Supplemental information:

Supplemental figure legends:

Supplemental Figure 1: Phylogenetic tree constructed using the program clustalw (http://clustalw.genome.jp/) showing the similarity between the different classes of chitin synthases among Basidiomycetes and Ascomycetes. Abbreviations: Af=*Aspergillus fumigatus*; An=*Aspergillus nidulans*; Ao=*Aspergillus oryzae*; Um=*Ustilago maydis*; Cn=*Cryptococcus neoformans*; Bf=*Botryotinia fuckeliana*; Fo=*Fusarium oxysporum*; Ed=*Exophiala dermatitidis*.

Supplemental Figure 2: Targeted replacement of *A. fumigatus CSMA* and *CSMB* genes. **A.** Physical maps of the genomic regions and strategy for the construction of the different disruption vectors designated as pCJ-E1, pCJ-Eb2, pCJ-D2 and pCJ-F4. (**B).** Analysis of the transformants A30 (Δ*csmA*), B1 and B7 (Δ*csmB*), D22 and D78 (Δ*csmA*/Δ*csmB*) by Southern blotting.

Supplemental Figure 3: Construction of the Δ*csmA*/*ΔcmsB* strain by using the self excising β-rec/six blaster cassette in a Δ*akuB*^{KU80} background**: (A).** Schematic representation of genotypes after *CSMA* replacement, subsequent marker excision and *CSMB* replacement. Restriction sites and hybridizing probe (black bar) are schematically indicated; **(B)**. Southern analysis of the genomic DNA isolated from corresponding Δ*csmA* and Δ*csmAx* strains. The calculated sizes of hybridization signals by using the probe 1 are specified of DNA fragments are specified; **(C)**. Southern analysis of the genomic DNA isolated from corresponding Δ*csmA*x/Δ*csmB* strains. The calculated sizes of hybridization signals by using the probe 2 are specified.

Supplemental Figure 4: Ectopic integration of the *CSMA* and *CSMB* genes in the single and double mutants. R1: mutant Δ*csmA* transformed with *CSMA*; S5: mutant Δ*csmB* transformed with *CSMB;* pB1, pB4 y pB7: double mutant Δ*csmA*/Δ*cmsB* transformed with *CSMB* (genotype Δ*csmA/CSMB*); pA1, pA3 double mutant Δ*csmA*/Δ*cmsB* transformed with *CSMA* (genotype *CSMA/*Δ*csmB*). Panel A is hybridized with a *CSMB* probe and Panel B with a *CSMA* probe after digestion of the genomic DNA with *Xho*I, *Eco*RI and *Spe*I.

Supplemental Figure 5: Expression of all chitin synthase genes in the vegetative mycelium grown for 16 h in YG medium.

Supplemental Figure 6: Expression of all chitin synthase genes in the conidiating mycelia (grown for 24 h in aerial conditions) of the parental, Δ*csmA* and Δ*csmA*/Δ*csmB* strains**.**

Supplemental Figure 7: (A). Sizes of the colonies of the Δ*csmA*, Δ*csmB*, Δ*csmA/*Δ*csmB* and parental strains when 10-months old (1-3) or 2-weeks old conidia (4-6) were spotted on the malt-agar at three different concentrations (per spot: 1 & 4 – 5000, 2 & 4 – 2500 and 3 & 6 – 1250 conidia, respectively). Note that the colony diameter of the Δ*csmA/*Δ*csmB* double mutant was highly reduced with 10-month old conidia due to the loss of viability of the conidia over storage **(B).** Survival of the conidia during storage of the slants over time at RT.

Supplemental Figure 8: (A). Ten-fold conidial dilutions ($2x10^6$) of the Δ*csm* mutants and the parental strains were spotted on YG plates with 0.1 µg/ml of caspofungin (CAS), anidulafungin (ANF) and micafungin (MCF). Plates were incubated 3-days at 28°C; **(B)**.

Sensitivity of the Δ*csm* mutants and the parental strains to other drugs (itraconazole, voriconazole and amphotericin B (AMB)) and calcofluor white. Selected concentrations showing an inhibitory effect are presented. YG plates were incubated 48 h at 37°C.

Supplemental Figure 9: Lack of correlation between the phenotypes of the *CHS* mutant and their affiliation to a family defined by BLAST homologies. The bar-graph showed the number of Δ*chs* mutants generated in the Ascomycetes, Basidiomycetes, yeasts and these mutants with phenotypes.

