

## Supplemental Material

# The Heat Shock Repressor HspR Directly Controls Avermectin Production, Morphological Development, and H<sub>2</sub>O<sub>2</sub> Stress Response in *Streptomyces avermitilis*

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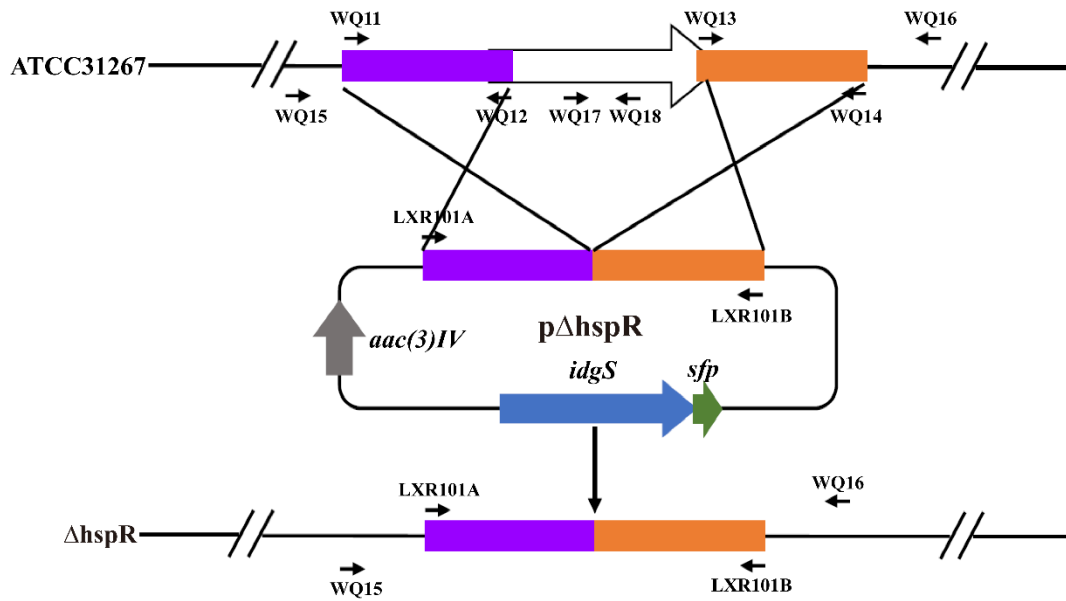
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# These two authors made equal contributions to the study.

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**Running title:** HspR function in *Streptomyces avermitilis*

**FIG S1**



**FIG S1** Method for *hspR* deletion (schematic). Large arrows: genes and their directions. Small arrows: positions of primers used for amplifying exchange regions and confirming gene deletions. Blocks: homologous exchange regions used for gene deletion.

