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Research Article

RESEARCH ARTICLE

Analysis of the effect of temperature on protein abundance in *Demodex*-associated *Bacillus oleronius*

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One sentence summary: The elevated temperature of rosacea skin may alter protein production by a bacterium implicated in its induction.

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ABSTRACT

A potential role for bacteria in the induction of rosacea has been suggested. The aim of this work was to characterise the effect of temperature on the production of immunostimulatory proteins by *Bacillus oleronius*—a bacterium to which rosacea patients show sera reactivity and which was originally isolated from a *Demodex* mite from a rosacea patient. The affected skin of rosacea patients is at a higher temperature than unaffected skin, and it was postulated that this might alter the protein expression pattern of *B. oleronius*. *B. oleronius* growth was reduced at 37°C compared to 30°C but resulted in increased expression of the immune-reactive 62kDa protein (1.65 fold [$P < 0.05$]). Proteomic analysis revealed increased abundance of a wide range of proteins involved in the stress response (e.g. stress proteins [21.7-fold increase], phosphocarrier protein HPr [438.5-fold increase], 60 kDa chaperonin [12.6-fold increase]). Proteins decreased in abundance after growth at 37°C included ferredoxin (325-fold decrease) and peptidase (244-fold decrease). This work indicates that the increased skin temperature of rosacea patients may alter the growth and protein production pattern of *B. oleronius* and lead to the greater production of immuo-stimulatory proteins.

Keywords: *Bacillus*; *Demodex*; growth temperature; proteomics; rosacea

ABBREVIATIONS

BP: biological process
CC: cellular components
PCA: principal component analysis

INTRODUCTION

Rosacea is a chronic inflammatory disease which predominantly affects the skin of the face and the eyes (Jarmuda *et al.* 2012). Rosacea is a complex disease and a potential role for bacteria in its induction has been postulated (Yamasaki and Gallo 2009; Margalit *et al.* 2016; Woo *et al.* 2016). The condition can be subdivided into four subtypes (papulopustular, erythematotelangiectatic, phymatous and ocular) and is characterised by erythema, papules and telangiectasia (Wilkin *et al.* 2004). Rosacea occurs in all skin types but is most apparent in those with Fitzpatrick type II–III (O'Reilly, Menezes and Kavanagh 2012). Symptoms are generally confined to the central regions of the face and it is common for patients to experience more than one of the subtypes simultaneously (Del Rosso 2004). Patients experience stinging, pain and facial flushing and the exacerbation of symptoms may be induced by a range of trigger factors such as alcohol, heat and exercise. Rosacea can be treated with a range of antimicrobials (e.g. erythromycin) and is characterised by cycles of relapse and remission (Gupta and Chaudhry 2005).

Rosacea may arise in patients who display a genetic predisposition associated with abnormal dermal immune responses that over-react to a variety of triggering factors (Woo *et al.*

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2016). Analysis of the immune responses in erythematotelangiectatic rosacea patients indicated significant influx of pro-inflammatory cells to affected regions and elevated production of IFN-γ and IL-17 (Buhl *et al.* 2015). Characterisation of the chemokine production pattern in rosacea patients indicated a Th1/Th17 response (Buhl *et al.* 2015). The Th17 pathway is associated with autoimmune disease but also in the response to fungal and bacterial infection (Patel and Kuckroo 2015).

Rosacea patients display a higher density of *Demodex* mites in their skin than unaffected controls (Bonnar, Eustace and Powell 1993; Woo *et al.* 2016). The role of the elevated *Demodex* density in the induction of rosacea is unclear but therapies that reduce their density (e.g. Soolantra) lead to a reduction in symptoms which may return after the cessation of therapy along with a return in the *Demodex* population. *Demodex* mites can act as vectors for the transmission of bacteria around the face and may serve to bring bacteria on their surface into the pilosebaceous unit (Jarmuda *et al.* 2012).

