

## IN VITRO EVALUATION OF THE ANTIMICROBIAL POTENTIAL OF BETULINIC ACID AND ANALYSIS OF MECHANISMS OF ACTION WITH MOLECULAR MODELING

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**Table S1.** Access codes of selected protein sequences obtained from the GenBank database.

	DNA gyrase	beta-lactamase	PBP
<i>Staphylococcus aureus</i>	BAR07518.1	KSA62127.1	BBA24197.1
<i>S. epidermidis</i>	KSZ68723.1	RUN10939.1	EJE17909.1
<i>Pseudomonas aeruginosa</i>	OHQ53593.1	KJJ11413.1	TEF07458.1
<i>Escherichia coli</i>	KHJ14714.1	KXG94406.1	ARB43848.1
<i>Mycobacterium tuberculosis</i>	AIH53390.1	KMY17933.1	QRX86232.1
	CYP51	SAP-2	DHFR
<i>Candida albicans</i>	AIQ80983.1	AAM21051.1	AAC05610.1
<i>C. tropicalis</i>	QXT50470.1	AAD33216.1	XP_002550914.1
<i>C. glabrata</i>	KTB23913.1	-	KTB19277.1

<i>Aspergillus flavus</i>	QOR32183.1	-	RMZ41744.1
<i>Penicillium citrinum</i>	-	-	-
<i>Trichophyton rubrum</i>	-	-	OAL62276.1
<i>Microsporium canis</i>	-	-	EEQ31600.1

**Table S2.** PDB codes of template proteins and degree of identity proteins of interest.

Species	Protein	ID PDB molde	Identity
<i>S. epidermidis</i>	DNA gyrase	5NPP	93.83%
	beta-lactamase	1XA1	94.90%
	PBP	3VSL	83.18%
	SAP-2	2H6S	51.20%
<i>C. tropicalis</i>	DHFR	1AOE	81.25%
<i>A. flavus</i>	CYP51	6CR2	64.03%
<i>Trichophyton rubrum</i>	DHFR	6DRS	48.97%

**Table S3.** Percentage of amino acids present in the allowed and favored regions of the Ramachandran plot for each model and percentage of the degree of compatibility between the 3D structure and the 1D amino acid sequence, based on the models generated by Verify 3D.

Species	Protein	Ramachandran	Verify 3D
<i>S. epidermidis</i>	DNA gyrase	99.3%	89.63%
	beta-lactamase	97.8%	100%
	PBP	98.5%	90.52%
	SAP-2	98.7%	93.15%
<i>C. tropicalis</i>	DHFR	100%	92.23%
<i>A. flavus</i>	CYP51	98.8%	93.02%
<i>Trichophyton rubrum</i>	DHFR	100%	97.51%

**Table S4.** RMSD values for the proteins selected in the study.

Species	Protein	PDB ligand	RMSD
<i>S. aureus</i>	DNA gyrase	E32	-
	beta-lactamase	benzylpenicillin	0.29
	PBP	cefotaxime	0.30
<i>P. aeruginosa</i>	DNA gyrase	EZ6	0.25
	beta-lactamase	imipenem	0.32
	PBP	imipenem	0.44
<i>E. coli</i>	DNA gyrase	6G9	0.25
	beta-lactamase	ácido borônico	0.23
	PBP	-	-
<i>M. tuberculosis</i>	DNA gyrase	MDP	0.29
	beta-lactamase	amoxicilin	0.42
	PBP	ampicilin	0.30
<i>C. albicans</i>	CYP	posaconazole	0.83
	SAP-2	benzamidine	0.19

	DHFR	N22	0.61
<i>C. tropicalis</i>	CYP	-	-
<i>C. glabrata</i>	CYP	-	-
<i>A. flavus</i>	DHFR	H8A	0.32

**Figure S1.** Alignment of DNA gyrase enzyme sequences from selected bacteria in the study. The gray regions correspond to non-similar and non-identical amino acids. The red regions correspond only to identical amino acids. The yellow regions are similar amino acids. The black boxes represent the active site amino acids.