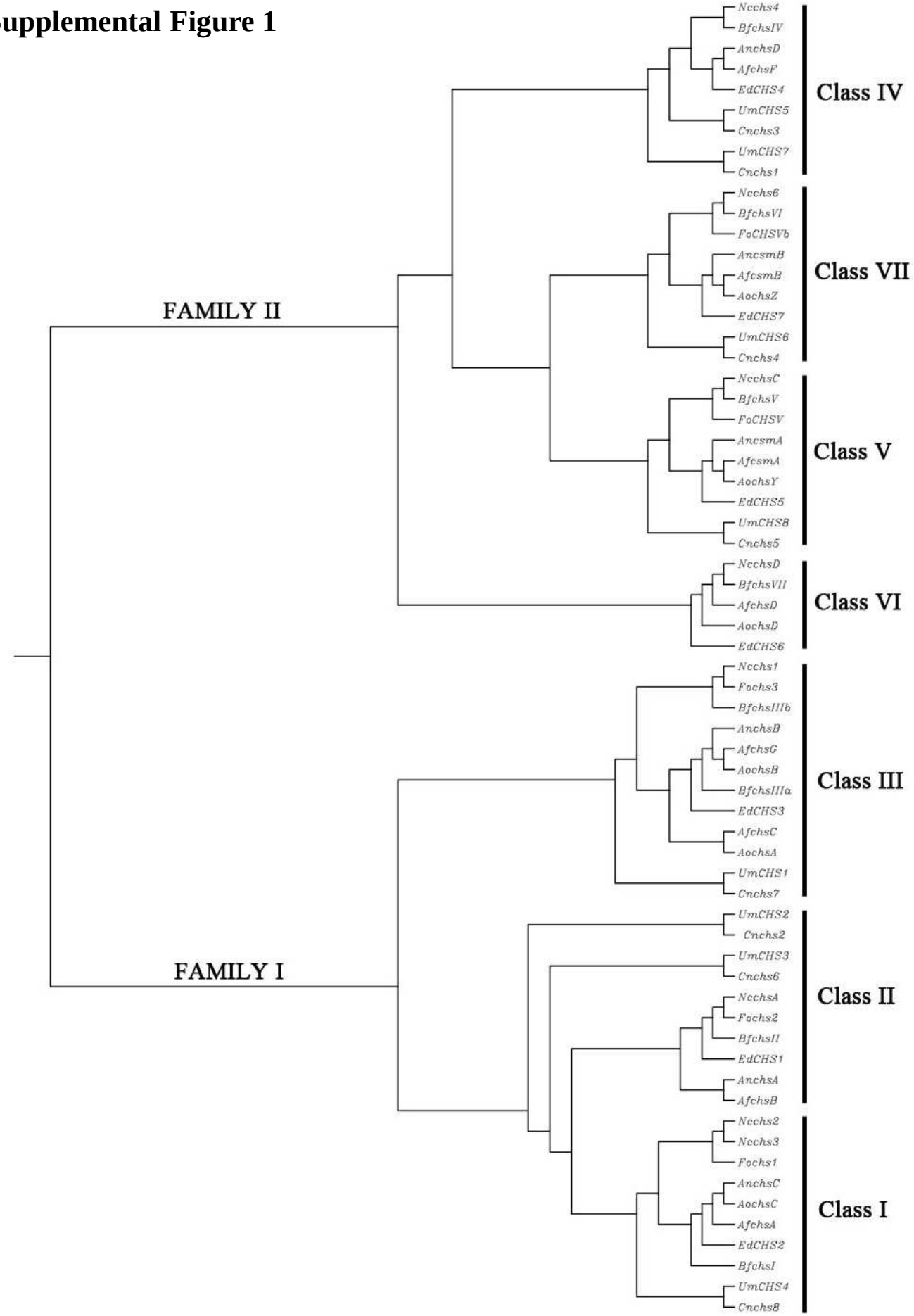
Supplemental Table 1: Primers used to construct the Δ*cms* mutant strains and to verify the complementation of the Δ*cmsA*/Δ*csmB* double mutant strain

Primers used for the deletion of *CSMA* and *CSMB* (Strategy 1)	
AfV-A1	5'-TAGCGGCCGCGTCCCTAGCACCTAATATTCC-3' *Not*I
AfV-A2	5'-TCGATGGATCCTATCTAGAGAGAAATGCCGCGTAAGATAG-3' *Bam*HI *Xba*I
AfV-A5	5'-GCTAAGGATCCATGTTAACGATTCCAATGCAACAGCTTTG-3' *Bam*HI *Hpa*I
AfV-A6	5'-AAGCGGCCGCGGATACATTAGCCGCTGC-3' *Not*I
AfVB-B1	5'-TTGCGGCCGCGACCTAGGTCTGAAGCGTTTGG-3' *Not*I
AfVB-B2	5'-CGATCGGATCCAGTCTAGAGTACCTGATCTACCGTGTCGC-3' *Bam*HI *Xba*I
AfVB-B5	5'-GCTAAGGATCCATGTTAACGAGGACGTCACAAGATA-3' *Bam*HI *Hpa*I
AfVB-B6	5'-AAGCGGCCGCCAAAGTCTGGTACTGTCC-3' *Not*I
Primers used for the deletion of *CSMA* and *CSMB* (Strategy 2)	
5'csmAforw	5'-AATTCGAGCTCGGTACTGCGCACCTAATATTCCTAAGAGTTC-3'
5'csmArev	5'-GGACCTGAGTGATGCTTCAAATCTGAGTACGAATAATG-3'
csmA3'forw	5'-TGGTCCATCTAGTGCATCGCCTCATCCACTGAGAATGG-3'
csmA3'rev	5'-GCCAAGCTTGCATGCCTGCGCAGAATGGAAAGATGACATCGTCC-3'
forw csmA	5'TCAGATTTGAAGCACCTCCG3'
5'csmBforw	5'AATTCGAGCTCGGTACTGCGCACGATCCGCACCCCAAAC3'
5'csmBrev	5'GGACCTGAGTGATGCTTGGCTTTCACGATGACCC3'
csmB3'forw	5'TGGTCCATCTAGTGCCATGCGATTTGTGGTACTGG3'
csmB3'rev	5'GCCAAGCTTGCATGCCTGC GCAGCCGTTGTTCCACC3'
forw csmB	5'TGTCTATTCCAGTCATTCAGC3'
Sv630	5'-TCGGAGAACACCTTGCTGACG3'
Primers used to verify complementation of the Δ*cmsA*/Δ*csmB* mutant	
sE_F	GATACCTACAACTTCCTACC
sE_R	CATCTCTAGCTCCAGCGG
sEb_F	CAGCCGTGATCTCGATGG
sEb_R	GTTCATAGAACCAGTCAGG