FIG S2

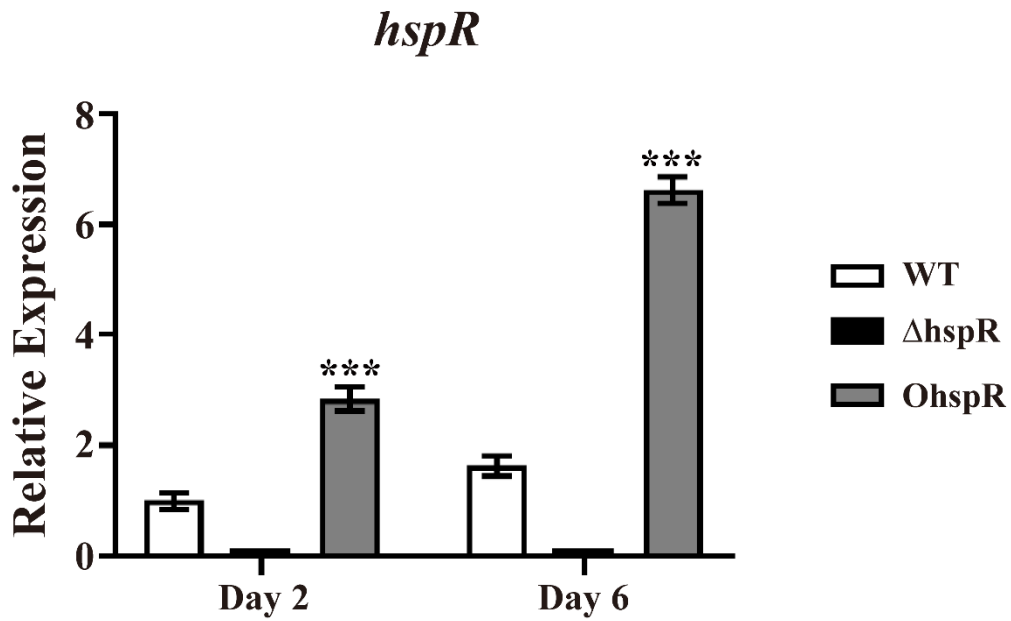
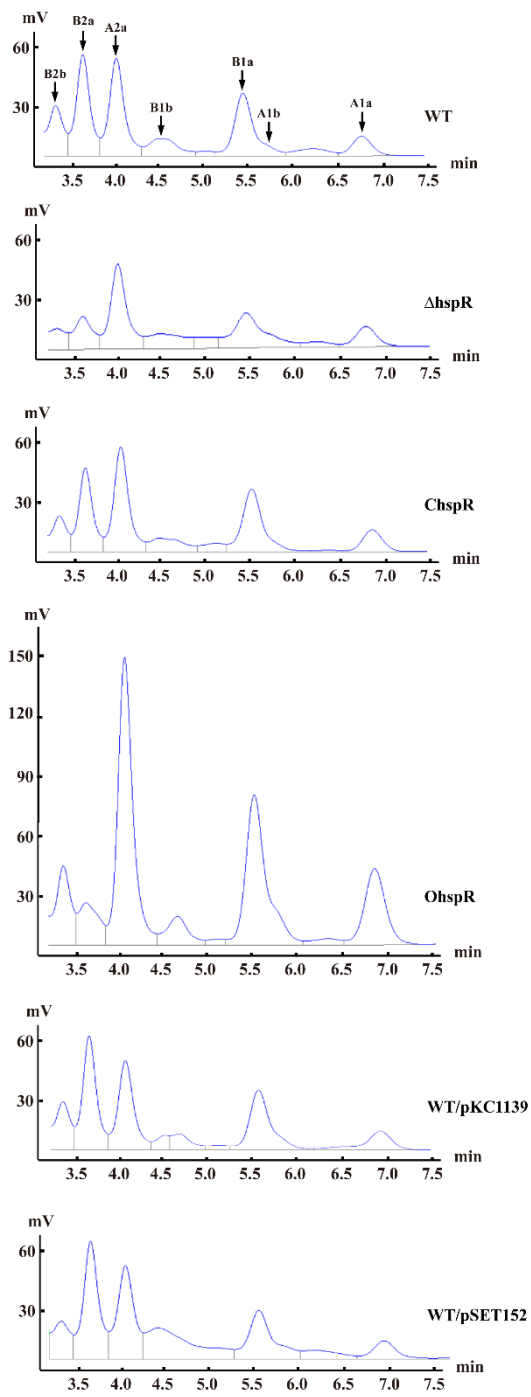


FIG S2 RT-qPCR analysis of *hspR* in WT,  $\Delta$ hspR, and OhspR grown in FM-I for 2 or 6 days.

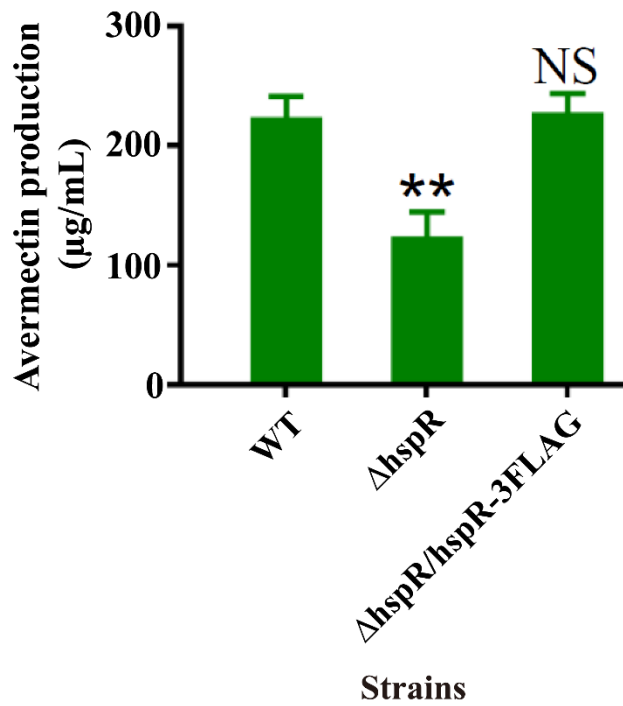
Transcription level was calculated relative to WT level on day 2 (set to 1). Error bars: SD for three replicates. \*\*\*,  $p < 0.001$  ( $t$ -test).

