Table S1 Oligonucleotide primers used for qPCR

Name	Sequence
brlA-F	5′-TATCCAGACATTCAAGACGCACAG-3′
brlA-R	5′-GATAATAGAGGGCAAGTTCTCCAAAG-3′
abaA-F	5′-GAGTGGCAGACCGAATGTATGTTG-3′
abaA-R	5′-TAGTGGTAGGCATTGGGTGAGTTG-3′
aflA-F	5′-CCTATAAGTGCTTCAAAGATCGTGATCG-3′
aflA-R	5′-CGTACATGGATGACACGTTGTCCCAG-3′
aflC-F	5′-CCTATTCTAGCCGCCTTTCTTGAC-3′
aflC-R	5′-CATGTTGCCAGATTCCTCATATTCC-3′
aflD-F	5′-TGTATGCTCCCGTCCTACTGTTTC-3′
aflD-R	5′-TGTAGTCTCCTTAGTCGCTTCATC-3′
aflM-F	5′-GCGGAGAAAGTGGTTGAACAGATC-3′
aflM-R	5′-CAGCGAACAAAGGTGTCAATAGCC-3′
aflP-F	5′-CGATGTCTATCTTCTCCGATCTATTC-3′
aflP-R	5′-TCTCAGTCTCCAGTCTATTATCTACC-3′
aflR-F	5′-GCAACCTGATGACGACTGATATGG-3′
aflR-R	5′-TGCCAGCACCTTGAGAACGATAAG

