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Phytother Res. Author manuscript; available in PMC 2013 March 11.

Published in final edited form as:
Phytother Res. 2013 March ; 27(3): 390–396. doi:10.1002/ptr.4738.

Tea Tree Oil-Induced Transcriptional Alterations in *Staphylococcus aureus*

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Abstract

Tea tree oil (TTO) is a steam distillate of *Melaleuca alternifolia* that demonstrates broad-spectrum antibacterial activity. This study was designed to document how TTO challenge influences the *Staphylococcus aureus* transcriptome. Overall, bioinformatic analyses (*S. aureus* microarray meta-database) revealed that both ethanol and TTO induce related transcriptional alterations. TTO challenge led to the down-regulation of genes involved with energy-intensive transcription and translation, and altered the regulation of genes involved with heat shock (e.g. *clpC*, *clpL*, *ctsR*, *dnaK*, *groES*, *groEL*, *grpE* and *hrcA*) and cell wall metabolism (e.g. *cwrA*, *isaA*, *sle1*, *vraSR* and *vraX*). Inactivation of the heat shock gene *dnaK* or *vraSR* which encodes a two-component regulatory system that responds to peptidoglycan biosynthesis inhibition led to an increase in TTO susceptibility which demonstrates a protective role for these genes in the *S. aureus* TTO response. Agene (*mmpL*) encoding a putative resistance, nodulation and cell division efflux pump was also highly induced by TTO. The principal antimicrobial TTO terpene, terpinen-4-ol, altered ten genes in a transcriptional direction analogous to TTO. Collectively, this study provides additional insight into the response of a bacterial pathogen to the antimicrobial terpene mixture TTO.

Keywords

tea tree oil; *Staphylococcus aureus*; transcriptomics; heat shock; *vra*

INTRODUCTION

Tea tree oil (TTO) extracted from *Melaleuca alternifolia* has been shown to exhibit broad-spectrum antimicrobial activity *in vitro* (Carson *et al.*, 1995) and is comprised of hundreds of hydrocarbon components (Southwell and Lowe, 1999), including the major antimicrobial terpene, terpinen-4-ol (Carson and Riley, 1995). Preliminary trials suggest that TTO

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Conflict of Interest
All authors declare no financial/commercial conflicts of interest.

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formulations may be effective in the treatment of acne and fungal infections, and bacterial pathogen decolonization protocols (Carson *et al.*, 2006). The overall bactericidal activity of TTO is attributed to its ability to denature proteins and alter membrane and cell wall structure and function (Carson *et al.*, 2002; Carson *et al.*, 2006; Carson and Riley, 1995; Cox *et al.*, 1998; Cox *et al.*, 2000; Gustafson *et al.*, 1998; Sikkema *et al.*, 1995).

Infections caused by the bacterial pathogen *Staphylococcus aureus* are attributed to a mortality rate comparable to the deaths caused by HIV/AIDS, tuberculosis and viral hepatitis combined in the United States (Boucher and Corey, 2008). The relatively high incidence of infections caused by multiply antibiotic-resistant methicillin-resistant *S. aureus* (MRSA) strains remains a major concern within the medical community (Bubacz, 2007). For instance, there were an estimated 14 million healthcare visits for suspected skin and soft tissue infections caused by *S. aureus* in 2005 (Hersh *et al.*, 2008), and MRSA can cause a high percentage of these infections (Moran *et al.*, 2006). *S. aureus* and MRSA are susceptible to TTO and have been targeted in TTO decolonization clinical trials (Dryden *et al.*, 2004).

In an effort to better understand the anti-staphylococcal activity of TTO, we have performed a transcriptional profiling experiment with *S. aureus* exposed to a growth inhibitory concentration of TTO. To our knowledge, this is the first report of a bacterial pathogen's genome-wide TTO transcriptional response.