**FIG S3**



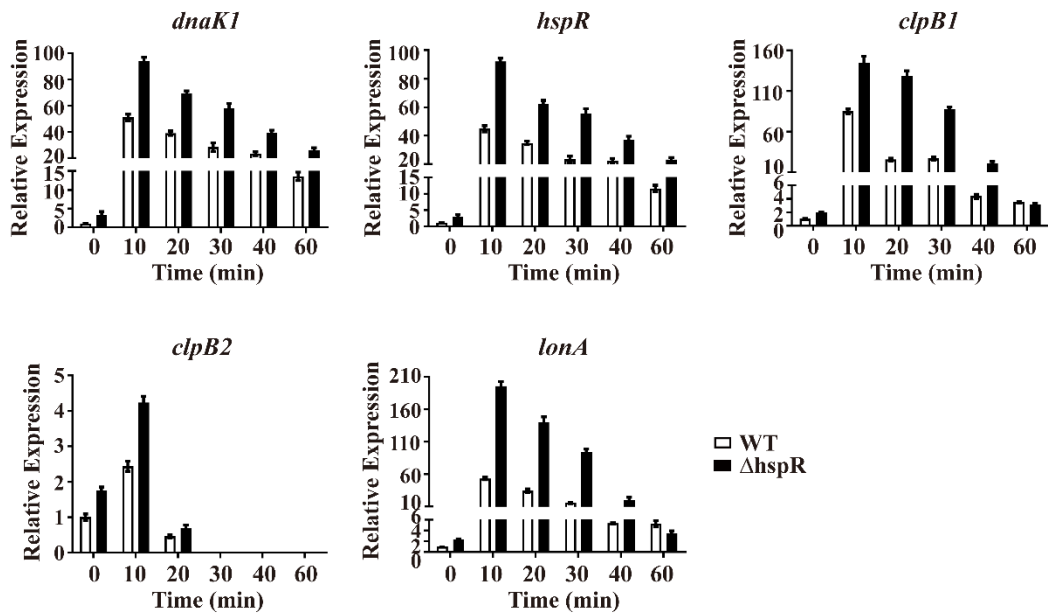
**FIG S3** HPLC analysis of avermectin profiles in WT,  $\Delta$ hspR, ChspR, OhspR, and control strains (WT/pKC1139, WT/pSET152) after 10-day culture in FM-I.

**FIG S4**



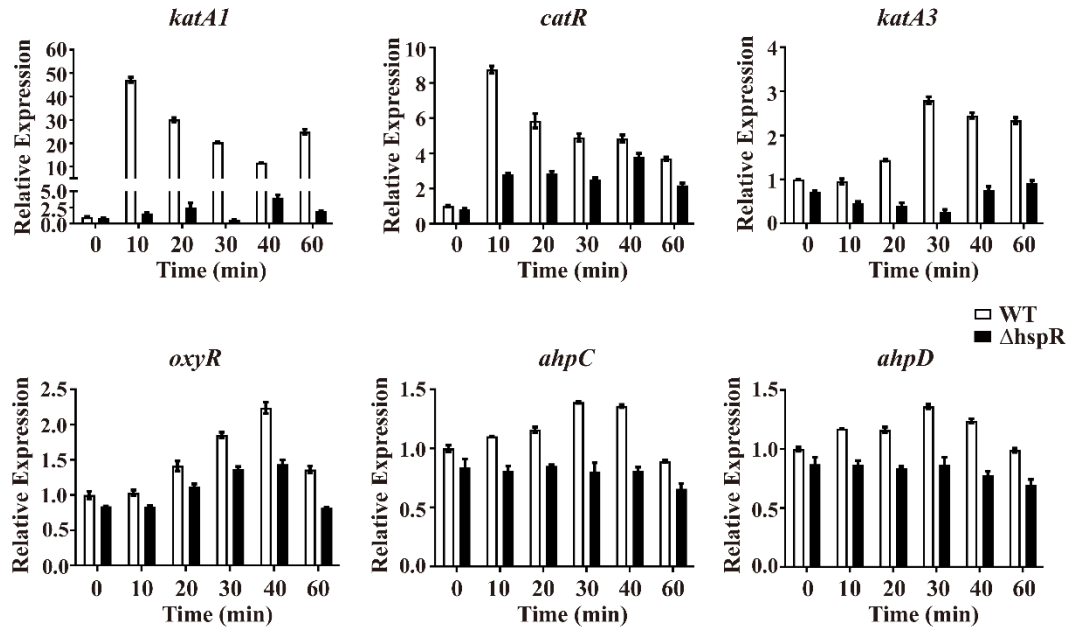
**FIG S4** Avermectin yield in *S. avermitilis* WT, ΔhspR, and ΔhspR/hspR-3FLAG (ΔhspR carrying pSET152-hspR-3FLAG) cultured in FM-I for 10 days. Error bars: SD for three replicates. NS, not significant; \*\*,  $p < 0.01$  (*t*-test).

