

Identification and functional characterization of a putative alternative oxidase (AOX) in *Sporisorium reilianum* f. sp. *zeae*

Hector Mendoza ¹, Caroline D. Culver ¹, Emma A. Lamb ¹, Luke A. Schroeder ¹, Sunita Khanal ² and Michael H. Perlin ^{1,*}

¹Department of Biology, Program on Disease Evolution, University of Louisville, Louisville, KY, USA;

²Division of Cardiovascular Medicine, School of Medicine, University of Maryland, Baltimore, MD;

*Correspondence: michael.perlin@louisville.edu

Supporting Information

Figure S1 – Growth inhibition assay of complemented SRZ Δ aox strains

Figure S2 - Fold change differences of AOX expression in teliospores and haploid cells of SRZ in reference to SRZ2

Table S1 – Strains used and generated in this study

Table S2 – Primers used in this study

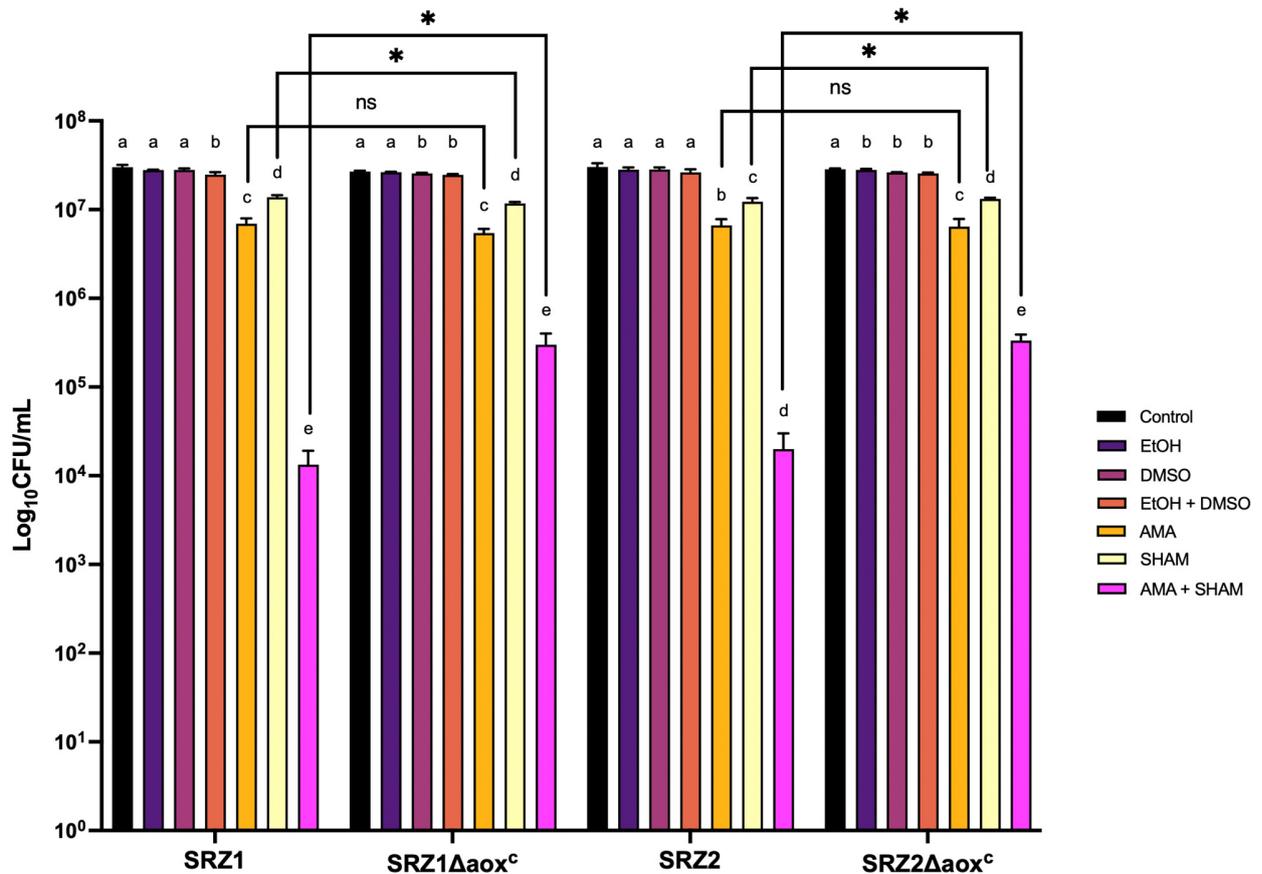
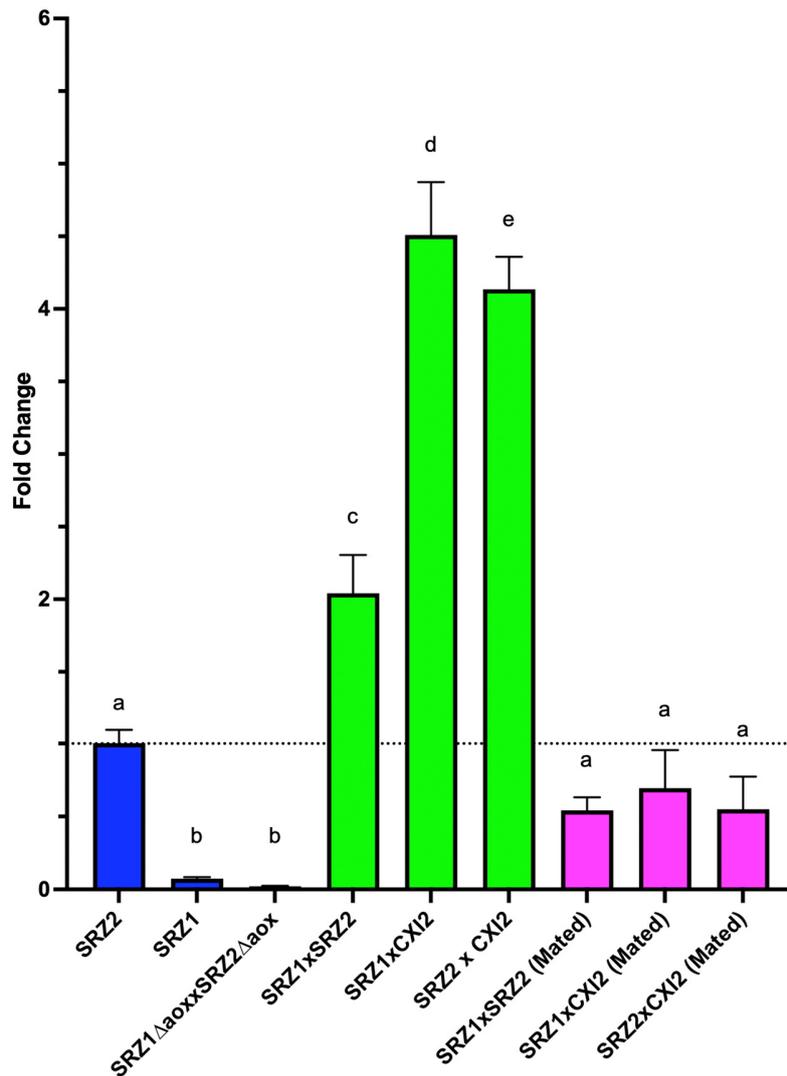