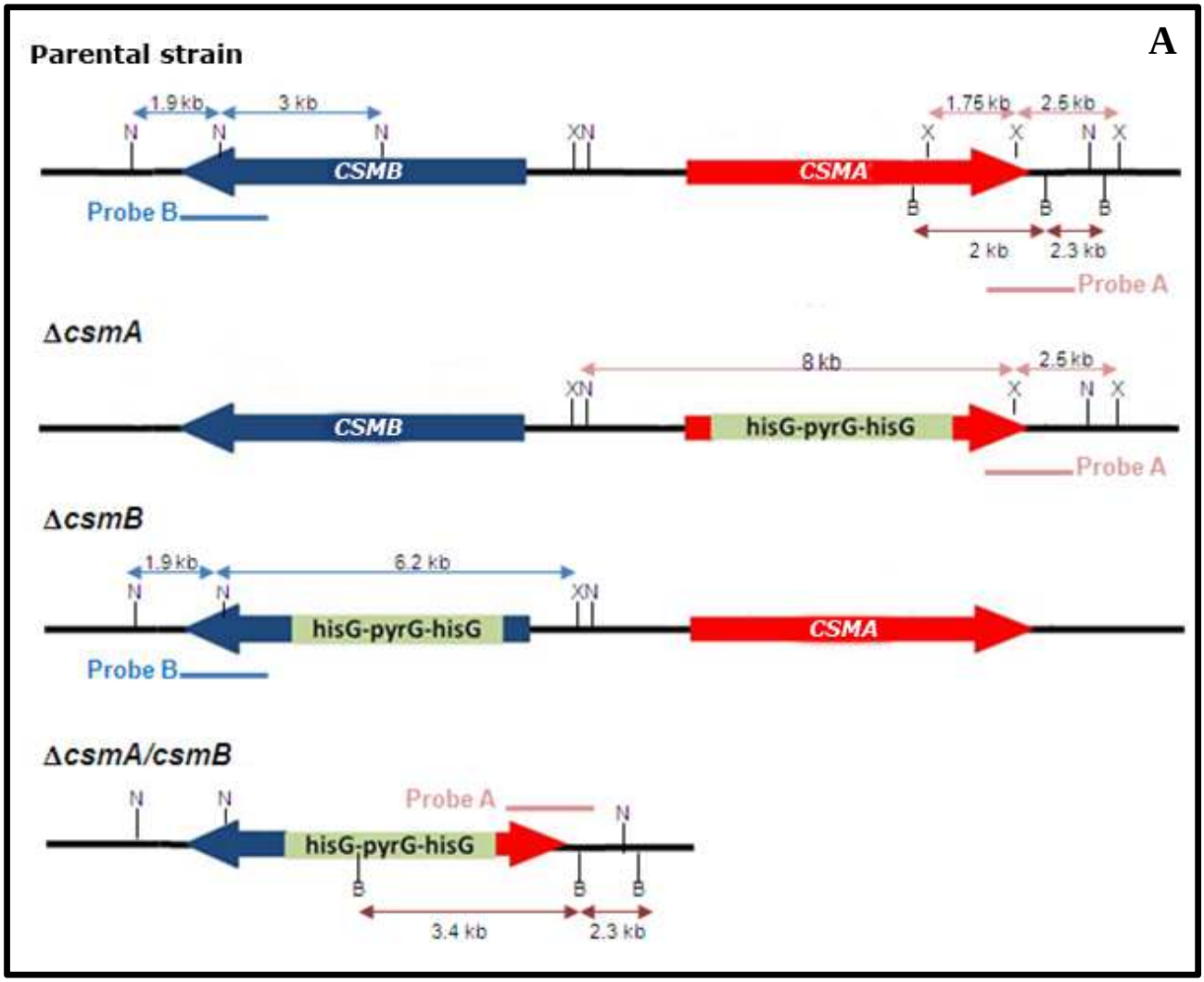
Supplemental Table 2: Primers used for Quantitative RT-PCR