**FIG S5**



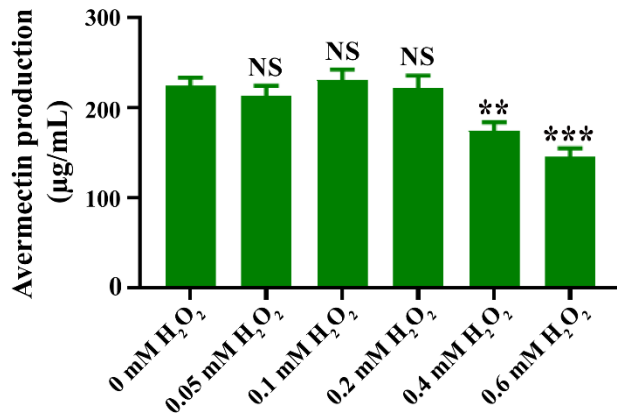
**FIG S5** Induction of five HSGs by heat treatment (50°C) in WT and  $\Delta$ *hspR* grown in FM-II. For each gene, transcription level in WT before temperature rise (0 min) was set to 1. Error bars: SD for three replicates.

**FIG S6**



**FIG S6** Induction of six H<sub>2</sub>O<sub>2</sub> stress-related genes by 0.6 mM H<sub>2</sub>O<sub>2</sub> in WT and  $\Delta$ *hspR* grown in FM-II. For each gene, transcription level in WT before H<sub>2</sub>O<sub>2</sub> treatment (0 min) was set to 1. Error bars: SD for three replicates.

**FIG S7**



**FIG S7** Avermectin yield in WT strain after 10-day culture in FM-I containing H<sub>2</sub>O<sub>2</sub> at indicated concentrations. Error bars: SD for three replicates. NS, not significant; \*\*,  $p < 0.01$ ; \*\*\*,  $p < 0.001$  for comparison with control (no H<sub>2</sub>O<sub>2</sub> addition) ( $t$ -test).



