

**The Regulatory Effect of Nitrogen Metabolism Regulator AreB on
Lipid Accumulation in Oleaginous Fungus *Mucor circinelloides***

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Table S1. Primers and their sequences used in this study.

Primers	Sequences (5'-3')	annotation
<i>areB</i> -out-F	CGTGAGCAAACGATGAAGATAATG	PCR for <i>areB</i> knockout checking
<i>areB</i> -out-R	CTTCATTCCTCTGAGTTCGTTGTG	PCR for <i>areB</i> knockout checking
<i>CarRP</i> -out-F	GAGGCAAGAGATTGTGATAAGC	PCR for <i>areB</i> overexpression checking
<i>CarRP</i> -out-R	GTATCTGACATAGTCGAGCTTG	PCR for <i>areB</i> overexpression checking
actin-F	GATGAAGCCCAATCCAAGA	RT-qPCR for <i>actin</i> gene
actin-R	TTCTCACGGTTGGACTTGG	RT-qPCR for <i>actin</i> gene
<i>areB</i> -RT-F	GTTGGCTATTTTGATCAGGTAAGAGC	RT-qPCR for <i>areB</i> gene
<i>areB</i> -RT-R	CAGCAGGTTTTGAACTTACTTTGCG	RT-qPCR for <i>areB</i> gene
<i>areA</i> -RT-F	GTCCCAAGATGCGTTATTGACG	RT-qPCR for <i>areA</i> gene
<i>areA</i> -RT-R	CATCGCCCTCCAATGTACCTAC	RT-qPCR for <i>areA</i> gene
<i>acl</i> -RT-F	GGAAAAGAAATCAAGATTGAAAGAACGACTG	RT-qPCR for <i>acl</i> gene
<i>acl</i> -RT-R	AGCCTTGGCATCAACATCACC	RT-qPCR for <i>acl</i> gene
<i>acc1</i> -RT-F	GATATGCATTCCCGTGTTCCG	RT-qPCR for <i>acc1</i> gene
<i>acc1</i> -RT-R	ACCGAGTAATTCATCAGCTGGG	RT-qPCR for <i>acc1</i> gene
<i>acc2</i> -RT-F	GCTGAAGCTGGTGGTATTGCC	RT-qPCR for <i>acc2</i> gene
<i>acc2</i> -RT-R	GATACGAGCATTAGGCCACATC	RT-qPCR for <i>acc2</i> gene
<i>g6pdh1</i> -RT-F	ATTGTTTCACAGAGGAAGAGACTTTCC	RT-qPCR for <i>g6pdh1</i> gene
<i>g6pdh1</i> -RT-R	TGTTATCAATGTTGGAACGGTTCAG	RT-qPCR for <i>g6pdh1</i> gene
<i>g6pdh2</i> -RT-F	TGTCATGCAAAATCATTGCTGCAG	RT-qPCR for <i>g6pdh2</i> gene

<i>g6pdh2</i> -RT-R	GACATACTGTCCCAAGAGAGAATCTTCC	RT-qPCR for <i>g6pdh2</i> gene
<i>g6pdh3</i> -RT-F	TGCACTCTTCACTGAAAATGAGATTATCG	RT-qPCR for <i>g6pdh3</i> gene
<i>g6pdh3</i> -RT-R	TTATCAATATAGGTGCGATCCCATGC	RT-qPCR for <i>g6pdh3</i> gene
<i>6pgdh1</i> -RT-F	GATGGTTCACAACGGTATTGAATACGGA	RT-qPCR for <i>6pgdh1</i> gene
<i>6pgdh1</i> -RT-R	CGATCAAGAAAGAATCCAATTCACCCTTG	RT-qPCR for <i>6pgdh1</i> gene
<i>6pgdh2</i> -RT-F	GCACAACGGTATTGAGTACGGC	RT-qPCR for <i>6pgdh2</i> gene
<i>6pgdh2</i> -RT-R	CCCTTATTCCATTTCATCAAAGACATC	RT-qPCR for <i>6pgdh2</i> gene
<i>cme1</i> -RT-F	TGATTCCAAGGGTCTTGTTACTACTACACG	RT-qPCR for <i>cme1</i> gene
<i>cme1</i> -RT-R	GGACTTGAGTATTAAAGGCACCTGTAGTAGATG	RT-qPCR for <i>cme1</i> gene
<i>cme2</i> -RT-F	TTCCAAGGGTCTTG TGACAATGAACC	RT-qPCR for <i>cme2</i> gene
<i>cme2</i> -RT-R	GTGTGTTGAATGCGTTAGGTGTAGAGG	RT-qPCR for <i>cme2</i> gene
<i>fas1</i> -RT-F	GGTCTTCGTTTCATTGACCCCACTG	RT-qPCR for <i>fas1</i> gene
<i>fas1</i> -RT-R	GACATCAGCCTTGTCACCTTGTTGG	RT-qPCR for <i>fas1</i> gene
<i>fas2</i> -RT-F	ACTGGTATCCGTTTCATTGAGCCTG	RT-qPCR for <i>fas2</i> gene
<i>fas2</i> -RT-R	AAATGTCTGCCTTTTCTCCCTGTTGA	RT-qPCR for <i>fas2</i> gene

Table S3. List of abbreviations used in Figure 7.