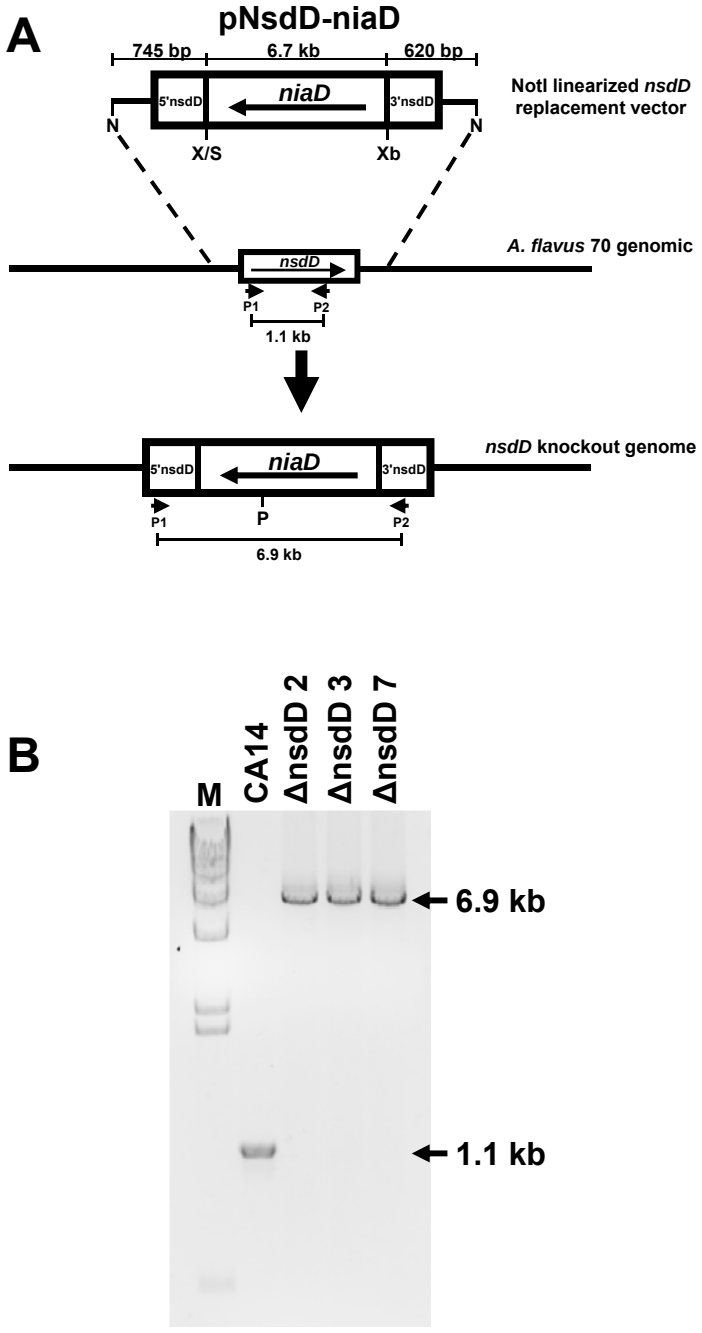

Fig. S1. Preparation of Δ*nsdD* knockout mutants. (A) Schematic diagram of the knockout vector pNsdD-*niaD* used to generate the Δ*nsdD* knockout mutants. The dashed lines show the region expected to undergo recombinational replacement of the wild-type DNA with DNA containing the *nsdD* gene disrupted by the *niaD* selectable marker gene. Direction of transcription is indicated by horizontal arrows. P1 and P2 denote oligonucleotide primers used to confirm identity of Δ*nsdD* transformants by PCR of genomic DNA. The lengths of expected PCR products of either wild-type CA14 or Δ*nsdD* transformant DNA are shown under horizontal lines. (B) Results of PCR of CA14 and putative Δ*nsdD* transformant DNAs. Primers P1 and P2 amplification of DNA from CA14 DNA generated a product of 1.1 kb that was the expected size for the wild-type *nsdD* gene. All three of the putative Δ*nsdD* mutants demonstrated a product of 6.9 kb that was of the expected size for recombinational inactivation of the *nsdD* gene by the pNsdD-*niaD* plasmid.

