Ecological functions and environmental fate of exopolymers of Acidobacteria

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Chapter 4

Impact of different trace elements on the growth and proteome of two strains of Granulicella

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Abstract

Members of the phylum *Acidobacteria* are difficult to isolate and grow. For some isolated strains, recent studies suggested that trace elements are needed in culture media for proliferation but the impact of these trace elements on their growth and metabolism is not known to date. Here we evaluated the effect of the trace element solution SL-10 on the growth of two strains (5B5 and WH15) of *Granulicella* sp. and studied changes in the proteome in response to manganese (Mn). Growth of *Granulicella* species was enhanced in nutrient media amended with trace element solution SL-10. When trace elements were tested separately, mangenese was shown to enhance growth of *Granulicella* species which was associated with a higher tolerance to this metal compared to seven other metal ions. Variations in tolerance to metal ion concentrations among the two strains suggest different mechanisms to cope with metal ion homeostasis and stress. Comparative proteome analysis revealed different responses to manganese for the two *Granulicella* strains. Strain 5B5 had more upregulated proteins (57), while strain WH15 had more downregulated proteins (112). Further comparisons demonstrated that no upregulated or downregulated proteins were shared between the two strains. In strain 5B5, a higher number of upregulated proteins that can use Mn^{2+} as co-factor was detected. Genome analyses of the two strains also revealed that the most common transcriptional regulator of Mn homeostasis *mntR* was not present. Instead several candidate transporters were found that could be involved in Mn homeostasis of *Granulicella*. We postulate that these transporters may enhance the adaptive ability of *Granulicella* to metal-enriched environments, such as the Mn-rich decaying wood environment from which these strains were isolated.

Keywords: *Acidobacteria*, *Granulicella*, Genome, Proteome, Manganese, Metabolism.

1. Introduction

Despite being widespread and dominant in soil ecosystems (Kuramae *et al.*, 2012, Navarrete *et al.*, 2013, Pereira de Castro *et al.*, 2016), the phylum *Acidobacteria* has a low number of cultivated representatives, due to difficulties in isolation and propagation under laboratory conditions (Dedysh & Yilmaz, 2018). Most *Acidobacteria* isolates are slow growers and can take weeks to months to develop colonies (Eichorst *et al.*, 2011, de Castro *et al.*, 2013). Recently, changes in traditional culture methods and application of unconventional culture media composition have increased the number of new *Acidobacteria* isolates considerably. Currently, 62 species have been described (NCBI Resource Coordinators, 2016), while in 2011 only 14 species had been isolated and characterized (de Castro, 2011). Modifications in culture media and cultivation conditions, such as low concentration of nutrients (Janssen *et al.*, 2002, Stevenson *et al.*, 2004), higher CO_2 concentrations (Stevenson *et al.*, 2004), unusual or complex polysaccharides as carbon sources (Pankratov *et al.*, 2008, Eichorst *et al.*, 2011), longer incubation periods (de Castro *et al.*, 2013), addition of humic acids and quorum-sensing molecules (Stevenson *et al.*, 2004), employment of soil solution equivalents and inhibitors for unwanted microorganisms (de Castro *et al.*, 2013, Foesel *et al.*, 2013), are strategies that have been applied for the enrichment and isolation of new *Acidobacteria* species.

Once the isolates are obtained, better cell proliferation can be achieved with richer culture media, containing higher concentrations of nutrients (de Castro *et al.*, 2013). For instance, trace elements can be used to improve microbial growth and biomass in laboratory conditions, even though the specific requirements among strains and species are variable (Banerjee *et al.*, 2009, Merchant & Helmann, 2012). Metal ions, such as Fe, Mn, Zn, and Cu are fundamental for microbial metabolism, being required at low concentrations (Abbas & Edwards, 1990). They play an important role in biological processes, acting as co-factors of enzymes (Wintsche *et al.*, 2016), activating metalloregulators and trace element dependent proteins (Hantke, 2001, Zhang *et al.*, 2009), forming functional complexes with secondary metabolites (Morgenstern *et al.*, 2015, Locatelli *et al.*, 2016) and promoting the detoxification of reactive oxygen species (Kehres & Maguire, 2003, Locatelli *et al.*, 2016).

Although some culture media used for *Acidobacteria* growth and isolation are supplemented with trace elements (de Castro *et al.*, 2013, Navarrete *et al.*, 2013), the impact of these metals on their growth and metabolism is not yet known. Although metal ions are essential for many biological processes, they can be toxic at high concentrations (Puri *et al.*, 2010). Metal ions cannot be synthesized or degraded, therefore cellular homeostasis of metals relies mostly on transport, which involves several mechanisms that sense, uptake, immobilize or pump metals out of the cell (Chandrangsu *et al.*, 2017). In the present study, we evaluated the effect of trace elements and particularly Mn on the growth of two strains of *Granulicella* sp. WH15 and 5B5, derived from decaying wood, *Acidobacteria* subgroup 1 (class *Acidobacteriia*) (Valášková *et al.*, 2009). We used the optimized culture medium PSYL5 (Campanharo *et al.*, 2016), in order to boost the growth of the two strains, evaluated the impact of Mn through

proteome studies and performed genomic analyses on both strains.

2. Material and Methods

2.1. Acidobacteria strains

Two strains of *Acidobacteria*, 5B5 and WH15 belonging to *Granulicella* genus of subdivision 1 were used in this study. Both strains belong to the culture collection of the Netherlands institute of Ecology (NIOO-KNAW), department of Microbial Ecology. They were isolated from wood in advanced decay stage, in association with the white-rot fungus *Hypholoma fasciculare*, in the Netherlands (Valášková *et al.*, 2009). The genome of strain WH15 is deposited at NCBI with accession number CP042596 while the genome of 5B5 was sequenced in this study.

2.2. Trace elements solution (SL10) and individual trace elements effect on bacterial growth

The effect of trace element solution SL 10 (Atlas, 2010) on the growth of both bacterial strains was evaluated for two different concentrations (1 ml and 10 ml) of the solution per L of PSYL5 culture medium. PSYL5 medium was composed of (g/L): 1.8 KH_2PO_4, 0.2 $MgSO_4.7H_2O$, 30 sucrose and 1.0 yeast extract; pH was adjusted to 5.0 (Campanharo *et al.*, 2016). Culture medium without the amendment of SL10 solution was used as a control. Seven-day-old cell suspensions of both strains were inoculated in 70 ml of culture medium to an OD_{600nm} 0.01. The cultures were incubated under aeration for 7 days at 30 °C and a constant rotation rate of 50 rpm. Every 24 hours the optical density of the cultures was measured with an Eppendorf photometer at a wavelength of 600 nm (Eppendorf, Hamburg, Germany). For the evaluation of the different trace elements on the growth of both strains, individual trace element stock solutions and growth curves were executed for each metal separately, using the same growth conditions described above. The composition of the trace element solution SL10 and the final concentration of each trace element in culture medium is shown in Table 1. The metal ion that produced a significantly higher growth in comparison with the control was selected for further experiments. All experiments were executed in triplicates.

Statistical analysis was performed using Sigmaplot v14. Normality of the data was checked using Shapiro-Wilk test. Two Way Repeated Measures ANOVA was used to test the effect of SL10 solution and individual trace elements on the growth rate of WH15 and 5B5 strains.

