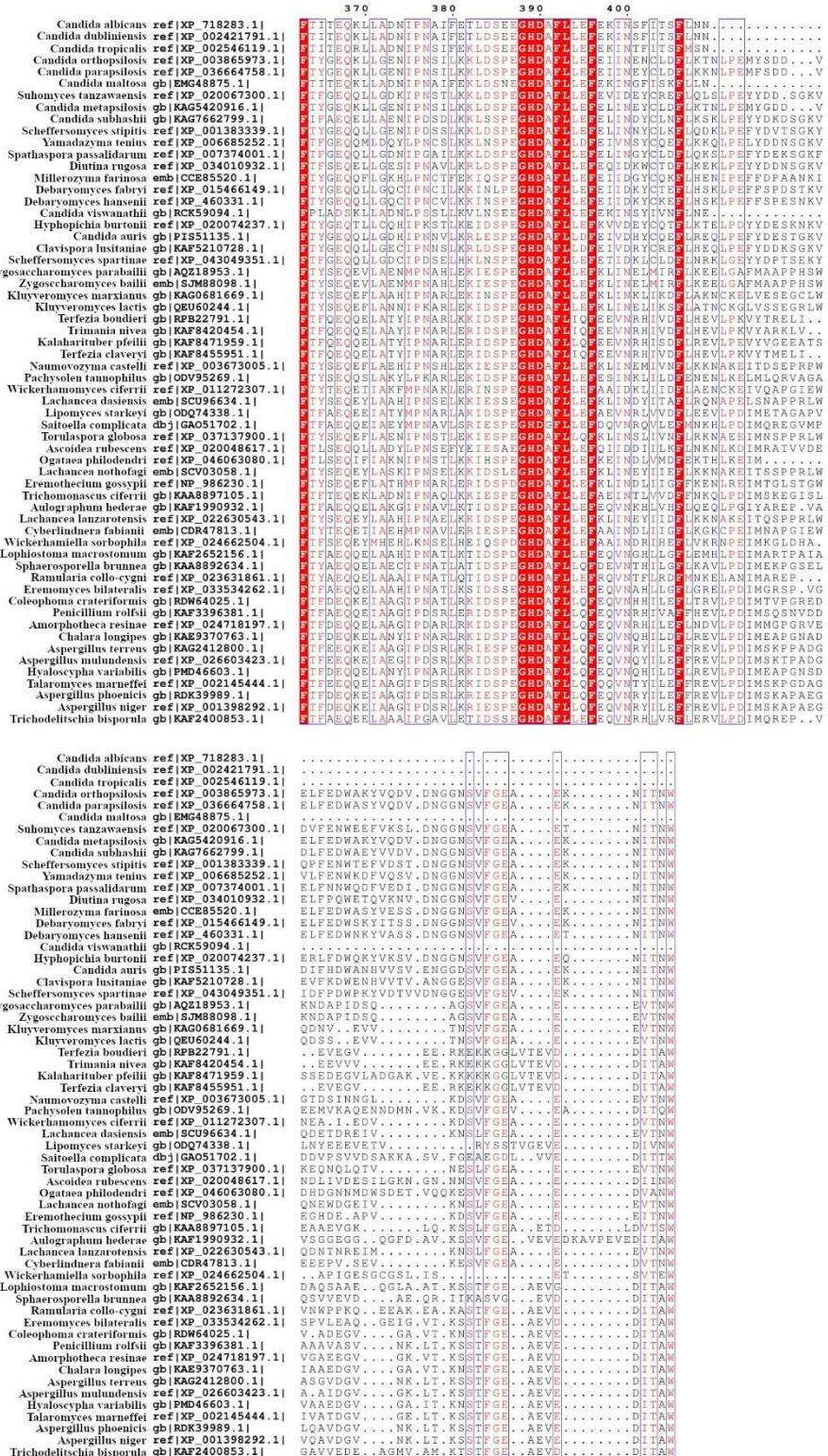
1. The multiple sequence alignment of amino acids sequences from 60 microorganisms from the <i>Ascomycota</i> group .....	2
2. Phylogenetic tree of amino acid sequences from 60 microorganisms from the <i>Ascomycota</i> group .....	7
3. Conservation of characteristic amino acid motifs in L-homoserine O-acetyltransferase sequences.....	8
4. Overproduction of <i>CaMet2p</i> and <i>CaMet2NHP</i> in <i>E. coli</i> cells.....	8
5. SDS-PAGE or Western blot analysis of enzymes overproduction and purification.....	9
6. Phylogenetic tree of Met2p from various organisms.....	9
7. References.....	9