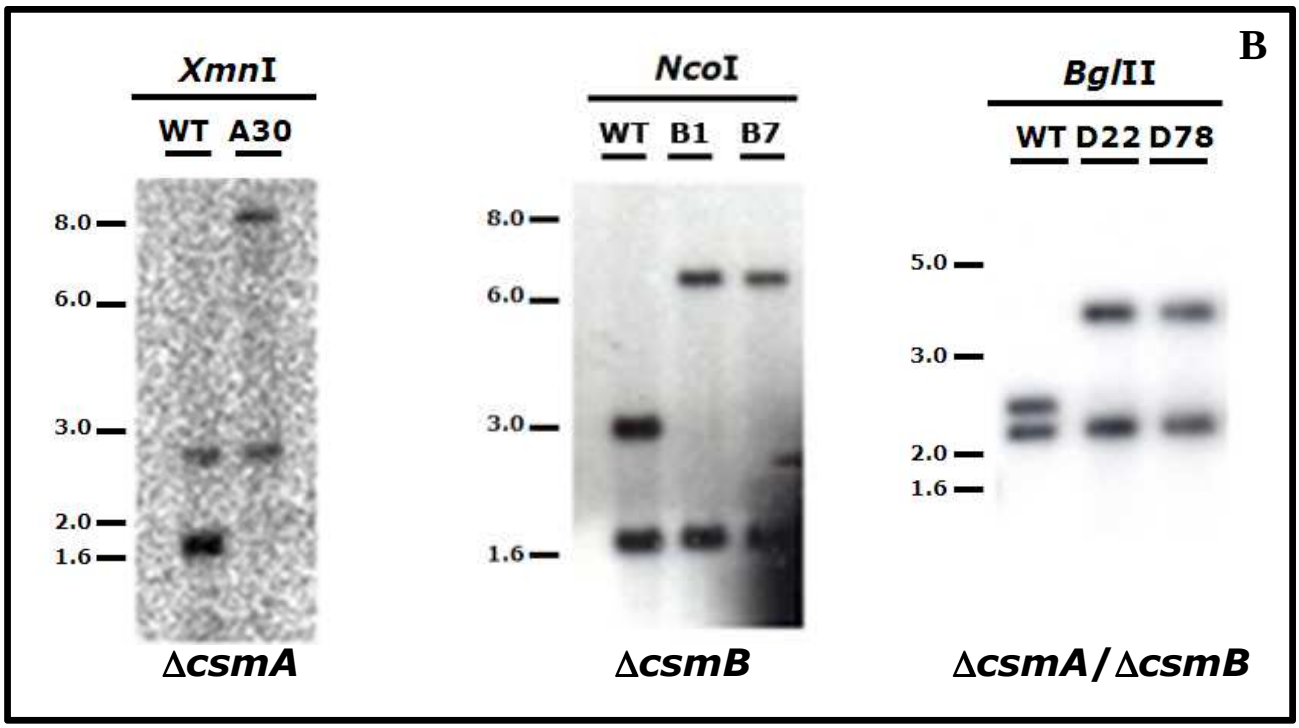
Name	**Sequence**
CHSAa (AFUA_2G01870)	ATGCGACGGATGATGACAGG
CHSAb (AFUA_2G01870)	ACGACCAGGAACCACATTGC
CHSBa (A FUA_4G04180)	GCGGCGGACTGGACAAGG
CHSBb (A FUA_4G04180)	ACAATGGAGCAGGTGGCAAGG
CHSCa (AFUA_5G00760)	GGTGCCGAGTGCGATTCAG
CHSCb (A FUA_5G00760)	CGTAGGTTGTAGCCGTTGCG
CHSDa (A FUA_1G12600)	GGACCGAGAGCCGATGCC
CHSDb (A FUA_1G12600)	GCCTTGAGCCTTAGCCAGTTC
CSMAa (A FUA2G13440)	TGTCATTGTCAAGGTCGGAAAGC
CSMAb (A FUA_2G13440)	CACGGTTCAGGAATCTCATCAGC
CSMBa (A FUA_2G13430)	AGGAGGGCGGAGGATGGATG
CSMBb (A FUA_2G13430)	CAAGTGCGGTGAAGGCTATGC
CHSFa (A FUA_8G05630)	ACTTTGACCTTCTGAACTGGCTTG
CHSFb (A FUA_8G05630)	TCCTCTTATCTTCTCCCGCTTGG
CHSGa (A FUA_3G14420)	TGGTGCGTGCCTTCAGTGG
CHSGb (A FUA_3G14420)	ACCGAATGTAGCAGCGAGAGC
TEFa (A FUA_1G06390)	CCATGTGTGTCGAGTCCTTC
TEFb (A FUA_1G06390)	GAACGTACAGCAACAGTCTGG

Supplemental Figure 1



Supplemental Figure 2





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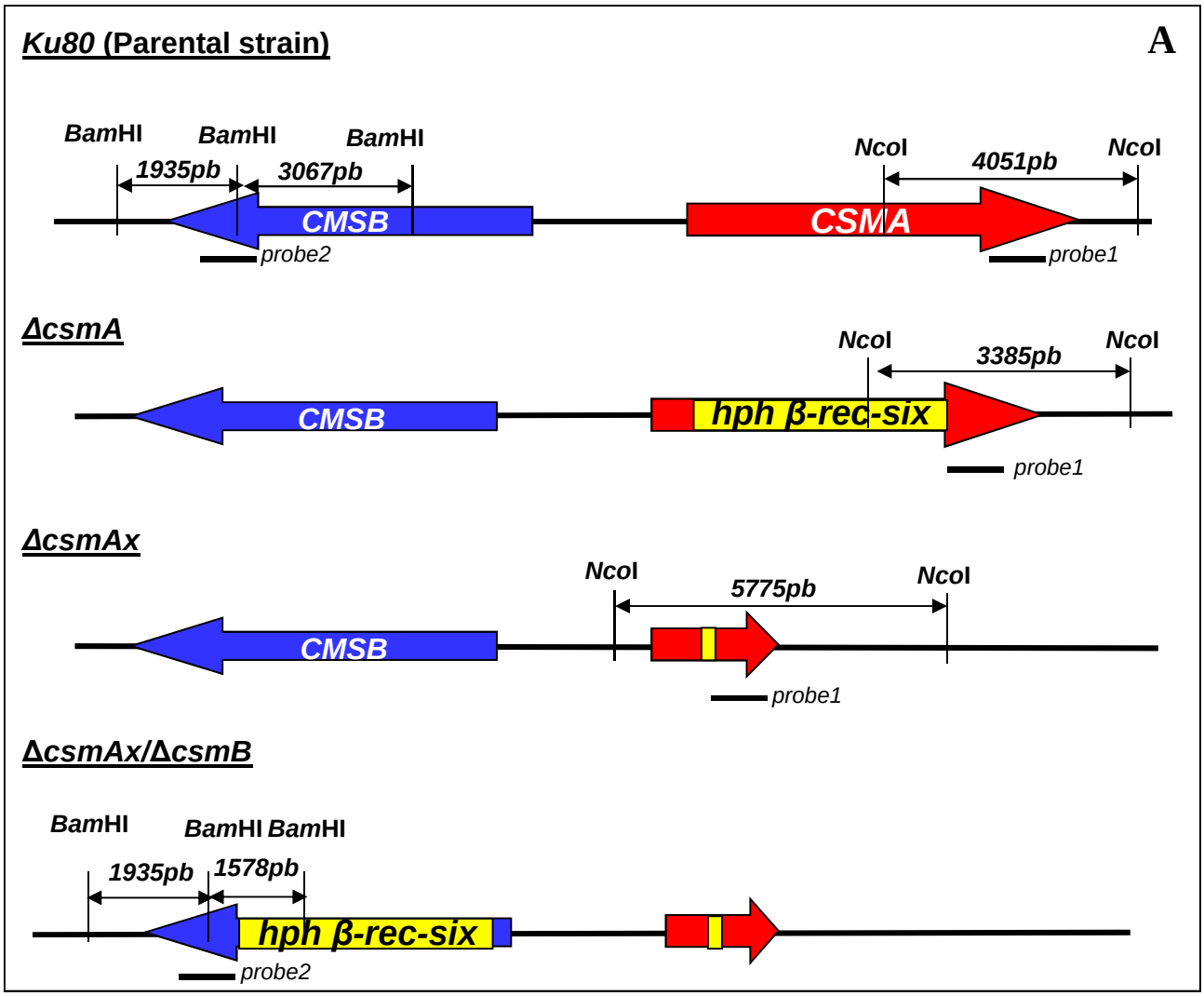