<i>M. tuberculosis</i>	MTDTTLPPDDSLDRLEPVDIQQMQRSYIDYAMSVIVGRALPEVRDGLKPVHRRVLYAMF	60
<i>S. aureus</i>	MKEELLMAELPQSRINERNITSEMRESFLDYAMSVIVARALPDVRDGLKPVHRRILYGLN	60
<i>S. epidermidis</i>	-----MAELPQSRINERNITSEMRESFLDYAMSVIVSRALPDVRDGLKPVHRRILYGLN	54
<i>P. aeruginosa</i>	-----MGELA-KETLPVNIEDELKQSYLDYAMSVIVGRALPDARDGLKPVHRRVLYAMS	53
<i>E. coli</i>	-----MSDLA-REITPVNIEEELKSSYLDYAMSVIVGRALPDVRDGLKPVHRRVLYAMN	53
<i>M. tuberculosis</i>	DSFRFPDPSHAKSARSVAETMGNHYHPHGDASITLVRMAQPSWLSRYPLVDGQGNFGSPG	120
<i>S. aureus</i>	EQGMTFPDKSYKKSARIVGDVMGKYHHPHGDSSIIEAMVRMAQDFSRYRPLVDGQGNFGSMD	120
<i>S. epidermidis</i>	EQGMTFPDKPYKKSARIVGDVMGKYHHPHGDSSIIEAMVRMAQDFSRYRPLVDGQGNFGSMD	114
<i>P. aeruginosa</i>	ELGNDWNKPYKKSARVVGDVIGKYHHPHGDIAVDTIVRMAQPFSLRYMLVDGQGNFGSV	113
<i>E. coli</i>	VLGNDWNKAYKKSARVVGDVIGKYHHPHGDIAVDTIVRMAQPFSLRYMLVDGQGNFGSID	113
<i>M. tuberculosis</i>	NDPPAMRYTEARLTPLAMEMLREIDEETVDFIPNYDGRVQEPTVLSRFPNLLANGSGG	180
<i>S. aureus</i>	GGGAAMRYTEARMTKITLELLRDINKDTIDFIDNYDGNEREPSVLEAFRFPNLLANGASG	180
<i>S. epidermidis</i>	GGGAAMRYTEARMTKITLELLRDINKDTIDFIDNYDGNEREPSVLEAFRFPNLLVNGAAG	174
<i>P. aeruginosa</i>	GNNAAMRYTEVMAKLAHELLADLEKETVDWVPNYDGTEQIEAVMPTKIPNLLVNGSSG	173
<i>E. coli</i>	GSAAAMRYTEIRLAKIAHELLMADLEKETVDFVDNYDGTEKIPDMPTKIPNLLVNGSSG	173
<i>M. tuberculosis</i>	IAVGMATNIPPHNIRELADAVFWALENHDADEEETLAAVMGRVKGPDFFPTAGLIVSQGT	240
<i>S. aureus</i>	IAVGMATNIPPHNITELINGVLSLSKNPD----ISIAELMEDIEGPDFFPTAGLILKSGI	236