	1	10	20	30
<i>Candida albicans</i> ref XP_718283.1	M..	YKDVTTEEOLQ..INPYVKLVPLPQCTIVEPENTLE.		
<i>Candida dubliniensis</i> ref XP_002421791.1	MT..	YKDVTTEEOLQ..INPYVKLVPLPQCTIVEPENTLE.		
<i>Candida tropicalis</i> ref XP_002546119.1	MV..	FKDVTTEEQCQ..SNPYARLVPQCTIVEPQDLE.		
<i>Candida orthopsis</i> ref XP_003865973.1		..MT..NVYKDJITEEQRA..TNSYASLVPQCTIVEPENTLE.		
<i>Candida parapsilosis</i> ref XP_036617581.1		..MT..NVYKDJITEEQRA..TNSYASLVPQCTIVEPENTLE.		
<i>Candida metapsilosis</i> gb EM24075.1	MT..	..FDTTTRDITAEONE..TNPYARLUVNPLPQCTIVEPNTLE.		
<i>Suhouya tanzawaensis</i> ref XP_0207567300.1	MT..	..TTSTTYRDTAEONE..TNPYARLUVNPLPQCTIVEPNTLE.		
<i>Candida metapsilosis</i> gb KAG5420916.1	MT..	..MT..NVYKDJITEEQRA..TNSYASLVPQCTIVEPENTLE.		
<i>Candida subshashii</i> gb KAG7662799.1	M..	..AMT.DSGYKDUITEQRN..SNPPAKLVPQCTIVEPQOLE.		
<i>Schefersomyces stipitis</i> ref XP_001383339.1	M..	..TEN.NQVXEITAEQKR..TNSYASLVPQCTIVEPNTLE.		
<i>Yamadazyma teitou</i> ref XP_006685252.1	MT..	..THI.NAHYRDTIREQCK..ONPYAALVPQCTIVEPKVELE.		
<i>Sporthaspora passalidiana</i> ref XP_007374001.1	MV..	..PCK..YKDVTTEEQRQ..TNSPFARLVPQCTIVEPKVELE.		
<i>Diatina rugosa</i> ref XP_034010932.1		..MT..SYKDUTDKERS..VNAPAGNVPQCTIVEPNLKLE.		
<i>Millerozyma farinosa</i> embl CEB55501.1	MT..	..VILSKSTYRDTTEEQRN..LNPYAKLVPQCTIVEPSEEL.		
<i>Debaromyces fabri</i> ref XP_015466149.1	MT..	..LLSPNPIYKDTTEEQRN..SNPPAKLVPQCTIVEPNTLE.		
<i>Debaromyces hanseae</i> ref XP_460331.1		..SSLPNPIYKDTTEEQRN..SNPPAKLVPQCTIVEPNTLE.		
<i>Candida viswanathii</i> gb RCK59094.1	M..	..YKDVTTEEQF..TNPYARLUVNPLPQCTIVEPYHBL.		
<i>Hypophichia burtonii</i> ref XP_020074237.1		..M..NPKFDVSEEQMR..SNPYAAMVPQCTIVEPKVELE.		
<i>Candida auris</i> gb PI51135.1	MK..	..NPVFKDVSEEQMR..SNPYAAMVPQCTIVEPKVELE.		
<i>Clavispora lusitania</i> gb KAF5210728.1	MS..	..NPKIYKDTTEETNR..TNPFAAMVPQCTIVEPFQOLE.		
<i>Schefersomyces spartinae</i> ref XP_043049351.1	MS..	..IEIFVSTKYDVKTEQL..ONPLARLVPQCTIVEPFQOLE.		
<i>Zygosaccharomyces parasabii</i> gb AQ218953.1	M..	..KTLREJDAAELSQTN..FMKLVPLPQCTIVEPENTLE.		
<i>Zygosaccharomyces parasabii</i> gb S2088098.1	M..	..MTLREJDAAELSQTN..FMKLVPLPQCTIVEPENTLE.		
<i>Khyveromyces laetic</i> gb KAG621669.1	M..	..SSTVK..TNPYARLUVNPLPQCTIVEPNTLE.		
<i>Terfezia boudieri</i> gb RPB22791.1	MIVLGNHSNES.	..SSLSPDDEELSKTN..TPKIVHHSQCTIVEPNTLE.		
<i>Trimania nivea</i> gb KAF8420454.1	MV..	..VAPMKYERINTQF..ENPFPTLISDQSVAV..PSFTL.		
<i>Kalaharituber pfeifferi</i> gb KAF8471959.1	MPEV..NGRL.	..VAPMKYERINTQF..ENPFPTLISDQSVAV..PSFTL.		
<i>Terfezia clavari</i> gb KAF8455951.1	MTVGNSNES.	..VAPMKYERINTQF..ENPFPTLISDQSVAV..PSFTL.		
<i>Naumovozyma castelli</i> ref XP_03673005.1	M..	..VAPMKYERINTQF..ENPFPTLISDQSVAV..PSFTL.		
<i>Pachyhamomyces ciferrii</i> ref XP_02260572307.1	M..	..MT..FNLRKLDVEETIKRENPPFTKLVLQCTIVEPFQOLE.		
<i>Lipomyces starkeyi</i> gb ODV95269.1		..NKLKRLQESD..ENPFPTLISDQSVAV..PSFTL.		
<i>Saitoella complicata</i> dbj GRO51702.1	M..	..GOYKREJESEF..ENPFPTLISDQSVAV..PSFTL.		
<i>Torulaspora globosa</i> ref XP_037137900.1	M..	..PRPSYRILYEP..ONPFVSIWSQCTAV..PSFTL.		
<i>Ascoidea rubescens</i> ref XP_02048617.1	M..	..RRLKHDVGEIAEINPVFKLVLDNDRIVE..PEMVL.		
<i>Ogataea philodendri</i> ref XP_0460363080.1	M..	..H..YHYDSDQ..ENPFSLVNBNOQKIL..PSFTL.		
<i>Lachancea nothofagi</i> embl SCV03058.1	M..	..AKTVEPTNKAELIACOSIMH..PSFTL.		
<i>Eremothecium gossypii</i> gb 986230.1	M..	..G..FKVIEQCT..ENPFSLVNBNOQKIL..PSFTL.		
<i>Trichomycetes ciferrii</i> gb KAA88705.1	M..	..TA..APTQFETLQSQ..ENPFSLISQCRIAI..PRVTL.		
<i>Lachancea lanzenstorferi</i> gb KAG66334.1	M..	..MELKEJDVVTIILAKANPQVQWQCTIVEPFQOLE.		
<i>Cyberlindnera fabianii</i> embl CDR47813.1	M..	..NVEGLKQCP..ENPFPTLISDQSVAV..PSFTL.		
<i>Wickerhamella sorbophilia</i> ref XP_024662504.1	M..	..GALPQKQD..ENPFPTLISDQSVAV..PSFTL.		
<i>Lophiostoma macrostomum</i> gb KAF2652156.1	M..	..A..LKHIDSEF..ENPFCKLVLNKSQRIAI..PSFTL.		
<i>Sphaeroporella brunnei</i> gb KAA8892634.1	M..	..AKTVEPTNKAELIACOSIMH..PSFTL.		
<i>Ramularia collo-cygri</i> ref XP_023613861.1	M..	..GSLIVERAMAATAE..APTQFETLQSQ..ENPFSLISQCRIAI..PRVTL.		
<i>Eremomyces bilobata</i> ref XP_033543262.1	M..	..MTAHPGLVHTSN..R..TPFKRIPKEDPQR..ENPVYHILPQDQIAI..PENTLE.		
<i>Coleophoma crateriformis</i> gb P0642605.1	M..	..MLISNPDSDFPMEN..G..YKYYKVRTAQ..ENPFLINVLQQIAI..PENTLE.		
<i>Amorphothecium resiniae</i> ref XP_024718197.1	M..	..MASACGNGTDLARNQGRYGMVSEYYQDRSHQCP..ENPFAGLIPQCTTAI..PSFTL.		
<i>Chalara longipes</i> gb KAG9370763.1	M..	..MARPQ..D..GALPQKQD..ENPFPTLISDQSVAV..PSFTL.		
<i>Aspergillus terreus</i> gb KAG2412800.1	M..	..MSSESPDQG..SAOPLKYERTHOP..ENPFPTLISDQSVAV..PSFTL.		
<i>Aspergillus muhlenius</i> ref XP_026603423.1	M..	..ADTSPLSNGS..ATVSAPKVRTRQCP..ENPFPTLISDQSVAV..PSFTL.		
<i>Hyaloscypha variabilis</i> gb PMD46603.1	M..	..A..LKHIDSEF..ENPFCKLVLNKSQRIAI..PSFTL.		
<i>Talaromyces marnetti</i> ref XP_002144444.1	M..	..AKTVEPTNKAELIACOSIMH..PSFTL.		
<i>Aspergillus phoenicis</i> gb RDK39989.1	M..	..MEYVQ..APTQFETLQSQ..ENPFSLISQCRIAI..PSFTL.		
<i>Aspergillus niger</i> ref XP_001398292.1	M..	..VAQATA..PGOQRQDASQP..ENPFPTLISDQSVAV..PSFTL.		
<i>Trichodelitschia bisporula</i> gb KAF2400853.1	M..	..VAQATA..PGOQRQDASQP..ENPFPTLISDQSVAV..PSFTL.		
	40	50	60	70