MATERIALS AND METHODS

Chemicals, TTO and bacterial strains utilized

All chemicals were purchased from Sigma-Aldrich Co. (St Louis, MO) except TTO, which was obtained as a gift from Paul Bolster (P. Guinane Pty. Ltd. New South Wales, Australia; *M. alternifolia* oil sample A354) (Table 1). Mueller–Hinton broth (MHB) and bacteriological grade agar were purchased from Becton Dickinson and Company (Sparks, MD). *S. aureus* strains SH1000, SH1000*dnaK::kan*, N315, N315*vraSR::cat*, COL, and clinical MRSA strains LP9 and MM66 have been previously described (Delgado *et al.*, 2007; Horsburgh *et al.*, 2002; Gustafson *et al.*, 1992; Kuroda *et al.*, 2003; Singh *et al.*, 2007).

TTO and ethanol susceptibility testing, TTO kill curve and whole cell autolysis

Overnight, *S. aureus* cultures were incubated for 24 h (37 °C, 200 rpm) and used to initiate growth in all cultures required for the following experiments. TTO minimum inhibitory concentration (MIC) measurements were performed by adding 1ml of diluted overnight culture (final OD_{580} = 0.01) into 1ml of MHB containing 0.01% (v/v) Tween 80 and 0.05% to 0.65% TTO (v/v), and after 24 h incubation at 37 °C, MICs were determined. Ethanol MICs were performed as described above except; MHB containing 3% to 15% filter sterilized (0.22 μm) (Nalge Nunc International, Rochester, NY) ethanol (v/v) without Tween 80 addition was utilized. Minimum bactericidal concentrations (MBC) were determined by streaking 100 μl aliquots of the MIC tube, and all MIC tubes containing higher drug concentrations, onto MH agar and scoring for the lowest drug concentration with no observable growth following 24 h incubation (37 °C). The TTO kill curve was carried out utilizing early exponential SH1000 cultures (OD_{580} = 0.4) and the same culture challenged with 0.25% TTO (v/v). Colony forming units per ml (CFUs/ml) were then determined in untreated and TTO-challenged cultures 15, 30, 60 and 120 min after TTO exposure. Triton X-100-induced whole cell autolysis was performed as previously described (Gustafson *et al.*, 1992). Briefly, individual 25 ml mid-exponential phase SH1000 cultures (OD_{580} = 0.4) were subjected to no challenge or 0.25% TTO challenge for 15 min. The cells were then harvested by centrifugation (8200 × g, 4 °C, 10 min), washed once with 5ml of ice-cold water and

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repelleted. The cell pellets were then suspended in 25 ml of Tris–HCl (0.05 M, pH 7.2) or the same buffer containing 0.05% Triton X-100. The cell suspensions were then incubated with gentle agitation at 37 °C, and OD_{580} was read every h for 8 h.

Microarray, quantitative real-time polymerase chain reaction and *S. aureus* microarray meta-database

Total RNA was isolated as previously described (Riordan *et al.*, 2007), and DNA microarrays (TIGR version 6) were used for transcriptional profiling of laboratory strain SH1000 (MHB cultures OD_{580}= 0.4 at 37 °C, 200 rpm) following 0.25% TTO v/v upshock for 15 min using protocols provided by Pathogen Functional Genomics Resource Center (http://pfgrc.jcvi.org/index.php/microarray/array_description/staphylococcus_aureus/version6.html). RNA isolated from untreated and TTO-challenged SH1000 cultures were then converted to fluorescently labeled cDNA and hybridized to *S. aureus* microarrays version 6 as previously described (Riordan *et al.*, 2007; Delgado *et al.*, 2008). Duplicate microarrays of SH1000 were hybridized and analyzed, and ORFs altered 1.5-fold and above were determined to be significant (p<0.05) (Riordan *et al.*, 2007). The *S. aureus* TTO transcriptome data in this publication is deposited in NCBI's Gene Expression Omnibus accessible through GEO series accession number GSE31554 (http://www.ncbi.nlm.nih.gov/geo/). Based on our laboratory preference, when possible, all genes were given *S. aureus* strain COL locus ID numbers. The *S. aureus* microarray meta-database (SAMMD) analysis was then used to compare the SH1000 TTO stimulon to 93 publicly available experimental *S. aureus* transcriptional responses (Nagarajan and Elasri, 2007). Initially, the ORF IDs of genes differentially regulated following TTO challenge were converted to strain COL locus IDs, and non-redundant IDs were then used to search against SAMMD. Select genes altered in the TTO microarray data were then validated by quantitative real-time polymerase chain reaction (qRT-PCR) as previously described (Riordan *et al.*, 2007) using primers found in Table 2 in SH1000 cultures challenged with TTO as described above. In addition, qRT-PCR experiments were also performed with MHB cultures (OD_{580} = 0.4, 37 °C, 200 rpm) challenged with the major TTO antimicrobial component terpinen-4-ol (0.25% v/v final concentration) alone (Carson and Riley, 1995; Cox *et al.*, 2001). Critical cycle threshold values were normalized using 16 S rRNA as an internal reference, and changes in gene expression were reported using the $2^{-\Delta\Delta C_T}$ method (Livak and Schmittgen, 2001).