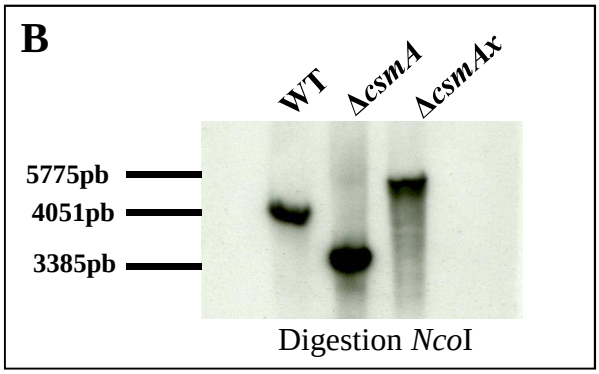

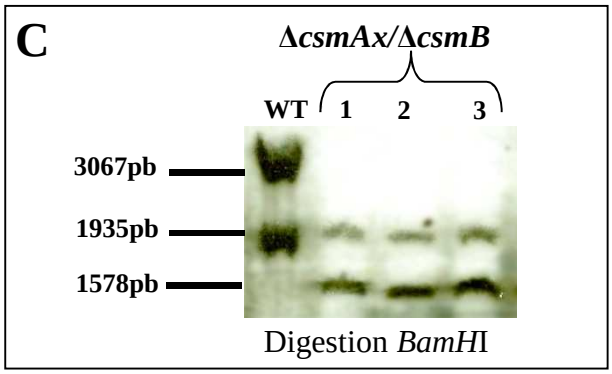

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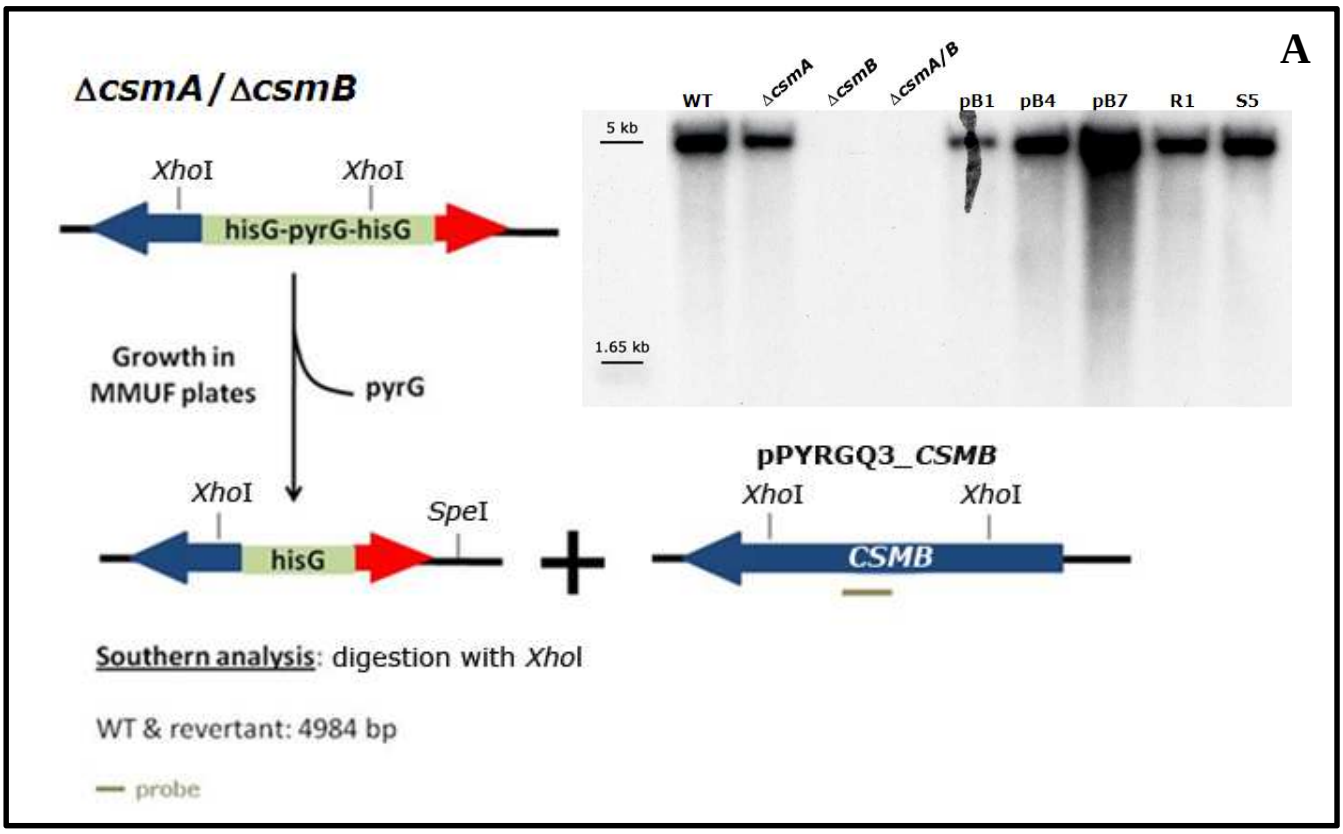