Table1: Composition of trace element solution SL10 and final concentration (µM) of each individual metal in culture medium.

Reagents	SL 10 composition (mg/l)	Final µM concentration in culture medium (1ml/L SL10)
$FeCl_2.4H_2O$	1.500	7.54
$ZnCl_2$	0.070	0.51
$MnCl_2.4H_2O$	0.100	0.51
H_3BO_3	0.006	0.10
$CoCl_2.6H_2O$	0.190	0.80
$CuCl_2.2H_2O$	0.002	0.01
$NiCl_2.6H_2O$	0.024	0.10
$Na_2MoO_4.2H_2O$	0.036	0.15

2.3. Genome of *Granulicella* sp. 5B5

The *Granulicella* sp. 5B5 strain obtained from the collection of the Netherlands Institute of Ecology (NIOO-KNAW) was grown on 1/10 TSB agar medium (Valášková *et al.*, 2009) at pH 5.0 for 3 days at 30 °C. The bacterial cells were harvested and the genomic DNA was extracted using MasterPure™ DNA Purification Kit (Epicentre, Madison, WI) according to manufacturer's instructions. A total of 10 mg of DNA was sent to the Genomics Resource Center (Baltimore, USA) for a single long insert library (15kb-20kb), that was constructed and sequenced in one SMRTcell using the PacBio RS II (Pacific Biosciences, Inc.) sequencing platform. *De novo* assembly was performed with the help of SMRT Analysis software v2.2.0 (Pacific Biosciences) featuring HGAP 2 (Chin *et al.*, 2013), and subsequent correction with Pilon 1.16 (Walker *et al.*, 2014) to reveal a circular replicon: a 3,928,701 bp chromosome (G+C content 61,1%; 58× coverage). Automatic gene prediction and annotation was performed by using Prokka (Seemann, 2014) and RAST genome annotation server (http://rast.nmpdr.org/) (Aziz *et al.*, 2008). Genes were mapped to COG and KEGG IDs using the COG database (2014 release) (Galperin *et al.*, 2015) and KEGG database (release 2013) (Kanehisa, 2000), using eggNOG mapper. The CAZyme contents of 5B5 genome were determined by identifying genes containing CAZyme domains using the dbCAN2 meta server (cys.bios.niu.edu/dbCAN2) (Zhang *et al.*, 2018), according to the CAZy (Carbohydrate-Active Enzyme) database classification (Lombard *et al.*, 2014). Only CAZyme domains predicted by at least two of the three algorithms (DIAMOND, HMMER and Hotpep) employed by dbCAN2 were kept. Circular genome map was drawn using CGView software (Stothard & Wishart, 2004). Average Nucleotide Identity (ANI) between strains 5B5 and WH15 was calculated using the webtool ANI calculator, available at https://www.ezbiocloud.net/tools/ani (Yoon *et al.*, 2017). The *Granulicella* sp. 5B5 strain genome is deposited at NCBI with accession number CP046444.

2.4. Heavy metal resistance assays and Metal Resistance Gene (MRG) annotation

The resistance of strains 5B5 and WH15 to varied metal ion concentrations was tested in solid culture medium PSYL5 pH 5. Five concentrations (0.5 mM, 1 mM, 2 mM, 5 mM, 10 mM) of 9 metal ion sources were tested: $ZnCl_2$, $NiCl_2$, $MnCl_2$, $CoCl_2$, $CuCl_2$, $NaMoO_4$, $AlCl_2$, $CdCl_2$ and

$C_8H_4K_2O_{12}Sb_2$. As a control, an *Escherichia coli* DH5α strain, with known low metal resistance was used. Six colonies of each strain previously grown on PSYL5 solid medium without metal were inoculated in the culture media with each different metal concentration. After 7 days of growth at 30 °C, colonies were reinoculated on a new plate with the same metal concentration, in order to confirm growth. If colonies did not develop within 7 days, plates were incubated for extra 7 days. Colonies were reinoculated 3 times for confirmation. When the strains were resistant to the highest concentration of metal used (10 mM), we performed additional tests using higher metal concentrations (15 mM, 20 mM, 25 mM, 30mM, and 40 mM). In order to identify genes that could be involved in metal ion homeostasis, we searched the genomes of both strains against the experimentally confirmed and predicted BacMet databases using BacMet Scan (Pal *et al.*, 2014) with less strict parameters (40% similarity), due to the high quantity of hypothetical proteins in the genomes of both bacteria.

2.5. Acquisition of cytosolic proteome with and without manganese treatment by mass spectrometry and data analysis

For the proteome analysis, we analysed the effects of the metal ion which significantly improved the growth yield of both strains in comparison to the control without metal ion. Therefore, Mn was selected for further experiments. The growth curves of WH15 and 5B5 with added manganese ($MnCl_2$) and controls without trace elements were repeated, using the same parameters as described above. Cells were collected at day 4 of the growth curve, when the differences in the OD_{600nm} between manganese treatment and control treatment started to be statistically significant. A total of 3 ml of bacterial cells per replicate (n=6 for each strain) were harvested by centrifugation at 10,015 x g at 4 °C for 10 min. Pellets were washed twice with 1 mL of TE buffer and finally resuspended in 1 ml TE buffer. A volume of 500 µL of cell suspension was transferred into 2 mL screw cap tubes filled with 500 µL glass beads (0.1 mm in diameter; Sarstedt, Germany) and mechanically disrupted using Fastprep (MP Biomedicals) for 3 x 30 sec at 6.5 m/s; with on ice incubation for 5 min between cycles. To remove cell debris and glass beads, samples were centrifuged for 10 min at 4 °C at 21,885 x g, followed by a second centrifugation (30 min at 4 °C at 21,885 x g) to remove insoluble and aggregated proteins. The protein extracts were kept at -20 °C. Protein concentration was determined using RotiNanoquant (Carl Roth, Germany). Proteins were separated by SDS-PAGE. Protein lanes were cut into ten equidistant pieces and in-gel digested using trypsin as described earlier (Grube *et al.*, 2014). Tryptic peptides were separated on an EASY-nLC II coupled to an LTQ Orbitrap Velos using a non-linear binary 76 min gradient from 5 – 75 % buffer B (0.1 % acetic acid in acetonitrile) at a flow rate of 300 nL/min and infused into an LTQ Orbitrap Velos (Thermo Fisher Scientific, USA) mass spectrometer. Survey scans were recorded in the Orbitrap at a resolution of 60,000 in the m/z range of 300 – 1,700. The 20 most-intense peaks were selected for CID fragmentation in the LTQ. Dynamic exclusion of precursor ions was set to 30 seconds; single-charged ions and ions with unknown charge

were excluded from fragmentation; internal calibration was applied (lock mass 445.120025). For protein identification resulting MS/MS spectra were searched against a database containing protein sequences of *Granulicella* sp. strain 5B5 or *Granulicella* sp. strain WH15 and common laboratory contaminants (9,236 entries or 7,782 entries, respectively) using Sorcerer-Sequest v.27, rev. 11 (Thermo Scientific) and Scaffold v4.7 (Proteome Software, USA) as described earlier (Stopnisek *et al.*, 2016). Relative quantification of proteins is based on normalized spectrum abundance factors (NSAF (Zhang *et al.*, 2010)). The mass spectrometry proteomics data were deposited to the ProteomeXchange Consortium (http://proteomecentral.proteomexchange.org) via the PRIDE (Perez-Riverol *et al.*, 2019) partner repository with the dataset identifier PXD016551.