<i>S. epidermidis</i>	IAVGMATNIPPHNITEVIDGVLSLSKNPD----ITINELMEDIQGPDPPTAGLVLGKSGI	230
<i>P. aeruginosa</i>	IAVGMATNIPPHNLGEVIDGCLALMDNPD----LTVDELMLQYIPGDPFPPTAGIINGRAGI	229
<i>E. coli</i>	IAVGMATNIPPHNITEVINGCLAYIDDED----ISIEGLMEHIPGDPFPPTAAIINGRGI	229
<i>M. tuberculosis</i>	ADAYKTGRGSIQMSRAVIE--EDSRGRTSLVITELFYQVNHDFNTSIAEQVRDGLKLAG	298
<i>S. aureus</i>	RRAYETGRGSIQMSRAVIE--ERGGGRQRIIVTEIFQVKNKARMEKIAELVRDCKIDG	294
<i>S. epidermidis</i>	RRAYETGRGSIQMSRAVIE--ERGGGRQRIIVTEIFQVKNKARMEKIAELVRDCKIDG	288
<i>P. aeruginosa</i>	IEAYRTGRGRIYIRARAVVEEMEGGREGQIIITELEFYQLNKARLEKIAELVKEKKIEG	289
<i>E. coli</i>	EEAYRTGRGKVYIRARAEVEV-DAKTGRETIIVHETIFYQVKNKARLEKIAELVKEKRV	288
<i>M. tuberculosis</i>	ISNIEDQSSDRVGLIVIEIKRDAVAKVWINNLYKHTQLQTSFGANMLAIVDGVPTLR	358
<i>S. aureus</i>	ITDLREETSLRTGVFVVIDVRKDANASVILNNLYKQTPLQTSFGVMIALVNGRPKLINL	354
<i>S. epidermidis</i>	ITDLREETSLRTGVFVVIDVRKDANASVILNNLYKQTPLQTSFGVMIALVNGRPKLINL	348
<i>P. aeruginosa</i>	ISELRDES-DKDGMRVVIELRRGEVGEVVLNNLYAQTLQSVFGINVVVALVDGQPTLR	348
<i>E. coli</i>	ISALRDES-DKDGMRVVIIEVKRDAVGEVVLNNLYSQTLQVSEGINMVALHHCQPKIMNL	347
<i>M. tuberculosis</i>	DQLIRYVVDHQLDVIVRRTTYRLRKANERAHILRGLVKALDALDEVIALIRASETVDIAR	418
<i>S. aureus</i>	KEALVHYLEHQKTVVRRTTYRLRKAKDRAHILEGLRIALDHIDEITSTIRESDTKVAM	414
<i>S. epidermidis</i>	KEALVHYLEHQKTVVRRTTYRLRKAKDRAHILEGLRIALDHIDEITSTIRESDTKIAM	408
<i>P. aeruginosa</i>	KDMLVFRHRREVTTRRTTYRLRKARERGHILEGQAVALSNIQVIELIKSSPTPAEAK	408
<i>E. coli</i>	KDIIAFAVFRHRREVTTRRTTYRLRKARDRAHILEALAVANIDPIIELIRHAPTPAEAK	407

