Overexpression of a *Metarhizium robertsii* HSP25 gene increases thermotolerance and survival in soil

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Fig. S1  

**a** Alignment of *M. robertsii* 2575 HSP25 with seven fungal small heat shock proteins (sHSPs). Sequences were aligned using the CLUSTAL X algorithm. Identical amino acids are shaded in black. Domains IbpA and α-crystallin are underlined with continuous and discontinuous lines, respectively.

**b** Neighbor-joining (NJ) phylogenetic tree of HSP25. The NJ tree was constructed with the MEGA4 software. Bootstrap values are adjacent to each internal node, representing the percentage of 1,000 bootstrap replicates. Amino acid sequences were obtained from *B. bassiana* (EJP69022.1), *F. lichenicola* (ACP18866.1), *G. zae* (XP_381334.1), *H. lixii* (AAX55622.1), *M. oryzae* (XP_360786.1), *N. crassa* (XP_963774.1) and *T. virens* (ABO32163.1). Bar represents two substitutions per 100 amino acids.
**Fig. S2** Southern blot analysis of *hsp25* gene in *M. robertsii* 2575 wild-type strain (wt) and two transformants (hsp25-2 and hsp25-5). 10 µg of genomic DNA was digested with *Eco*RI (EI) or *Xba*I (X), electrophoresed on 0.7% agarose gel and transferred to a Hybond-N+ membrane (Amersham, Piscataway NJ). The *hsp25* ORF fragment was amplified as probe. Probe preparation, membrane hybridization and visualization were according to the manufacturer’s instructions (DIG High Prime DNA Labeling and Detection Starter Kit II, Roche).
Fig. S3 Expression of HSP25 and its effect on viability of *E. coli* under heat stress. **a** Lysate of *E. coli* cells from bacterial cultures with (+) or without (-) 1 mM IPTG at 28 °C for 8 h. Proteins were analyzed on a 12% SDS-PAGE gel. pET-28a(+)/HSP25, *E. coli* strain BL21 DE3 cells transformed with *hsp25* expression vector; pET-28a(+), *E. coli* cells transformed with pET-28a(+) vector. **b** Cells harboring pET-28a(+)/HSP25 or pET-28a(+) were induced with 1 mM IPTG at 28 °C for 8 h, then incubated at 45 °C for 4 h. The aliquots (100 µL) sampled at 1 h interval were grown on Lysogeny Broth (LB, Sigma-Aldrich) agar plate at 37 °C overnight. Cell survival was plotted as percentage of heat-treated colony forming units relative to number of untreated colonies. Values are means of three replicates with the corresponding standard error.
Fig. S4 Kinetics of insect survivorship in bioassays. Shown is the survival of *Galleria mellonella* larvae following topical application with suspensions of $1 \times 10^7$ conidia/ml of *M. robertsii* 2575 wild-type (wt) and two hsp25 overexpressing strains (hsp25-2 and hsp25-5). Control insects were dipped in water. Values are means of three replicates with the corresponding standard deviation.