<i>Candida albicans</i> ref XP_718283.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Candida dubliniensis</i> ref XP_002421791.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Candida tropicalis</i> ref XP_002546119.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..NKKTD.			
<i>Candida parapsilosis</i> ref XP_036617581.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Candida maltoza</i> gb EM4847875.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..NKKTD.			
<i>Subomyces tanzawaensis</i> ref XP_0207567300.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..NKKTD.			
<i>Candida metapsilosis</i> gb KAG5420916.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Candida subshashii</i> gb KAG7662799.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Schefersomyces stipitis</i> ref XP_001383339.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..GKTFD.			
<i>Yamadazyma teitou</i> ref XP_006685252.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..GKTFD.			
<i>Sporthaspora passalidiana</i> ref XP_007374001.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..GKTFD.			
<i>Diadiplosis luteola</i> ref XP_02260532.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..NKKTD.			
<i>Millerozyma farinosa</i> embl CEB55501.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..NKKTD.			
<i>Debaromyces fabri</i> ref XP_015466149.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..GKTFD.			
<i>Debaromyces hanseae</i> ref XP_460331.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Candida viswanathii</i> gb RCK59094.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Hypophichia burtonii</i> ref XP_020074237.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Candida auris</i> gb PI51135.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Clavispora lusitania</i> gb KAF5210728.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Schefersomyces spartinae</i> ref XP_043049351.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Zygosaccharomyces parasabii</i> embl S2088098.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Khyveromyces laetic</i> gb KAG621669.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Terfezia boudieri</i> gb RPB22791.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Trimania nivea</i> gb KAF8420454.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Kalaharituber pfeifferi</i> gb KAF8471959.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Terfezia clavari</i> gb KAF8455951.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Naumovozyma castelli</i> ref XP_03673005.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Pachyhamomyces ciferrii</i> ref XP_02260572307.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Lachancea daniensis</i> embl SCV06634.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Lipomyces starkeyi</i> gb ODV95269.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Saitoella complicata</i> dbj GRO51702.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Torulaspora globosa</i> ref XP_037137900.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Ascoidea rubescens</i> ref XP_02048617.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Ogataea philodendri</i> ref XP_0460363080.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Lachancea nothofagi</i> embl SCV03058.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Eremothecium gossypii</i> gb 986230.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Trichomycetes ciferrii</i> gb KAA88705.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..DRAFD.			
<i>Autographum hederae</i> gb K1990932.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..EGKAB.			
<i>Lachancea lanzenstorferi</i> embl CDR47813.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PDLAB.			
<i>Wickerhamella sorbophilia</i> ref XP_024662504.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Lophiostoma macrostomum</i> gb KAF2652156.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Sphaeroporella brunnei</i> gb KAA8892634.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Ramularia collo-cygri</i> ref XP_023613861.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Eremomyces bilobata</i> ref XP_03554262.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Coleophoma crateriformis</i> gb P0642605.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Penicillium roltzii</i> gb KAF339631.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Amorphothecium resiniae</i> ref XP_024718197.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Chalara longipes</i> gb KAG9370763.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Aspergillus terreus</i> gb KAG2412800.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Aspergillus muhlenius</i> ref XP_026603423.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Hyaloscypha variabilis</i> gb PMD46603.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Talaromyces marnetti</i> ref XP_002144444.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Aspergillus phoenicis</i> gb PMD46603.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Aspergillus niger</i> ref XP_001398292.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			
<i>Trichodelitschia bisporula</i> gb KAF2400853.1	CETIHNPFVAYKTIKGNLNETA..NALVICHALSQSDVDWGLC..T..PGRAD.			