**Figure S2.** Alignment of beta-lactamase enzyme sequences from selected bacteria in the study. The gray regions correspond to non-similar and non-identical amino acids. The red regions correspond only to identical amino acids. The yellow regions are similar amino acids.

<i>S. aureus</i>	-----MAKLLTMSVVSFCFIFLLLVF-----	21
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<i>S. epidermidis</i>	-----MAKLLIMSVVSFCFIFLLLVF-----	21
<i>P. aeruginosa</i>	-LVWARGFGYADRQH---RINASEHTAFHAGDLSLLIASATLQLAERGQLSLDAPLQD	119
<i>M. tuberculosis</i>	-TLYAKGFGVRDVGKGGGPDNKVDADTVFQLASVSKSVGATVVAHAVTDNVVTWDTTPVVS	177
<i>E. coli</i>	RQRF--VYGVDVAS---QKANTLDTVYELGSMKAFTGLVVQILIQEGRLRQGGDIIT	104
<i>S. aureus</i>	-----FRYI-----LKRYFNYSLNKVVWYLTMLAGL	47
<i>S. epidermidis</i>	-----FRYI-----LKRYFNYSLNKVVWYLTMLAGL	47
<i>P. aeruginosa</i>	TLREFYVRSRFHADQSEADRAITFRRLLSHQSGLPGEHLPLFGERPNSLQQLPAKVSGV	179
<i>M. tuberculosis</i>	KLPWFALRDPYV-----TGQVTIADLYSHRSGLPDHAGDLLEDLG--YDRRQVLQRLKY	229
<i>E. coli</i>	YLPFMRLNYQGK-----PASLTVADFLYHTSGLPFSTLARLENPMPGSAVAQQLRNENL	158
<i>S. aureus</i>	IPFIPIKFSFIKFNVNQ-APTVE---SKSHDLNHNINTTKPIQEFTTDIHKFNWDSID	103
<i>S. epidermidis</i>	IPFIPIKFSFIKFNVNQ-SPTVE---SRSHDLNHNINTTKPVQEFTTDIHKFNWDSID	103
<i>P. aeruginosa</i>	WLSNPPGTQVAHSNLGYELVGAAIERNTGKHFEQHMREHLLDPLQMTRSS-----	229
<i>M. tuberculosis</i>	LPLAPFRI SYAYTFGVTAAGAAVAAAAGQSWEDLSDEVLYRPLGMGSTS-----	279
<i>E. coli</i>	LFAPGAKFSYASANYDVL--GAVIENVTKTFTTEVIAERLTQPLGMSATV-----	206
<i>S. aureus</i>	NICTVIWIVLVIIISFKFLKALLYLKYLKQSLYLNENEKNKIDTILFNHQYKKNIVIRK	163
<i>S. epidermidis</i>	NICTVIWIVLVIIISFKFLKSLLYLKYLKQSLYLNENEKNKIDTILFNHQYKKNIVIRK	163
<i>P. aeruginosa</i>	-----FAR-----NAL-----PQAQRAHGYSGG-----	247
<i>M. tuberculosis</i>	-----SRF-----TDFLARPN---HAVNHVKVADRWEAR-----	305
<i>E. coli</i>	-----AVK-----GDEIIVNK-----ASGYKLG-----	224
<i>S. aureus</i>	AEAIQSPITFWYGKYIILIPSSYFKVIDKRLKYI--ILHEYAHAKNRDTHLIIIFNIFS	221
<i>S. epidermidis</i>	AETIQSPITFWYGKYIILIPSSYFKVIDKRLKYI--ILHEYAHAKNRDTHLIIIFNIFS	221
<i>P. aeruginosa</i>	---G-R-----PGSASDLFVNDLWSSPVDLSRFVRMLFANGRHKERQ-----	285
<i>M. tuberculosis</i>	---YQR-----DPDAQSPAGGVSSSLNDMTHWLMVADGVYNGRR-----	343
<i>E. coli</i>	---FGKPVLFHAPLARNHVEAAYIHHTLPLDMEIWI DAWLHRKALPATL-----	269
<i>S. aureus</i>	IIMSYNPLVHVIVKRKIIHDNEVEADRFVLNNINKNEFKTYAESIMDSVL-----KTPFS	275
<i>S. epidermidis</i>	IVMSYNPLIHIVKRKIIHDNEVEADRFVLNNINKNEFKTYAESIMDSVL-----NIPFF	275
<i>P. aeruginosa</i>	-LLRKHSVEEMFRQQNAG-NAL---DFD-----CQVGLAWF-LSPCGSAPLEGGIRHY	332
<i>M. tuberculosis</i>	-ITSPEALLPVYTPQVISRHPVSPRARA-----SFYGYGFN-VGVTSSEGR---TEYS	390
<i>E. coli</i>	---REAMSNSWRG--NSDVPLAADN-R-----ILYASGWFIQDN---Q---GPYI	307

**Figure S3.** Alignment of PBP enzyme sequences from selected bacteria in the study. The gray regions correspond to non-similar and non-identical amino acids. The red regions correspond only to identical amino acids. The yellow regions are similar amino acids.