Statistical analysis was done using MeV (Saeed *et al.*, 2003); t-test was applied for proteins that were identified in at least two replicates of the respective condition. T-test of z-transformed normalized data were performed with the following parameters: unequal group variances were assumed (Welch approximation), P-values based on all permutation with P=0.01, significance determined by adjusted Bonferroni correction. Only significantly changed proteins showing at least 1.5-fold changes between conditions were considered for further analysis. Furthermore, so-called on/off proteins, that were only identified in one condition were analysed. Functional classification of *Granulicella* sp. strain 5B5 and WH15 proteins was carried out using eggNOGmapper (http://eggnogdb.embl.de/#/app/emapper) (Huerta-Cepas *et al.*, 2017), COG (Galperin *et al.*, 2015) and KEGG databases (Kanehisa, 2000). In order to identify proteins that could be involved in metal ion homeostasis, we searched proteins with significantly changed amounts against the experimentally confirmed and predicted BacMet databases using BacMet Scan (Pal *et al.*, 2014) with less strict parameters (40% similarity), due to the high quantity of hypothetical proteins in the genomes of both bacteria. Voronoi treemaps for visualization of proteome data were generated with Paver software (Decodon GmbH, Germany).

3. Results

3.1. Effects of the trace element solution SL10 on growth

The addition of trace element solution SL10 in liquid culture medium produced a significant effect ($p<0.001$) on the growth of both strains of *Granulicella* sp. Both concentrations (1X and 10X) of trace element solution (SL10) significantly increased 5B5 strain growth, with the highest growth recorded for 1X and 10X concentrations of SL10 (Figure 1a). In addition, the cultures showed a longer lag phase for 10X concentration of SL10 (Figure 1a).

WH15 strain had a significantly higher growth rate with the addition of 1X SL10 compared to control and 10X SL10 (Figure 1b), except at day one of incubation. Differently from strain 5B5, 10X SL10 did not enhance the growth of WH15, having instead the opposite effect (Figure 1b).

3.2. Effect of individual trace elements on growth

Of all the trace elements, manganese (Mn) and copper (Cu) significantly increased the growth of strain 5B5 compared to control starting from day three of the incubation period until the end ($p<0.001$) (Figure 1c). Iron (Fe) significantly increased the growth of 5B5 strain only at day six, when compared with the control. Boron (B) Zinc (Zn), cobalt (Co), nickel (Ni) and molydbdate (Mo) did not have any significant effect on the growth of the strain throughout the duration of the experiment (Figure 1c and d).

Throughout the incubation period, Mn was the only trace element that significantly ($p<0.001$) increased the growth of strain WH15 in comparison with the control with no metal (Figure

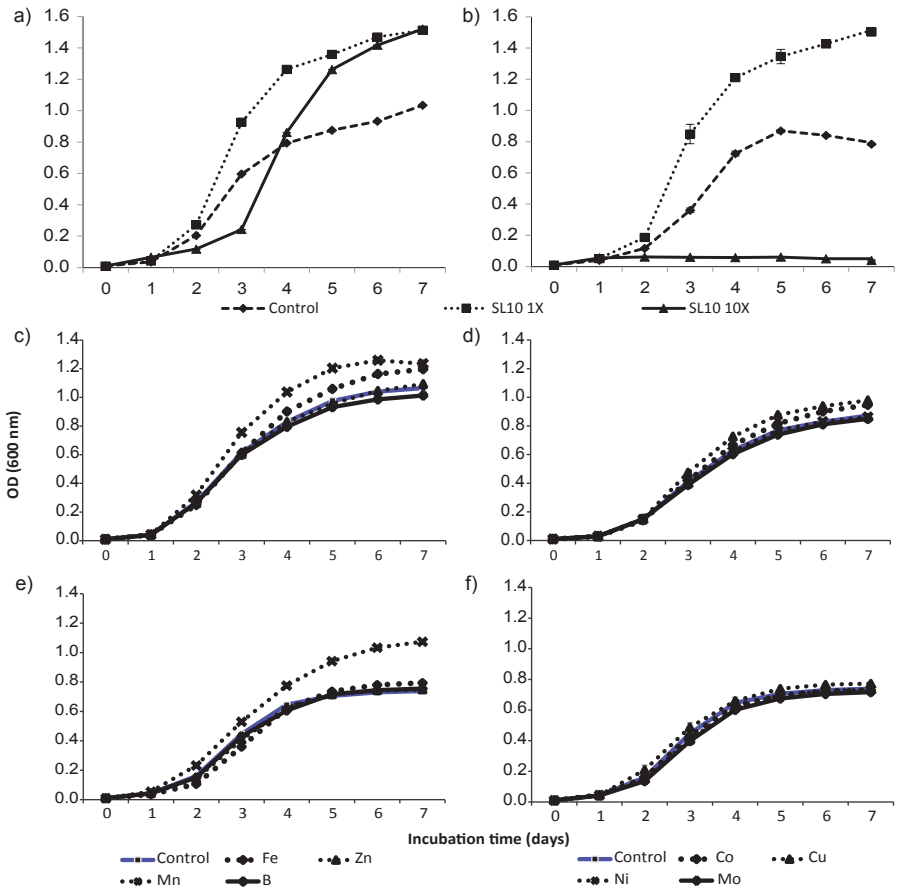

Figure 1: Growth curves of *Granulicella* sp.5B5 and WH15 strains on PSYL5 liquid culture medium with different concentrations of trace element solution (SL10) and individual metal ions. a) Strain 5B5 with SL10 solution: control (no SL10), 1X SL10 and 10X SL10; b) Strain WH15 with SL10 solution: control (no SL10), 1X SL10 and 10X SL10; c) 5B5 with individual metal ions: control (no metal), Fe, Zn, Mn, B; d) 5B5 with individual metal ions: control (no metal), Co, Cu, Ni, Mo; e) WH15 with individual metal ions: control (no metal), Fe, Zn, Mn, B; f) WH15 with individual metal ions control (no metal)), Co, Cu, Ni, Mo. The error bar is the standard error of the mean (n=3) and indicates differences in response variable between different treatments. Fe: $FeCl_2.4H_2O$; Zn $ZnCl_2$; Mn: $MnCl_2.4H_2O$; B: H_3BO_3; Co: $CoCl_2.6H_2O$; Cu: $CuCl_2.2H_2O$; Ni: $NiCl_2.6H_2O$; Mo: $NaMoO_4.2H_2O$.

1e). Fe, Zn, Co, Cu, Ni, Mo and Bo did not have any significant effect on the growth of WH15 as compared to the control (Figure 1e and 1f).