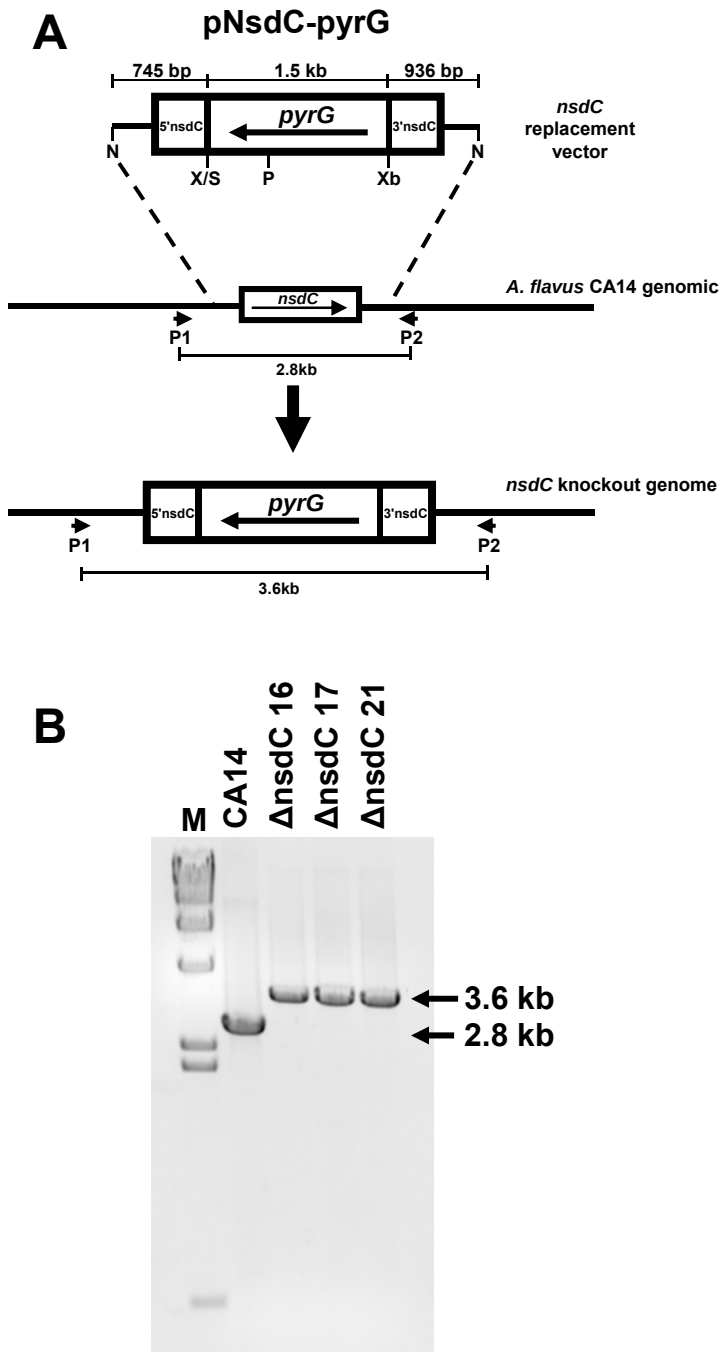

Fig. S2. Preparation of Δ*nsdC* knockout mutants. (A) Schematic diagram of the knockout vector pNsdC-pyrG used to generate the Δ*nsdC* knockout mutants. The dashed lines show the region expected to undergo recombinational replacement of the wild-type DNA with DNA containing the *nsdC* gene disrupted by the pyrG selectable marker gene. Direction of transcription is indicated by horizontal arrows. P1 and P2 denote oligonucleotide primers used to confirm identity of Δ*nsdC* transformants by PCR of genomic DNA. The lengths of expected PCR products of either wild-type CA14 or Δ*nsdC* transformant DNA are shown under horizontal lines. (B) Results of PCR of CA14 and putative Δ*nsdC* transformant DNAs. Primers P1 and P2 amplification of DNA from CA14 DNA generated a product of 2.8 kb that was the expected size for the wild-type *nsdC* gene. All three of the putative Δ*nsdC* mutants demonstrated a product of 3.6 kb that was of the expected size for recombinational inactivation of the *nsdC* gene by the pNsdC-pyrG plasmid.