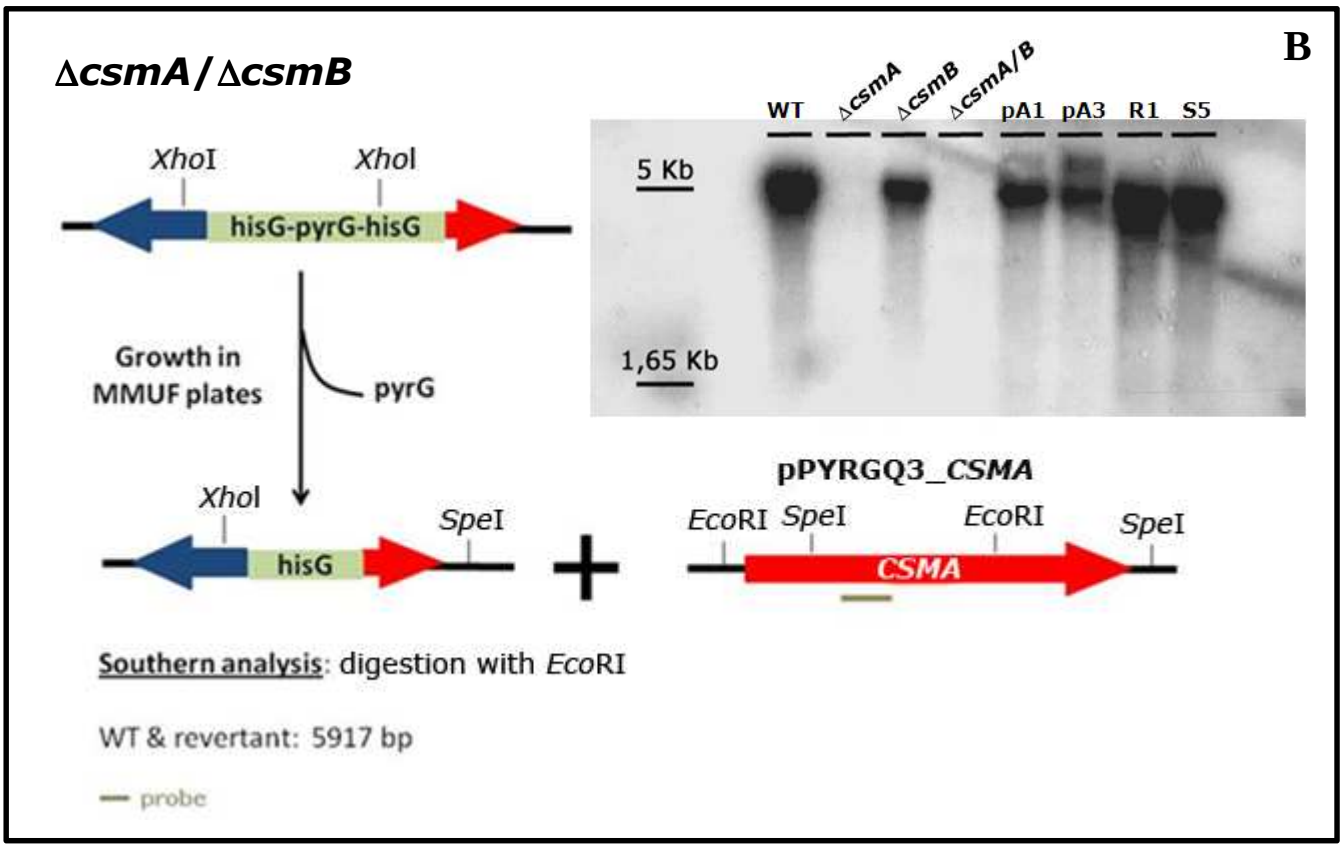

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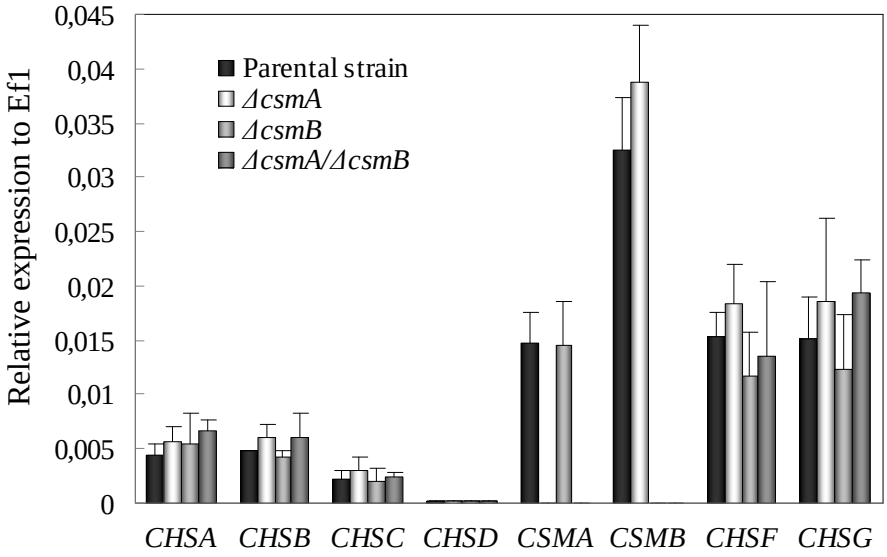