A number of studies suggest a potential role for bacteria in the induction of rosacea (Jarmuda *et al.* 2012). *Staphylococcus epidermidis* isolated from the skin of rosacea patients were consistently beta-hemolytic while those from controls were non-hemolytic. Growth of the hemolytic *S. epidermidis* at 37°C leads to greater production and secretion of immuno-stimulatory proteins (Dahl, Ross and Schlievert 2004). Recent research has demonstrated a wide range of bacteria in *Demodex* mites and that the type and abundance of such bacteria vary depending upon the type of rosacea from which the mites were extracted (Murillo, Aubert and Raoult 2014). In cases of papulopustular rosacea, *Demodex* mites contain large populations of *Proteobacteria* and *Firmicutes* while bacterial populations in mites from erythematotelangiectatic patients contained higher proportions of *Actinobacteria*. A bacterium (*Bacillus oleronius*) isolated from a *Demodex* mite from the face of a papulopustular rosacea patients produced two proteins (62 and 83 kDa) to which sera from papulopustular (Lacey, Kavanagh and Tseng 2009), ocular (O'Reilly *et al.* 2012b) and erythematotelangiectatic (McMahon *et al.* 2014) rosacea patients reacted suggesting a possible role for this bacterium in the induction and persistence of the condition (Jarmuda *et al.* 2012). This bacterium was first isolated from the digestive tract of a termite (Kuhnigk *et al.* 1995) where it may act as an endosymbiont. It has also been isolated from eye lashes and eyelids (Szkaradkiewicz *et al.* 2012). These *Bacillus* proteins were capable of activating neutrophils (O'Reilly *et al.* 2012a) via the IP3 pathway (McMahon *et al.* 2016) leading to the secretion of MMP 9 and cathelicidin (O'Reilly *et al.* 2012a). These proteins also induced an aberrant wound healing response in a corneal epithelial cell line (hTCEpi) (McMahon *et al.* 2014). It has been postulated that bacteria in the digestive tract of *Demodex* mites may be released upon the death of the mite and that proteins associated with these trigger an immune response within the skin which may lead to the erythema and tissue damage associated with rosacea (O'Reilly, Menezes and Kavanagh 2012).

The aim of the work described here was to assess the effect of temperature on the growth and production of immuno-stimulatory proteins by *B. oleronius*. It was postulated that the elevated temperatures of inflamed skin (37°C–39°C) of rosacea patients (Dahl, Ross and Schlievert 2004) might alter the growth of *B. oleronius* and lead to the enhanced production of immuno-stimulatory proteins.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents were of the highest purity and were purchased from Sigma Aldrich Chemical Company Ltd (Dorset, UK) unless otherwise stated.

Bacteria culture conditions

Bacillus oleronius was isolated from the hindgut of a *Demodex folliculorum* mite isolated from the face of a papulopustular rosacea patient (Lacey *et al.* 2007). Cultures of *B. oleronius* were maintained on nutrient agar plates, and cultures grown overnight in nutrient broth (Oxoid, Ltd) at 30°C 200 rpm to stationary phase. This culture was then used to inoculate fresh media and grown at 30°C or 37°C under aerobic conditions to assess the effect of temperature on protein expression. Growth was quantified by measuring the optical density at 600 nm on a spectrophotometer (Eppendorf Biophotometer).

Protein extraction and western blotting

Cultures of *B. oleronius* were grown for 72 h at 30°C or 37°C. Cultures (100 mL) were harvested by centrifugation at 3000× *g* for 15 min (Beckman GS-6 Centrifuge). The supernatant was discarded and cells were washed twice with PBS. Cells were re-suspended in lysis buffer (20 mM piperazine, 5 mM NaCl, 0.2% v/v Trition x100, pH 7.2) with protease inhibitors (10 μg/ml of Aprotinin, Leupeptin, Pepstatin A, TLCK) and stirred for 1 h at 4°C. Protein suspension was obtained by centrifugation at 6000× *g* for 2 min. Supernatant was removed and protein was quantified using the Bradford method. Protein lysate was re-suspended in denaturing sample buffer [1 μg/μl] for 1D SDS-PAGE. After electrophoresis, resolved proteins were western blotted as described by McMahon *et al.* (2014) and probed using the anti-62 kDa antibody (O'Reilly, Menezes and Kavanagh 2012).

Label-free proteomic analysis

Protein was extracted from *B. oleronius* grown at 30°C or 37°C as described and the Bradford method was applied to quantify protein for acetone precipitation overnight. Samples were centrifuged at 10 000× *g* for 10 min, and the pellet was resuspended in 25 μl of 6 M urea, 2 M thiourea and 0.1 M Tris-HCl buffer (pH 8.0). Protein was reduced with dithiothreitol (0.5 M DTT), alkylated with idoacetamide (0.55 M IAA) and digested with sequence grade trypsin (Promega, Ireland) at a trypsin: protein ratio of 1:40, overnight at 37°C. Tryptic peptides were purified for mass spectrometry using C18 spin columns (Medical Supple Company, Ireland) and 1 μg of peptide mix was eluted onto a QExactive (ThermoFisher Scientific, USA) high resolution accurate mass spectrometer connected to a Dionex Ultimate 3000 (RSLCnano) chromatography system. Peptides were separated by an increasing acetonitrile gradient form 2%–40% on a Biobasic C18 Picofrit column (100 mm length, 75 mm ID), using a 180-min min reverse phase gradient at a flow rate of 250 nl/min. A full MS scan of range 200–2000 was followed to select the 15 most intense ions prior to MS/MS. Protein identification from this data was performed using the Andromeda search engine in Max-Quant (version 1.2.2.5) to correlate against a combined *B. subtilis* and *B. vietnamensis* database downloaded from http://www.uniprot.org/. Selected parameters and results

