

Supplementary Information

For

A New Gene *SCY3* Homologous to *Scygonadin* Showing Antibacterial Activity and a Potential Role in the Sperm Acrosome Reaction of *Scylla paramamosain*

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Figures and Tables

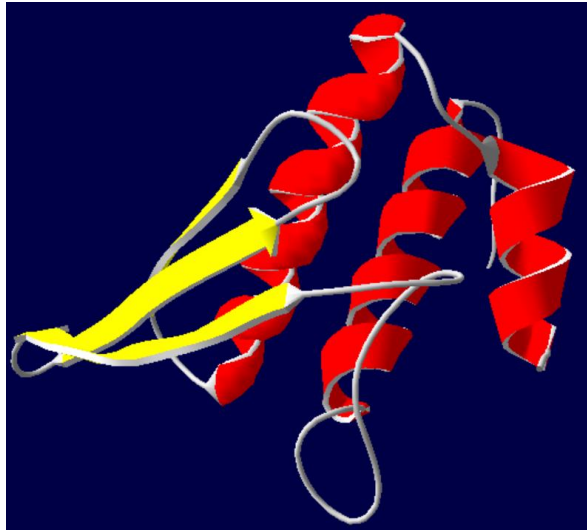


Figure S1. Prediction of the tertiary structure of SCY3 by PHYRE2.

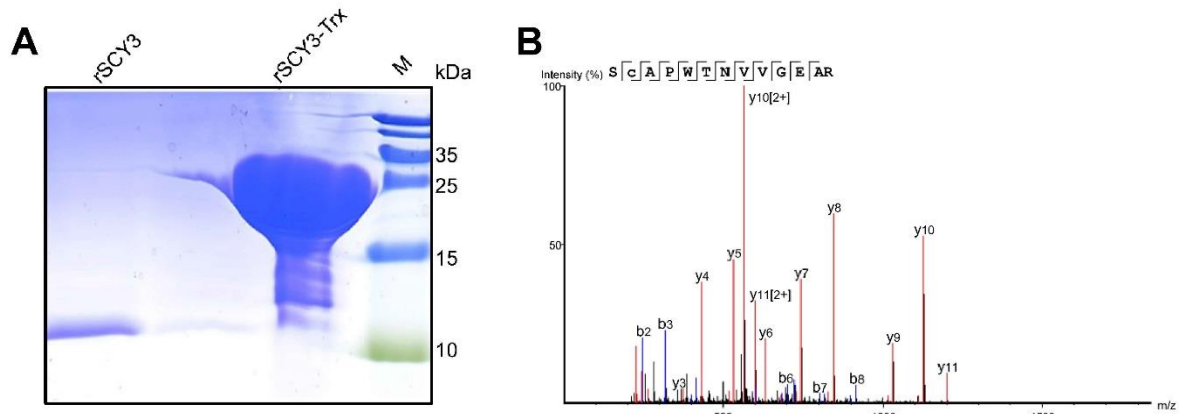


Figure S2. The expression and purification of SCY3 in eukaryotic expression system and prokaryotic expression system, and the mass spectrometry result. (A) SDS-PAGE analysis of purification results of rSCY3 in eukaryotic expression system and prokaryotic expression system. M, marker; 1, purified protein rSCY3 in eukaryotic expression system; 2, purified protein rSCY3 in prokaryotic expression system. (B) The mass spectrometry result of eukaryotic expression product rSCY3.