<i>M. tuberculosis</i>	MVTKTT-----LASATSGLLLLAVAMSGCTPRPQGPAAEKFFAAIA	44
<i>S. aureus</i>	ML--KRLKEKSNDEIVQNTINKRINFIFGVIVFIFAVLVRLG-----YLQ	44
<i>S. epidermidis</i>	ML--KRLKEKTNDEKMRNTMNKRINFIFGFIVFIFAVLVRLG-----YLQ	44
<i>P. aeruginosa</i>	-----MKLNYFQGALYPWRFVIVCLLLAMVGATVWRIV-----DLH	37
<i>E. coli</i>	MKAAAKTQKPKRQEEHANFISWRFALLCGCILLALAFLLGRVA-----WLQ	46
<i>M. tuberculosis</i>	IGDTASAAQLSDNPNEAREALNAAWAGLQAAHLDAQVLSAKYAEDETTVAYRFSWHLPKD	104
<i>S. aureus</i>	IAQGSHYKQIIKNDENITV-----NESVPRGRILDRNGK---VLVDNASKM	87
<i>S. epidermidis</i>	IAQGSHYKQLIKNDENITV-----NESVPRGRILDRNGK---VLVDNASKM	87
<i>P. aeruginosa</i>	VIDHDFLKGQGDARSVRHI-----AIPAHRLITDRNGE---PLAVSTPVT	80
<i>E. coli</i>	VISPDMLVKEGDMRSLRVQ-----QVSTSRGMITDRSQR---PLAVSVPVK	89
<i>M. tuberculosis</i>	RIWTYDGQL-----KMA-----RDEGRWHVRWTTSGLLHPKLGEHQT	140
<i>S. aureus</i>	AITYTRGRKTTQSEMLDTAEKLSKLIKMDTKKITERDKKDFWIQ-----LHPKKAKAMM	141
<i>S. epidermidis</i>	SITYTRNRKTSQKEMLNTAKKLTDLIKMDTDKITERDKKDFWIQ-----MYPSSAKKLM	141
<i>P. aeruginosa</i>	TLWAN-----PKELM-----A-----AKERWPQ-----LAAALGQ---	105
<i>E. coli</i>	AIWAD-----PKEVH-----DA---GGISVGDWRKA-----LANALNI---	119
<i>M. tuberculosis</i>	----FALR-----ADPPRRASVNEVGGTD--VLVP---GYLYHYSLDAGQAGRELFGTAH	186
<i>S. aureus</i>	TKEQAMLADGSIKQDQYDKQLLSKIGKSQDLDELSSKDLQVLAIFRE--M-----	188
<i>S. epidermidis</i>	RKEQLMLEDGSIQEQFDTQLRNKIGKKQLKQLTKKDLQVLAIFYRE--M-----	188
<i>P. aeruginosa</i>	-----DTKLFA---DRIEQN-----AERE---FIYLVVRG-----	128
<i>E. coli</i>	-----PLDQLS---ARINAN-----PKGR---FIYLARQ-----	142
<i>M. tuberculosis</i>	AVVGALHPFDDTLNDFQLLAEQASSSTQPLDLVTLHADDSNRVAAAIGQLPGVVITPQAE	246
<i>S. aureus</i>	-----NAGTVLDFQMIKNEDVSE-----KEYAAVSQQLSKLPGVNNTSMWD	229

*S. epidermidis* -----NAGSTLDEQTIKNEVDSE-----KEYAASVQQLSKLPGVNTTMDWD 229  
*P. aeruginosa* -----LTPEQ-----GEGVIALKVPGVYSIEEFR 152  
*E. coli* -----VNEEDM-----ADYIKKLKLPGLHLEERSR 166

*M. tuberculosis* L-LSTD-----KHFAP-----AVLNDVKKAVVDELDKAG 275  
*S. aureus* RKYFYGDTLRGIFGDVSTPAEGIPKELTEHYLSKGYSRNDRVGKSYLEYQYEDVLKGGKKK 289  
*S. epidermidis* RKYFYGDTLRGIFGDVSTSTEGIPKELTEQYLSKGYSRNDRVGKSYLEYQYEDVLKGGTKK 289  
*P. aeruginosa* RFYFAGEVVAHAVGF-----TDVDDRREGIELAFDEWLAGVPG 191  
*E. coli* RYFESGEVTAHLIGF-----TNVDSQGIQEGVEKSFDKWLTGQPG 205