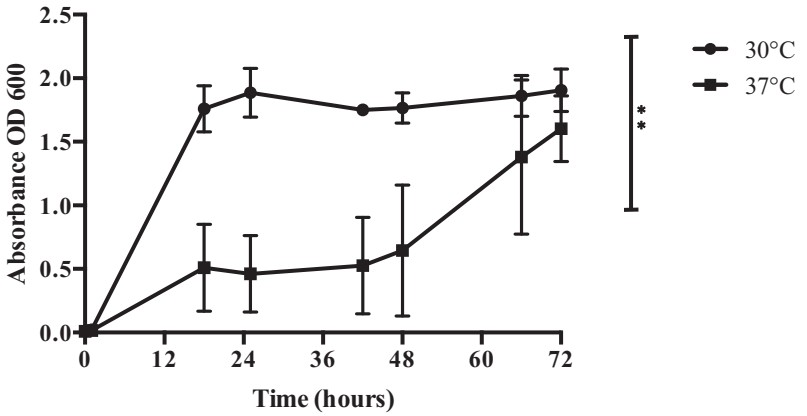

Figure 1. Growth of *B. oleronius* cultured at 30°C and 37°C for 72 h. Growth at 37°C is significantly less than at 30°C (**$P < 0.01$). All values are the mean ± SE of three independent determinations.

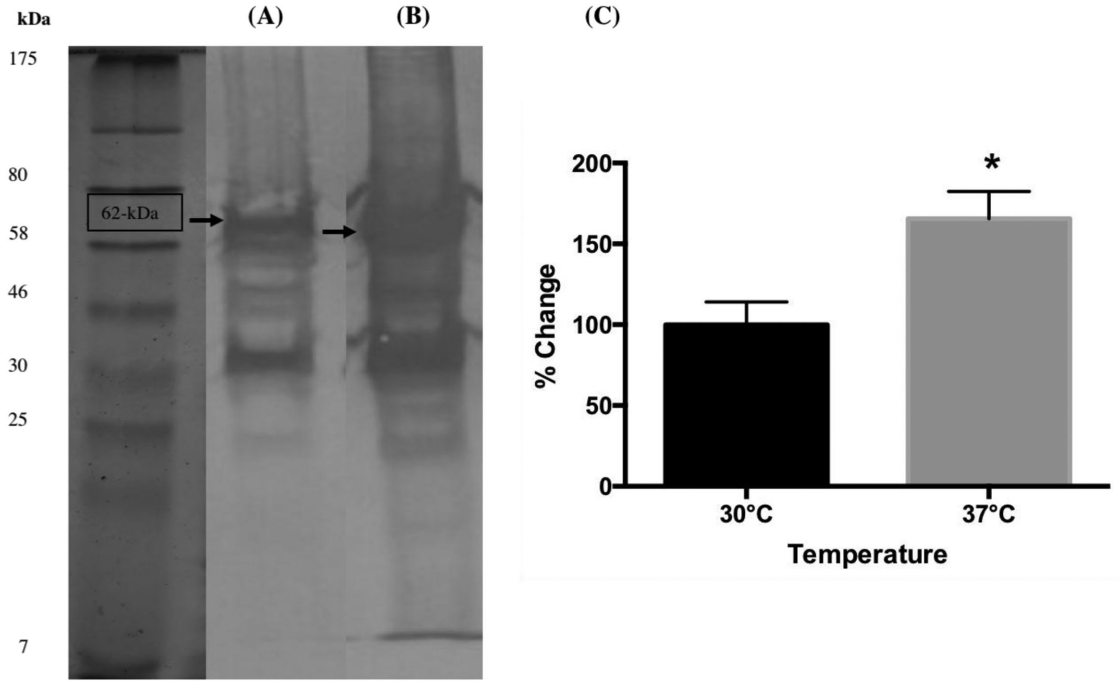

Figure 2. *Bacillus oleronius* proteins were resolved by SDS-PAGE as described and western blot analysed with anti-62kDa protein antibody. An increase of the immune-reactive 62 kDa is observed at 37°C **(B)** compared to 30°C **(A)**. Image J analysis (version 1.50i) indicating significant 1.65-fold increase of reactive 62 kDa at 37°C in comparison to 30°C ($P < 0.05$) **(C)**.

processing were applied as described in detail by Maguire *et al.* (2017).