RESULTS AND DISCUSSION

SH1000 viable cell counts were determined following the addition of TTO at various time points. At 15 min, SH1000 cell population numbers had not decreased following TTO challenge (control 2.2×10^6 vs TTO challenged 2.0×10^6 CFUs/ml). Therefore, the cell population used in the microarray experiment described here does not represent a population that is experiencing events leading to immediate cell death. At all future time points investigated, the TTO-challenged cultures demonstrated a significant fall in surviving CFUs/ml only after 30min (30min – 1.3×10^6, 60 min – 5.2×10^5 and 120 min – 6.9×10^4 CFUs/ml).

Following 15 min of 0.25% TTO challenge, 312 genes were up-regulated, and 324 genes were down-regulated 1.5-fold (Table 3). The directional alteration in expression of ten of these genes (*betB*, *dnaK, mmpL*, *purA*, *purM*, *rpmI, tenA*, *vraR*, *vraS* and *vraX*) was confirmed by qRT-PCR (Table 3). qRT-PCR performed with cultures challenged with 0.25% v/v terpinen-4-ol also resulted in a similar direction of altered gene expression of these ten genes as TTO challenge (Table 3).

Overall SAMMD analysis revealed the highest overlap between the TTO stimulon and the response of two unrelated MRSA strains (MM66 and LP9) (Delgado *et al.*, 2008) to a

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growth-inhibiting ethanol concentration (10% v/v) (GEO series accession number GSE17391 http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE17391). The SH1000 TTO transcriptional response shared 425 and 469 altered genes with the LP9 and MM66 ethanol transcriptional responses, respectively. It is of interest to note that *S. aureus* strains demonstrating relatively high TTO (% v/v) MICs (SH1000 = 0.28 ± 0.01, LP9 = 0.2 ± 0) also demonstrated relatively high ethanol (%v/v)MICs (SH1000 = 9.0 ± 1.0, LP9 = 9.1 ± 1.0). Conversely, *S. aureus* strains that exhibited relatively low TTO MICs (COL= 0.15 ± 0, MM66 = 0.15 ± 0.03) also displayed relatively low ethanol MICs (COL= 6.9 ± 1.2, MM66 = 7.2 ± 1.0).

A total of 19 genes involved with transcription were down-regulated (−2.3- to −24.2-fold) following TTO-challenge including *rpoA*, *rpoB*, *rpoC* and *rpoE* which encode the DNA-directed RNA polymerase alpha-, beta- and delta-subunits, as well as a sigma factor. In addition, of the 149 translational genes found within *S. aureus*, 73 are down-regulated (−1.5- to −18.6-fold) in SH1000 following TTO challenge (Fig. 1). These down-regulated genes included 27 genes (−1.6-fold) of the large 29 gene ribosomal operon (Wang *et al.*, 2004). Many translation genes were similarly downregulated by ethanol exposure (Fig. 1).

