

**A novel stress response mechanism, triggered by indole, involved in
quorum quenching enzyme MomL and iron-sulfur cluster in
*Muricauda olearia Th120***

Yan Wang¹, Hui Li¹, Xinxin Cui¹, Xiao-Hua Zhang^{1,2*}

1. College of Marine Life Sciences, Ocean University of China, Qingdao, 266003, China

*2. Laboratory for Marine Ecology and Environmental Science, Qingdao National Laboratory for Marine Science
and Technology, Qingdao 266071, China*

** Author to whom correspondence should be addressed; E-Mail: xhzhang@ouc.edu.cn (X.-H.Z.); Tel/Fax:
+86-532-82032767.*

Running title: Study of a novel stress response mechanism

Figure S1. The OD₆₀₀ value comparison between with (black) or without indole (transparent) conditions. Error bars represent the standard deviations of three replicates. For statistical analysis, ***/ ** /* means P<0.001, P<0.01, and P<0.05, respectively.

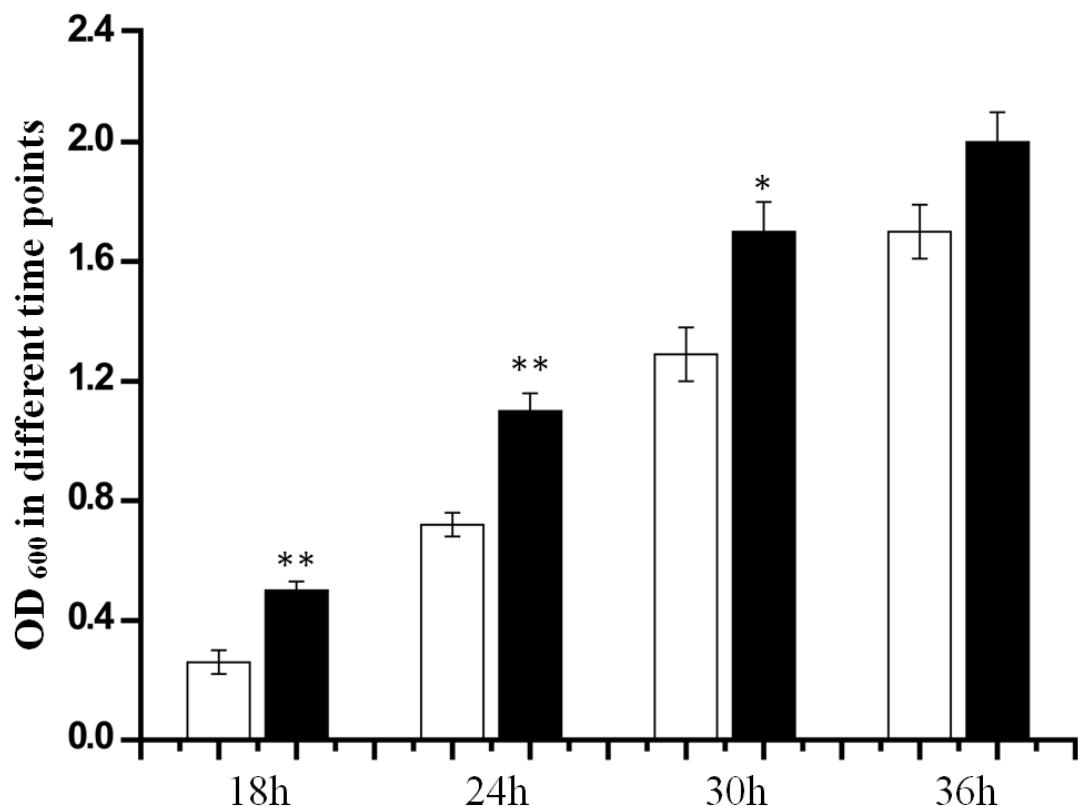


Figure S2. The gene cluster of *suf-momL* of *Muricauda olearia* Th120.