Figure S1: Growth inhibition assay of complemented SRZ Δ aox strains. 10^5 cells/mL were treated as indicated on the right and incubated at 28°C for 24 hours. Control groups consisted of untreated cells grown in PD broth. AMA and SHAM were used at concentrations of 50 μ M and 2 mM, respectively. Cultures were then plated onto PD agar to determine the number of surviving colonies. Bars represent averages of biological triplicates with standard errors indicated. One-way ANOVA followed by Tukey's Multiple Comparison Test was performed in Graphpad 9.0. Letters above bars represent significant differences ($p < 0.05$) between the different treatments in reference to the corresponding control group of each strain. Comparisons of treatments between different strains are indicated by connecting black line brackets, with $p < 0.05$ represented by "*" (significant) and $p > 0.05$ represented by "ns" (not significant).



S2 – Fold change differences of *aox* expression in teliospores and haploid cells of SRZ in reference to SRZ2. Analysis was done relative to *gapdh* expression as endogenous control. Relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method and were done in reference to strain SRZ2. Teliospores produced in a cross between *aox* deletion mutants and mated cells were included as controls. Colored bars correspond to cell type analyzed: blue = haploid cells, green = teliospores and pink = mated cells. One-way ANOVA followed by Tukey's Multiple Comparison Test was performed in Graphpad 9.0. Significant differences are indicated with letters above bars ($p < 0.05$).

Table S1 – SRZ strains used or generated in this study.

| Strain | Genotype | Selectable marker | Source |
|-------------------------|---------------------------|-------------------|--|
| SRZ2 | a2b2 | WT | [2] |
| SRZ1 | a1b1 | WT | [2] |
| SRZCXI2 | a3b3 | WT | Unpublished, from teliospores collected in China |
| SRZ2 Δ aox #1 | a2b2 Δ aox::hph | Hygromycin | This study |
| SRZ1 Δ aox #2 | a1b1 Δ aox::hph | Hygromycin | This study |
| SRZCXI2 Δ aox #4 | a3b3 Δ aox::hph | Hygromycin | This study |
| SRZ2 Δ aoxc #1 | a2b2 Δ aox::aoxsdh | Carboxin | This study |
| SRZ1 Δ aoxc #1 | a2b2 Δ aox::aoxsdh | Carboxin | This study |
| SRZ2aox-eGFP #1 | a2b2aox::hph | Hygromycin | This study |
| SRZ2aox-eGFP #1 | a1b1aox::hpc | Hygromycin | This study |

Table 2 – Primers used in this study.

| Running # | Sequence (5'→3') | Amplicon |
|-----------|-------------------------------------|--|
| oHM33 | ACCATCCCTCTAAAACGACGGCCAGTGAAT | <i>hph</i> (2027 bp) |
| oHM34 | AGTTTCGATTCTGTGGAATTGTGAGCGGATA | |
| oHM35 | CGTCGTTTTAGAGGGATGGTTGTGAAATGG | Upstream flanking region of <i>aox</i> (1285 bp) |
| oHM36 | TTTTGATATCAAGCATGGTGACGAGGAGAT | |
| oHM37 | CAATTCCACAGAATCGAACTGGCGAATGTC | Downstream flanking region of <i>aox</i> (1270 bp) |
| oHM38 | TTCAATATTAATTAAGGTGATGAAGGAACGAACG | |
| oHM39 | CATGCTTGATATCAAAAGGCCGCGTTGCTG | <i>amp^r</i> , <i>ori</i> (1688 bp) |
| oHM40 | ATCACCTTAATTAATATTGAAAAAGGAAGAG | |
| oHM61 | GAAAAGACCGTGGCTCTCC | <i>aox</i> for qRT-PCR |
| oHM62 | GTGCTTCACTGGCATCGTC | |
| oHM70 | TTTTGATATCCGACTTTTCGGGTGATTTTC | For AOX-eGFP fusion construct |
| oHM102 | GAAGACCGCCATGGTGAGCAAGGGCGAG | |
| oHM103 | CGTCGTTTTATTCTTGTGATTCGGGGACTC | |
| oHM104 | TGCTCACCATGGCGGTCTTCTCAGCAGC | |
| oHM105 | ATCACAAGAATAAAACGACGGCCAGTGAAT | |
| oHM146 | CGAAAAGTCGGATATCAAAAGGCCGCGTTG | For AOX complementation construct |
| oHM147 | TTTTGATATCCGACTTTTCGGGTGATTTTC | |
| oHM148 | GCTCGATATTGGTGGTAAGGGTATCGGACA | |
| oHM149 | CCTTACCACCAATATCGAGCACGTTGATGG | |
| oHM155 | CCACAATCGTGAATCGAACTGGCGAATGTC | |
| oHM156 | AGTTTCGATTCACGATTGTGGCGAATCGCGG | <i>gapdh</i> for qRT-PCR[1] |
| oHM30 | TTCAATATTAATTAAGGTATGCCTCAGCTCAAAGG | |
| oYZ58 | GGATTTTCATCGGCAACTCAC | |
| oYZ59 | TACCACGAGACGAGCTTGAC | |