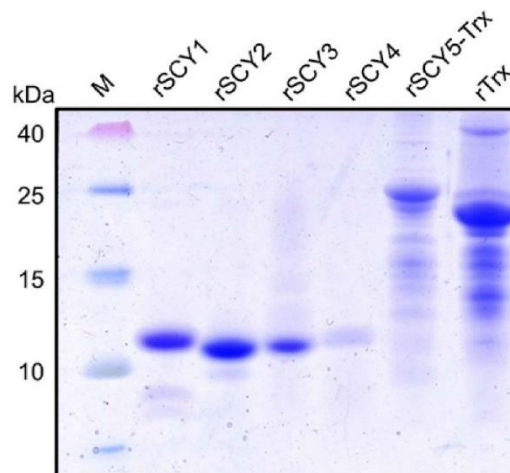


Figure S3. The expression and purification of rSCY1, rSCY2, rSCY3, rSCY4 in eukaryotic expression system and rSCY5, rTrx in prokaryotic expression system. M, marker; 1, purified

protein rSCY1 in eukaryotic expression system; 2, purified protein rSCY2 in eukaryotic expression system; 3, purified protein rSCY3 in eukaryotic expression system; 4, purified protein rSCY4 in eukaryotic expression system; 5, purified protein rSCY5 in prokaryotic expression system; 6, purified protein rTrx in prokaryotic expression system.

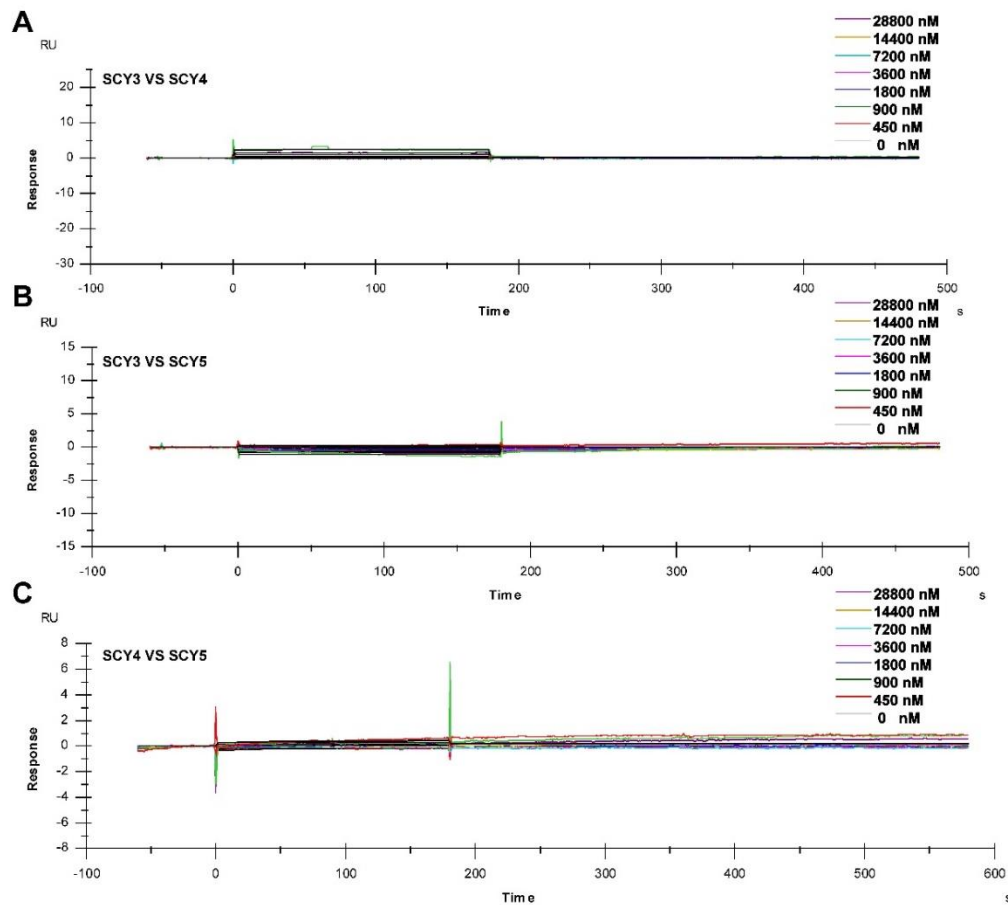


Figure S4. Binding kinetic between rSCY3 and rSCY4 (A), rSCY3 and rSCY5 (B), rSCY4 and rSCY5 assessed by surface plasmon resonance technology.