The classic heat shock genes encode proteases and molecular chaperones which collectively contribute to the continued maintenance and remodeling of denatured proteins following exposure to stress, such as ethanol or heat treatment (Chastanet *et al.*, 2003; Gottesman *et al.*, 1997; Qoronfleh *et al.*, 1990). The processing of denatured proteins is energetically costly and powered by ATP hydrolysis (Kenniston *et al.*, 2003; Lin and Rye, 2006). Some of the highest TTO up-regulated genes in SH1000 included the heat shock genes *dnaK*, *groEL*, *groES, dnaJ and grpE* (Table 3), which can be controlled by the synergistically acting HrcA and CtsR DNA-binding proteins (Chastanet *et al.*, 2003). Both *hrcA* and *ctsR* were also up-regulated following TTO challenge (Table 3). DnaK is important to the general stress response, assists in denatured protein refolding and may be involved in membrane assembly, maintenance and structure (de Crouy-Chanel *et al.*, 1999). It is also important in the response of *S. aureus* to TTO, since an insertionally inactivated *dnaK* mutant of SH1000 (SH1000*dnaK::kan*) displayed a lower TTO (% v/v) MIC (0.25 ± 0) and MBC (0.30 ± 0), compared to parent strain SH1000 (MIC = 0.28 ± 0.01, MBC= 0.32 ± 0.01, $p<0.05$). CtsR regulates the expression of the heat shock responsive *clp* ATP-binding protease genes (Derre *et al.*, 1999), and *clpB*, *clpC*, *clpL* and *clpP* were all up-regulated following TTO challenge (Table 3). Two additional heat-responsive genes within the *ctsR* operon (SACOL0568 and SACOL0569) (Wang *et al.*, 2004) were also up-regulated following TTO challenge (Table 3).

Many of the genes that demonstrated the greatest down-regulation following TTO challenge include those required for purine biosynthesis (*purA, purC, purD, purE, purF, purH, purK, purL, purM, purN, purQ* and *purS*) (Table 3). Nucleotide metabolism in bacteria is affected by the activity of adenylate kinase Adk and di-phosphate kinase Ndk (Willemoes and Kilstrup, 2005), and both *adk* and *ndk* were also down-regulated following TTO challenge (Table 3). SACOL2242, which encodes a xanthine/uracil permease family protein, was also down-regulated by TTO challenge (Table 3). Following TTO exposure, one of the thiamine (vitamin B1) biosynthetic operons (*tenA-thiM-thiD-thiE*) (Muller *et al.*, 2009) was also down-regulated in SH1000. Bacterial purine biosynthetic pathways have been linked to thiamine biosynthesis (Petersen *et al.*, 1996), so the TTO-induced down-regulation of both purine and thiamine biosynthetic genes might be linked.

The gene that demonstrated the greatest increase in expression following TTO challenge was *vraX*, which encodes a 55 aa protein (McAleese *et al.*, 2006; Scherl *et al.*, 2006). The function of VraX is presently unknown; however, it has been proposed that the first 19 N-