3.3. Granulicella sp. strain 5B5 genome annotation and CAZymes

The assembled genome of *Granulicella* sp. 5B5 is 3,928,701 bp, with 61.1% GC content, 3,306 proteins and only one rRNA operon. Functional annotation using COG (Cluster of Ortholog Groups) and RAST analysis resulted in the classification of 2,615 genes into 20 COG functional groups and the annotation of 1,260 genes to RAST subsystems. The properties of the genomes of strains 5B5 and also WH15 (sequenced previously (Chapter 3), Costa et al., submitted) are listed in Table 2. A circular genome map of 5B5 is depicted in Figure 2, together with that of strain WH15. The distribution of genes into COGs/RAST functional categories for strain 5B5 genome is depicted in Figure 3. Average Nucleotide Identity (ANI) (Figueras *et al.*, 2014) between strains WH15 and 5B5 was 72.75%, showing that the strains do not belong to the same species.

RAST analysis showed that only 37% of the annotated genes (1,260/3,374) could be assigned to subsystems. Among the subsystem categories present in the genome, carbohydrates, dormancy and sporulation had the highest and lowest feature counts, respectively (Figure 2b).

Table 2. Genomic features of *Granulicella* sp. strains 5B5 and WH15.

Genome	***Granulicella* sp. 5B5**	***Granulicella* sp. WH15**
Size (bp)	3,928,701	4,673,153
G+C content (%)	61.1	60.7
Number of coding sequences	3306	3,939
Number of features in Subsystems	1,260	1,496
Number of RNA genes	51	51
Number of contigs	1	1

Analysis with ANTISMASH v4.2.0 revealed the presence of 5 biosynthetic gene clusters (Table 3). The identified clusters showed potential for the production of terpenes, betalactone, type III polyketide synthases (T3PKS) and bacteriocin. Annotation with dbCAN (table 4) revealed the presence of 92 carbohydrate-associated enzymes, distributed in four classes: seven carbohydrate esterases (CE), 63 glycosyl hydrolases (GH), 20 glycosyl transferases (GT) and two polysaccharide lyases (PL), but no carbohydrate binding modules (CBM) or auxiliary activities (AA) were observed. Further evaluation of the CAZymes demonstrated the potential for the degradation of a wide range of carbohydrates, as the genome of strain 5B5 possessed CDSs for 48 CAZyme families, including as α- and β-glucosidases (GH1, GH13, GH3, GH31), α- and β-galactosidases (GH2, GH27, GH35, GH57), α- and β-mannosidases (GH1, GH38, GH125), rhamnosidases (GH28, GH106), fucosidases (GH29), xylosidases (GH39, GH43, GH54), arabinofuranosidase (GH43, GH54) and amylases (GH13, GH77). The cellulose synthase genes observed in other *Granulicella* genomes (Rawat *et al.*, 2013, Rawat *et al.*, 2013) were

not observed in the genome of strain 5B5.

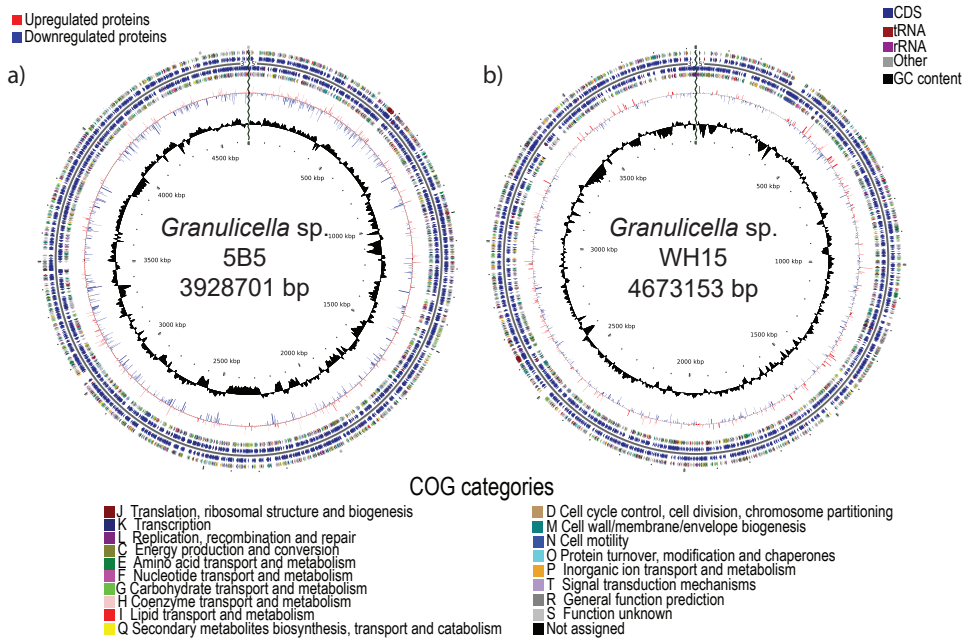

Figure 2: Graphical circular genome map of *Granulicella* sp. strains a) 5B5 and b) WH15. Rings indicate coding sequences and COG categories, GC content, upregulated (red) and downregulated (blue) proteins upon the addition of Mn to bacterial cultures.

Table 3: Biosynthetic gene clusters in *Granulicella* sp. strain 5B5 genome revealed by analysis with ANTISMASH.

Cluster	**Type**	**Most similar known cluster**
Cluster1	Terpene	Malleobactin (NRPS 11% similarity)
Cluster2	betalactone	
Cluster3	t3pks	
Cluster 4	Bacteriocin	
Cluster 5	Terpene	

Table 4: Number of genes from different CAZyme families observed in the genomes of strains 5B5 and WH15.

CAZyme family	***Granulicella* sp. 5B5**	***Granulicella* sp. WH15**
Auxiliary activity (AA)	0	13
Carbohydrate binding module (CBM)	0	22
Carbohydrate esterase (CE)	7	41
Cohesin	0	1
Glycoside hydrolase (GH)	63	86
Glycosyl transferase (GT)	20	52
Polyssacharide lyase (PL)	2	2
Total	**92**	**217**