Statistical analysis

All experiment were carried out on three independent occasions, and results were expressed as the mean ± SE. Significant differences were considered at $P < 0.05$.

RESULTS

Effect of temperature on growth and production of stimulatory antigens by *B. oleronius*

Bacillus oleronius was grown for 72 h in nutrient broth at 30°C and 37°C and monitored as described. The results indicate the bacteria cultured at 37°C demonstrated reduced growth and that growth accelerated at 48 h (Fig. 1). At 30°C, the stationary phase is reached at 18 h while at 37°C the culture failed to reach the stationary phase by 72 h.

Cultures of *B. oleronius* were grown at 30°C or 37°C for 72 h. Protein was extracted from cells and resolved by 1D SDS-PAGE prior to western blotting as described using the anti-62kDa antibody. The result demonstrated statistically significant ($P < 0.05$) increased reactivity by the anti-62 antibody (1.65-fold increase) to proteins from the cells grown at 37°C in particular (Fig. 2).

Variations in proteomic response of *B. oleronius* following growth at elevated temperature

Label-free quantitative proteomics was performed on cell lysate from *B. oleronius* grown at 30°C and 37°C. Principal component

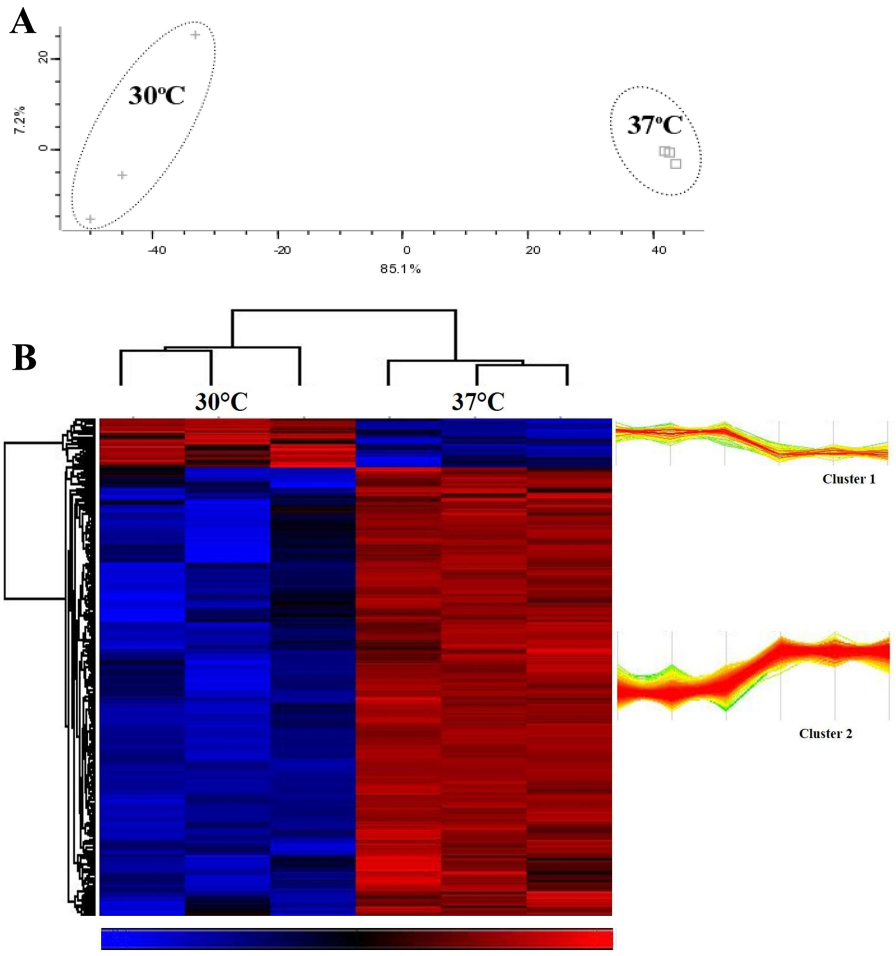

Figure 3. **(A)** PCA of three replicates of each treatment included in LFQ analysis. Dashed circles surround the two sample groups. **(B)** Heat map based on hierarchical clustering of *B. oleronius* proteomic profiles cultured at 37°C versus 30°C. This heat map represents the median protein expression values of all statistically significant differentially and uniquely detected proteins. Hierarchical clusters resolved two distinct columns comprising the replicates from the original sample groups and cluster rows based on expression profile similarities.