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terminal aa sequence of this protein is a signal peptide, suggesting that VraX is exported (Scherl *et al.*, 2006). SAMMD analysis revealed that *vraX* expression is also up-regulated by multiple cell wall and/or membrane active compounds (bacitracin, d-cycloserine, oxacillin, tunicamycin, flavomycin, fosfomycin, teicoplanin, vancomycin, daptomycin, lysostaphin, epicatechin gallate, ranalexin and antimicrobial peptides) (Bernal *et al.*, 2010; Dengler *et al.*, 2011; Overton *et al.*, 2011; Pietiainen *et al.*, 2009; Utaida *et al.*, 2003). Our results and those previously reported support the notion that *vraX* up-regulation follows all forms of cell membrane and/or cell wall metabolism insult. Genes encoding the two-component system VraSR (vancomycin-resistance associated sensor/regulator) are both up-regulated following vancomycin exposure (Kuroda *et al.*, 2003) and in *S. aureus* strains exhibiting decreased susceptibility to vancomycin (Kuroda *et al.*, 2000). Our data indicated that *vraSR* is also up-regulated following TTO insult (1.7- and 3.9-fold) (Table 3). Since *vraR* is required for the expression of *vraX* (Dengler *et al.*, 2011), it is possible that the up-regulation of this gene contributes to increased *vraX* expression following TTO challenge. The expression of additional genes whose regulation is altered by *vraSR* (e.g. *ctpA*, *drp35*, *fmtA*, *opuD*, *pbp2*, *prsA* and *sgtB*) (McAleese *et al.*, 2006; Utaida *et al.*, 2003) was also altered in expression following TTO challenge (Table 3). In support of a hypothesis that *vraSR* plays a protective role in the the response of *S. aureus* to TTO, we determined that N315 *vraSR::cat* demonstrated a reduced TTO (% v/v) MIC (0.15 ± 0) and MBC (0.20 ± 0), compared to parent strain N315 (MIC = 0.23 ± 0.01, MBC= 0.26 ± 0.01, $p<0.05$). Two genes encoding known peptidoglycan hydrolases or autolysins (*sle1* and *isaA*) (Kajimura *et al.*, 2005; Stapleton *et al.*, 2007) were also down-regulated, and *cwrA*, a gene that is induced by cell wall active antimicrobials (Balibar *et al.*, 2010), was up-regulated by TTO challenge (Table 3).

Since cell wall metabolism genes were affected by TTO challenge, we performed whole cell autolytic assays with TTO-treated and untreated SH1000 cell populations. While we detected a slight reduction in unstimulated whole cell autolysis in SH1000 challenged with TTO compared to untreated SH1000, Triton X-100 stimulated autolysis occurred at a similar rate for TTO-treated and untreated cells (data not shown).

One of the most highly TTO-induced genes (*mmpL*) (Table 3) encodes a protein that is a member of the resistance, nodulation and cell division (RND) family of proteins. *mmpL* produces a product that demonstrates the greatest identity (31%) across its entire length with the *Mycobacterium tuberculosis* RND family protein MmpL7 that is required for virulence (Perez *et al.*, 2006). It is of interest to note that the *Pseudomonas aeruginosa* RND-protein MexB is part of the MexAB-OprM efflux pump that is required for the full expression of TTO tolerance by this organism (Papadopoulos *et al.*, 2008).

CONCLUSIONS

Our SAMMD findings indicate that both the commonly employed antiseptic ethanol and TTO induce analogous transcriptional responses which may be related to the ability of these substances to cause similar damage to both membrane and protein structures (Carson *et al.*, 2006; Gustafson *et al.*, 1998; McDonnell and Russell, 1999). This finding strengthens the idea that these two substances have similar mechanisms of anti-staphylococcal activity.

Another major finding is that TTO challenge leads to the down-regulation of a large cadre of the genes involved with both transcription and translation. The down-regulation of these genes may occur since these processes become futile in a TTO-challenged cell population. Since the synthesis of ribosomal components and translation consumes the majority of energy in growing cells (Dethlefsen and Schmidt, 2007; Russell and Cook, 1995), we propose that the down-regulation of these genes during TTO challenge allows the cell to

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conserve and/or relocate energy resources to processes designed to protect the cell from TTO cidal activity, such as the heat shock response. A protective role for the heat shock response against TTO challenge is supported by the finding that the inactivation of *dnaK* in SH1000 leads to an increase in TTO susceptibility.

The TTO up-regulation of *vraX* and *vraSR*, and the altered regulation of other genes involved with cell wall metabolism following TTO challenge indicates that cell wall metabolism is affected by TTO and terpinen-4-ol as was previously suggested for TTO at least (Carson *et al.*, 2002; Gustafson *et al.*, 1998). This finding led us to investigate the importance of *vraSR* in the response of strain N315 to TTO. *vraSR* inactivation in N315 led to an increase in TTO susceptibility, demonstrating that this two-component regulatory system is required for the TTO protective response. Interestingly, whole cell autolysis experiments suggest that autolysin production and/or activity is not irreversibly altered following TTO challenge.