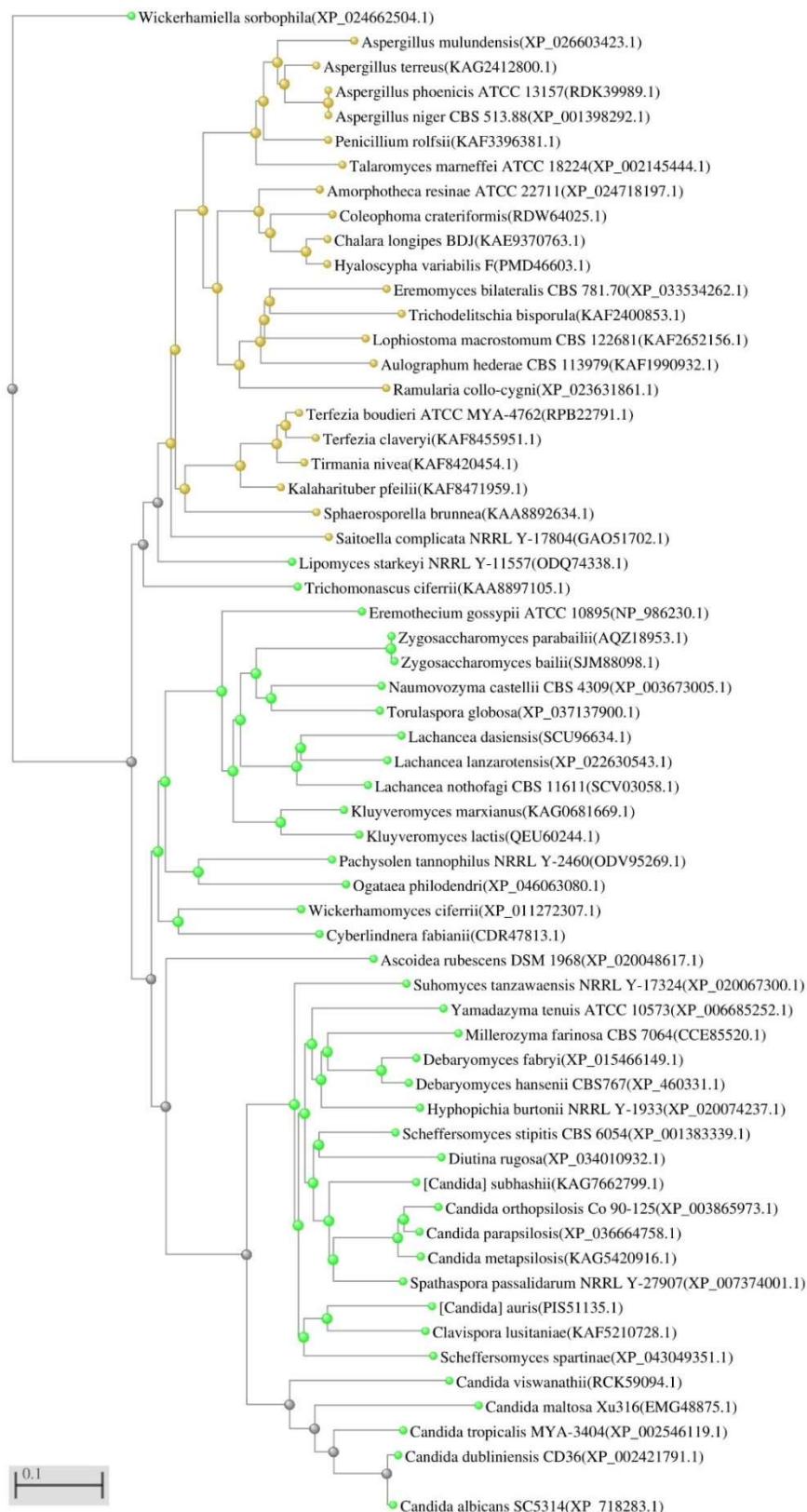


	210	220	230	240	250	
<i>Candida albicans</i> ref XP_718283.1	YDDGGYYET..GNKPDGSGLSAARMARALTYRSRNFSERFGRKLGPSP.....NDG					
<i>Candida dubliniensis</i> ref XP_002421791.1	YDDGGYYET..GNKPDGSGLSAARMARALTYRSRNFSERFGRKLGPSP.....SDG					
<i>Candida tropicalis</i> ref XP_00546119.1	YDDGGYYET..GNKPTDGSLSAARMARALTYRSRNFSERFGRKLGVRF.....SSA					
<i>Candida parapsilosis</i> ref XP_008865973.1	YDDGGYYET..GNKPTDGSLSAARMARALTYRSRNFSERFGRKLGSLS.....SLS					
<i>Candida maltosa</i> gb EMG48875.1	FEDGGYYOT..SNRDPDGSLSAARMARALTYRSRNFSERFGRKLFPKTQ.....DD.					
<i>Subomyces tanzawaensis</i> ref XP_02067300.1	YDDGGYYET..NNKPDGSGLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Candida metapsilos</i> gb KAG5420916.1	YDDGGYYEN..YENENKGLRLPDSGLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Candida subshashii</i> gb KAG7662799.1	YDDGGYYEN..NGIRPDGSLSAARMARALTYRSRNFSERFGRKLGPSP.....NRIGSE					
<i>Schefersomyces stipitis</i> ref XP_001383339.1	YDDGGYYEN..YENENKGLRLPDSGLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Yamadazyma tenina</i> ref XP_006685252.1	YDDGGYYESPNVMPDGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Spathaspora passalidiformis</i> ref XP_007374001.1	YDDGGYYEN..DEPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Milleromyces farinosa</i> emb CCE95520.1	YDDGGYYEN..YEREDGARDLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Dobaryomyces fabri</i> ref XP_015466149.1	YDDGGYYEN..YENENKNCIKPDSGLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Dobaryomyces hanseii</i> ref XP_460331.1	YDDGGYYEN..DDPLAALGAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Candida viswanathii</i> gb RCK59094.1	YNGGGYYDF..DDPLAALGAAARMARALTYRSRNFSERFGRKLGPSP.....KDE					
<i>Hypophichia burtonii</i> ref XP_02074237.1	YDDGGYYERSDNVRKPLSGLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Candida auris</i> gb PI51135.1	YDDGGYYENPKLRLPDLGGLAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Clavispora</i> sp. gb KAF5210728.1	YDDGGYYETPNLSPNPGGLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Schefersomyces spathinae</i> ref XP_001493511.1	YDDGGYYEES..GEPNPGNSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Zygosaccharomyces bailii</i> gb SJM8098.1	YDDGGYYEES..HESLN..DEPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Kluveromyces marxianus</i> gb KAG0681669.1	YDDGGYYEES..EPPTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Kluveromyces lactis</i> gb OEU60244.1	YLDGGYPLE..EPPTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Terfezia boudieri</i> gb RPB22791.1	YDDGGYPDF..DAPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Trimanita nivea</i> gb KAF8420454.1	YDDGGYPDF..DAPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Kalaharituber pfeffii</i> gb KAF8471595.1	YDDGGYPDF..DAPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Terfezia clavarii</i> gb KAF8455951.1	YDDGGYPDF..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Nomozyma castellii</i> ref XP_001493005.1	YDDGGYPDF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Pachysolenotus</i> sp. gb ODV95269.1	YDDGGYPDF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Wickerhamomyces ciferrii</i> ref XP_011273007.1	Lachancea dasicens emb SCU96634.1	YDDGGYPDF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....				