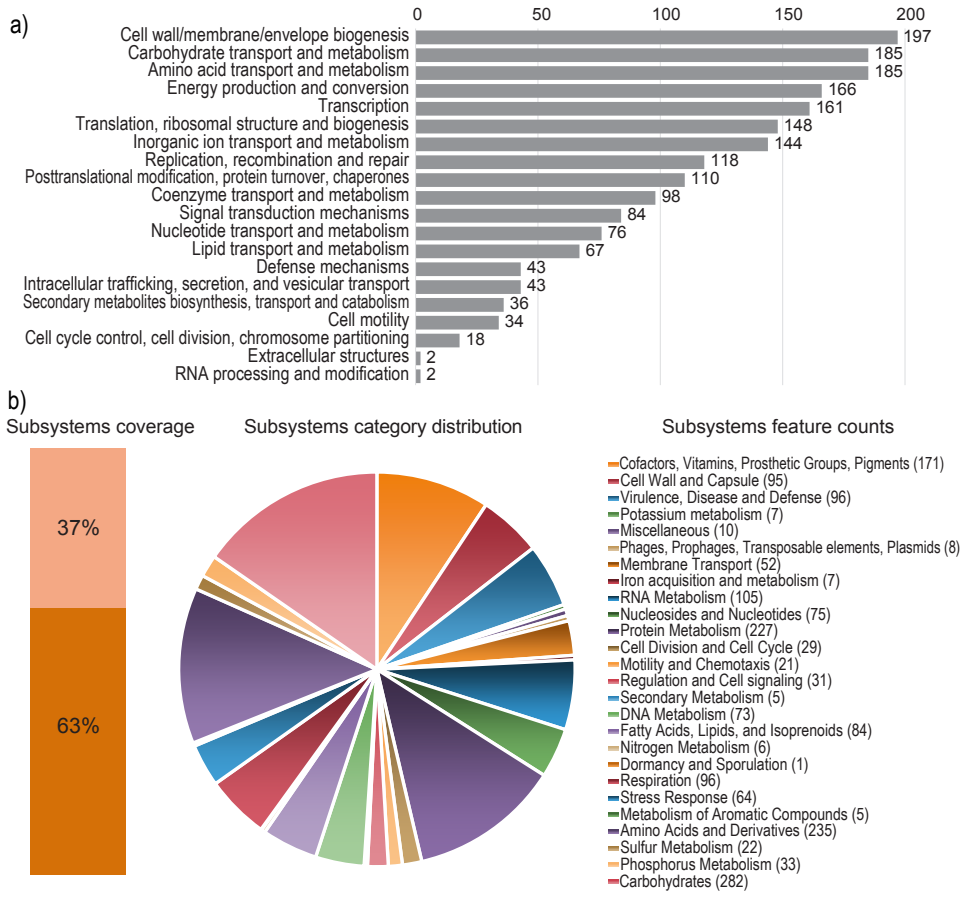

Figure 3: Statistics of COG and RAST subsystems annotations of *Granulicella* strain 5B5. a) COG categories distribution, showing number of genes annotated in each category. b) Subsystem category distribution. The light orange bar represents the percentage of proteins that could be annotated by RAST Server and the dark orange bar represents the proteins that were not annotated. The pie chart represents the percentage of proteins annotated to each subsystem category.

3.4. Metal resistance assays and Metal Resistance Gene (MRG) annotation

Metal resistance tests on agar medium demonstrated that both *Granulicella* strains were able to grow on $NiCl_2$ (max 2 mM) and $NaMoO_4$ (max 1 mM). Strain 5B5 was able to grow on 0.5 mM $ZnCl_2$, strain WH15 grew on 0.5 $AlCl_2$, and both strains could grow at concentrations of $MnCl_2$ up to 40 mM (Table 5).

WH15 genome search against BacMet experimentally-confirmed and predicted resistance genes databases revealed 28 ORFs and 78 ORFS, respectively, with hits similar (>45% identity) to genes involved in resistance of a wide range of metals such as As, Cd, Zn, Co, Cu, Fe, Mn, Mo, Ni and Zn, multidrug and metal efflux transporters and DNA binding response regulators (Table S1).

Table 5: Growth of *Granulicella* sp. strains WH15 and 5B5 in solid culture medium with different metal concentrations.

Metal source	Strain	concentration in mM					
		0.5	1	2	5	10	15-40
$ZnCl_2$	WH15	-	-	-	-	-	-
	5B5	+	-	-	-	-	-
$NiCl_2$	WH15	+	+	+	-	-	-
	5B5	+	+	+	-	-	-
$MnCl_2$	WH15	+	+	+	+	+	+
	5B5	+	+	+	+	+	+
$CoCl_2$	WH15	-	-	-	-	-	-
	5B5	-	-	-	-	-	-
$CuCl_2$	WH15	-	-	-	-	-	-
	5B5	-	-	-	-	-	-
$NaMoO_4$	WH15	+	+	-	-	-	-
	5B5	+	+	-	-	-	-
$AlCl_2$	WH15	+	-	-	-	-	-
	5B5	-	-	-	-	-	-
$CdCl_2$	WH15	-	-	-	-	-	-
	5B5	-	-	-	-	-	-
$C_8H_4K_2O_{12}Sb_2$	WH15	-	-	-	-	-	-
	5B5	-	-	-	-	-	-

+ : positive colony formation; -:no growth. Comparisons made with the control without metal.

In addition, strain WH15 possessed two copies of Mn transporter MntH, and two ORFs (GWH15_19170 and GWH15_03225), with 60.2 and 44% identity with the Mn transcriptional regulator *mntR*, respectively.

We obtained a similar profile for the 5B5 genome, with 65 ORFs that had hits higher than 45% identity against the experimentally confirmed database and 23 ORFs that had hits higher than 45% identity against the predicted database (Table S2). For both searches, genes involved in resistance to several metal ions, as well as multidrug and metal efflux transporters and transcription regulators were observed (Table S2). The genome of strain 5B5 also contains 2 copies of the *mntH* transporter, and 3 ORFs related to Mn transcriptional regulator *mntR*, as well as 3 ORFs similar to Mn ABC transporters *mntA*/*ytgA* and Mn efflux pump *mntP* (Table S2).

3.5. Manganese-responsive proteome of *Granulicella*

Since Mn had a significant effect on the growth of both *Granulicella* sp. strains, we further investigated the effects of Mn on cellular metabolism by a proteomics. At day 4, the differences in growth between control and Mn treatment started to be statistically significant for both strains (Figure 4), and therefore samples were collected at this particular time point for proteome analysis.

Proteome data for strain 5B5 showed that 1,028 proteins were detected in both treatments in at least two out of three replicates each. Overall, 216 proteins showed significantly different abundances, with 14 so-called on/off proteins, which were present in only one condition (Figure S1a). The proteome patterns of strain 5B5 under control and Mn treatments are depicted in Figure 5a. A total of 46 proteins were upregulated 1.5-fold and 11 proteins were

"on", while 43 proteins were downregulated 1.5-fold and 3 proteins were "off" in the Mn treatment. Among these differentially expressed proteins, 90 could be assigned to COG categories and 67 could be annotated to KEGG orthologs (Figure 6).

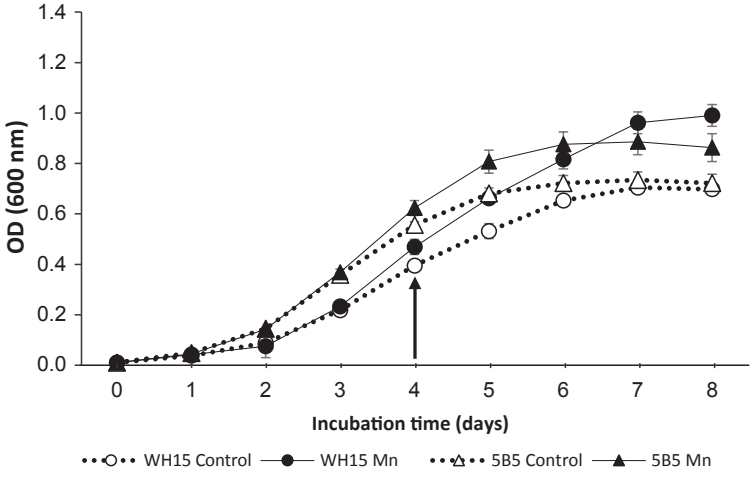

Figure 4: Growth curves of *Granulicella* sp. strains 5B5 and WH15 in PSYL 5 liquid culture medium with addition of Mn and control without addition of metal. The arrow indicates the timepoint when samples were collected for proteomics analysis. The error bar is the standard error of the mean and indicates differences in response variable between different treatments.