**TABLE S1** Putative targets of HspR

#	Accession number	Gene	Function	Score
<b>Regulatory function (23)</b>				
1	SAV_223		putative DNA-binding protein	8.4
2	SAV_343		putative regulatory protein	10.2
3	SAV_554		putative LacI-family transcriptional regulator	14.1
4	SAV_570	<i>ssgY</i>	putative cell division regulatory protein	8.8
5	SAV_711		putative transcriptional regulator	8.6
6	SAV_729		putative LysR-family transcriptional regulator	8.8
7	SAV_1503		putative TetR-family transcriptional regulator	9.4
8	SAV_2601		putative PadR-like family transcriptional regulator	10.7
9	SAV_3053	<i>catR</i>	putative hydrogen peroxide sensitive repressor	11.8
10	SAV_3231	<i>oxyR</i>	putative hydrogen peroxide sensing regulator	12.7
11	SAV_3481		putative two-component system response regulator	9
12	SAV_3571		putative MarR-family transcriptional regulator	9.1
13	SAV_3757		putative ArsR-family transcriptional regulator	9.1
14	SAV_3949		putative regulatory protein	9.4
15	SAV_4274	<i>abaB1</i>	putative LysR-family transcriptional regulator	8.8
16	SAV_4997	<i>wblB</i>	putative WhiB-family transcriptional regulator	9.4
17	SAV_4999	<i>sig44</i>	putative RNA polymerase ECF-subfamily sigma factor	8.8
18	SAV_5042	<i>whiB</i>	putative WhiB-family transcriptional regulator	8.8
19	SAV_5626		putative DNA-binding protein	8.2
20	SAV_5653		putative ROK-family transcriptional regulator	8.7
21	SAV_5792		putative MerR-family transcriptional regulator	8.5
22	SAV_7041		putative transcriptional regulatory protein	9.9
23	SAV_7292	<i>sig56</i>	putative RNA polymerase ECF-subfamily sigma factor	8.4
<b>Secondary metabolism (2)</b>				
24	SAV_938	<i>aveA1</i>	type I polyketide synthase AVES 1	14.4
25	SAV_2991	<i>ptlH</i>	1-deoxypentalenic acid 11-beta hydroxylase	9.4
<b>Metabolism of amino acids and related molecules (6)</b>				
26	SAV_2583	<i>gabT</i>	putative 4-aminobutyrate aminotransferase	10.1
27	SAV_2730	<i>serA</i>	putative D-3-phosphoglycerate dehydrogenase	8.7
28	SAV_2733	<i>ilvB1</i>	acetolactate synthase subunit large	8.3
29	SAV_3325	<i>hutH1</i>	putative histidine ammonia-lyase	8.4
30	SAV_3648	<i>cysK2</i>	putative cysteine synthase	8
31	SAV_6005	<i>glnA3</i>	putative glutamine synthetase	8.2
<b>Carbohydrate metabolism (13)</b>				
32	SAV_564		putative 2-hydroxyhepta-2,4-diene-1,7-dioate isomerase	8.1
33	SAV_628	<i>agaA1</i>	putative alpha-galactosidase, secreted	8.3
34	SAV_828		putative rhamnosidase	10.4
35	SAV_1854	<i>celA2</i>	putative secreted endo-1,4-beta-glucanase	10.8
36	SAV_1855	<i>guxA3</i>	putative secreted cellulose 1,4-beta-cellobiosidase	10.8
37	SAV_2000	<i>aceB1</i>	putative malate synthase	11.4

38	SAV_2159	<i>amyA2</i>	putative alpha-amylase	9.2
39	SAV_2427	<i>citA3</i>	putative citrate synthase	12
40	SAV_2859	<i>abpS</i>	putative cellulose-binding protein	8
41	SAV_3258	<i>chiA2</i>	putative chitinase A, secreted	8.9
42	SAV_3295		putative glycosyltransferase	9.9
43	SAV_3570		putative trans-aconitate methyltransferase	9.1
44	SAV_6020	<i>aceE2</i>	putative pyruvate dehydrogenase E1 component	10.2
<b>Fatty acid and lipid metabolism (2)</b>				
45	SAV_1384	<i>fadA5</i>	putative acetyl-CoA acetyltransferase	8.2
46	SAV_2856	<i>fadA1</i>	putative acetyl-CoA acetyltransferase	9.2
<b>Nucleotide metabolism (2)</b>				
47	SAV_3329	<i>cyaA</i>	putative adenylate cyclase	9.5
48	SAV_4146	<i>purC</i>	putative phosphoribosylaminoimidazole-succinocarboxamide synthetase	9.1
<b>Protein synthesis, folding, and modification (12)</b>				
49	SAV_1956	<i>map1</i>	putative methionyl aminopeptidase	10.2
50	SAV_3994	<i>pkn8</i>	putative serine/threonine protein kinase	9.3
51	SAV_4725	<i>trpS2</i>	putative tryptophanyl-tRNA synthetase	8
52	SAV_4957	<i>rplM</i>	putative ribosomal protein L13	8.4
53	SAV_5155		putative protease, secreted	8
54	SAV_5300		putative tripeptidyl aminopeptidase, secreted	8.6
55	SAV_5565	<i>prpK</i>	putative magnesium or manganese-dependent protein phosphatase	8.7
56	SAV_6025	<i>pepA</i>	putative leucyl aminopeptidase	9.7
57	SAV_6799	<i>pkn30</i>	putative serine/threonine protein kinase	8.5
<b>DNA synthesis, repair, recombination, modification, and packaging (3)</b>				
58	SAV_6486		putative 3-methyladenine DNA glycosylase	9.3
59	SAV_6714		putative 5'-3' exonuclease	12.3
60	SAV_7571	<i>ttrA2</i>	putative helicase	9.4
<b>RNA synthesis and modification (1)</b>				
61	SAV_3892		putative 2'-5' RNA ligase	8
<b>Membrane bioenergetics (1)</b>				
62	SAV_1507	<i>fprB</i>	putative NAD(P)H-ferredoxin reductase	8.7
<b>Detoxification and adaptation to atypical conditions (8)</b>				
63	SAV_1302	<i>axe1</i>	putative acetyl xylan esterase	8.2
64	SAV_1427	<i>terA</i>	putative tellurium resistance protein	8.2
65	SAV_2026	<i>katA3</i>	putative catalase	8.5
66	SAV_2966	<i>lonA</i>	putative lon class III heat-shock ATP-dependent protease	14.8
67	SAV_3052	<i>katA1</i>	putative catalase	11.8
68	SAV_3232	<i>ahpC</i>	putative alkyl hydroperoxide reductase	12.7
69	SAV_4154	<i>cspD3</i>	putative cold shock protein	8.7
70	SAV_4484	<i>dnaK1</i>	putative heat shock protein Hsp70	17.4
71	SAV_4514	<i>clpB1</i>	putative ATP-dependent Clp protease	17.2
72	SAV_6608	<i>pacB3</i>	putative penicillin acylase, secreted	9.6

