

Scientific Reports

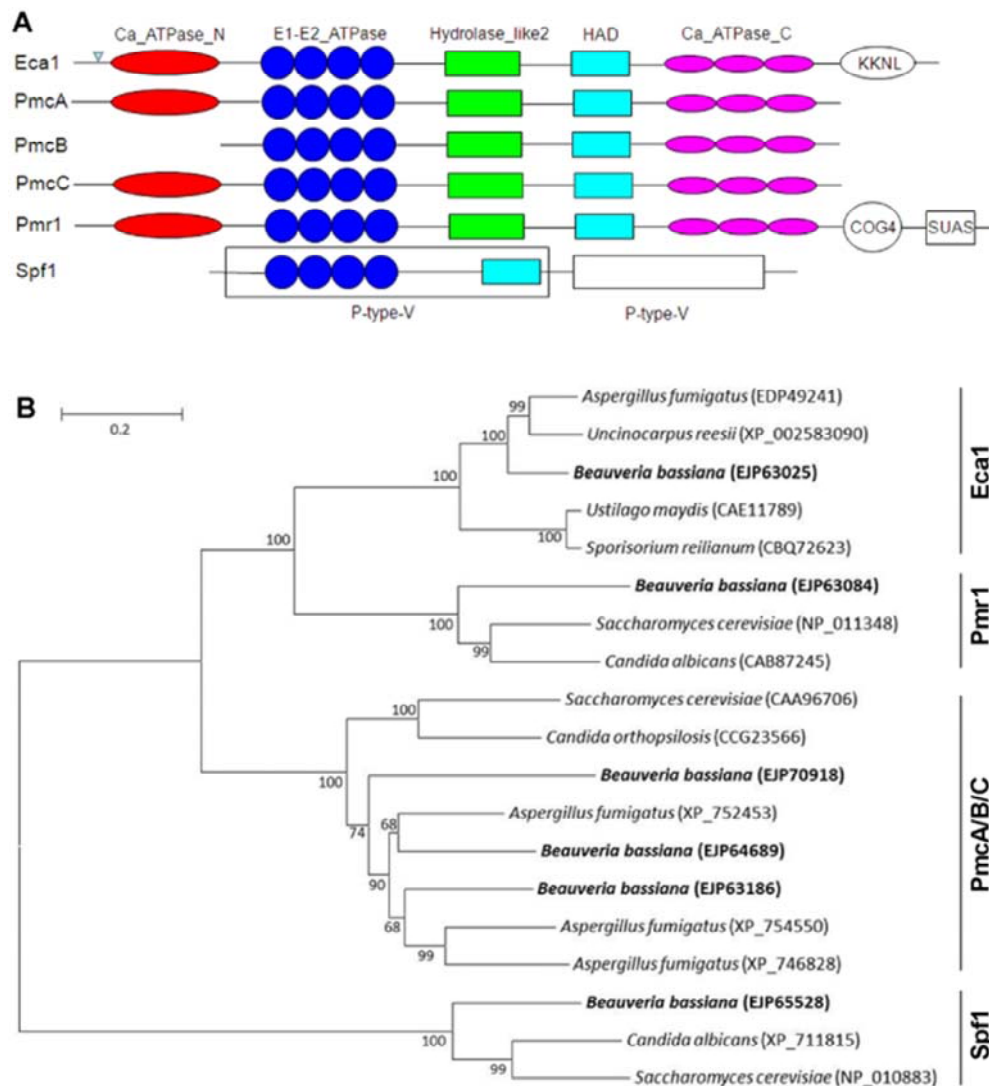
Supplementary Information

**Differential Roles for Six P-Type Calcium ATPases in Sustaining Intracellular  $\text{Ca}^{2+}$  Homeostasis, Asexual Cycle and Environmental Fitness of *Beauveria bassiana***

