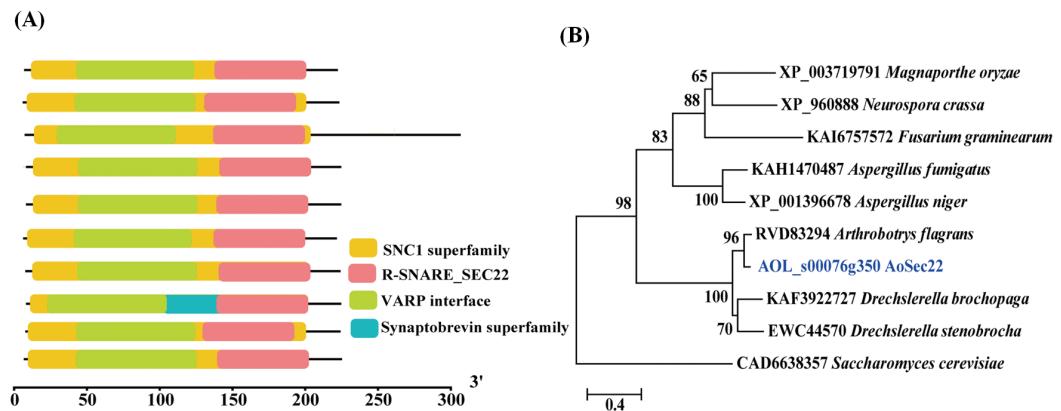
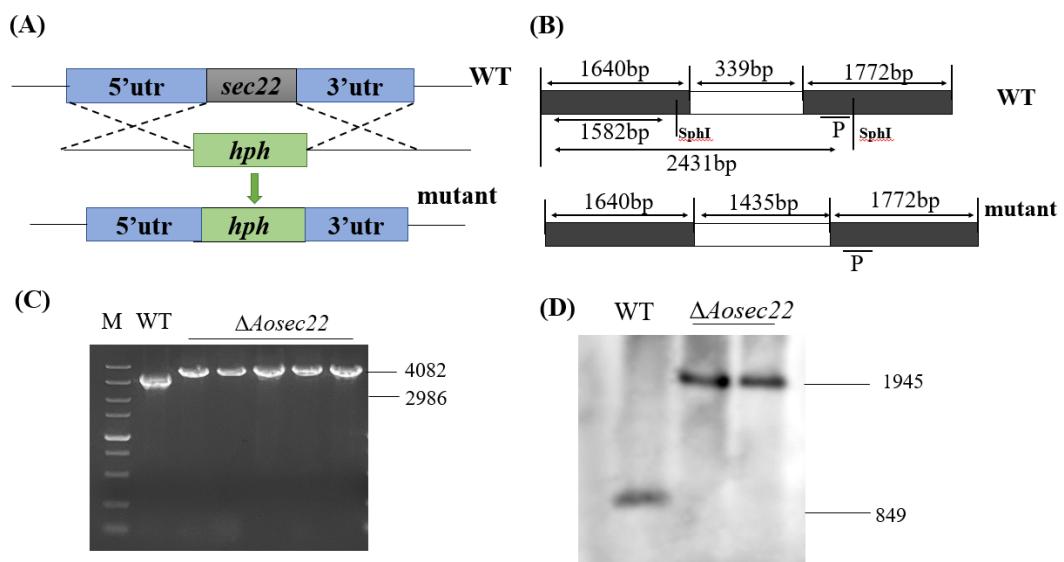