analysis (PCA) was performed with normalised intensity values and resolved a clear difference in the proteomes of bacteria grown at 30°C and 37°C (Fig. 3A). All statistically significant proteins were visualised in a hierarchical cluster (Fig. 3B), performed using Z-score normalised intensity values for differentially abundant proteins. In total, 905 peptides were identified representing 900 proteins with two or more peptides and 560 proteins were determined to be differentially abundant with a fold change > 1.5-fold (ANOVA, $P < 0.05$) (Fig. 5). At 37°C, 506 proteins were found in higher abundance (426 non-imputated proteins and 80 imputated) (Table 1), and 54 proteins were found in lower abundance (9 non-imputated and 45 imputated) (Table 2) when compared against 30°C. These proteins were statistically analysed following imputation of zero values using a number close to the lowest value of the range of proteins plus or minus standard deviation. The protein showing the highest increase in abundance at 37°C was alanine dehydrogenase with a fold change of 94.3 ($P < 0.05$) (Fig. 4). Proteins identified at 37°C with imputated values and higher abundances were phosphocarrier protein HPr (483.5-fold increase), putative phosphoesterase (385.5-fold increase), iron transporter FeoA (323.8-fold increase), cold-shock protein (260.2-fold increase), nucleoside diphosphate kinase (163.3-fold increase) (Table 1).

The protein zinc metalloprotease was identified as the protein most decreased in abundance at 37°C with a fold change of 19.3 ($P < 0.05$). Imputated proteins with a lowered abundance included ferredoxin (325.2-fold decrease), peptidase S8 (244.5-fold decrease), protein prkA (57.2-fold decrease), stage IV sporulation protein A (33.4-fold decrease) and protein translocase subunit SecY (9.7-fold decrease) (Table 2).

The Blast2GO annotation software was applied to group proteins together based on conserved and GO terms in order to identify pathways and processes potentially associated with temperature stress. GO terms were categorised by biological processes (BP; Fig. 5A) and cellular components (CC; Fig. 5B). The greatest change in protein proportion of BP were proteins labelled as cellular process (189 proteins in 30°C; 562 proteins in 37°C), single-organism process (151; 428) and metabolic process (194; 562). Proteins grouped as CC with increased abundance at 37°C were labelled under cell part (148 proteins in 30°C; 444 proteins in 37°C), macromolecular complex (59; 157) and cell (149; 445).

DISCUSSION

The aim of the work presented here was to characterise the effect of temperature on the abundance of immune stimulatory

Table 1. Relative fold change of proteins 'increased' in abundance in *Bacillus oleronius* cultured at 37°C. Only proteins that had over two matched peptides with a *t*-test probability <0.5 and that were found to be differentially expressed at a 1.5-fold change were considered to be significantly higher in abundance at 37°C.

Protein annotation (* = non-imputated protein)	Peptides	Sequence coverage %	PEP	Overall intensity	Fold difference
Phosphocarrier protein HPr	13	98.9	3.39E-62	1.90E + 11	483.5
Putative phosphoesterase	15	59.3	1.68E-172	1.01E + 11	385.5
Iron transporter FeoA	9	57.8	9.09E-130	9.44E + 10	323.8
Cold-shock protein	7	100	3.74E-76	8.26E + 10	260.2
Nucleoside diphosphate kinase	16	75	5.23E-112	3.57E + 10	163.3
Ethyl tert-butyl ether	7	40.2	0	2.84E + 10	153.4
Cold-shock protein	11	100	5.91E-24	1.68E + 11	150.8
Elongation factor Ts	29	57	1.67E-98	4.80E + 10	139.0
Thiol-disulfide oxidoreductase	15	69.5	4.60E-65	2.67E + 10	134.6
Phosphocarrier protein Chr	9	48.2	0	5.84E + 10	131.5
Acetyl-CoA acetyltransferase	27	71.1	2.27E-175	2.18E + 10	109.8
*Alanine dehydrogenase	29	80.1	4.30E-86	7.11E + 10	94.3
Peroxiredoxin	9	67.1	0	2.53E + 10	90.3
Peroxiredoxin	13	57.8	0	2.02E + 10	85.9
Universal stress protein	10	54.7	1.79E-186	4.59E + 10	84.0
Serine/threonine protein kinase	12	70.7	1.14E-188	1.60E + 10	78.8
Iron ABC transporter ATP-binding protein	20	78.5	2.51E-38	2.11E + 10	75.3
Cold-shock protein	5	97	1.01E-82	3.38E + 10	74.2
Transition state regulator	11	77.4	5.08E-12	1.97E + 10	71.0
ABC transporter substrate-binding protein	28	58.1	1.44E-199	1.78E + 10	70.8
Nitrogen fixation	10	93.6	3.13E-232	3.86E + 10	69.2
Carbonic anhydrase	8	45.9	2.30E-97	1.92E + 10	67.7
Alkyl hydroperoxide reductase	17	71.7	6.41E-64	1.96E + 10	67.3
50S ribosomal protein L11	9	46.8	1.35E-89	2.41E + 10	65.7
XRE family transcriptional regulator	7	49.6	2.02E-198	1.40E + 10	64.9
Flagellar basal body rod protein	4	14.9	6.40E-80	1.98E + 10	63.8
Sugar ABC transporter substrate-binding protein	32	61.3	1.23E-98	8.06E + 10	60.4
Branched-chain alpha-keto acid dehydrogenase subunit E2	25	62.9	0	2.09E + 10	59.9
Translation initiation factor IF-1	7	88.9	1.01E-76	3.46E + 10	57.9
Chemotaxis protein CheY	10	81.7	5.10E-42	1.61E + 10	56.6