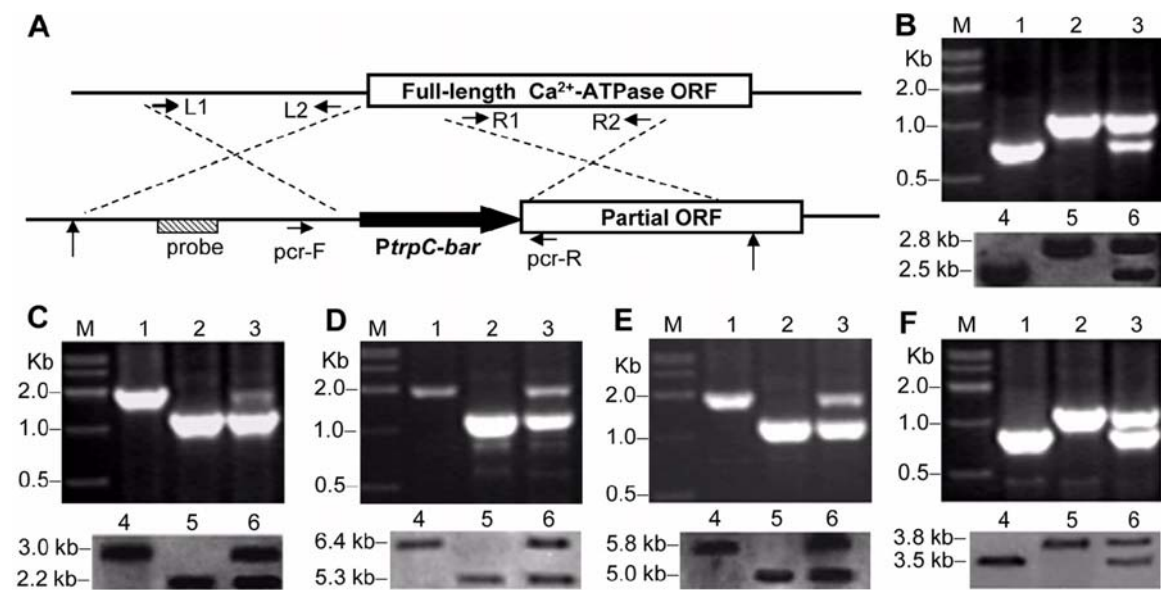
**Jie Wang<sup>1,2</sup>, Xiao-Guan Zhu<sup>1</sup>, Sheng-Hua Ying<sup>1</sup>, and Ming-Guang Feng<sup>1\*</sup>**

<sup>1</sup> Institute of Microbiology, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, 310058, China (\*Corresponding at: [mgfeng@zju.edu.cn](mailto:mgfeng@zju.edu.cn))

<sup>2</sup> Key Laboratory of Tropical Marine Bio-resources and Ecology, RNAM Center for Marine Microbiology, Guangdong Key Laboratory of Marine Material Medical, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, 510301, China



**Figure S1 Structural and phylogenetic features of six P-type calcium ATPases found in the genome database of *B. bassiana*.** (A) Conserved domains predicted from the calcium ATPases via sequence alignment and online blast analysis at <http://blast.ncbi.nlm.nih.gov/blast.cgi>. (B) Phylogenetic tree constructed for the calcium ATPases in *B. bassiana* and other fungi (NCBI accession codes given in parentheses following the fungal names) using a neighbor-joining method in MEGA7 software at <http://www.megasoftware.net>. Scale bar: branch length proportional to genetic distance.



**Figure S2 Constructing and identifying the deletion/complement mutants of calcium ATPase genes in *B. bassiana*.** (A) Schematic diagram for the deletion strategy of each target gene. The upward arrows indicate the sites of restriction enzymes used for Southern blotting of *eca1* (*EcoRI/EcoRI*), *pmcA* (*EcoRV/EcoRV*), *PmcB* (*EcoRV/EcoRV*), *pmcC* (*EcoRI/EcoRI*) and *spf1* (*NdeI/SpeI*). (B–F) The mutants of *eca1*, *pmcA*, *pmcB*, *pmcC* and *spf1* identified via PCR (lanes 1–3) and Southern blot hybridization (lanes 4–6) respectively. Lanes 1 and 4: wild-type strain. Lanes 2 and 5: deletion mutant of each gene. Lanes 3 and 6: complemented mutant of each gene.