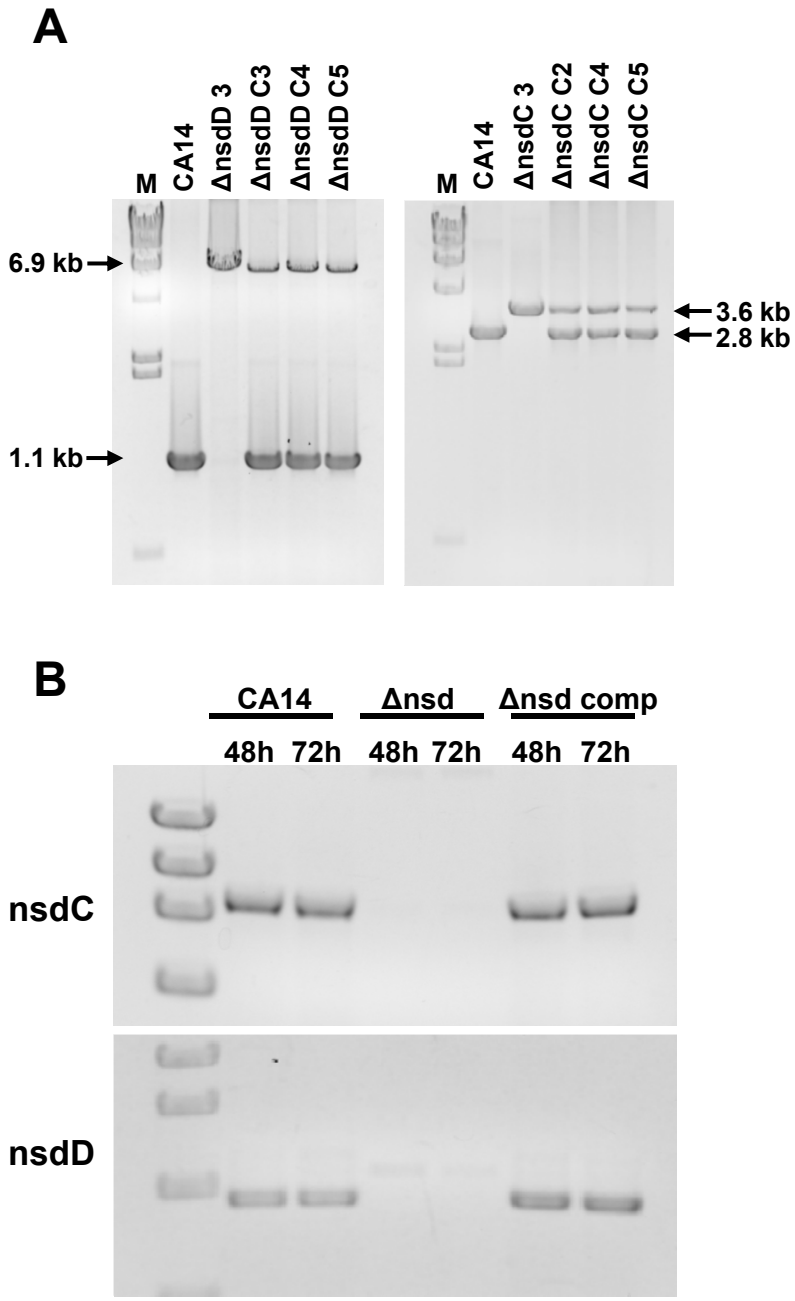

Fig. S3. PCR and RT-PCR analysis of Δ*nsd* complementation strains. (A) Results of PCR of genomic DNAs from the wild-type CA14, Δ*nsd* mutants, and Δ*nsd* complementation (comp) strains. All three of the Δ*nsdD* and Δ*nsdC* complementation strains demonstrated PCR products of 1.1 kb and 2.8 kb respectively, as was observed for the wild-type CA14 amplification product. (B) Expression of *nsdC* and *nsdD* from 48 and 72 h cultures of the CA14, Δ*nsd* mutants, and Δ*nsdC* C5 and Δ*nsdD* C4 complementation strains. Results of RT-PCR demonstrate that the Δ*nsd* complementation strains are generating their respective *nsd* transcripts as observed from PCR of the CA14 but not the Δ*nsd* mutant cDNAs.

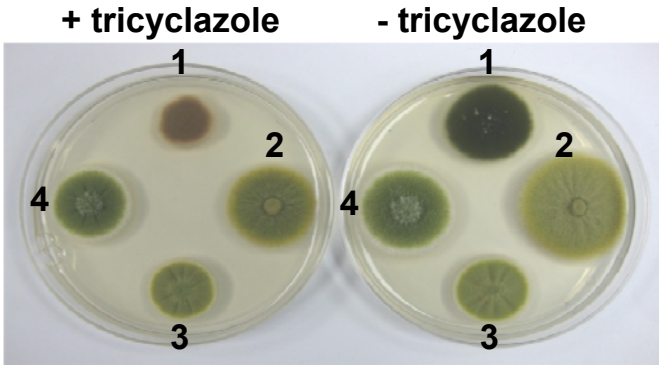

Fig. S4. Effect of the melanin biosynthesis inhibitor, tricyclazole, on pigment production in the Δ*nsd* mutants. 1) *A. alternata*; 2) CA14 Δ*nsdC* 17; 3) CA14 Δ*nsdD* 3; 4) CA14. Spores of the wild-type CA14, Δ*nsdC* 17 and Δ*nsdD* 3 mutants, and *Alternaria alternata* were point inoculated onto YGT-U agar supplemented with 100 µg/ml tricyclazole. Plates were incubated for 5 days at 30°C under illumination. Note that there is no significant change of pigmentation of the CA14 or Δ*nsd* strains in the presence of tricyclazole. However the *A. alternata* colony pigmentation has been altered from a black to reddish-brown color indicative of inhibition of melanin biosynthesis.

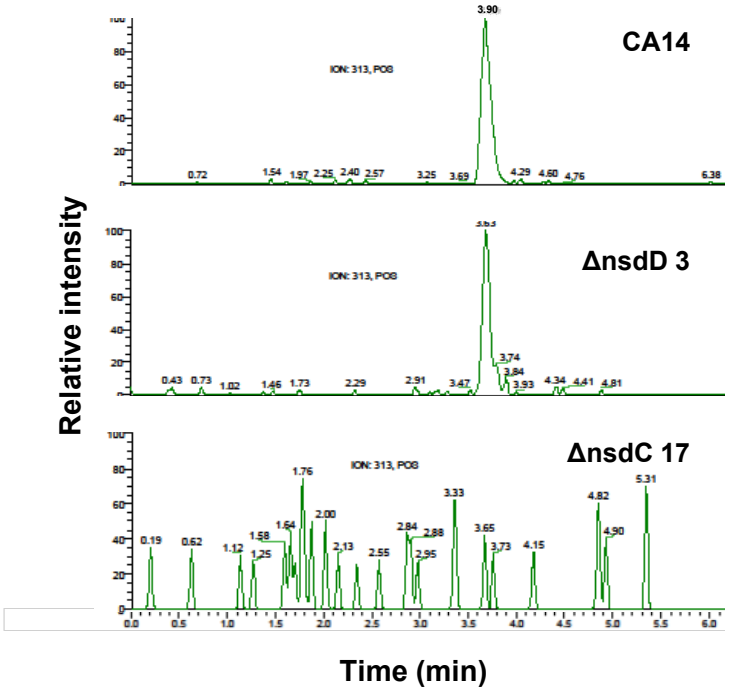

Fig. S5. LC/MS results confirming that little to no AFB1 (*m/z*=313 positive ion and *m/z* 311 neg ion) is present in extracts of the Δ*nsdC* strain but is present in the Δ*nsdD* mutant and the wild-type *A. flavus* CA14.