proteins by *B. oleronius*. It was hypothesised that the elevated skin temperature associated with this chronic dermatological condition may lead to an increase in the production of *B. oleronius* immuno-stimulatory proteins, thus heightening the cutaneous immune response. The results presented here indicate that *B. oleronius* grows slower at 37°C than at 30°C (Fig. 1) but produces more of the immune-stimulatory 62 kDa protein at this higher temperature (Fig. 2)

Label-free quantitative analysis identified a substantial number of proteins increased in abundance when *B. oleronius* was grown at 37°C. Proteomic analysis revealed the increased abundance of many stress-related proteins including phosphocarrier protein HPr (483.5-fold increase), putative phosphoesterase (385.5-fold increase), cold-shock protein (260.2-fold increase), universal stress protein (84.0-fold increase), general stress protein (21.7-fold increase), 10 kDa chaperonin (18.1-fold increase) and 60 kDa chaperonin (12.6-fold increase). This heat shock protein (HSP) 60 kDa protein is a member of the GroEL family. Stress proteins and HSPs are highly immunogenic and can act as an early trigger of the innate immune response by the recognition of macrophages (Horváth *et al.* 2008). Increased antigenic load has been shown to recruit neutrophils to the site of infection (O'Reilly *et al.* 2012a). The heightened immune response of rosacea patients highlights the abnormal activation of neutrophils which subsequently contributes to erythema and inflammation (McMahon *et al.* 2016). Neutrophil activation leads to the secretion of pro-inflammatory cytokines, such as tumour necrosis factor (TNF-α) and IL-8, *in vivo* and also induces further neutrophil migration (Holmes 2013; Jarmuda *et al.* 2014). Neutrophils also secrete matrix metalloprotease-9 and cathelicidin, which degrade collagen and act as an antimicrobial (O'Reilly *et al.* 2012a). The downstream effects of the innate immune defense lead to inflammation and tissue degradation in the vicinity of the sebaceous unit which is commonly observed in rosacea, particularly in cases of papulopustular rosacea where inflammation is localised at the site of papules and pustules (Jarmuda *et al.* 2012).

The most significantly abundant protein at 37°C was phosphocarrier protein HPr (483.5-fold increase) which is involved in the phosphotransferase system responsible for the uptake of carbohydrates (Siebold *et al.* 2001). The phosphocarrier protein HPr is required by some Gram-negative bacteria for virulence and the HPr regulon consists of many membrane-associated proteins which have been implicated in host interaction and stress response (Antunes *et al.* 2016). Iron transporter FeoA (323.8-fold increase) is involved in ferrous iron transport which is essential for bacterial virulence; however, an overload of iron effectors has been implicated with the inflammatory response (Cartron *et al.* 2006; Wessling-Resnick 2010). It has been shown that rosacea patients are immune-reactive to the *B. oleronius* antigens; once the serum reactive antigens escape from the pilosebaceous unit, the innate immune system may be activated due to the presence of pathogenic foreign material. If the antigenic load reaches a critical level, this may induce an

Table 2. Relative fold change of proteins 'decreased' in abundance in *Bacillus oleronius* cultured at 37°C. Only proteins that had over two matched peptides with a t-test probability <0.5 and that were found to be differentially expressed at a 1.5-fold change were considered to be significantly lower in abundance at 37°C.