It is possible that the up-regulation of *mmpL* represents an effort by *S. aureus* to reduce TTO and antimicrobial terpene accumulation. Research is presently underway to determine if *mmpL* inactivation leads to a reduction in susceptibility to TTO and its antimicrobial terpenes.

Furthermore, qRT-PCR experimentation revealed that ten genes altered by TTO challenge also responded in a similar transcriptional direction to the major TTO antimicrobial terpinen-4-ol. These genes included the TTO and terpenin-4-ol up-regulated *dnaK, mmpL*, *vraR*, *vraS* and *vraX*, and the down-regulated *betB, purA*, *purM*, *rpmI* and *tenA*. These equivalent transcriptional responses indicate that terpinen-4-ol alone probably contributes to the TTO-induced transcriptome alterations observed.

Acknowledgments

All authors wish to acknowledge the former and ongoing support from the National Institutes of Health: SC1GM083882-01 (JEG); S06 GM61222-05 (JC, NMSU-MBRS-RISE PROGRAM); R25 GM07667-30 (NMSU-MARC PROGRAM) and 1R15AI084006 (BJW). This project was also supported by grants from the National Center for Research Resources (5P20RR016480-12) and the National Institute of General Medical Sciences (8 P20 GM103451-12) from the National Institutes of Health. Special thanks to Professor Thomas V. Riley, Dr. Christine Carson (University of Western Australia) and Paul Bolster (P. Guinane Pty. Ltd. New South Wales, Australia) for their help in the acquisition of TTO utilized in this study.

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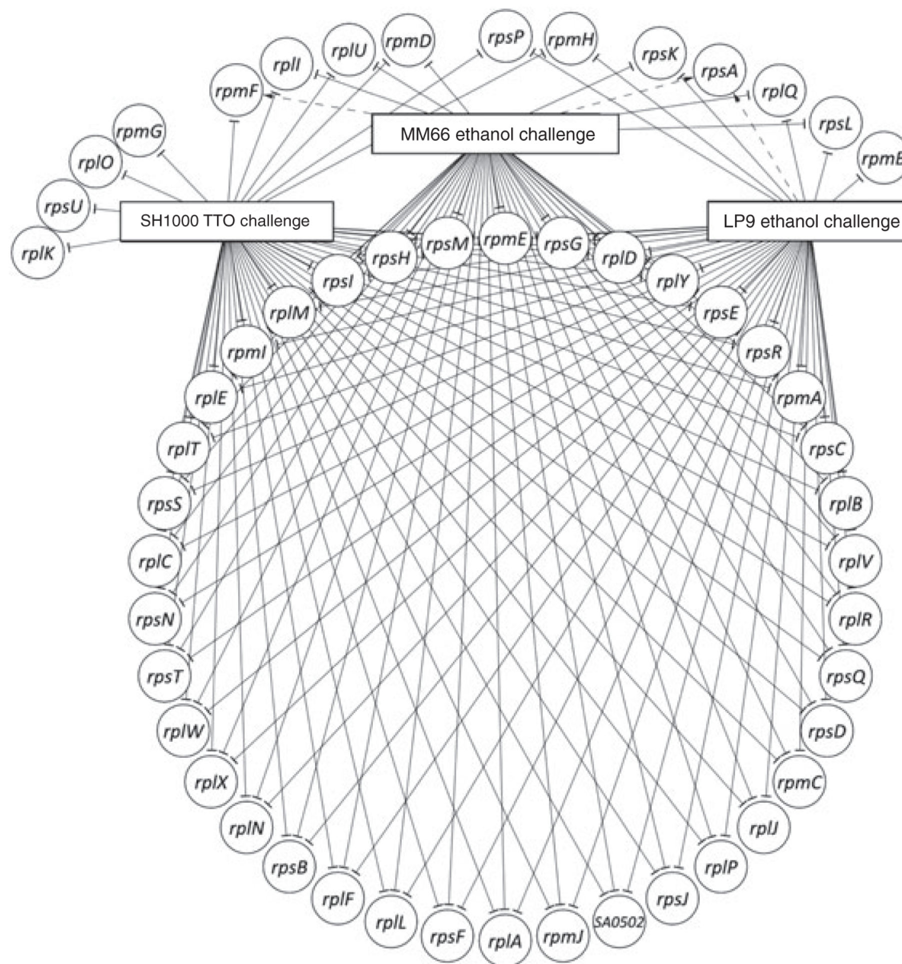

Figure 1.