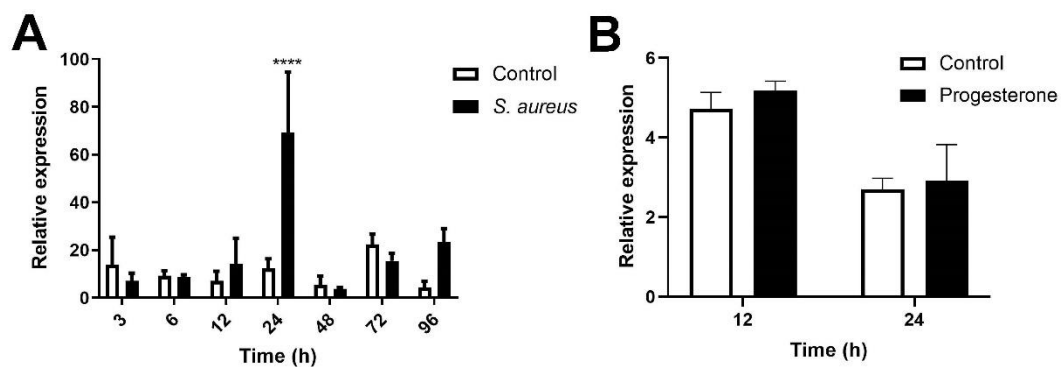


Figure S5. The expression pattern of SCY3 in hemocyte (A) after challenge with *S. aureus* and (B) in *ejaculatory duct* stimulated with progesterone. The asterisks indicated a significant difference compared with the control group (****: $P < 0.0001$).

Table S1: Microbial strains information

<i>Microorganisms</i>	<i>CGMCC No.^a</i>
<i>Gram-negative bacteria</i>	
<i>Pseudomonas fluorescens</i>	1.0032
<i>Pseudomonas stutzeri</i>	1.1803
<i>Shigella flexneri</i>	1.1868
<i>Escherichia coli</i>	1.2389
<i>Aeromonas hydrophila</i>	1.2017
<i>Vibrio fluvialis</i>	1.1609
<i>Vibrio harveyi</i>	1.1593
<i>Vibrio alginolyticus</i>	1.1833
<i>Vibrio parahaemolyticus</i>	1.1615
<i>Gram-positive bacteria</i>	
<i>Listeria monocytogenes</i>	1.10753
<i>Staphylococcus epidermidis</i>	1.4260
<i>Pseudomonas stutzeri</i>	1.1868
<i>Micrococcus lysodeikticus</i>	1.0634
<i>Micrococcus luteus</i>	1.634
<i>Bacillus subtilis</i>	1.108
<i>Fungi</i>	
<i>Candida albicans</i>	2.2411
<i>Cryptococcus neoformans</i>	2.1563
<i>Fusarium graminearum</i>	3.349
<i>Fusarium solani</i>	3.584
<i>Fusarium oxysporum</i>	3.6785
<i>Aspergillus niger</i>	3.0316
<i>Aspergillus ochraceus</i>	3.583
<i>Aspergillus fumigatus</i>	3.5835

^aChina general microbiological culture collection number.

Table S2: Primers used in the present study.