Protein annotation (* = non-imputated protein)	Peptides	Sequence Coverage %	PEP	Overall intensity	Fold difference
Ferredoxin	3	26.8	1.02E-22	1.70E + 10	325.2
Peptidase S8	11	7.5	1.14E-72	1.66E + 09	244.5
Protein prkA	9	19.2	1.33E-63	2.54E + 08	57.2
Stage IV sporulation protein A	6	12.8	6.74E-22	1.13E + 08	33.4
*Zinc metalloprotease	15	38.3	8.08E-177	1.72E + 09	19.3
Protein translocase subunit SecY	4	7.9	2.47E-11	3.05E + 08	9.7
Glyoxalase	4	39.2	4.87E-51	5.08E + 08	9.0
*NAD(P)H nitroreductase	17	47.8	0	5.48E + 10	6.1
*Osmotically inducible protein C	8	68.5	3.59E-194	1.67E + 09	6.0
GMP synthase [glutamine-hydrolyzing]	6	14.2	1.94E-78	5.05E + 07	5.4
ABC transporter ATP-binding protein	2	5.7	2.73E-22	6.07E + 07	5.0
*Superoxide dismutase	13	79.7	0	3.57E + 11	4.8
SAM-dependent methyltransferase	3	13.6	1.18E-16	8.63E + 07	4.7
*Glutamine amidotransferase	8	64.2	1.47E-118	3.10E + 09	4.5
Asparaginase	3	16.6	1.68E-41	9.32E + 07	4.2
Thiamine pyrophosphokinase	3	16.3	6.09E-30	1.07E + 08	4.0
*Oligoendopeptidase F	17	26.1	0	3.10E + 09	3.9
Transcriptional regulator	3	26.8	1.22E-13	8.73E + 07	3.7
Hydrolase	5	23	6.66E-57	1.07E + 08	3.6
4-hydroxy-tetrahydrodipicolinate reductase	3	22.3	1.77E-20	8.26E + 07	3.2
*Peptide deformylase	9	54.3	2.24E-189	1.31E + 09	3.1
Segregation and condensation protein B	3	14.2	1.86E-07	8.30E + 07	3.1
Energy-coupling factor transporter transmembrane protein EcfT	3	14.3	1.61E-06	9.12E + 07	3.1
Glycerol-3-phosphate acyltransferase	1	7.3	8.78E-25	9.54E + 07	3.1
Gluconeogenesis factor	3	13.8	5.68E-28	1.58E + 08	2.9
Diguanylate phosphodiesterase	6	13	2.58E-19	1.13E + 08	2.8
Alcohol dehydrogenase	4	13.7	5.46E-64	1.49E + 08	2.8
Alcohol dehydrogenase	4	13.7	5.34E-41	1.49E + 08	2.8
SAM-dependent methyltransferase	4	12.6	8.63E-11	1.29E + 08	2.7
Oxidoreductase	3	13	2.67E-61	1.22E + 08	2.5

inflammatory cascade resulting in the erythemic symptoms of rosacea (Lacey, Kavanagh and Tseng 2009; Li *et al.* 2010; Szkaradkiewicz *et al.* 2012).

The two proteins most significantly decreased in abundance were ferredoxin (325.2-fold) and peptidase S8 (244.5-fold) at 37°C (Table 2). Ferredoxin is an iron sulphur protein from the flavoprotein superfamily and acts as one of two electron acceptors in the electron bifurcation mechanism (Mock *et al.* 2015; Peters *et al.* 2016; Seo *et al.* 2016). Most bacteria produce extracellular proteases at the stationary growth phase; however, elevated growth temperature may act as a stress for *B. oleronius* and affect the production and transcription of proteases, such as peptidase S8. This protein is a subtilisin-like protease with catalytic mechanisms and biological activity (Di Cera 2009; Morya *et al.* 2012; Liu, Huang and Feng 2015). Protein prkA (57.2-fold) and stage IV protein A (33.4-fold) have key roles in bacterial sporulation and both are significantly reduced in abundance at 37°C (Table 2). Protein prkA is a sigma^{E}-dependent sporulation protein functioning as a general marker protein for different stress factors (Tam *et al.* 2006). Although sporulation allows bacteria to survive during stress, it also creates an opportunity to remain dormant in environments that are temporarily undesirable. Lowered abundance of sporulation proteins at 37°C inhibits *B. oleronius* from protection during temperature stress. Stage IV protein A is involved with the development of the cortex and germ wall to form a peptidoglycan structure, all of which is essential for spore dormancy (Waites *et al.* 1970; Driks 2002). This work studied the proteomic changes of *B. oleronius* in response to elevated temperature and demonstrated increased abundance of proteins associated with stress responses, energy metabolism and biological processes. A similar increase in abundance of general stress proteins has been identified in *B. subtilis* in response to various stress and starvation conditions (Hecker and Völker 2001). Importantly, at 37°C a number of significant proteins were decreased in abundance (Table 2) resulting in reduced extracellular proteolytic activity and catalytic mechanisms (e.g. peptidase S8) as well as minimising the opportunity for sporulation (e.g. stage IV protein A).