*M. tuberculosis* WRVSVNQNGVDVSVLHEV-APSPASSVSITLDRVVQNA-----QHAVNT---RG----G 323  
*S. aureus* EMKYTTDKSGKVTSSSEVLN-PGARGQDLKLTIDIDLQKEVEALLDKQIKKLRSQGAKDMD 348  
*S. epidermidis* QMKYTTDKSGRVISSEVLN-PGSRGHDQLQTLTIDIDLQKKVESLLEKQISKLRSQGAKDMD 348  
*P. aeruginosa* KRQVLKDRRGRVIKDVQVTKNAPKGTALALSIDLRLQYLAHRELNRALLE---NGA---K 245  
*E. coli* ERIVRKDRYGRVIEDISSTD-SQAHNLAALSIDERLQALVYRELNNAVAF---NKA---E 258

**Figure S4.** Alignment of CYP51 enzyme sequences from selected fungi in the study. The gray regions correspond to non-similar and non-identical amino acids. The red regions correspond only to identical amino acids. The yellow regions are similar amino acids. The black boxes represent the active site amino acids.

*A. flavus* LVFHWIPPIGSTITHYGMDPYGFFFSCKREKYGDIETITLIGRPTTVYLGTCQNEFIFLNGKL 97  
*C. glabrata* LVFYNIPIVWGSAPYGTTPYEFEDCQKKYGDIFSTMLIGRIMTVYLGPKGHEFIFNAKL 118  
*C. albicans* LVFYNIPIVWGSAAHYGQDPYEFESCRQKYGDVFSFMLIGKIMTVYLGPKGHEFVFNAKL 109  
*C. tropicalis* MVFYNIPIVWGSAAHYGQDPYEFETKRLKYGEVFSFMLIGKIMTVYLGPKGHEFIYNAKL 109

*A. flavus* KDVNAEEVYSPLTTPVFGSDVYVDCPNSKLLIEQKKETNFGLSQAALAEAHVPLTEKEVED 157  
*C. glabrata* ADVSAEAAYSHLTTPVFGKGVYIYDCPNHRLMEQKKFVKGALTKEAFVRYVPLIAEETIKY 178  
*C. albicans* SDVSAEDAYKHLTTPVFGKGVYIYDCPNSRLMEQKKFAKFALTDSFKRYVPKIREEILNY 169  
*C. tropicalis* SDVSAEAAYSHLTTPVFGKGVYIYDCPNSRLMEQKKFAKFALTDSFKTYVPKIREEVLNY 169

*A. flavus* LAMSPNEHG---TSCQVDIPAAAMITITFTAGSALQCEEVRSKLTTEFAVLYHDLDKQET 214  
*C. glabrata* FRNSKNFKINENNSCIVDVMVSQPEMTITFTASRSLGKEMRDKLDTFAYLYSDLDKQET 238  
*C. albicans* FVTDESEKLEKETHGVANVMKTQPEITITFTASRSLFGDEMRRIFDRSFAQLYSDLKQET 229  
*C. tropicalis* FVNDVSEKTKERDHEVASVMKTQPEITITFTASRSLFGDEMRSFDRSFAQLYADLDKQET 229

*A. flavus* EINEVFFNLPLPYNKRRDAAHARMRSIYIDITNKRENAGDNVPEKLDMIGNLMQCTYKNG 274  
*C. glabrata* EINEVFFNLPLPYYRKRDAHAQQAISGTYSMLIKEREKNDIQ-NRDLIDELMKNSTYKDG 297  
*C. albicans* EINEVFFNLPLPHYWRRDAAQKKISATYMKIKSRDRDGDIDPNRDLIDSLLIHSTYKDG 289  
*C. tropicalis* EINEVFFNLPLPHYWRRDAAQRKISAHYMKIKRRRESGDIDPKRDLIDSLLVNSTYKDG 289

*A. flavus* QPLPKETIAHIMTLLMAGQHSSSSISWIMIRLASQFAVVEELYQEQLANLERTGPNGS 334  
*C. glabrata* TKMTDQEIALLGVLMGGQHTSAATSANCLLHLAERPDVQEELYQEQMRVLN-----ND 352  
*C. albicans* VKMTDQEIALLGVLMGGQHTSASTSANFLLHLGEKHLQDVIYQEVVELLKEKG--GD 347  
*C. tropicalis* VKMTDQEIALLGVLMGGQHTSASTSANFLLHLAEQQLQDDLYEELTNLLKEKG--GD 347