**Table S1** Paired primers designed for the deletion, complementation and detection of calcium ATPase genes in *B. bassiana*.

| Primer     | Paired sequences (5'–3')*                                                                                                        | Purpose                                                          |
|------------|----------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------|
| Eca15'-F/R | <u>AAAAAGGATC</u> CTTCGGTCAGGTTTGGTGGAT / <u>AAAAAAGCTT</u> TTGGGGGATGCGTCTTGTTG                                                 | Cloning <i>eca1</i> 5'-end (1551 bp)                             |
| Eca13'-F/R | <u>AAAAAAGTAGT</u> TATCATCTCCGTCCTCCGTCG / <u>AAAAAGTTAA</u> CACGGGAGAACAAGAGGC                                                  | Cloning <i>eca1</i> 3'-end (1565 bp)                             |
| PmcA5'-F/R | <u>AAAAAGAATT</u> CTCGAAGACTGGGATAGCCT / <u>AAAAAGGATC</u> CGATGACAACGATGACGCCTC                                                 | Cloning <i>pmcA</i> 5'-end (1927 bp)                             |
| PmcA3'-F/R | <u>AAAAAAGATCT</u> GACTAAAAAGTGATGGCTG / <u>AAAAAGTTAA</u> CAGTGTGTTGAAGAACCCAG                                                  | Cloning <i>pmcA</i> 3'-end (2103 bp)                             |
| PmcB5'-F/R | <u>AAAAAGAATT</u> CTGCCTTGTTCAGAGTTGGTG / <u>AAAAAGGATC</u> CGAGTTTGTGGAAGAGGCT                                                  | Cloning <i>pmcB</i> 5'-end (2065 bp)                             |
| PmcB3'-F/R | <u>AAAAATCTAGA</u> CCGCCAAAAAGGTCAGATG / <u>AAAAAGTTAA</u> CGCCGAAGAAGACGATGAGGAC                                                | Cloning <i>pmcB</i> 3'-end (1995 bp)                             |
| PmcC5'-F/R | <u>AAAAAGAATT</u> CTAGAGTCGTTGGTGGAAGTAT / <u>AAAAAGGATC</u> GTGGGTGTAGTCTTCTTCAT                                                | Cloning <i>pmcC</i> 5'-end (1846 bp)                             |
| PmcC3'-F/R | <u>AAAAAACTAGT</u> AATCCGTCGCCATCAACTCG / <u>AAAAAGTTAA</u> CGTTTCTCATCTCTCGTTTCTC                                               | Cloning <i>pmcC</i> 3'-end (1913 bp)                             |
| Spf15'-F/R | <u>AAAAAGAATT</u> CTTCCGAGAATGAGTCAGTCCC / <u>AAAAAGGATC</u> TTGGGTCAAGAAAATAAAGGGC                                              | Cloning <i>spf1</i> 5'-end (1913 bp)                             |
| Spf13'-F/R | <u>AAAAAAGATCT</u> ACCACTATGGCACCAACACCTT / <u>AAAAAGTTAA</u> CACGGCAGTCAGAGGATTGTCAC                                            | Cloning <i>spf1</i> 3'-end (1696 bp)                             |
| Eca1fl-F/R | <u>GGGGACCACTTTGTACAAGAAAGCTGGG</u> TTGGTGTTCGTCATCAGT / <u>GGGGACAAGTTTG</u><br><u>TACAAAAAAGCAGGCT</u> CTTTGCCAATCTGTCTCCT     | Cloning full-length <i>eca1</i> (5888 bp)<br>for complementation |
| PmcAfl-F/R | <u>GGGGACCACTTTGTACAAGAAAGCTGGG</u> TTCAAGAACATCCGACAGAAGC / <u>GGGGACAAGTTTG</u><br><u>TACAAAAAAGCAGGCT</u> AACAGAAACGCAAAAGAAC | Cloning full-length <i>PmcA</i> (6744 bp)<br>for complementation |
| PmcBfl-F/R | <u>GGGGACCACTTTGTACAAGAAAGCTGGG</u> TCAGAGTTCGTCCTTTTATTC / <u>GGGGACAAGTTTG</u><br><u>ACAAAAAAGCAGGCT</u> ATGGAGAAGACCTGGACAAG  | Cloning full-length <i>PmcB</i> (7288 bp)<br>for complementation |
| PmcCfl-F/R | <u>GGGGACCACTTTGTACAAGAAAGCTGGG</u> TAAATGAATCAGAGCGAGTGG / <u>GGGGACAAGTTTG</u><br><u>TACAAAAAAGCAGGCT</u> CACATCCACACGAACTC    | Cloning full-length <i>PmcC</i> (6552 bp)<br>for complementation |
| Spf1fl-F/R | <u>GGGGACCACTTTGTACAAGAAAGCTGGG</u> TCCTTCACAAACAGAGATGC / <u>GGGGACAAGTTTG</u><br><u>TACAAAAAAGCAGGCT</u> CGTTGCTGTTGTTTGACTC   | Cloning full-length <i>Spf1</i> (6545 bp)<br>for complementation |
| pEca1-F/R  | TGTTTCATCGTCAGCCAGCCT/CAATGACACGGCAATCGGC                                                                                        | PCR detecting <i>eca1</i>                                        |