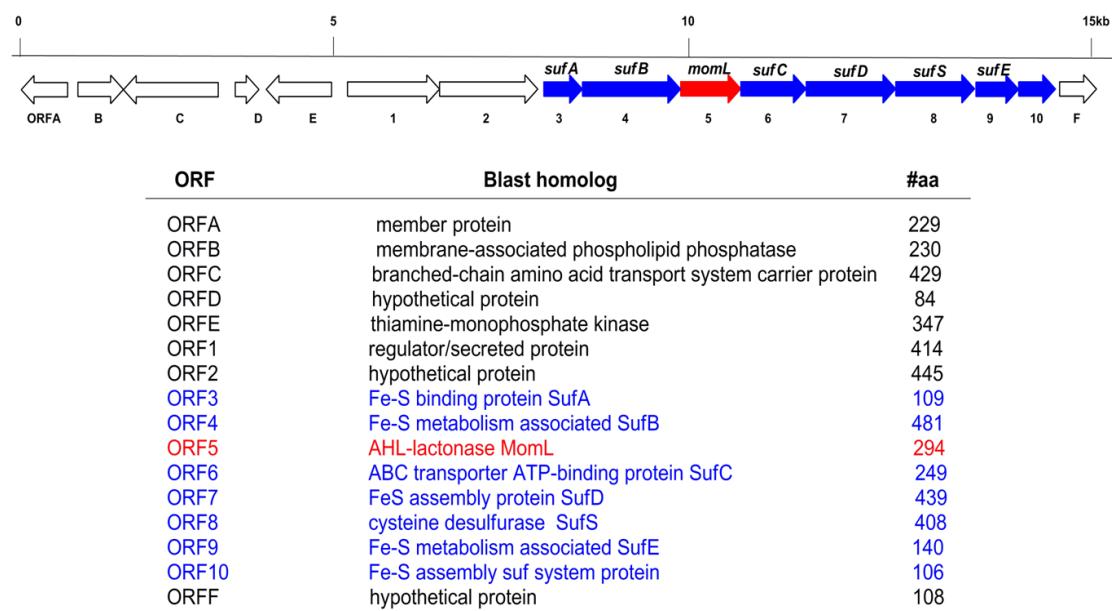


Figure S3. Sequence alignment of *oxyR* genes.

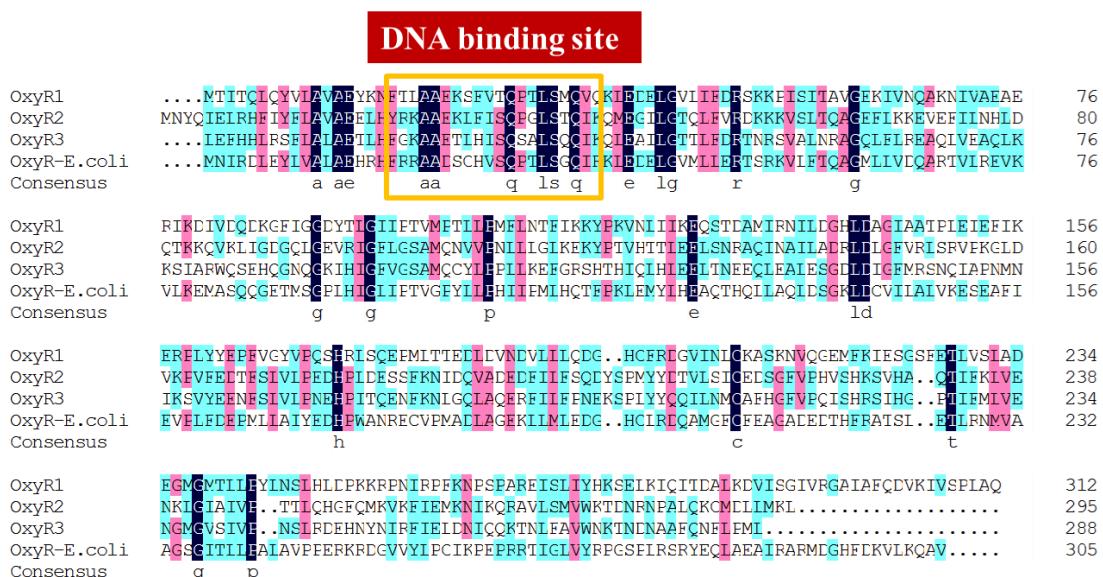


Figure S4. the R^2 value (Pearson correlation coefficient) of the transcriptome assay and real-time PCR.