*A. flavus* LAPQYKDFDNLPLHQNVIETLRLHSSIHSLLRKVKNPLPVGTPPVIFITSHVLLAAPG 394  
*C. glabrata* TKEITVDDLQNMPLLNQMIKETLRLHPLHSLFRKVMRDVAIENTSVVVERDYHVLVSPG 412  
*C. albicans* LNDITYEDLQKLPSVNNTIKETLRLMPLHSIFRKVTNPLRIEENNVIFKGYHVLVSPG 407  
*C. tropicalis* LNDITYEDLQKLPLVNNTIKETLRLMPLHSIFRKVMNPLRVENKVIKGYHVLVSAQ 407

**Figure S5.** Alignment of SAP-2 enzyme sequences from selected fungi in the study. The gray regions correspond to non-similar and non-identical amino acids. The red regions correspond only to identical amino acids. The yellow regions are similar amino acids.

*C. albicans* -----MFLKNIFALALALLVDATPTT-----KRSAGFVALDFSIVK 38  
*C. tropicalis* MVWVIGLMKQVFISIVFGLTVEGLAISKLNDRGAAPGLMGNFFDKRSTPGVLSLELYVNR 60

*C. albicans* TPKAFPVTNG---QEGKTSKRQAVPTLHNEQVTYAADITVGSNNQKLNIVIDTGSSDI 94  
*C. tropicalis* NHDDSNFTIGPHFVNEYSKRDDYISVELYNEQVTYSANFTVGSNSQKQNVIVDTGSSDI 120

*C. albicans* WVPVNVDCQVTVSDQTADEFCKQKSTYDPSGSSASQDINTPEKIGYGDGSSSQGTLYKDT 154

<i>C. tropicalis</i>	WVVSANCOEK-EGYSSDYCFSGSTYDPSSSSSTIEELGKSENRIRYGDGSSSSSGTWVKDT	179
<i>C. albicans</i>	VGFGGVSIKNOVLADVDSTSIDQGILGVGYKTNEAG-GSYDNVPVTLNKKQCVIAKNAYSL	213
<i>C. tropicalis</i>	VGINGAILNQQFGDVNSTSVSQGILGIGLDTNESTDITIVENFFPINLKEQGFINTNAYSL	239
<i>C. albicans</i>	YLNSEDAATGQIIFGGVDNAKYSSESLIALPVTSDRELRLISLGSVEVSGKTIINTDNVDVIV	273
<i>C. tropicalis</i>	YLNAPSATSGTIIFGGIDHAKYTGSLLTTLPLTSNREFTIQTN SATVGTSTID-INTGLIL	298
<i>C. albicans</i>	DSGTTITTYLQQDLAQIIFAFNGKLTQDSNGNSFIEVDCNLSGDVVFNFSSKNAKISVFEAS	333
<i>C. tropicalis</i>	DSGTTITTYLPOSVVSIANAIGCDIYNRP-IGAYIWSNCRNGKVTYNEFQGLNIDIFYS	357
<i>C. albicans</i>	EFVASSLQGDDEQPYDKQLLF-DVNDANILGDNFLRSAYIVYDLDNEISLAQVKYTSAS	392
<i>C. tropicalis</i>	DLAVPLYYNSGAVAGFCAIGILYGENFNLGDNFLRHAYVVYNLDALTISLAPVVTSDS	417
<i>C. albicans</i>	SISALT	398
<i>C. tropicalis</i>	NVTIV-	422

**Figure S6.** Alignment of DHFR enzyme sequences from selected fungi in the study. The gray regions correspond to non-similar and non-identical amino acids. The red regions correspond only to identical amino acids. The yellow regions are similar amino acids.