Abbreviation	Description
GLC	glucose
G6P	glucose 6-phosphate
F6P	Fructose 6-phosphate
G3P	glyceraldehyde 3-phosphate
6PG	6-phosphogluconate
Ru5P	ribulose 5-phosphate
PYR	pyruvate
AcCoA	acetyl coenzyme A
CIT	citrate
ISOCIT	isocitrate
AKG	α -ketoglutarate
SuCoA	succinyl coenzyme A
SUC	succinate
FUM	fumarate
MAL	malate
OAA	oxaloacetate
MaCoA	malonyl coenzyme A
Pal	palmitic acid
Ste	stearic acid
Ole	oleic acid
Lin	linoleic acid
γ Lin	γ -linolenic acid
DHAP	dihydroxyacetonephosphate
LPA	lysophosphatidic acid
PA	phosphatidic acid
DAG	diacylglycerol
TAG	triacylglycerol
FA	fatty acid
Orn	Ornithine
Cit	Citrulline
ASA	Argininosuccinate
Arg	Arginine
GLU	glutamate
GLN	glutamine
G6PDH	glucose-6-phosphate dehydrogenase
6PGDH	6-phosphogluconate dehydrogenase
ME	malic nzyme
CS	citrate synthase
ACO	aconitase
ICDH	isocitrate dehydrogenase
KDH	2-ketoglutarate dehydrogenase
SUCLG	succinyl-CoA ligase

SDH	succinate dehydrogenase
FH	fumarate hydratase
MDH	malate dehydrogenase
FAS	fatty acid synthase
ELO	fatty acid elongase
$\Delta 9$	fatty acid delta 9 desaturase
$\Delta 12$	fatty acid delta 12 desaturase
$\Delta 6$	fatty acid delta 6 desaturase
ACSL	acyl-CoA synthetase
GDH	glutamate dehydrogenase
GLT	glutamate synthase
GS	glutamine synthetase
NR	nitrate reductase
OTC	Ornithine transcarbamoylase
ASS	Argininosuccinate synthetase
ASL	Argininosuccinate lyase
ARG	Arginase

Figure S1. The map of *areB*-knockout (pCRC172) and *areB*-overexpression (pCRC173) plasmids.

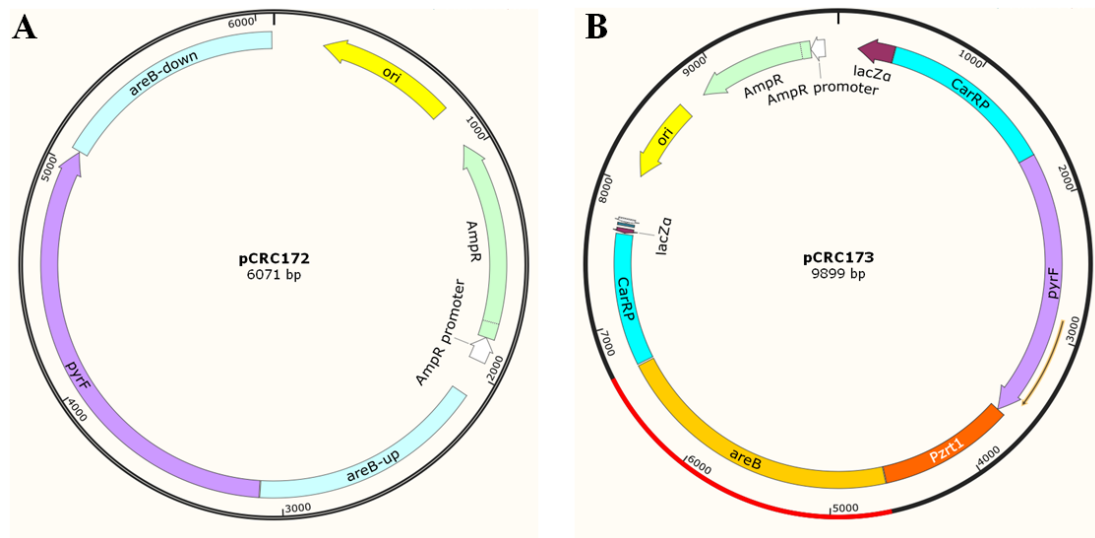


Figure S2. Sequence analysis of AreB in *M. circinelloides*.