		SCY3 (5'-3')
For RACE PCR	3-3R1	CCCTCAACAAGCTTCTCCCT
	3-3R2	AGATGTCGTGTGCTCCTTGG
	3-5R1	AGCCAGTCCGTGACTTCTTC
	3-5R2	TGCAGTGTAGGAGCCGTTTT
		CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAA
For Introns PCR	UPM-Long	CGCAGAGT
	UPM-short	CTAATACGACTCACTATAGGGC
	3geneF	ATGCGTCCATCTCTCTTGGTCAGTC
	3geneR	TTAGTAGGAAGCTAGCCAGTCCGTG
For RT-PCR	3-DL-F	TGAACTCCAGGCAACTAA
	3-DL-R	TCCGTGACTTCTTCTATGG
	GAPDH-F	CTCCACTGGTGCCGCTAAGGCTGTA
	GAPDH-R	CAAGTCAGGTCAACCACGGACACAT
For recombinant expression	3-ZH-F	GGAATTCGGCTCCACCCTCAACAAGCTTCTCC
		ATAAGAATGCGGCCGCTTAATGGTGATGGTGATGATG
	3-ZH-R	GTAGGAAGCTAGCCAGTCCGTG
	3-YH-F	GGAATTCGGCTCCACCCTCAACAAGCTTCTCC
		ATAAGAATGCGGCCGCTTAGTAGGAAGCTAGCCAGTC
	3-YH-R	CGTG
	T7	TAATACGACTCACTATAGGG
	T7TER	TGCTAGTTATTGCTCAGCGG
	5'AOX	GACTGGTTCCAATTGACAAGC
	3'AOX	GGCAAATGGCATTCTGACAT

The underlined sequences represent restriction sites, the double underscore represents His purification label.

Table S3: Antibacterial activity of rSCY3.

Microorganisms	CGMC C No. ^a	<i>E. coli</i> -derived		<i>P. pastoris</i> -derived		rTrx	
		SCY3		SCY3		MIC	MBC
		^b MIC	^b MBC	MIC	MBC		
Gram-negative bacteria							
<i>Pseudomonas fluorescens</i>	1.0032	>48	>48	>48	>48	>48	>48
<i>Pseudomonas stutzeri</i>	1.1803	>48	>48	>48	>48	>48	>48
<i>Shigella flexneri</i>	1.1868	>48	>48	>48	>48	>48	>48
<i>Escherichia coli</i>	1.2389	>48	>48	>48	>48	>48	>48
<i>Aeromonas hydrophila</i>	1.2017	>48	>48	>48	>48	>48	>48
<i>Vibrio fluvialis</i>	1.1609	>48	>48	>48	>48	>48	>48
<i>Vibrio harveyi</i>	1.1593	>48	>48	>48	>48	>48	>48
<i>Vibrio alginolyticus</i>	1.1833	>48	>48	>48	>48	>48	>48
<i>Vibrio parahaemolyticus</i>	1.1615	>48	>48	>48	>48	>48	>48
Gram-positive bacteria							
<i>Listeria monocytogenes</i>	1.10753	>48	>48	>48	>48	>48	>48
<i>Staphylococcus epidermidis</i>	1.4260	>48	>48	>48	>48	>48	>48
<i>Pseudomonas stutzeri</i>	1.1868	>48	>48	>48	>48	>48	>48
<i>Microcococcus lysodeikticus</i>	1.0634	>48	>48	>48	>48	>48	>48
<i>Microcococcus luteus</i>	1.634	24-48	24-48	24-48	24-48	24-48	24-48
<i>Bacillus subtilis</i>	1.108	>48	>48	>48	>48	>48	>48
Fungi							
<i>Candida albicas</i>	2.2411	>48	>48	>48	>48	>48	>48
<i>Cryptococcus neoformans</i>	2.1563	>48	>48	>48	>48	>48	>48
<i>Fusarium graminearum</i>	3.349	>48	>48	>48	>48	>48	>48
<i>Fusarium solani</i>	3.584	>48	>48	>48	>48	>48	>48
<i>Fusarium oxysporum</i>	3.6785	>48	>48	>48	>48	>48	>48
<i>Aspergillus niger</i>	3.0316	>48	>48	>48	>48	>48	>48
<i>Aspergillus ochraceus</i>	3.583	>48	>48	>48	>48	>48	>48
<i>Aspergillus fumigatus</i>	3.5835	>48	>48	>48	>48	>48	>48

^aChina general microbiological culture collection number; ^bMIC and ^bMBC: All the concentrations showed in this table were in μM . The values of MIC (minimal inhibitory concentration) and MBC (minimal bactericidal concentration) were expressed as the lowest concentration yielding no detectable microbial growth or that killed more than 99.9 % of microorganism.