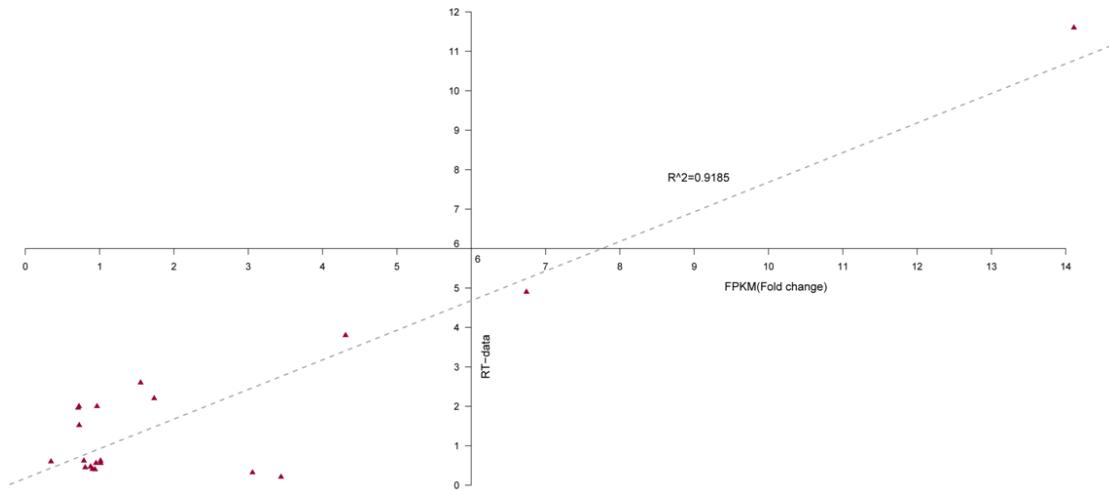


Table S1. Primer information for transcriptional analysis of target genes.

16S-forward	TACGGGAAGAAACCACC
16S-reverse	GCCAGTATCAAAGGCAAT
<i>orfE</i> -forward	GCCTTGCTTCCCTTTC
<i>orfE</i> -reverse	CCACCCAAGTCACTGTTTC
<i>orfI</i> -forward	TGCCTTGGTGGGTCTG
<i>orfI</i> -reverse	TGCCGTGATTACTGCTT
<i>sufA</i> -forward	TCGGAACAGCCAAGC
<i>sufA</i> -reverse	CACGGCATTATCCTCAAA
<i>sufB</i> -forward	TGCCGTGGATATTGTCA
<i>sufB</i> -reverse	CCCTTCGGAATGTAGC
<i>momL</i> -forward	TCTTCCAGAGCCCTTAC
<i>momL</i> -reverse	GGTTGTCCTCGCTTGTC
<i>sufC</i> -forward	GTCCAGAAAGGCAAGGG
<i>sufC</i> -reverse	CAGAACGGTCTCATCCA
<i>sufE</i> -forward	TGTCAGAGTAAGGTTGGTA
<i>sufE</i> -reverse	AGTTGCTTACCATACTCACC
<i>orf10</i> -forward	GAAGAAACCACCATAGATAACAC
<i>orf10</i> -reverse	GGCAAACTTCCGCTAC
<i>orfF</i> -forward	CTTGGAAACTGCGCTCTA
<i>orfF</i> -reverse	GATTGGCACCGAGGAA
<i>oxyR1</i> -forward	TGCTACTACAGGACGGACAT
<i>oxyR1</i> -reverse	TTGAGGGCATCGGTTAT
<i>oxyR2</i> -forward	TATCCTACCGTGCATACC
<i>oxyR2</i> -reverse	AAATCTTCATCGGCTACT
<i>oxyR3</i> -forward	TTGGCAATCCGAACACC
<i>oxyR3</i> -reverse	GGTTGGACCGCATAAAA
<i>fur</i> -forward	CGTGTCACTGCCTTCC
<i>fur</i> -reverse	GCTTGCCTACCAAGTTG
<i>himA</i> -forward	ATCTTCTGGAAATGTAG
<i>himA</i> -reverse	TCAATTCTGCTCCTGCT
<i>himD</i> -forward	CTTGGAGAATGGAGACA
<i>himD</i> -reverse	TTACGCCTCTACAAAAA

Table S2. Survival rate of *Pcc* under Km condition.