(A) The amino acid sequence of *M. circinelloides* AreB. The conserved GATA-type zinc finger domain (Cys-X₂-Cys-X₁₇-Cys-X₂-Cys) is underlined. (B) Multiple sequence alignment of fungal GATA factors: Asd4 from *Neurospora crassa* (AAG45180.1), AreB from *Aspergillus nidulans* (AAG49353.1), Gzf3 from *Saccharomyces cerevisiae* (CAA89405.1), and Dal80 from *Saccharomyces cerevisiae* (CAA82107.1).

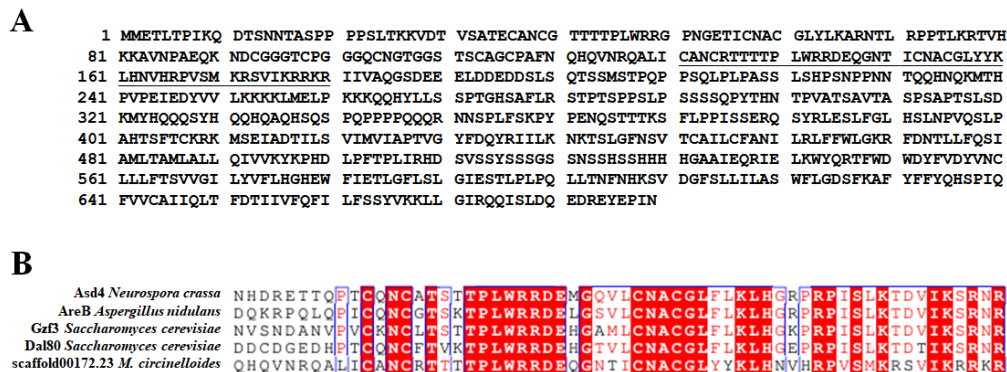


Figure S3. The hydrophilicity and hydrophobicity profile of the deduced AreB.

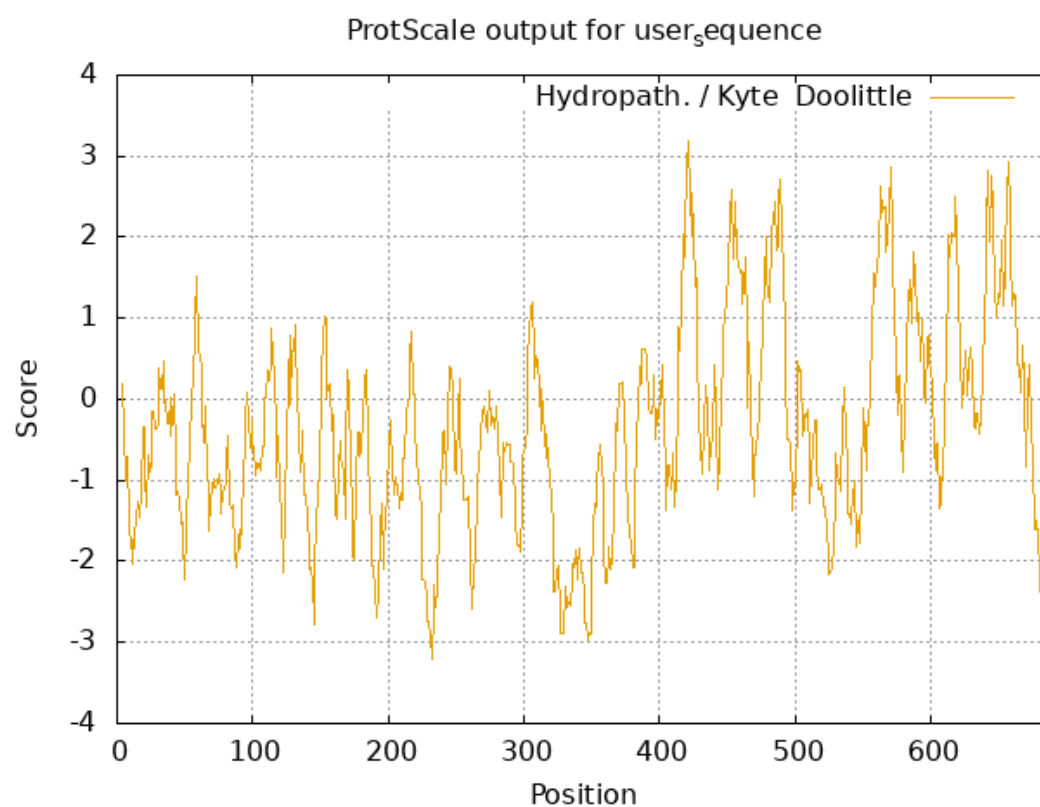


Figure S4. PCR verification of the Δ areB and OE-areB strains.