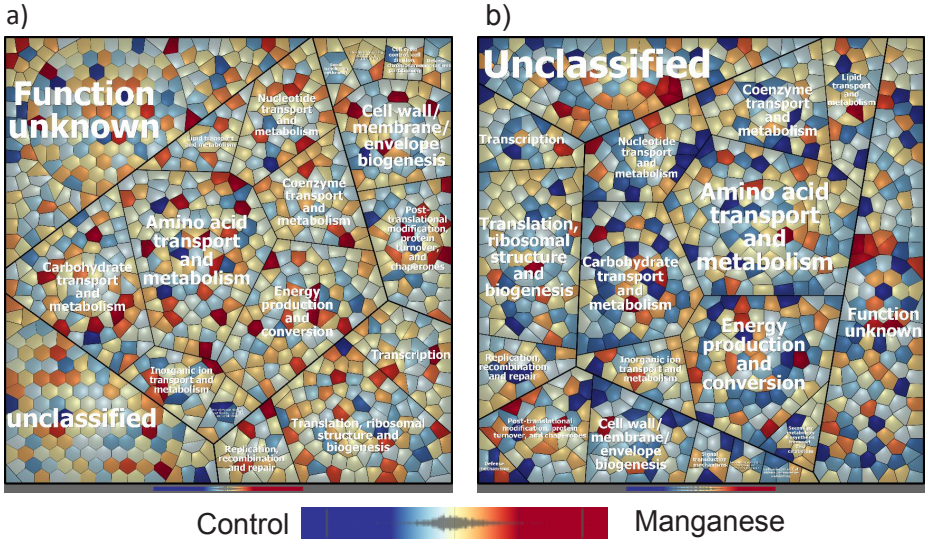

Figure 5: Voronoi treemap visualization of protein expression patterns of *Granulicella* sp. strains a) 5B5 and b) WH15 spectrum under control and manganese treatments. Functional classification was done using Prophane 2.0 (www.prophane.de) and is based on eggNOG database, B function level "subrole". Each cell represents a quantified protein; proteins are clustered according to their function. Proteins with higher amount under control conditions (no metal) are depicted in blue, proteins with higher amount in manganese treatment are depicted in red.

The qualitative analysis of the proteomic data for strain WH15 demonstrated that, overall, 909 proteins were identified in both conditions in two out of three replicates each. In total, 171 proteins showed significant differences between Mn and control conditions (t-test, p=0.01) (Figure S1b). The proteome patterns of strain WH15 under control and manganese treatments are depicted in Figure 5b.

Comparisons between treatments showed that 16 proteins were upregulated at least 1.5-fold, while 93 proteins were downregulated at least 1.5-fold. In addition, 19 proteins were

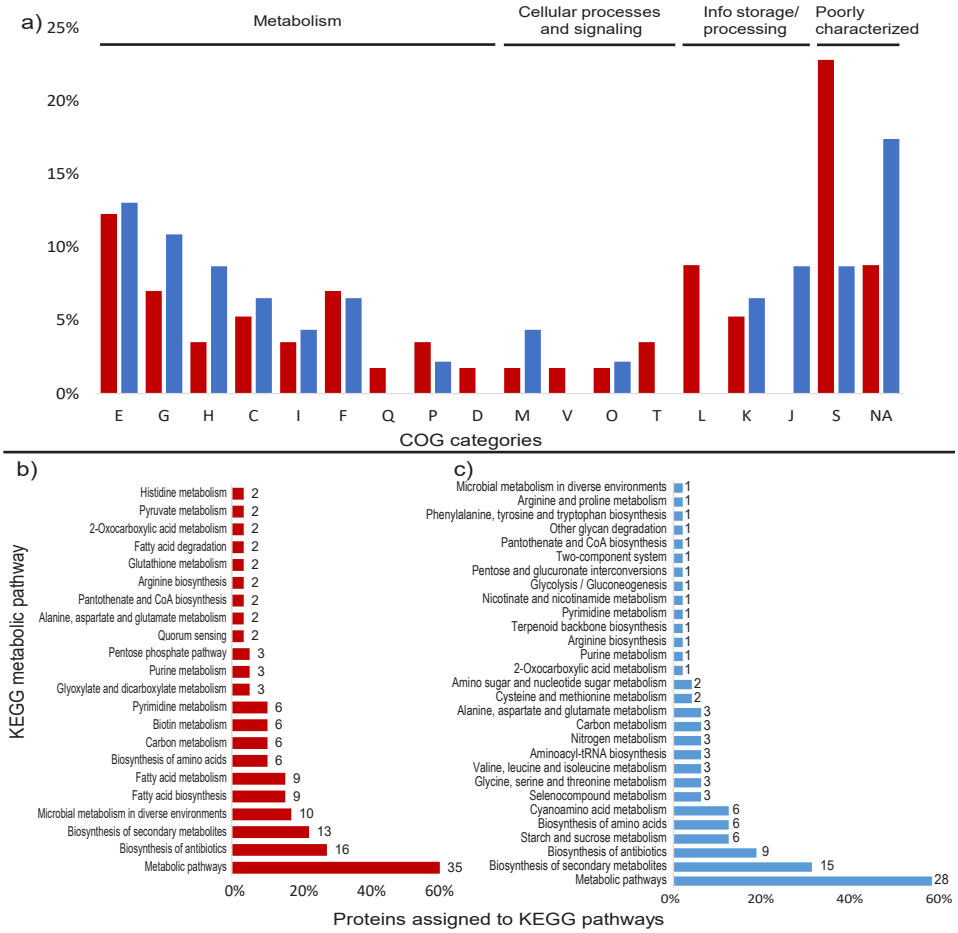

Figure 6: Differentially expressed proteins assigned to COG categories and KEGG pathways in the proteomic profile of *Granulicella* strain 5B5 with the addition of Mn. a) Percentage of upregulated (red) and downregulated (blue) proteins assigned to COG categories; b) Number of upregulated proteins assigned to KEGG pathways (only pathways with more than one protein mapped are shown); c) Number of downregulated proteins assigned to KEGG pathways. E-Amino acid transport and metabolism; G- Carbohydrate transport and metabolism; H-Coenzyme transport and metabolism; C-Energy production and conversion; I-Lipid transport and metabolism; F-Nucleotide transport and metabolism; Q- Secondary metabolites; D-Cell cycle; N-Cell motility; M-Cell wall/membrane/envelope biogenesis; V-Defence mechanisms; P-Inorganic ion transport and metabolism; U-Intracellular trafficking; O-Post translational modification; T-Signal transduction mechanisms; L-Replication, recombination and repair; K-Transcription; J-Translation; S-Function unknown; R-General function and prediction; X-Mobilome.; NA-not assigned.

"off" in the Mn treatment and present only in control conditions. Among the significantly different proteins, 112 were assigned to COG categories and 89 were annotated to KEGG orthologs (Figure 7).