<u>73</u>	<i>SAV_7241</i>	<i>clpB2</i>	putative ATP-dependent Clp protease	12.1
<b>Cell wall and cell envelope (3)</b>				
74	<i>SAV_3393</i>	<i>nagZ2</i>	putative beta-N-acetylhexosaminidase, secreted	8.7
75	<i>SAV_5302</i>	<i>nagZ5</i>	putative beta-N-acetylhexosaminidase, secreted	10
76	<i>SAV_5913</i>	<i>dacF</i>	putative D-alanyl-D-alanine carboxypeptidase	9
<b>Cell division and differentiation (1)</b>				
<u>77</u>	<i>SAV_4666</i>	<i>ftsH</i>	putative cell division protein FtsH	9.6
<b>Transport/binding proteins and lipoproteins (16)</b>				
78	<i>SAV_1133</i>		putative membrane transport protein	8
79	<i>SAV_1338</i>	<i>ssuA1</i>	putative ABC transporter substrate-binding protein	9.6
80	<i>SAV_2185</i>		putative ABC transporter ATP-binding protein	9.3
81	<i>SAV_2603</i>		putative multi-drug efflux transporter	8.3
82	<i>SAV_2657</i>	<i>araE</i>	putative L-arabinose permease	12.1
83	<i>SAV_2769</i>	<i>oppC1</i>	putative peptide ABC transporter permease protein	9
84	<i>SAV_3149</i>	<i>oppA4</i>	putative peptide ABC transporter substrate-binding protein	9.8
85	<i>SAV_3150</i>	<i>bldKA1</i>	putative peptide ABC transporter permease protein	9.8
86	<i>SAV_3482</i>		putative di-tripeptide transporter	9
87	<i>SAV_3897</i>	<i>pbuG1</i>	putative hypoxanthine/guanine permease	8.1
88	<i>SAV_4053</i>		putative ABC transporter ATP-binding protein	9.1
89	<i>SAV_5783</i>		putative lipoprotein	8
90	<i>SAV_5912</i>		putative ABC transporter permease protein	9
91	<i>SAV_6715</i>	<i>opuAA</i>	putative glycine betaine ABC transport ATP-binding protein	12.3
92	<i>SAV_7200</i>	<i>glpF3</i>	putative glycerol uptake facilitator protein	9.5
93	<i>SAV_7293</i>		putative lipoprotein, secreted	8.4
<b>Mobile and extrachromosomal element functions (2)</b>				
94	<i>SAV_3722</i>		putative IS701 family ISAzvi8-like transposase	8.7
95	<i>SAV_7558</i>		putative IS5 family IS493-like transposase	8.3
<b>Unknown or unclassified genes (60)</b>				
96	<i>SAV_105</i>		hypothetical protein	9.1
97	<i>SAV_197</i>		hypothetical protein	9.5
98	<i>SAV_267</i>		hypothetical protein	8
99	<i>SAV_293</i>		putative oxidoreductase	8.2
100	<i>SAV_500</i>		hypothetical protein	8.5
101	<i>SAV_550</i>		hypothetical protein	14.1
102	<i>SAV_553</i>		hypothetical protein	14.1
103	<i>SAV_622</i>		hypothetical protein	8.9
104	<i>SAV_804</i>		putative secreted protein	8.5
105	<i>SAV_812</i>		putative small hydrophilic protein	8
106	<i>SAV_872</i>		putative oxidoreductase	8.1
107	<i>SAV_903</i>		putative membrane protein	9.3
108	<i>SAV_1201</i>		putative dehydrogenase	10.8
109	<i>SAV_1428</i>		hypothetical protein	8.2
110	<i>SAV_1478</i>		hypothetical protein	9.4
111	<i>SAV_1506</i>		hypothetical protein	8.7