Translational genes similarly affected by TTO and ethanol challenge generated with SAMMD data and Cytoscape (Shannon *et al.*, 2003). Solid lines ending in a perpendicular line denote gene down-regulation, dashed lines ending in an arrow denote gene up-regulation.

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Table 1

TTO chemical composition

Component	A354 (%)	ISO4730 (% range)
α-pinene	2.4	1–6
sabinene	0.4	trace–3.5
α-terpinene	9.8	5–13
limonene	0.8	0.5–1.5
p-cymene	2.2	0.5–8
1,8-cineole	1.7	trace–15
γ-terpinene	20.6	10–28
terpinolene	3.4	1.5–5
terpinen-4-ol	41.5	30–48
α-terpineol	2.9	1.5–8
aromadendrene	1.5	trace–3
ledene	0.9	trace–3
δ-cadinene	1.1	trace–3
globulol	0.3	trace–1
viridiflorol	0.4	trace–1

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Table 2

Primers used for qRT-PCR experiments

Gene	Function	Locus ID	Primer sequence (forward and reverse)
betB	betaine aldehyde dehydrogenase	SACOL2628	5′-AATTGCTGTTGGTGGTAAACG-3′
			5′-TAACGACAGGTCCGAAAACC-3′
dnaK	DnaK protein	SACOL1637	5′-CCGGTGACAACAAACTTGG-3′
			5′-TCAGCAGCATCTTTCAAACG-3′
mmpL	MmpL efflux pump, putative	SACOL2566	5′-GGAATGACATCTACAGAAGTAGGC-3′
			5′-AACTGCTAGTCCAATCATTACGG-3′
purA	adenylosuccinate synthase	SACOL0018	5′-GAGGTTGGTCGTGAATACGG-3′
			5′-TGGGTACTCAGTAATTTCTTTACCG-3′
purM	phosphoribosylaminoimidazole synthetase	SACOL1080	5′-AATATGGGTATTGGCTATACGG-3′
			5′-CACAATATGACCAATTTGATAGGC-3′
rpmI	ribosomal protein L35	SACOL1726	5′-TGCCAAAAATGAAAACTCACC-3′
			5′-GAGATGTGAAAGCTCTTGAACG-3′
tenA	transcriptional regulator, TenA family	SACOL2086	5′-TAGGAGCTGACGCATTACGC-3′
			5′-CCCATTGTTCTAGTGTCATAGCC-3′
vraR	DNA-binding response regulator VraR	SACOL1942	5′-AAAGAAGCAATTGCCAAAGC-3′
			5′-TGAGTCGTCGCTTCTACACC-3′
vraS	histidine kinase sensor	SACOL1943	5′-AGTGCCGATGAAAGTTGTGC-3′
			5′-TTTTGTACCGTTTGAATGACG-3′
vraX	VraX protein	SACOL0625	5′-TCGACAGTATCACCATGAAGG-3′
			5′-TTTCAGTATCACTAAATGAATCGTCAC-3′