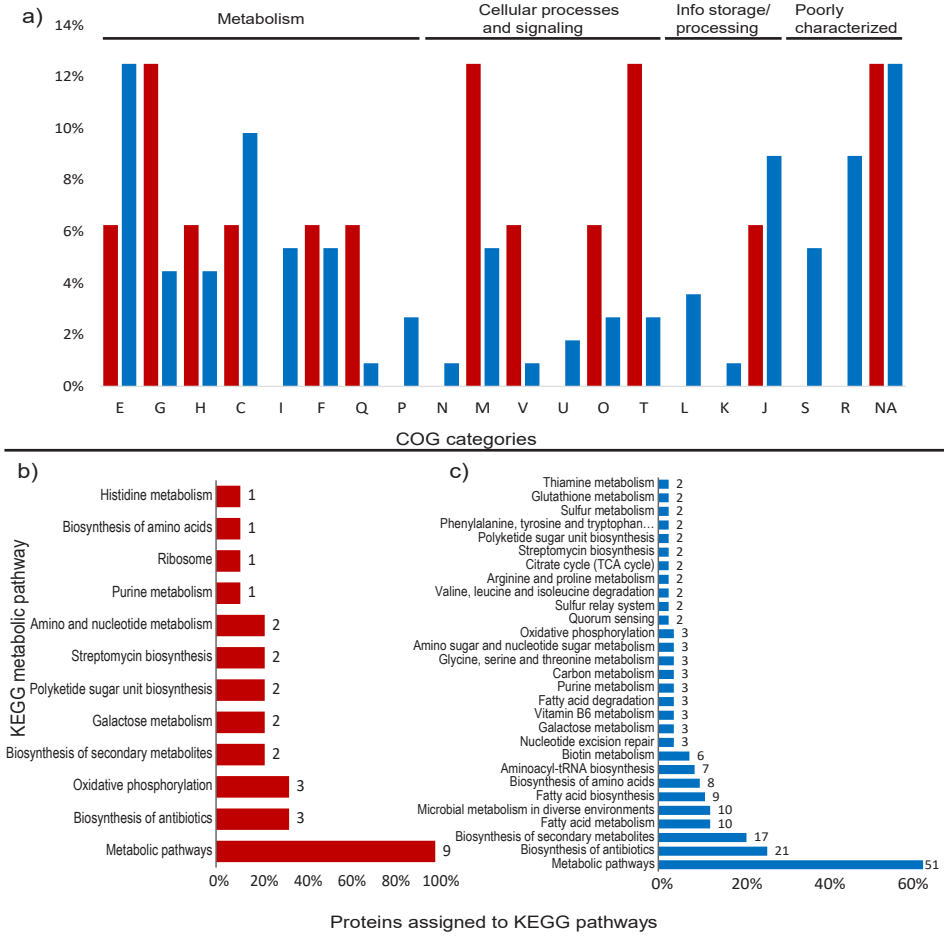

Figure 7: Differentially expressed proteins assigned to COG categories and KEGG pathways in the proteomic profile of *Granulicella* strain WH15 with the addition of Mn. a) Percentage of upregulated (red) and downregulated (blue) proteins assigned to COG categories; b) Number of upregulated proteins assigned to KEGG pathways; c) Number of downregulated proteins assigned to KEGG pathways (only pathways with more than one protein mapped are shown). E-Amino acid transport and metabolism; G- Carbohydrate transport and metabolism; H-Coenzyme transport and metabolism; C-Energy production and conversion; I-Lipid transport and metabolism; F-Nucleotide transport and metabolism; Q-Secondary metabolites; D-Cell cycle; N-Cell motility; M-Cell wall/membrane/envelope biogenesis; V-Defence mechanisms; P-Inorganic ion transport and metabolism; U-Intracellular trafficking; O-Post translational modification; T-Signal transduction mechanisms; L-Replication, recombination and repair; K-Transcription; J-Translation; S-Function unknown; R-General function and prediction; X-Mobilome.; NA-Not assigned.

Comparatively, proteome analysis revealed different responses to manganese for the two strains. Strain 5B5 had more upregulated proteins (57), while WH15 had more downregulated

proteins (112). Further comparisons demonstrated that no upregulated or downregulated proteins were shared between strains. In strain 5B5 a higher number of upregulated proteins that can use Mn^{2+} as co-factor was detected. For both strains, proteins annotated as Mn transporters were not detected in the proteomic profile.

3.5.1. Upregulated proteins

3.5.1.1. Strain 5B5

COG analysis showed that proteins were mainly distributed among the categories E-amino acid transport and metabolism (7), L-replication, recombination and repair (5), G-carbohydrate transport and metabolism (4) and F-nucleotide transport and metabolism (4) (Figure 6a). A total of 36 proteins were assigned to KEGG orthologs, that were mapped to 47 metabolic pathways, and some orthologs were mapped to more than one pathway. The majority of the proteins were mapped to 'general' metabolic pathways (35), biosynthesis of antibiotics (16), biosynthesis of secondary metabolites (13) and microbial metabolism in diverse environments (10) (Figure 6b), but no specific metabolic pathway was upregulated. Looking deeper into the upregulated proteins, we identified several enzymes that require Mn^{2+} or Mg^{2+} as cofactor, such as nucleoside diphosphate kinase Ndk, octaprenyl diphosphate synthase IspB, UDP-N-acetylmuramate--L-alanyl-gamma-D-glutamyl-meso-2,6-diaminoheptandioate ligase Mpl, adenine deaminase Ade, phosphate-specific transport system accessory protein PhoU, oxalate decarboxylase OxdD, phosphoenolpyruvate carboxykinase PckG and 3'-5' exoribonuclease YhaM (Table S3).

Search against BacMet Databases showed 25 proteins with hits (> 45% identity) against the experimentally confirmed database and 17 proteins with hits (> 45% identity) against the predicted metal resistance genes database (Table S4). The genes were mostly associated resistance/homeostasis of several metal ions, such as Fe, Cu, As, Ni, Co and Zinc. Interestingly, 4 ORFS were similar to metal ion transporters that could be involved in Mn homeostasis: ORF_05650 (hypothetical protein) had 43.5% identity with copper-translocating P-type ATPase CueA; ORF_03225 (hypothetical protein) had 31% identity with copper-translocating P-type ATPase CopA; ORF_06495 (TcrA) had 40% identity with copper-translocating P-type ATPase CopA and ORF_14875 (NatA_2) had 31% identity with metal ABC transporter ATP-binding protein TroB (Table S4).

3.5.1.2. Strain WH15

COG analysis showed that upregulated proteins distributed within several COG categories. The most common categories were: "G-carbohydrate transport and metabolism" (2), "M-cell wall/envelope/membrane biogenesis" (2) and "T-signal transduction mechanisms" (2) (Figure 7a). Within KEGG metabolic pathways, no specific pathway upregulation was observed.