Condition	Survival rate of <i>Pcc</i>
LB+20 µg/mL Km	0
LB+25 µg/mL Km	0
LB+50 µg/mL Km	0

Table S3. The affect of boiled inactivated MomL towards survival rate of *M. olearia* Th120 and *Pcc*.

Condition	Survival rate of <i>M. olearia</i> Th120	Survival rate of <i>Pcc</i>
Strains Co-culture	41%	39%
Strains Co-culture with 0.1U/ml inactivated MomL	40%	40%
Strains Co-culture with 1U/ml inactivated MomL	43%	42%

Table S4. List of *suf* related genes regulated by indole in the transcriptome assay.

Gene description	Fold change
<i>sufA-sufE</i>	5.1-8.7
<i>momL</i>	6.3
<i>oxyR1</i>	4.1

Table S5. Significantly enriched pathways and mapped genes regulated by indole based on KEGG pathway analysis.

gene categories	p value	enrich DEGs	NR function	KEGG function	KEGG detailed function
Lysine biosynthesis (ko00300)	0.0184	<i>gene301</i>	diaminopimelate epimerase	K01778 <i>dapF</i>	K01778(diaminopimelate epimerase)
		<i>gene1097</i>	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate-D-alanyl-D-alanyl ligase	K01929 <i>murF</i>	K01929(UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase)
		<i>gene2657</i>	UDP-N-acetylmuramoyl-L-alanyl-D-glutamate-2, 6-diaminopimelate ligase	K01928 <i>mure</i>	K01928(UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase)
		<i>gene3005</i>	aspartate kinase	K00928 <i>lysC</i>	K00928(aspartate kinase)
Vancomycin resistance (ko01502)	0.0247	<i>gene882</i>	D-alanyl-D-alanine dipeptidase	K08641 <i>vanX</i>	K08641(D-alanyl-D-alanine dipeptidase)
		<i>gene1097</i>	UDP-N-acetylmuramoylalanyl-D-glutamyl-2,6-diaminopimelate-D-alanyl-D-alanyl ligase	K01929 <i>murF</i>	K01929(UDP-N-acetylmuramoyl-tripeptide--D-alanyl-D-alanine ligase)
		<i>gene2656</i>	phospho-N-acetylmuramoyl-pentapeptide-trans ferase	K01000 <i>mraY</i>	K01000(phospho-N-acetylmuramoyl-pentapeptide-transferase)
C5-Branched dibasic acid metabolism (ko00660)	0.0323	<i>gene160</i>	3-isopropylmalate dehydrogenase	K00052 <i>leuB</i>	K00052(3-isopropylmalate dehydrogenase)
		<i>gene676</i>	succinyl-CoA synthetase subunit beta	K01903 <i>sucC</i>	K01903(succinyl-CoA synthetase beta subunit)
		<i>gene170</i>	acetolactate synthase large subunit	K01652 E2.2.1.6L , <i>ilvB</i> , <i>ilvG</i> , <i>ilvI</i>	K01652(acetolactate synthase I/II/III large subunit)
Two-component system (ko02020)	0.0577	<i>gene1134</i>	LuxR family transcriptional regulator	K11618 <i>liaR</i>	K11618(two-component system, NarL family, response regulator LiaR)
		<i>gene1255</i>	acriflavin resistance protein	K07787 <i>cusA</i> , <i>silA</i>	K07787(Cu(I)/Ag(I) efflux system membrane protein CusA/SilA)
		<i>gene2036</i>	copper/silver resistance-related transport membrane protein	K07787 <i>cusA</i> , <i>silA</i>	K07787(Cu(I)/Ag(I) efflux system membrane protein CusA/SilA)
		<i>gene882</i>	D-alanyl-D-alanine dipeptidase	K08641 <i>vanX</i>	K08641(D-alanyl-D-alanine dipeptidase)

Table S6. List of TonB transfer system genes regulated by indole.

Gene description	Fold change
TonB-dependent receptor-1	2.3
TonB-dependent receptor-2	3.4
TonB-1	6.5
TonB-2	7.8
TonB-3	7.2