112	<i>SAV_1950</i>	putative hydrolase	10.9
113	<i>SAV_2075</i>	putative transmembrane protein	10.7
114	<i>SAV_2158</i>	hypothetical protein	9.2
115	<i>SAV_2452</i>	hypothetical protein	8.1
116	<i>SAV_2582</i>	putative ATP/GTP-binding protein	10.1
117	<i>SAV_2599</i>	hypothetical protein	9.7
118	<i>SAV_2604</i>	putative secreted protein	8.3
119	<i>SAV_2706</i>	hypothetical protein	9
120	<i>SAV_3104</i>	hypothetical protein	8.6
121	<i>SAV_3239</i>	hypothetical protein	10
122	<i>SAV_3257</i>	hypothetical protein	8.9
123	<i>SAV_3333</i>	putative secreted protein	10
124	<i>SAV_3394</i>	hypothetical protein	8.7
125	<i>SAV_3717</i>	hypothetical protein	8.4
126	<i>SAV_3748</i>	hypothetical protein	9.4
127	<i>SAV_3758</i>	hypothetical protein	9.1
128	<i>SAV_3898</i>	putative integral membrane protein	8.1
129	<i>SAV_3916</i>	putative secreted protein	8.1
130	<i>SAV_4404</i>	hypothetical protein	13.3
131	<i>SAV_4490</i>	hypothetical protein	8.9
132	<i>SAV_4513</i>	hypothetical protein	17.2
133	<i>SAV_4578</i>	hypothetical protein	8.8
134	<i>SAV_4627</i>	putative secreted protein	8.2
135	<i>SAV_4632</i>	hypothetical protein	8.6
136	<i>SAV_4870</i>	hypothetical protein	9.1
137	<i>SAV_4956</i>	hypothetical protein	8.4
138	<i>SAV_5154</i>	putative secreted protein	8
139	<i>SAV_5191</i>	putative membrane protein	11.8
140	<i>SAV_5198</i>	hypothetical protein	8.9
141	<i>SAV_5215</i>	hypothetical protein	8.7
142	<i>SAV_5325</i>	hypothetical protein	10.3
143	<i>SAV_5347</i>	putative secreted protein	8.5
144	<i>SAV_5627</i>	hypothetical protein	8.2
145	<i>SAV_5652</i>	hypothetical protein	8.7
146	<i>SAV_5751</i>	hypothetical protein	13
147	<i>SAV_5772</i>	putative dehydratase	9.1
148	<i>SAV_5784</i>	hypothetical protein	8
149	<i>SAV_5816</i>	putative secreted protein	9.4
150	<i>SAV_5955</i>	hypothetical protein	9.4
151	<i>SAV_5964</i>	hypothetical protein	8.1
152	<i>SAV_5990</i>	putative secreted protein	8.2
153	<i>SAV_6246</i>	putative secreted protein	11.3
154	<i>SAV_6597</i>	putative secreted protein	9.2
155	<i>SAV_6946</i>	putative choline oxidase	8.1

**Total: 155**

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Underlining: putative targets confirmed by EMSAs to be bound by HspR.

Shading: putative targets confirmed by EMSAs to be not bound by HspR.