Previous work has demonstrated the upregulation of lipase and delta-hemolysin production by *S. epidermidis* cultured at 37°C in comparison to 30°C (Dahl, Ross and Schlievert 2004). In the work presented here, we demonstrate that this temperature inhibits the growth of *B. oleronius* but in response the bacterium produced an increased abundance of potentially immuno-stimulatory proteins which may antagonise the dermal immune response and lead to the symptoms of rosacea. A comprehensive understanding of the potential contribution of bacteria to the induction of rosacea may enable the development of more effective therapies for the control of this disfiguring condition.

FUNDING

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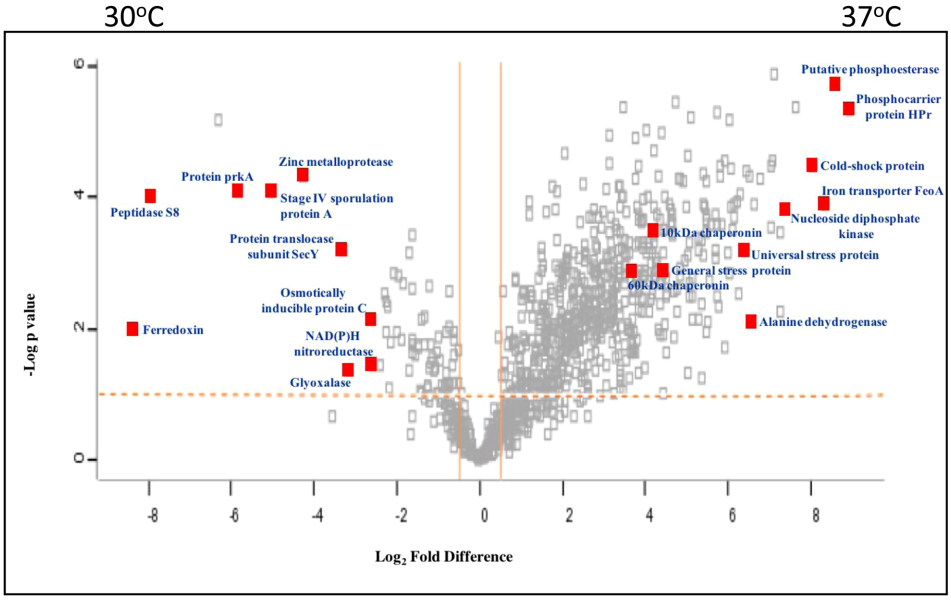

Figure 4. Volcano plot showing proteins altered in abundance in *B. oleronius* cultured at 37°C. Protein intensity difference (–$\log_2$ mean intensity difference) and significance in differences (–log *p*-value) based on a two-sided *t*-test. Proteins above the dashed line are considered statistically significant ($P < 0.05$) and those to the right and left of the vertical lines indicate >1.5-fold positive changes and fold negative changes at 37°C respectively, versus control at 30°C.

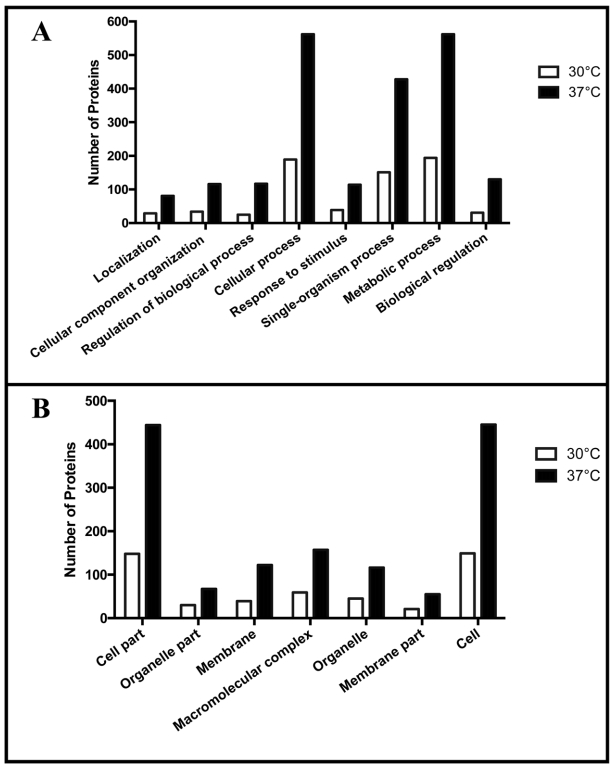

Figure 5. Comparative bar chart showing changes to number of proteins involved in selected biological processes **(A)** and cellular components **(B)** at level 2 ontology. Proteins based on percentage proportion of the total proteins found in the proteomic profile of *B. oleronius* grown at 30°C and 37°C.

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