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Table 3

Representative *S. aureus* genes altered by TTO challenge

Gene	Function	Locus ID	Fold change in gene expression		
			Microarray	qRT- PCR (TTO)	qRT- PCR (Terpinen-4-ol)
Up-regulated genes					
vraX	VraX protein	SACOL0625	39.0	3.0	2.3
mmpL	MmpL-like RND efflux pump	SACOL2566	14.7	2.8	1.6
	hypothetical protein (72 aa)	SACOL1033	13.1		
grpE	GrpE protein	SACOL1638	11.8		
	hypothetical protein (39 aa)	SAR1729a	10.7		
	putative short chain oxidoreductase	SACOL2594	10.7		
	hypothetical protein (138 aa)	SACOL2621	10.6		
hrcA	heat-inducible transcriptional repressor	SACOL1639	10.5		
cwrA	CwrA, conserved hypothetical protein	SACOL2571	10.4		
	hypothetical protein (188 aa)	SACOL0568	9.4		
	putative ATP:guanido phosphotransferase	SACOL0569	9.0		
csb7	alkyl hydroperoxidase	SACOL2484	8.7		
clpC	ATP-dependent Clp protease, ATP-binding subunit ClpC	SACOL0570	8.3		
	hypothetical protein (71 aa)	SAS1587	8.2		
	acetoin reductase	SACOL0111	8.2		
ctsR	putative DNA-binding protein	SACOL0567	8.1		
	hypothetical protein (146 aa)	SACOL0768	7.6		
epiE	epidermin immunity protein F	SACOL1872	7.4		
	alpha/beta fold family hydrolase	SACOL2597	7.0		
clpL	ATP-dependent Clp proteinase chain	SACOL2563	6.9		
groEL	GroEL protein	SACOL2016	4.9		
groES	GroES protein	SACOL2017	4.1		
vraR	DNA-binding response regulator	SACOL1942	3.9	2.2	2.0
dnaK	DnaK protein	SACOL1637	3.5	1.7	1.8
dnaJ	DnaJ protein	SACOL1636	3.0		
vraS	histidine kinase sensor	SACOL1943	1.7	1.8	1.9
Down-regulated genes					

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Gene	Function	Locus ID	Fold change in gene expression		
			Microarray	qRT- PCR (TTO)	qRT- PCR (Terpinen-4-ol)
purA	adenylosuccinate synthase	SACOL0018	−13.1	−2.2	−2.1
purM	phosphoribosylaminoimidazole synthetase	SACOL1080	−11.5	−1.7	−1.6
sle1	N-acetylmuramyl-L-alanine amidase	SACOL0507	−10.8		
isaA	lytic transglycosylase	SACOL2584	−10.8		
purL	phosphoribosylformylglycinamidine synthetase	SACOL1078	−10.5		
dltA	D-alanine-D-alanyl carrier protein ligase	SACOL0935	−10.1		
purF	amidophosphoribosyltransferase	SACOL1079	−9.8		
purK	phosphoribosylaminoimidazole carboxylase CO_2-fixation	SACOL1074	−8.9		
rplD	50S ribosomal protein L4	SACOL2238	−8.6		
purE	phosphoribosylaminoimidazole carboxylase, catalytic subunit	SACOL1073	−8.3		
purN	phosphoribosylaminoimidazole carboxylase, catalytic subunit	SACOL1072	−8.0		
tenA	transcriptional regulator, TenA family	SACOL2086	−7.9	−2.9	−1.8
purH	phosphoribosylaminoimidazolecarboxamide formyltransferase	SACOL1082	−7.8		
betA	choline dehydrogenase	SACOL2627	−7.6		
ndk	nucleoside diphosphate kinase	SACOL1509	−7.6		
purS	phosphoribosylformylglycinamidine synthase	SACOL1076	−7.2		
purF	phosphoribosylpyrophosphate amidotransferase	SACOL1079	−7.2		
	similar to xanthine/uracil permease family protein	SACOL2242	−7.1		
betB	betaine aldehyde dehydrogenase	SACOL2628	−7.0	−1.5	−1.6
	hypothetical protein (191 aa)	SACOL1086	−7.0		
rpmI	ribosomal protein L35	SACOL1726	−5.9	−2.0	−1.8
thiD	phosphomethylpyrimidine kinase	SACOL2085	−5.1		
purQ	phosphoribosylformylglycinamidine synthase I	SACOL1077	−4.9		
purC	phosphoribosylaminoimidazole-succinocarboxamide synthase	SACOL1075	−4.6		
thiE	putative thiamine-phosphate pyrophosphorylase	SACOL2083	−3.8		
thiM	hydroxyethylthiazole kinase	SACOL2084	−3.2		
adk	adenylate kinase	SACOL2218	−2.5		