<i>Lipomyces starkei</i> gb ODD74338.1	YDDGGYPDF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Saitoella complicata</i> dbj GAO51702.1	YDDGGYPDF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Torulaspora globosa</i> ref XP_037137900.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Ascoidea rubescens</i> ref XP_02004617.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Ogataea philodendri</i> ref XP_04603080.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Lachancea notofagi</i> emb SCU96634.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Trichocomaceae</i> sp. ref NP_986230.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Autographa hederae</i> gb KAF190932.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Lachancea lanzarotensis</i> ref XP_022630543.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Cyberlindnera fabianii</i> emb CDR47813.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Wickerhamiella sorbophilia</i> ref XP_024662504.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Lophiostoma macrostomonia</i> gb KAF2652156.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Sphaeroporella brunnea</i> gb KAA8892634.1	YDDGGYLSQ..DPPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Ramulariaello-cygnea</i> sp. ref NP_00131861.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Eremophilicella</i> sp. ref XP_00351262.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Coleophoma crateriformis</i> gb RDW64025.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Penicillium roltfii</i> gb KAF3396381.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Amorphotheca resinae</i> ref XP_024718197.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Chalara longipes</i> gb KAF9370673.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Aspergillus mulundensis</i> ref XP_026603423.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Hyaloscypha variabilis</i> gb PMF46603.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Talaromyces marnetti</i> ref XP_002145444.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Aspergillus phoenicis</i> gb RDX3998.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Aspergillus niger</i> ref XP_001398922.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Trichodelitschia bisporula</i> gb KAF2400853.1	YDDGGYTF..DPLAGLGSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
	260	270				
<i>Candida albicans</i> ref XP_718283.1	GASP.....RTKH.EEH.....SLLHNDGSKL.....IN					
<i>Candida dubliniensis</i> ref XP_002421791.1	SASP.....RTKH.EEH.....SLLHNDGSKL.....VN					
<i>Candida tropicalis</i> ref XP_00546119.1	SLSPOGENSGRSSPRPSKQ.EEH.....SLLHNDGSKS.....VN					
<i>Candida parapsilosis</i> ref XP_008865973.1	NR.DSON.DETGKVYPTKD.EEH.....WLLHNEQSOS.....					
<i>Candida maltosa</i> gb EMG48875.1	SGKDEE..DESGKVYPTKD.EEH.....WLLHNEQSOS.....					
<i>Subomyces tanzawaensis</i> ref XP_02067300.1	....TEES.VKEAVYRPTKD.EEH.....WLLHNEQSOS.....					
<i>Candida metapsilos</i> gb KAG5420916.1	EKLQKEE..VEKGIRYRPTKD.EEH.....WLLHNEQSOS.....					
<i>Candida subshashii</i> gb KAG7662799.1	EKLQKEE..VEKGIRYRPTKD.EEH.....WLLHNEQSRS.....					
<i>Schefersomyces stipitis</i> ref XP_001383339.1	....T.AEAERITYPTKD.EEH.....WLLHNEQSRS.....					