<i>C. glabrata</i>	-MSK-VPVVGII-----VAALLPEMGIGFQGNLFW-RLAKEMKYEREVTTLTNDNSK	48
<i>C. albicans</i>	-MLK-PNVAI I-----VAALKPALGIGYKSKMPW-RLRKEIRYFKDVTTRTTKPNT	48
<i>C. tropicalis</i>	MQSK-PVISII-----VAALKPSLGIGNNGKMPW-RLRKEIRYFKDVTSKTTVENS	49
<i>A. flavus</i>	MP-PTNPLTLIVATTPIPTRE---KTLGLGTLNGLTLPWPRIKADMSFARVTTTRPPRPGT	56
<i>T. rubrum</i>	MPAKLPPLTLVATTPITPTNPNGILKLIGIGKESTLWMPRIKKDMSFARVTTTRPPATAT	60
<i>C. glabrata</i>	-----QNVVIMGRKTTWESIEQKFRPLPKRINVVVSRSFDFGELRKVEDG-----	91
<i>C. albicans</i>	-----RNAVIMGRKTTWESIEQKFRPLPDRNLNIIILSRSYENEII--DDN-----	89
<i>C. tropicalis</i>	-----RNAVIMGRKTTWESIEQKFRPLPDRNLNIIILSRSYENEII--DEN-----	90
<i>A. flavus</i>	-----TNAMIMGRKTYDSVPSLRPLGKRINVIIVTRDVEGVSKRVAEE-LKEKR---	104
<i>T. rubrum</i>	ASGSASPAINAVIMGRKTYDSIEQKFRPLSKRLNVIITRDESGSVKERAIADWNASRKRE	120
<i>C. glabrata</i>	-----IYHSNSLRNCLTALQSSLA-----NENK	114
<i>C. albicans</i>	-----IIHASSIE-----SSLN-----LVSD	105
<i>C. tropicalis</i>	-----IIHANSVE-----SSLS-----FMSN	106
<i>A. flavus</i>	----AKMAAAAAAATSAGENKEEGPITDAIVSSGLEAALEDVEEKF-----KGG	149
<i>T. rubrum</i>	LEKQADQDQTDKAAATST----STEEPEVIVSSSLEDALSTLQRNFVISSSSDVQGKKR	175
<i>C. glabrata</i>	IERYIIGGGEIYRQSM-D--L-----ADHWLIITKIMPL-----PETTIPQMDT	155
<i>C. albicans</i>	VERVFIIGGAEIYNELINNSL-----VSHLLITEIE-H-----PSPESIEMDT	147
<i>C. tropicalis</i>	IDRVFIIGGAEIYNELINNSL-----VTQLLITEIE-H-----SNPESVEMDT	148
<i>A. flavus</i>	LGSVFVIGGAEIYATALG----LGGDRPVRIVMINVEKKGV-----DGEKAVFECDT	197
<i>T. rubrum</i>	LGNVYIMGGSEIYASSLRLTADALGENNPLRIVMDIRRRADGNAQCQDVEDLVDGFECDT	235
<i>C. glabrata</i>	FLQKQ---ELEQRFYDNSDKLVDFLPSIIQLEERLTSQEWNGELVKGIPVQEKGYQFYF	211
<i>C. albicans</i>	FLKFP----LESWTKQPKSELQKFVGDTV-LEDD-----IKEGDFTYNY	186
<i>C. tropicalis</i>	FLKFP----LEKWTQPKSELQKFVGDTV-LEDD-----INEGDFTYNY	188
<i>A. flavus</i>	FFPIDDELLMEKGWRKVSAAEVTEWVGEPVSGE----WKDEGE-----VRIQM	241
<i>T. rubrum</i>	CFPLDGKG-LKEGWNKVPSEKLAEWVGEAVSSD----WTWEGD-----IAMKI	278

**Figure S7.** Ramachandran plot of homology models generated for (A) *S. epidermidis* DNA gyrase, (B) *S. epidermidis* beta-lactamase, (C) *S. epidermidis* PBP, (D) *S. tropicalis* SAP-2, (E) DHFR from *S. tropicalis*, (F) CYP51 from *A. flavus* and (G) DHFR from *T. rubrum*. The colored regions represent the allowed and favored regions of secondary structures and the white regions represent the forbidden regions.