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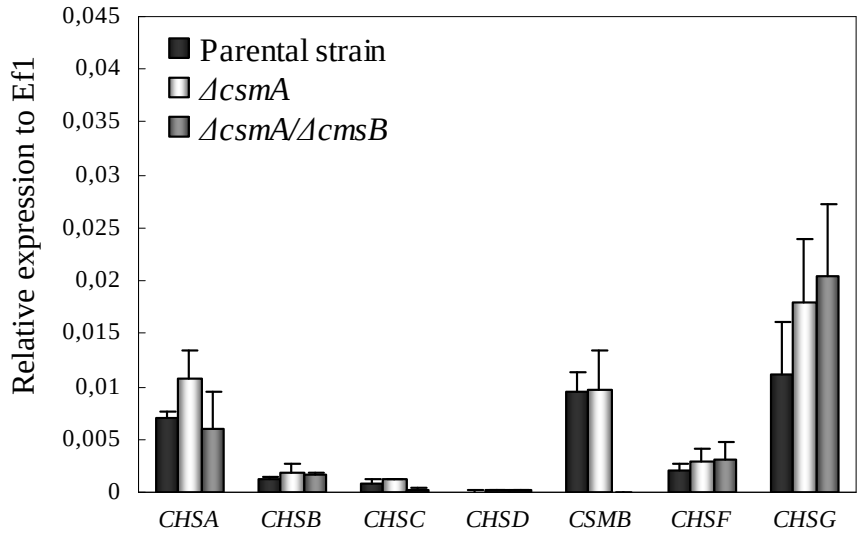