<i>Yamadazyma tenina</i> ref XP_006685252.1	YDDGGYYEES..GEPNPGNSLSAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Spathaspora passalidiformis</i> ref XP_007374001.1	YDDGGYYEES..HESLN..DEPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Milleromyces farinosa</i> emb CCE95520.1	YDDGGYYEES..DEPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Dobaryomyces fabri</i> ref XP_015466149.1	YDDGGYYEES..DEPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Dobaryomyces hanseii</i> ref XP_460331.1	YDDGGYYEES..DEPNTGCLASLAAARMARALTYRSRNFSERFGRKLGPSP.....					
<i>Candida viswanathii</i> gb RCK59094.1	EL.....					
<i>Hypophichia burtonii</i> ref XP_02074237.1	NDSLXLS..EQQGIRGLPFRD.EEH.....WLLHNEQSRS.....					
<i>Candida auris</i> gb PI51135.1	....SNKG...GGEISIRLPNKE.EEH.....WLLHNEQSRS.....					
<i>Clavispora</i> sp. gb KAF5210728.1	....TKRGV.QEENRVLPKNKE.EEH.....WLLHNEQSRS.....					
<i>Schefersomyces spathinae</i> ref XP_001493511.1	....SLSSS..VTESRVYPTKD.EEH.....WLLHNEQSRS.....					
<i>Zygosaccharomyces bailii</i> emb SJM8098.1	....TL..RKTPTVS.EEH.....WLLHNEQSRS.....					
<i>Kluveromyces marxianus</i> gb KAG0681669.1	QSQSQSOQ..LTSSKKLQ..DLN..EHH.....WLLHNEQSRS.....					
<i>Kluveromyces lactis</i> gb OEU60244.1	SESSONS..LTSSKKLQ..EHN..EHH.....WLLHNEQSRS.....					
<i>Terfezia boudieri</i> gb RPB22791.1	....QPS..RPTPTPO.EEH.....WLLHNEQSRS.....					
<i>Trimanita nivea</i> gb KAF8420454.1	....QPS..RPTPTPO.EEH.....WLLHNEQSRS.....					
<i>Kalaharituber pfeffii</i> gb KAF8471595.1	....QFY..RPTPTPO.EEH.....WLLHNEQSRS.....					
<i>Terfezia clavarii</i> gb KAF8455951.1	....QFY..RPTPTPO.EEH.....WLLHNEQSRS.....					
<i>Southernomyces polycarpae</i> ref NP_001318605.1	....QPT..RPTPTVS.EEH.....WLLHNEQSRS.....					
<i>Wickerhamomyces ciferrii</i> ref XP_011273007.1	Lachancea dasicens emb SCU96634.1	....QPT..RPTPTVS.EEH.....WLLHNEQSRS.....				
<i>Lipomyces starkei</i> gb ODD74338.1	....QLK..RPTPTVS.EEH.....WLLHNEQSRS.....					
<i>Saitoella complicata</i> dbj GAO51702.1	....AQF..RQENIL..EHN..EHH.....WLLHNEQSRS.....					
<i>Torulaspora globosa</i> ref XP_037137900.1	....PDT..RPTPTVS.EEH.....WLLHNEQSRS.....					
<i>Ascoidea rubescens</i> ref XP_02004617.1	....SSL..PPPTPS.EHS.....WLLHNEQSRS.....					
<i>Ogataea philodendri</i> ref XP_04603080.1	SESSSAV..SLRNQ.EHN..EHH.....WLLHNEQSRS.....					
<i>Lachancea notofagi</i> emb SCU96634.1	....EDS..EDSPSTK.EHN..EHH.....WLLHNEQSRS.....					
<i>Eremophilicella</i> sp. ref NP_986230.1	....KON..EHN..EHH.....WLLHNEQSRS.....					
<i>Trichocomaceae</i> sp. ref NP_00351262.1	....KPT..KPTPS.EHN..EHH.....WLLHNEQSRS.....					
<i>Coleophoma crateriformis</i> gb RDW64025.1	....EDO..RPTPS.EHN..EHH.....WLLHNEQSRS.....					
<i>Penicillium roltfii</i> gb KAF3396381.1	....AQF..NPSTPS..EHN..EHH.....WLLHNEQSRS.....					
<i>Amorphotheca resinae</i> ref XP_024718197.1	....GDO..NPSTPS..EHN..EHH.....WLLHNEQSRS.....					
<i>Chalara longipes</i> gb KAF9370673.1	....GDO..NPSTPS..DWH..EHH.....WLLHNEQSRS.....					
<i>Aspergillus terreus</i> gb KAG2412800.1	....GSE..KLPPTP..NEH..EHH.....WLLHNEQSRS.....					
<i>Aspergillus mulundensis</i> ref XP_026603423.1	....GTE..RPTPTPSNEH..EHH.....WLLHNEQSRS.....					
<i>Hyaloscypha variabilis</i> gb PMF46603.1	....EPQ..NPS..DWH..EHH.....WLLHNEQSRS.....					
<i>Talaromyces marnetti</i> ref XP_002145444.1	....CAA..CPSPS..S..DWH..EHH.....WLLHNEQSRS.....					
<i>Aspergillus phoenicis</i> gb RDX3998.1	....QOE..RSPTPP..NEH..EHH.....WLLHNEQSRS.....					
<i>Aspergillus niger</i> ref XP_001398922.1	....DQG..ARR..SA..EHH.....WLLHNEQSRS.....					
<i>Trichodelitschia bisporula</i> gb KAF2400853.1	....DQG..ARR..SA..EHH.....WLLHNEQSRS.....					

