

Structure-Function Relationship Study of an Amoebic Secretory Phosphatase: A Computational-Experimental Approach

Celina Terán-Ramírez, Rosa E. Mares-Alejandro*, Ana L. Estrada-González, Patricia L. A. Muñoz-Muñoz and Marco A. Ramos-Ibarra*

Biotechnology and Biosciences Research Group, Faculty of Chemical Sciences and Engineering, Autonomous University of Baja California, Tijuana, BCN, Mexico.

* Correspondence: rmares@uabc.edu.mx (R.E.M.A.); mramos@uabc.edu.mx (M.A.R.I.).

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EhHAPp49  MLWLLLLFQLSLGDLTYCEVPEFTFKPVDGYKPILOMVFRHGRSEWLTYKGDQATYNCDISQQLRFLSAQGMSEYEFHHIKTEVDKEK
HuPAP_1ND5  -----VTLVFRHGRSEIDTFPTDPIKE-----
HuLAP_4JOB  -----MVQVFRHGRSEHLKPLPLEEQV---EWNPQLLEVPPQTQFDYTVTNLAGGPKPY
LpHAP_5CDH  -----

EhHAPp49  M--VFAKDNMYGGSCQMGLTRKSLNQLATLGOKVRAMVGDNNFLETYMNISDIIMRSTKVWRVLCQAESELOHLYPSYSRDPD-----
HuPAP_1ND5  -----SSWPQGFQSLTOLGMECHYELGGEYIRKRYRK---FINESYKHEQVITRSTQVDRITMSAMTNLAALFEPGEVSIW-----
HuLAP_4JOB  PYDSQYHETTLKGGMFAGQLTKVGMQCMFALGERLRKNYVEDIFLSPTFNPQEVFIRSTNIFRNLESIRCLLAGLFCQCKEGEI-----
LpHAP_5CDH  -----LGQLTAEGMQCEYKMGVAFRKKYIEELHILPEHYEYGTITVIRSTDYARTIYSAQSLIMCLYVEGTGSPIPAGTS

EhHAPp49  -----ARRINIVPSEIIEYAVNNDHGMCPPEAELEKELFDKWFEELNLRHKEPYASILAKAERFFGVVNNYNWDSYFDILQEVQCCKNL
HuPAP_1ND5  NPILLWQPLPVEHIVLSEDLILYLPFRNCPFFOELSETLKSEEFCKR---LHPYKDFIATLGKLSCLHGQDLFGIWSKVYDPLYCESV
HuLAP_4JOB  -----IIHIDE-ADSEVLYPNYQSNLSLRQTRGRROTASLOPG-----ISEDLKKVKDRMGIDSSDK-VDFFIILLDNVAEQA
LpHAP_5CDH  ALPHAFQPLPVPFSAESKYDEVIIQQVDRKEKRLMEQYVESTREWCOK---NNELKDKYPLWSRLTGINIDTL-EDLETVGHTLYVHQI

EhHAPp49  EWPCIDTESGEKECFTEEFNKLVELVYHDGVYRFFDNDTIPLARLDAG---WFLRDTLSFQOMKYEGKTNVRYTHFAHDTTIIYPLVS
HuPAP_1ND5  HNFITL-----ESWATEDTMTKLRLELSELSSLGYGIHKQEKSRLOGG---VIVNELINHMKRATQIPSYKKLIMYSAHDTTVSGLQM
HuLAP_4JOB  HNL-----PSCPMLKRFAIRMIQORAVDTSLYILPKEDRESIQMAVCPFLHILESNLLKAMDSATAPDKIRKLYLYAHDTHFIFLLM
LpHAP_5CDH  HNAFM-----EGLASNDIETIINSAEWAFM---AQEKPOQIANVYSS---KMTNADYLNSSGSMKKSKLYVLLSAHDTTILASVLS

EhHAPp49  LLEGDYSSWPPYASYIIFEMYEK--ESEYYIRVGNINNEL-----DLNFCSDKENGMCKWKSFFDHMSKKVP--KSSDCNAKNQ
HuPAP_1ND5  ALDQVYNGLLPPYASCHLTLYFE--KGEYFVEMYRYNETQHEPYPLMLPGCSPP---SCPLERFAELVGPVLPQDWSTECMTTN-
HuLAP_4JOB  TLSIFDHKWFPEFDLTIMELYOHLESKEEFVQLYHYHQEQ-----VPRGCPD---GLCPFLMFLNAMS-----
LpHAP_5CDH  FLGAPLEKSEPPYASNVNFSLYDN--GANG-----MTVKITTYNGNPVLIPAGSG---SVCELPQQLVNLVHDSKNL-----

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Figure S1. Multiple sequence alignment of the protein sequences of EhHAPp49 and three structural homologs: HuPAP, human prostatic acid phosphatase (PDB Entry 1ND5); HuLAP, human lysophosphatidic acid phosphatase type 6 (PDB Entry 4JOB); and LpHAP, *Legionella pneumophila* HAP (PDB Entry 5CDH). The alignment was performed using Chimera's Multalign Viewer tool. Similar residues are shaded gray, while identical residues are boxed.