(A) Schematic of homologous recombination for *areB* gene knockout. The native *areB* locus (7.6 kb) was replaced by the knockout cassette, yielding a 4.7 kb fragment in the Δ areB strain. (B) PCR verification of the Δ areB strain. M: DNA marker; lane 1: WT control strain; lanes 2-4: the Δ areB transformants. (C) Schematic of homologous recombination for *areB* gene overexpression at the *carRP* locus. The native *carRP* gene was replaced by the overexpression cassette, producing a 7.6 kb fragment in the OE-areB strain. (D) PCR verification of the OE-areB strain. M: DNA marker; lanes 1-7: the OE-areB transformants. Three positive Δ areB transformants and seven positive OE-areB transformants were obtained. One positive clone from each construct was selected for subsequent experiments, and the WT strain was used as the control.

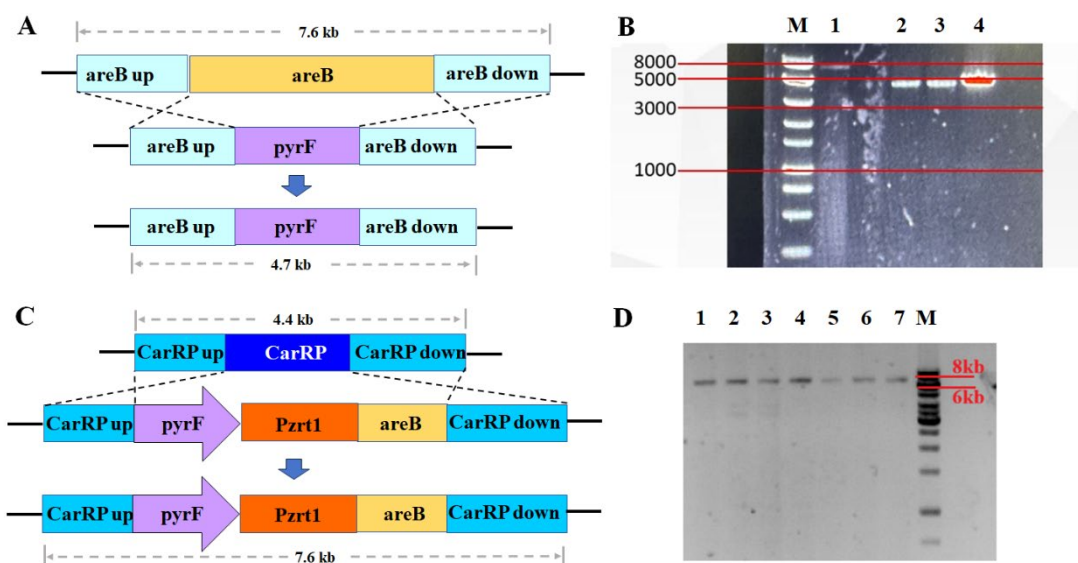
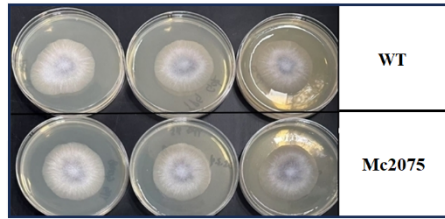


Figure S5. Phenotypic comparison of the WT and Mc2075 strains.

(A) Colony morphology of the WT and Mc2075 strains cultured on YPG agar medium for 72 h. (B) Biomass and total fatty acid content of the WT and Mc2075 strains after 72 h of fermentation.

A



B

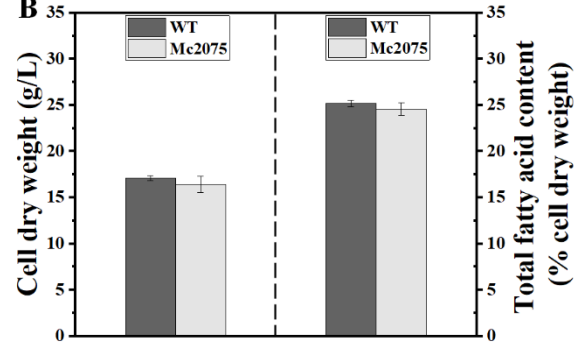


Figure S6. Principal component analysis (PCA) of transcriptomic data from the WT and Δ areB strains. QareB: areB-knockout strains; Qck: wild-type strains.