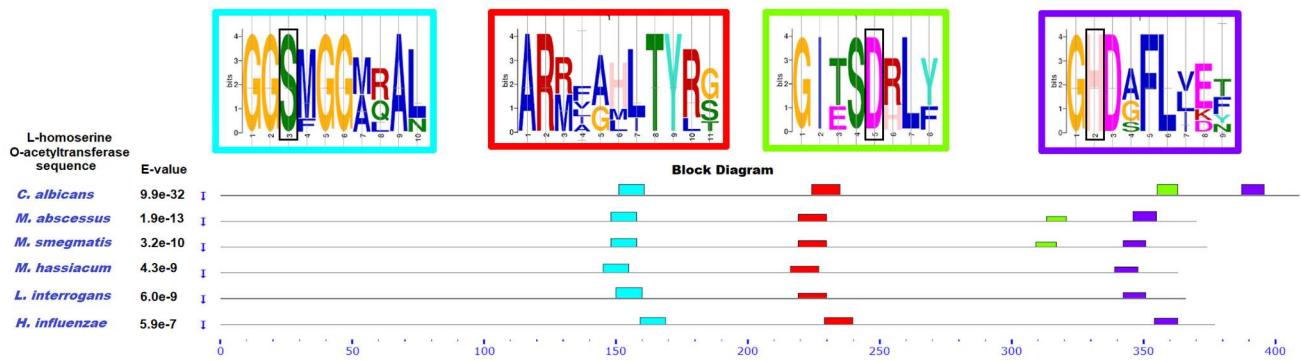




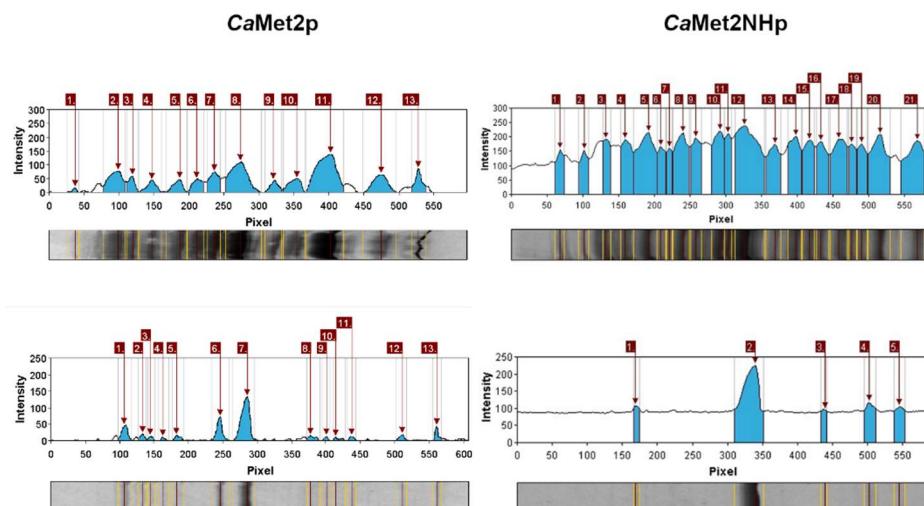
**Figure S1.** The multiple sequence alignment of amino acid sequences from 60 microorganisms from the *Ascomycota* group to the *CaMet2* amino acid sequence from *C. albicans*. Analysis performed using BLAST program [1].



**Figure S2.** Phylogenetic tree of amino acid sequences from 60 microorganisms from the Ascomycota group with high similarity ( $\geq 50.34\%$  of identity) to the Met2 amino acid sequence from *C. albicans*. Analysis performed using BLAST program [1].