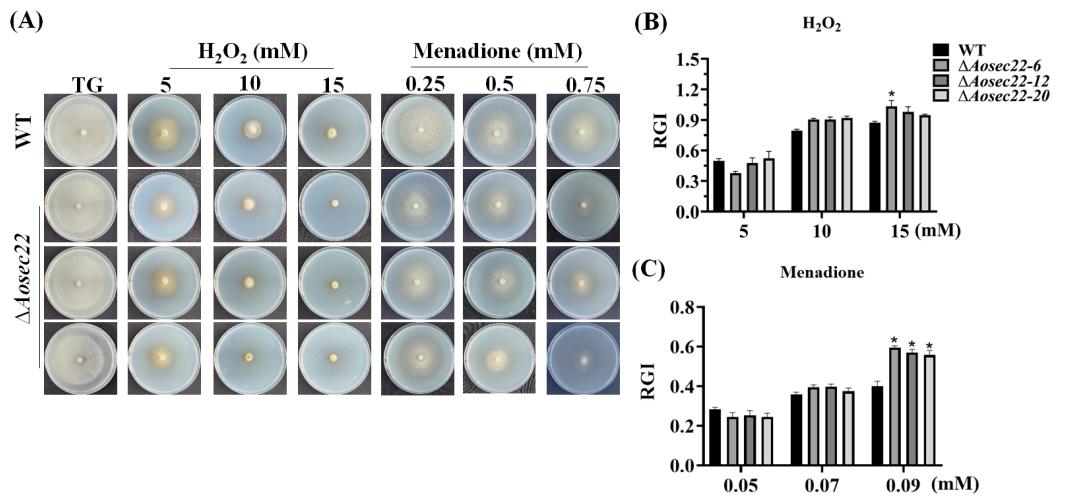
## Supporting Information



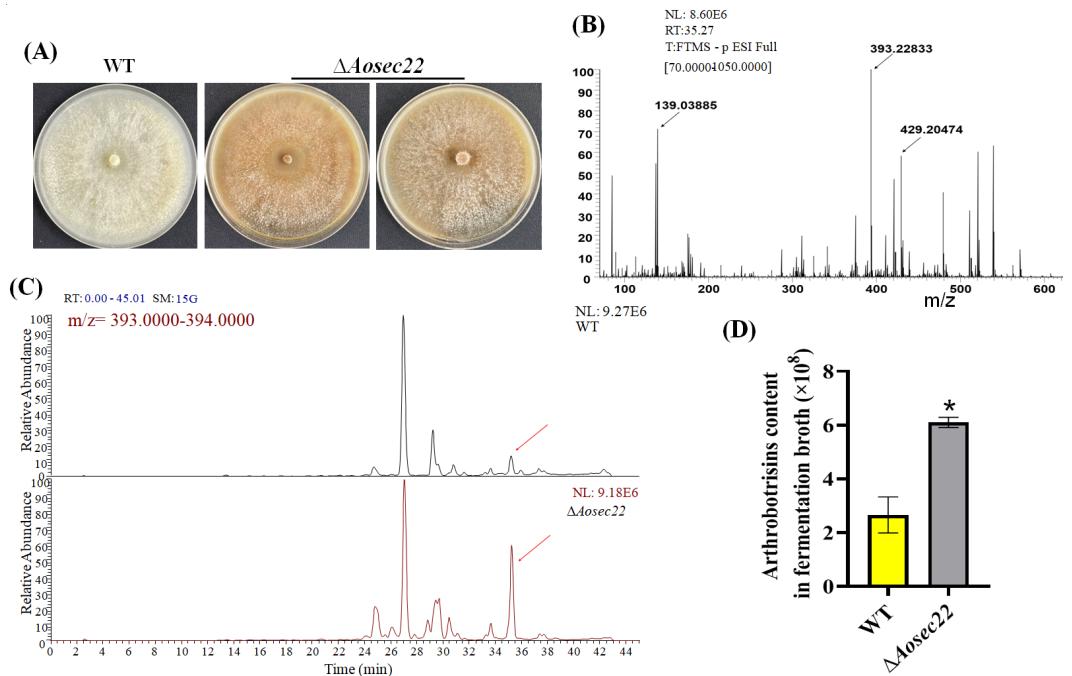
**Figure S1.** Multiple sequence alignment and phylogenetic analysis of WT and  $\Delta$ Aosec22 mutant strains. (A) Prediction of the functional domains of Sec22 proteins. (B) The phylogenetic tree of homologous Sec22 proteins from different fungi.



**Figure S2.** Knockout and verification of the genes *Aosec22* in *A. oligospora*. (A, B) Diagrammatic sketch of homologous recombination of gene *Aosec22*. The homologous flanking sequences of the target gene, Southern blot probe, and the restriction enzyme sites are marked. (C) Positive transformants verified by PCR amplification. M, the DNA marker; WT, the wild-type strain;  $\Delta$ Aosec22, the deletion mutants. (D) The  $\Delta$ Aosec22 mutants confirmed by Southern blots.



**Figure S3.** Comparison of oxidative stress responses between WT and  $\Delta\text{Aosec}22$  mutant strains. (A) Colonial morphology of fungal strains under oxidative stress. (B) Relative growth inhibition (RGI) of fungal colonies after being grown for 6 days at 28 °C on TG plates supplemented with different concentrations of  $\text{H}_2\text{O}_2$  and menadione. An asterisk indicates a significant difference between  $\Delta\text{Aosec}22$  mutant and the WT strain (Tukey's HSD,  $p < 0.05$ ).



