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**Differential Roles of Three  $\alpha$ -Crystallin Domain-Containing sHsps of *Beauveria bassiana* in Asexual Development, Multiple Stress Tolerance and Virulence**

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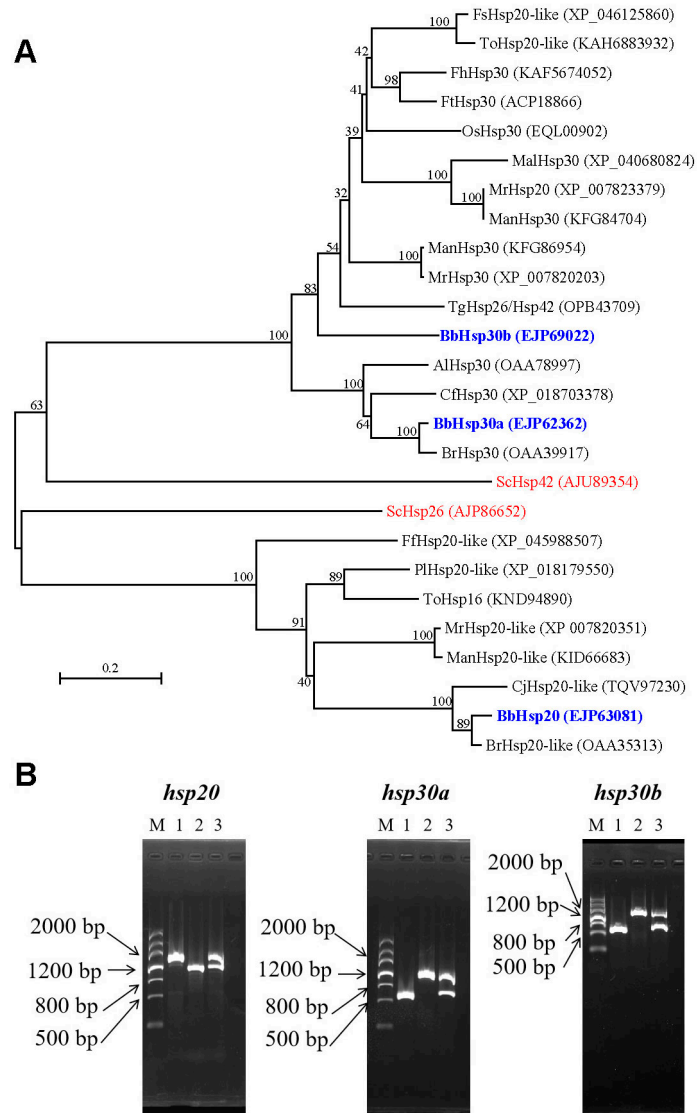
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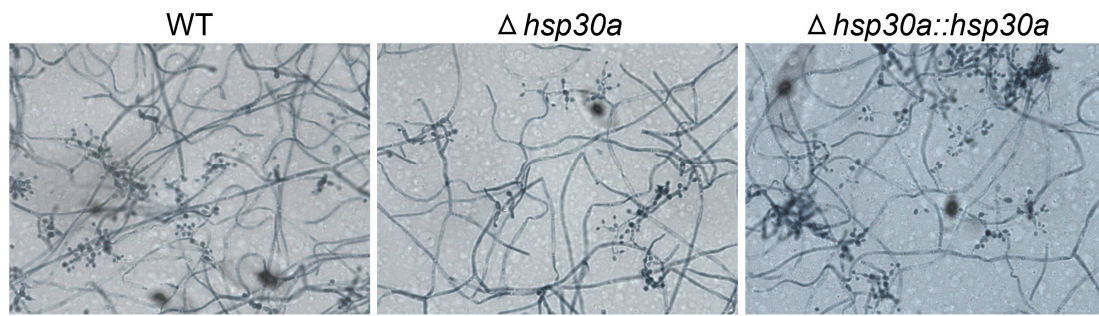
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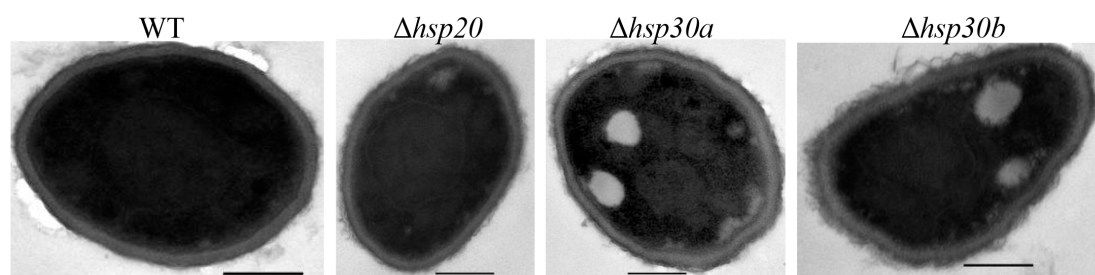
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**Figure S1.** Phylogenetic analysis of three  $\alpha$ -crystallin domain-containing sHsp genes found in the genome database of *B. bassiana* and identification of their mutants. **(A)** Phylogenetic tree for three sHsps of *B. bassiana* and other fungi based on a neighbor-joining method in MEGA7. The NCBI accession code of a given protein are given in the parentheses following each fungal name. Scale bar: branch length proportional to genetic distance. **(B)** The *hsp20*, *hsp30a* and *hsp30b* mutants identified via PCR. Lane 1: wild-type strain. Lane 2: deletion mutant. Lane 3: complemented mutant.