**Figure S3.** Conservation of characteristic amino acid motifs in L-homoserine O-acetyltransferase sequences. The catalytic triad residues are marked with a black frame. The analysis was performed using MAST , FIMO and TOMTOM tools from The MEME suite [2–4].



(a)

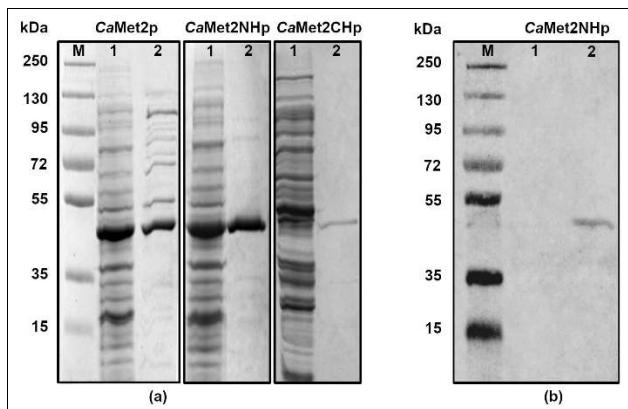
Protein name	CaMet2p	CaMet2NHP
Total amount of protein in cell extract	100 %	100%
Rate of protein expression	23 %	19 %
Volume of purified protein	10 mL	10 mL
Concentration of recombinant protein	0.114 mg mL <sup>-1</sup>	0.237 mg mL <sup>-1</sup>
Purity of recombinant protein	44 %	86 %

(b)

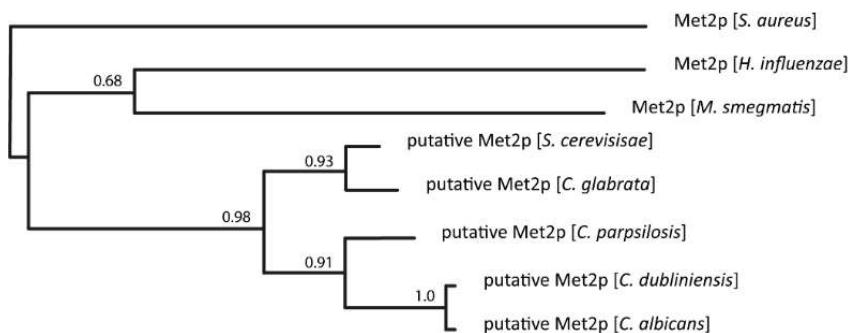
Protein name	CaMet2p	CaMet2NHP
Theoretical molecular mass	45.4 kDa	46.2 kDa
Calculated molecular mass after SDS-PAGE analysis	45.0 kDa	46.6 kDa

(c)

**Figure S4.** Overproduction of CaMet2p and CaMet2NHP in *E. coli* cells: (a) Densitometric analysis of SDS-PAGE electrophoresis analysis of cell extract (top) and purified fraction (bottom) from *E. coli* cells producing CaMet2p and CaMet2NHP recombinant protein. Gel Analyzer software [5]; (b) Mass balance of CaMet2p and CaMet2NHP purification developed on the base of the Gel Analyzer Program analysis results and experimental work; (c) Theoretical molecular mass (ProtParam analysis [6]) and determined by SDS-PAGE analysis molecular mass of the CaMet2p and CaMet2NHP.



**Figure S5.** (a) SDS-PAGE analysis of CaMet2p, CaMet2NHP and CaMet2CHP overproduction in *E. coli* cells and purification. Plus Prestained Protein Ladder (M), cell-free extract (1), CaMet2p-containing fraction (2) (b) Western blot analysis of CaMet2NHP overproduction. (1) no IPTG induction, (2) IPTG induced.



**Figure S6.** Phylogenetic tree of Met2p from various organisms. Analysis performed using BLAST program [1].

## References

1. Altschul, S.F.; Gish, W.; Miller, W.; Myers, E.W.; Lipman, D.J. Basic local alignment search tool. *J. Mol. Biol.* **1990**, *215*, 403–410, doi:10.1016/S0022-2836(05)80360-2.
2. Bailey, T.L.; Gribskov, M. Combining evidence using p-values: Application to sequence homology searches. *Bioinformatics* **1998**, *14*, 48–54, doi:10.1093/bioinformatics/14.1.48.
3. Gupta, S.; Stamatoyannopoulos, J.A.; Bailey, T.L.; Noble, W. Quantifying similarity between motifs. *Genome Biol.* **2007**, *8*, R24, doi:10.1186/gb-2007-8-2-r24.
4. Grant, C.E.; Bailey, T.L.; Noble, W.S. FIMO: scanning for occurrences of a given motif. *Bioinformatics* **2011**, *27*, 1017–1018, doi:10.1093/bioinformatics/btr064.
5. Lazar Jr., I.; Lazar Sr., I. GelAnalyzer 19.1 ([www.gelanalyizer.com](http://www.gelanalyizer.com)).
6. Gasteiger, E.; Hoogland, C.; Gattiker, A.; Duvaud, S.; Wilkins, M.R.; Appel, R.D.; Bairoch, A. Protein Identification and Analysis Tools on the ExPASy Server. In *The Proteomics Protocols Handbook*; Humana Press, 2005; pp. 571–607.