**Figure S4.** Comparison of the color of colony and the content of arthrobotrisins. (A) The WT and mutant were incubated on PDA medium for 10 days. (B) Mass spectrogram of arthrobotrisins in the WT strain (diagnostic fragments ion at  $m/z$  139, 393, and 429). (C) Mass spectrogram of arthrobotrisins in the WT and  $\Delta\text{Aosec}22$  strains (diagnostic fragments ion at  $m/z$  393.0000-394.0000). RT = 35.12 min. (D) The quantification data for arthrobotrisins.

**Table S1.** List of primers used for gene manipulation in this study.

AoSec22-5F	GTAACGCCAGGGTTTCCCAGTCACGACGACTCCTGCTGATTGCCT	Amplify the <i>AoSec22</i>
AoSec22-5R	ATCCACTAACGTTACTGAAATCTCAACCTTGACTGCTGGAGAT	gene 5' flank
AoSec22-3F	CTCCTTCAATATCATCTCTGTCTCCGACCAAGGGTTATGACGAAGA	Amplify the <i>AoSec22</i>
AoSec22-3R	GCGGATAACAATTACACAGGAAACAGCTCCGAAGTTGCGTGTCT	gene 3' flank
Hph-f	GTCGGAGACAGAACAGATGATATTGAAGGAGC	Amplify the <i>hph</i>
Hph-r	GTTGGAGATTCAGTAACGTTAAGTGGAT	cassette
YZ-Sec22-F	GCACTCCAGACACCACCC	Verify the
YZ-Sec22-R	CAGCCACATAACCGTCCC	transformants
Probe-Sec22-F	CAAGGGTTATGACGAAGA	Make Southern
Probe-Sec22-R	CTGAGTCAGATCCGTTCC	blotting probe

Table S2. Paired primers for RT-qPCR analysis of genes associated with phenotypes such as conidiation and fatty acid oxidation in *A. oligospora*.

Description	Gene name	Sequence (5'-3')
	AOL_s00007g157 ( <i>fbc</i> )	fbc-5F-CTCTCCGGCAAAGACAATCG fbc-3R-GTCGACTGAGGATAGTAGCT
	AOL_s00043g361 ( <i>fluG</i> )	fluG-5F-GATTCCAGTCCCGTGAATT fluG-3R-GCTAAGGAGAGGATGGGCAT
	AOL_s00080g63 ( <i>abaA</i> )	abaA-5F-AACTTTATGCGCCTTGT abaA-3R-TTGGCTAGGTGGCTGTACG
	AOL_s00054g811 ( <i>velB</i> )	velB-5F-ATTCGCAACTTCTCCCTCA velB-3R-GGCATTTGGATTCTGGGG
Sporulation-related genes	AOL_s00097g514 ( <i>brlA</i> )	brlA-5F-AACTCCATACCATCCGTA brlA-5R-CAGGATATTCCGGCACTCA
	AOL_s00173g221 ( <i>wetA</i> )	wetA-5F-CCCTGTGCTACTATTGCTAC wetA-5R-CCGTTGCGAGCATTCTT
β-tubulin gene	AOL_s00076g640 ( <i>tub</i> )	tubA-F-CCACCTTCGTCGGTAAC tubA-R-TCGTCCATACCCCTCACAG
	AOL_s00004g288	288-5F-AAGAAAATCCCACCTCAGAGAGG 288-3R-TACGTGTCCAGTAACATAGCTC
	AOL_s00081g51	51-5F-GCCGATCCTTACCAAATCATTC 51-3R-CCAATTCTTCCGTAGCTGAG
	AOL_s00210g122	122-5F-GCCGCACATATTGTTAACAGAT 122-3R-TGATCTTGCTGTTCTCAGTCAT
Genes related to fatty acid oxidation	AOL_s00110g113	113-5F-CTAACAGAAACTCAAGCATCGG 113-3R-GGAACCGGATTCATGAAATGAG
	AOL_s00079g276	276-5F-AACAATCCGTCGTTATTGTTCC 276-3R-GCGATCATGTAGTCTAGTCCTC
	AOL_s00054g29	29-5F-GGTATCTACGGAAATTGGCC 29-3R-GTGCAATATAATCGGGCTTGAG
	AOL_s00004g606	606-5F-TTCGGATTCTGTTATTACCTCCC 606-3R-TAACATGAGTCGCTTGTGTTGTG