**Figure S2.** Conidiophores and conidia after conidia were cultivated on SDAY plates for 3 days, and visualized at  $400\times$  magnification.



**Figure S3.** Images for the ultrathin sections of conidia observed under transmission electron microscopy (TEM).

**Table S1** The paired primers used for the manipulation of *hsp20*, *hsp30a*, and *hsp30b* in this study

Name	Paired sequences (5'-3')*	Purpose
hsp20-F/R	ATGGCCCCACGCAACAACC / TCACAGAAATGCCACCTTGCG	Amplification of <i>hsp20</i>
hsp30a-F/R	ATGTCTCTTTTCCCCG / GTTGATAGCAATGCGTCG	Amplification of <i>hsp30a</i>
hsp30b-F/R	ATGGCTTCCTTCTTCCCC / GTTGATGCTGATGCGGAC	Amplification of <i>hsp30b</i>
hsp20up-F/R	AAAAAGGATCCTCATTGCCTCCATCCATACCG / AAAAAAGCTTCGAGTTGGTTGTTGCGTGG	Cloning <i>hsp20</i> 5'-end (2135 bp)
hsp20dn-F/R	AAAAAAGATCTATGATGTGATGCGTGCTG / AAAAAAGTAACTCTGCCCCATCTGTCTACG	Cloning <i>hsp20</i> 3'-end (1537 bp)
hsp30aup-F/R	AAAAAGGATCCTCGCGCTAATTTTGGTACTAAGGT / AAAAAAGCTCTGTTTGCAAGTGGTTGATGCTTGAT	Cloning <i>hsp30a</i> 5'-end (1406 bp)
hsp30adn-F/R	AAAAAAGATCTTGTTCGGACAAGCAACCATC / AAAAAAGTAGTTCATATCTTCTTGGCCATCTCAC	Cloning <i>hsp30a</i> 3'-end (1374 bp)
hsp30bup-F/R	AAAAAGGATCCTGTCATACTCCGATTGATTGG / AAAAAAGTAACTCTGCCCCATCTGTCTACG	Cloning <i>hsp30b</i> 5'-end (1411 bp)
hsp30bdn-F/R	AAAAAAGATCTAGCAGCAGAACCCAGCAGC / AAAAAAGTAGTTCGTAATCCCATAGGAACAAG	Cloning <i>hsp30b</i> 3'-end (1543 bp)
hsp20fl-F/R	<u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> TGATTCCGCTCTTGTCTCTG <u>GGGGACAAGTTTGTACAAAAAGCAGGCT</u> TAGAAGAAGTGCCGACGAGT	/ Cloning full-length <i>hsp20</i> (3915 bp)
hsp30afl-F/R	<u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> GAATGCCTACACTGTGGACT <u>GGGGACAAGTTTGTACAAAAAGCAGGCT</u> TGATTGTAAGGAAGCCAGT	/ Cloning full-length <i>hsp30a</i> (2763 bp)
hsp30bfl-F/R	<u>GGGGACCACTTTGTACAAGAAAGCTGGGT</u> GATGACTCGGCACGAGGAAC <u>GGGGACAAGTTTGTACAAAAAGCAGGCT</u> TTTGTCTCAGTCCAGCGATGCC	/ Cloning full-length <i>hsp30b</i> (3478 bp)
pHsp20-F/R	CACAGGAAGTTGATACCGAAAT / GTCAAGATGGAGATAGTTGTGGTT	PCR detecting <i>hsp20</i>
pHsp30a-F/R	CAACATCAAGACTACAACCACTG / CTTAGTTGATAGCAATGCGTCG	PCR detecting <i>hsp30a</i>
pHsp30b-F/R	ACAACATCAACACACACCAC / TGTTTAGTTGATGCTGATGCGG	PCR detecting <i>hsp30b</i>
qHsp20-F/R	TCAGGAGATGAGAAGAAC / GCTGTGATGATGTTTGT	qRT-PCR detecting <i>hsp20</i>
qHsp30a-F/R	TCTTCCCTTGGTCGTGAT/GTATTGGTCGCTGCTCTC	qRT-PCR detecting <i>hsp30a</i>
qHsp30b-F/R	ATGGCTTCCTTCTTCCC/AGTATGTGTCAAACTCGTC	qRT-PCR detecting <i>hsp30b</i>

\* The underlined region indicates the restriction site (*hsp20*: *Bam*HI/*Hind*III and *Bgl*II/*Hpa*I; *hsp30a*: *Bam*HI/*Sac*I and *Bgl*II/*Spe*I; *hsp30b*: *Bam*HI/*Pst*I and *Bgl*II/*Spe*I; The italic underlined area indicates the recognition sequence of the gateway exchange reaction.