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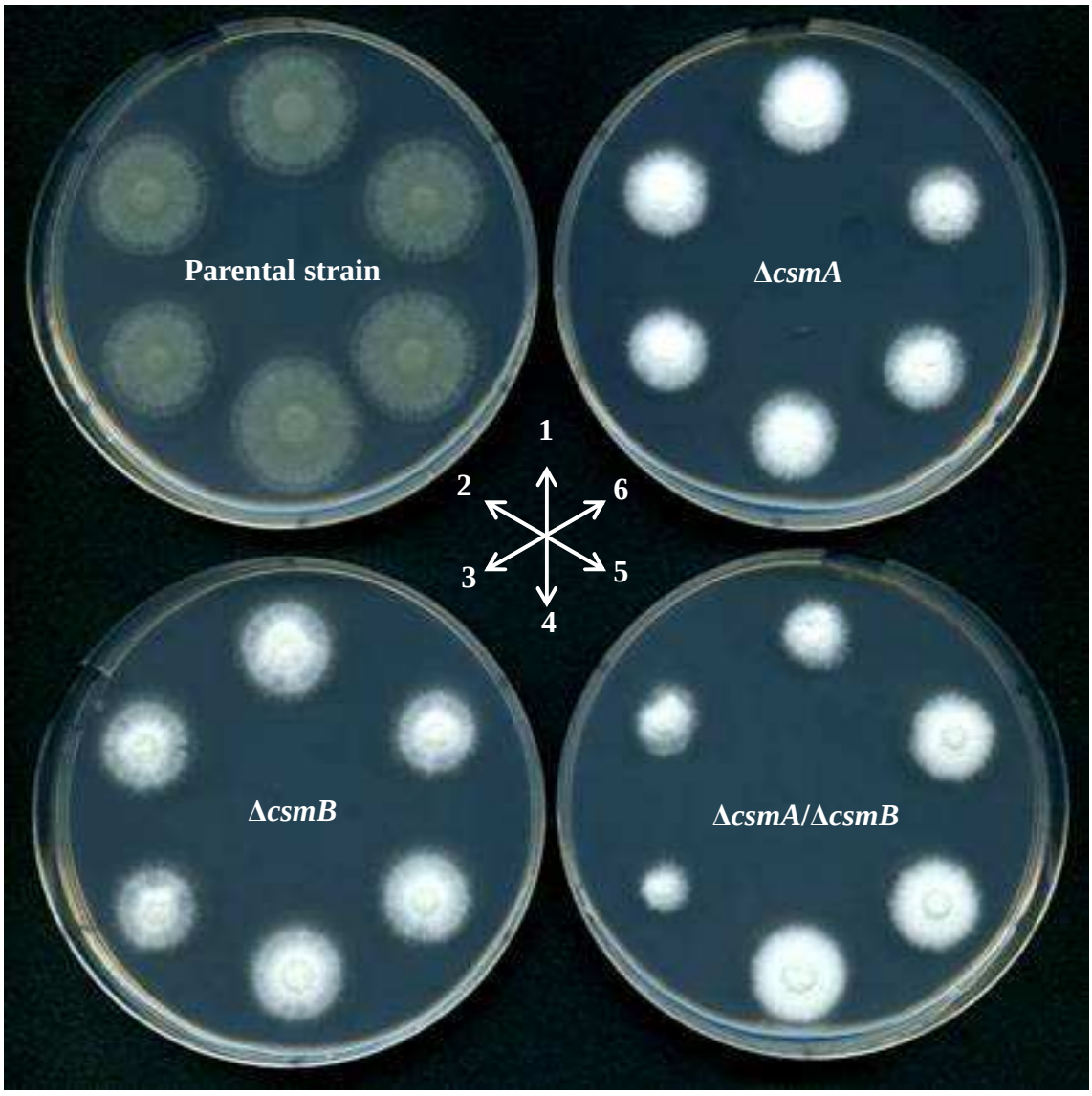
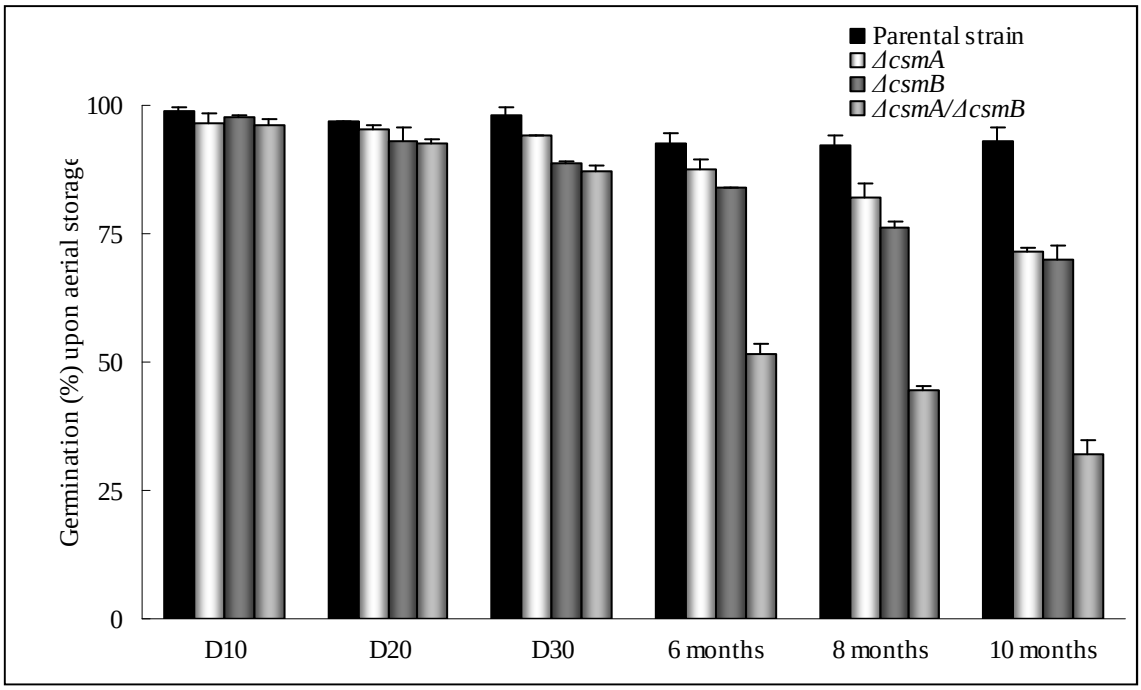
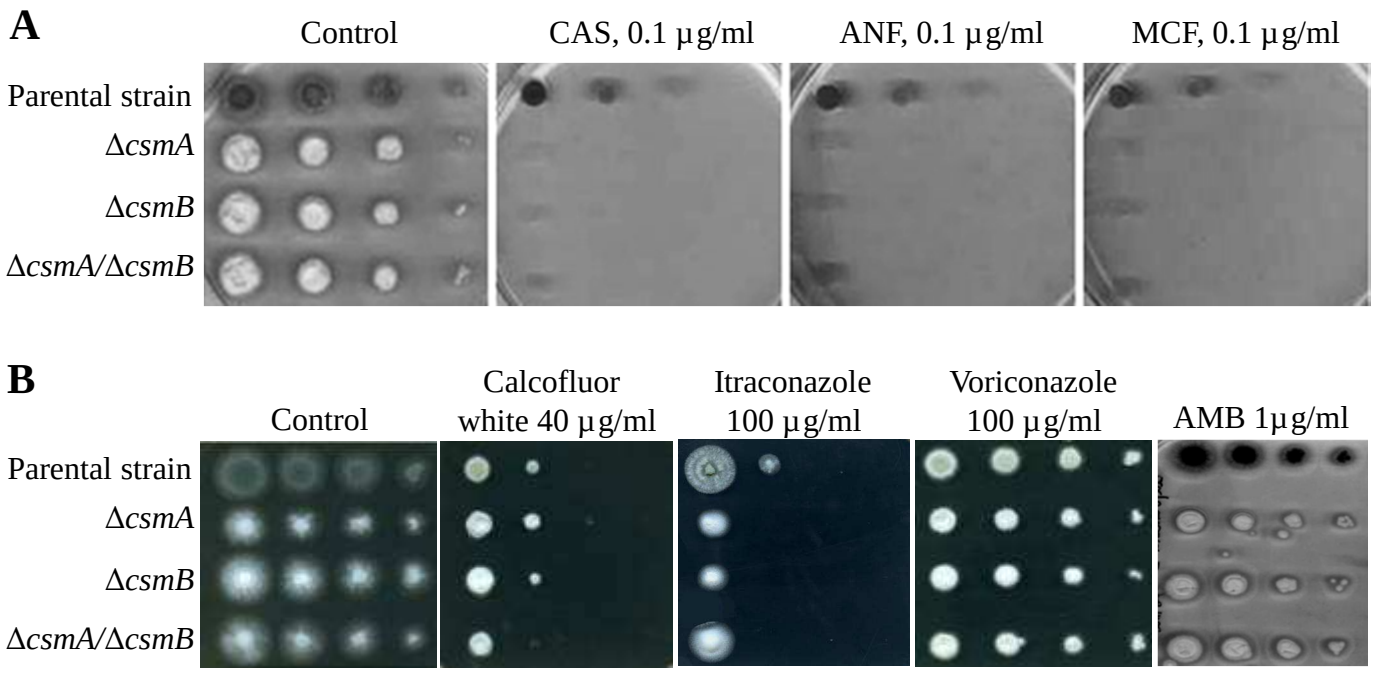




Supplemental Figure 8



Supplemental Figure 9