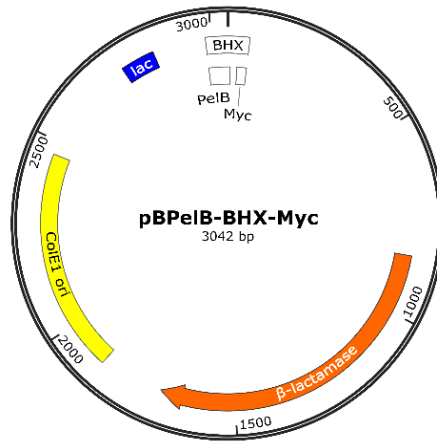


Figure S2. Schematic depiction of pBPelB-BHX-Myc, a pBluescript-based plasmid allowing gene fusion of a periplasmic targeting sequence (PelB) and a molecular tag peptide (Myc) to the N- and C-termini ends of recombinant proteins. BHX represents a mini MCS, which includes the BamHI, HindIII, and XhoI restriction sites. While ColE1 ori (yellow) functions as the autonomous replication sequence, encoded β -lactamase (orange) as the selection marker (Amp^R), and lac promoter (blue) as the regulatory region.

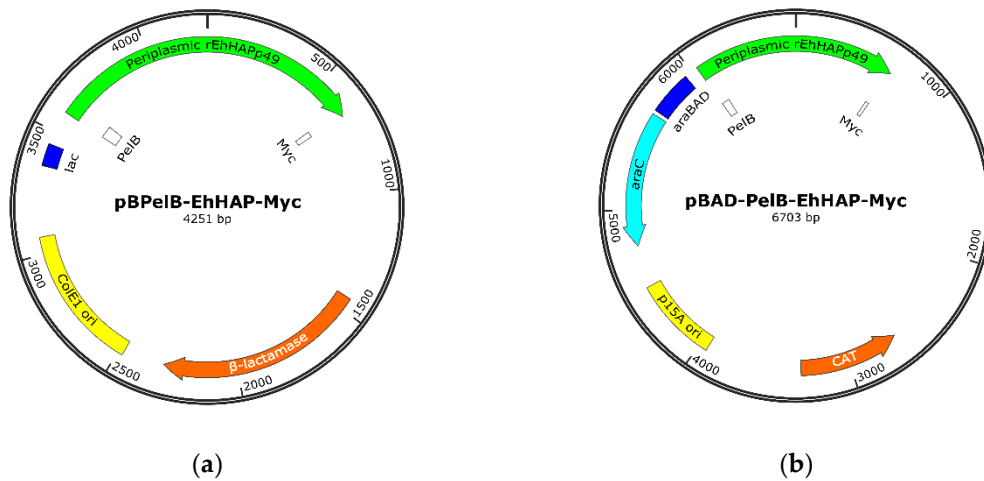


Figure S3. (a) Schematic depiction of pBPelB-EhHAP-Myc, a pBluescript-based plasmid encoding the *EhHAPp49* as a Myc-tagged periplasmic protein under the control of lac promoter (blue). ColE1 ori (yellow) and β -lactamase (orange) function as described for the parental plasmid, pBPelB-BHX-Myc (Supp. Fig. 1). (b) Schematic depiction of pBAD-PelB-EhHAP-Myc, a pBAD33-based plasmid encoding the *EhHAPp49* as a Myc-tagged periplasmic protein under the control of araBAD promoter (blue). While p15A ori (yellow) functions as the autonomous replication sequence, encoded chloramphenicol acetyl-transferase, CAT (orange), as the selection marker (Cm^R), and AraC (turquoise) as the regulatory protein.

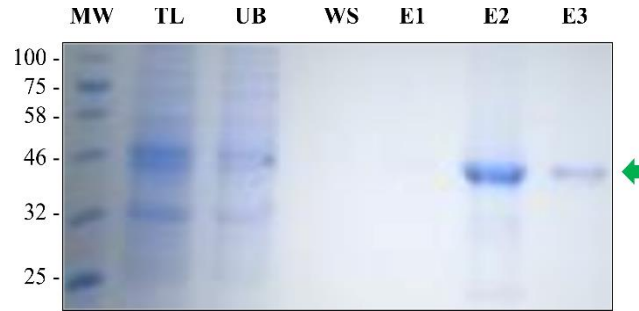


Figure S4. Analysis of IMAC fractions by 13.5% SDS-PAGE. Lanes: MW, molecular weight markers (kDa; on the left side); TL, total lysate; UB, unbound; WS, wash; E1-E3, elutions. Gel stained with Coomassie Brilliant Blue. On the right side, a green arrow indicates the relative mobility of *rEhHAPp49*.