| sbEca1-F/R | TCGTCTCTCATCTCGCAAG/AACGATTGAACCAAGGCTG                                                                                          | Southern blotting <i>eca1</i>                                    |
| qEca1-F/R  | TTGATTCTGCTCGGTTCT/ATAAGAATGGTGAGGATAACAG                                                                                        | qRT-PCR detecting <i>eca1</i>                                    |
| pPmcA-F/R  | CCTTCTTTCTGTCTCTTGCCG/AATGATGGTGACGACGACGAT                                                                                      | PCR detecting <i>pmcA</i>                                        |
| sbPmcA-F/R | AGCCAAAGTGCCGAGAATAG/GTGGCTTGAATGTCTGTGGT                                                                                        | Southern blotting <i>pmcA</i>                                    |
| qPmcA-F/R  | ATCGTAACATCTCTCCT/CTGTCTCTGCTTTCAAAC                                                                                             | qRT-PCR detecting <i>pmcA</i>                                    |
| pPmcB-F/R  | CCTTCTTTCTGTCTCTTGCCG/AATGATGGTGACGACGACGAT                                                                                      | PCR detecting <i>pmcB</i>                                        |
| sbPmcB-F/R | AGCCAAAGTGCCGAGAATAG/GTGGCTTGAATGTCTGTGGT                                                                                        | Southern blotting <i>pmcB</i>                                    |
| qPmcB-F/R  | ATCGTAACATCTCTCCT/CTGTCTCTGCTTTCAAAC                                                                                             | qRT-PCR detecting <i>pmcB</i>                                    |
| pPmcC-F/R  | TATTTTTCGGGGTTTTCCTC/TGCCATCTTCTCGCTCTCG                                                                                         | PCR detecting <i>pmcC</i>                                        |
| sbPmcC-F/R | ATTTTCCAACCTGGCGAGAG/TTGACCAACGGGAACACCAC                                                                                        | Southern blotting <i>pmcC</i>                                    |
| qPmcC-F/R  | CACCTCGTCCTGATCCTG/TGACTTTGGGTTCAAGAG                                                                                            | qRT-PCR detecting <i>pmcC</i>                                    |
| pSpf1-F/R  | CCGAATCACCATCAGGTCCAT/AACGCAGAAGATTGAAAGACG                                                                                      | PCR detecting <i>spf1</i>                                        |
| sbSpf1-F/R | CGAGTGACATTATTGCCAG/GCTGCTATCACCTACCTACC                                                                                         | Southern blotting <i>spf1</i>                                    |
| qSpf1-F/R  | CTGAATCCGCTTTCTTT/ATGTGTTTCTCGTAGAGC                                                                                             | qRT-PCR detecting <i>spf1</i>                                    |
| qPmr1-F/R  | GTAGTGTGCGAGATGAAT / GTAAGTCCGTGAGTGAGG                                                                                          | qRT-PCR detecting <i>Pmr1</i>                                    |
| q18S-F/R   | TGGTTTCTAGGACCGCCGTAA / CCTTGGCAAATGCTTTCGC                                                                                      | qRT-PCR detecting 18S rRNA                                       |

\* Underlined regions denote the sites of restriction enzyme for the deletion of each target gene (*Bam*HI/*Hind*III and *Spe*I/*Hpa*I for *eca1*, *Eco*RI/*Bam*HI and *Bgl*II/*Hpa*I for *pmcA*, *Eco*RI/*Bam*HI and *Xba*I/*Hpa*I for *pmcB*, *Eco*RI/*Bam*HI and *Spe*I/*Hpa*I for *pmcC*, and *Eco*RI/*Bam*HI and *Bgl*II/*Hpa*I for *spf1*) via homogenous recombination of 5' and 3' fragments separated by *bar* marker or the gateway exchange fragments for complementation of each target gene.