A total of 9 upregulated proteins were assigned to KEGG orthologs, which were mapped to 12 KEGG metabolic pathways, since some orthologs were mapped to more than one

pathway. Most of the annotated proteins were mapped to 'general' metabolic pathways (9), biosynthesis of antibiotics (3) and oxidative phosphorylation (3) (Figure 7b).
Some of the upregulated proteins were ATP synthase subunit b, and the carbohydrate-associated enzymes putative sugar phosphate isomerase YwlF, UDP-glucose 4-epimerase GalE and dTP-4-dehydrorhamnose 3,5 epimerase RmlC (Table S5). The search against BacMet databases showed that 3 ORFs had hits against genes related to metal ion homeostasis. ORF GWH15_13825 (*cysO*) had 42% identity with predicted resistance gene *copA*, encoding a copper-exporting P-type ATPase and 31% similarity with the experimentally confirmed cation/multidrug efflux pump AdeG, which is part of AdeFGH efflux system. ORF GWH15_17845 (hypothetical protein) had 30% similarity with predicted resistance gene *rcnB/yohN*, a nickel/cobalt homeostasis protein; ORF GWH15_19690 (hypothetical protein) had 36% identity with predicted resistance gene *mtrA*, a DNA-binding response regulator.

3.5.2. Downregulated proteins

3.5.2.1. Strain 5B5

Annotation with COG database demonstrated that most proteins were distributed within the categories E –amino acid transport and metabolism (6), G-carbohydrate transport and metabolism (5), H-coenzyme transport and metabolism (4) and J-translation, ribosomal structure and biogenesis (4) (Figure 6a). Overall, 31 proteins were assigned to KEGG identifiers, which were mapped to 29 KEGG metabolic pathways. Most of the proteins were mapped to 'general' metabolic pathways (29), biosynthesis of secondary metabolites (15) and biosynthesis of antibiotics (9) (Figure 6b). Several proteins linked to general metabolism were repressed, but no specific metabolic pathway seemed to be repressed. Among the repressed proteins we observed enzymes involved in various cellular functions, such as cysteine synthase CysM, L-threonine dehydratase TdcB, ribonucleoside-diphosphate reductase subunit beta NrdB, putative glucose-6-phosphate 1-epimerase YeaD and carbonic anhydrase CynT (Table S6).

3.5.2.2. Strain WH15

Within COG categories, most of the downregulated proteins belonged to the categories 'E-aminoacid transport and metabolism' (14), 'C-energy production and conversion' (11), 'J-Translation, ribosomal structure and biogenesis' (10) and 'R-General function prediction' (10) (Figure 7a). Overall, 80 proteins were assigned to KEGG orthologs, which were mapped to 52 KEGG metabolic pathways, and some orthologs were mapped to more than one type of pathway. The majority of the proteins were mapped to 'general' metabolic pathways (51), biosynthesis of antibiotics (21), biosynthesis of secondary metabolites (17), fatty acid metabolism (10) and microbial metabolism in diverse environments (10) (Figure 7b), with no pathway specifically stimulated. Several enzymes involved in amino acid biosynthesis and metabolism were identified, such as tyrosyl, leucyl, alanyl and glycyl-tRNA synthetases

(TyrS, LeuS, AlaS and GlyS), leucyl aminopeptidase PepA, threonine synthase ThrC, xaa-pro-dipeptidyl-aminopeptidase PepQ, aminopeptidase YpdF, cysteine desulfurase IscS and prolyl tripeptidyl peptidase PtpA (Table S7).

4. Discussion

In this study, we evaluated the effect of trace element addition on the growth of two strains of *Granulicella*, belonging to phylum *Acidobacteria* subgroup 1. We observed that the growth in liquid medium of both strains was enhanced by the addition of Mn, to which the strains tolerated higher concentrations than other metal ions. Furthermore, variations in tolerance to metal ion concentrations suggest that the *Acidobacteria* strains possess different mechanisms to deal with metal stress. Strain 5B5 is likely more adapted to survive in an environment with higher concentration of several metal ions when compared to strain WH15.

When evaluated separately, Mn had a more pronounced effect on growth than other metal ions, but the mix of metals was more effective in enhancing bacterial growth, reflecting wide physiological needs and the importance of different metal ions in bacterial metabolism. For instance, *Escherichia coli* BW25113 growth was maximized with a mixture of Ni and Fe, which had a better effect than each metal separately and other metal ion mixtures (Trchounian *et al.*, 2016). The amendment of Mn to fermentation medium improved the growth of *Lactobacillus bifermentans*, increasing the production and activity of the enzyme glucose isomerase, necessary for biotechnological applications (Givry & Duchiron, 2008). Manganese is also an essential growth factor for *L. casei* and other species of lactobacilli, which is attributed to its role as a co-factor of enzyme lactate dehydrogenase, enhancing cell growth rate and biomass concentration (Fitzpatrick *et al.*, 2001, Lew *et al.*, 2013). On the other hand, Mn had no significant impact in the growth of *Halobacterium* (Joshi *et al.*, 2015).

Among the metals used for metal ion resistance testing, *Granulicella* strains WH15 and 5B5 only showed tolerance to Mn, exhibiting growth at the concentration of 40 mM Mn, which is higher than other bacterial strains. For instance, a resistant *Serratia marcescens* strain, isolated from Mn mine waters in Brazil, could grow on a maximum concentration of 6 mM Mn (Barboza *et al.*, 2017). Mn tolerance can vary widely in microorganisms, with a minimal inhibitory concentration ranging from 0.1 mM to 228.9 mM Mn in certain marine bacterial strains (Gillard *et al.*, 2019).

Manganese is essential for the growth and survival of most living organisms. It is a co-factor of a wide range of enzymes, being vital in specific metabolic pathways, such as sugar, lipid and protein catabolism (Jensen & Jensen, 2014), oxygenic photosynthesis in cyanobacteria (Kehres & Maguire, 2003, Cvetkovic *et al.*, 2010), signal transduction, stringent response, sporulation, and pathogenesis (Kehres & Maguire, 2003, Jensen & Jensen, 2014). One of the most widely known and studied Mn functions is the detoxification of reactive oxygen

species (ROS), where it is a redox-active co-factor in free radical detoxyfing enzymes, such as Mn-superoxide dismutase (MnSod) and mangani-catalase (Jakubovics & Jenkinson, 2001, Jensen & Jensen, 2014). Additionally, the detoxifying capaticities of Mn are not only enzyme-mediated, since non-protein complexes of Mn can also work as antioxidants when enzymes are not sufficient (Jensen & Jensen, 2014). Both *Granulicella* strains were isolated from decaying wood material, in association with the white rot fungus *Hypholoma fasciculare* (Valášková et al., 2009), where topsoil-litter samples have higher Mn concentrations of 101920 µg Mn/kg (Chapter 5). Since high concentrations of Mn can be observed in wood decay environments, especially when decomposition is caused by white rot fungi (Blanchette, 1984), tolerance to higher manganese concentrations could be a strategy to assure the survival of the studied *Acidobacteria* in this environment.

The evaluation of the genes in both did not reveal the presence of common genes involved in Mn regulation, the transcriptional regulator mntR (Jensen & Jensen, 2014). This result implies that the homeostasis of Mn in *Granulicella* strains is under control of another transcriptional regulator. On the other hand, both strains possessed two copies of the Mn transporter gene *mntH*. MntH seems to be the main responsible transporter in Mn influx, but it was already observed that Mn has a significant repressive effect in the expression of Mn transporters under manganese sufficiency, keeping Mn homeostasis and optimal levels of Mn inside the cells (Guedon *et al.*, 2003, Jensen & Jensen, 2014). Furthermore, the search of the genomes against BacMet databases demonstrated that both genomes possessed a wide range of transporters that are linked to the homeostasis of diverse metal ions.

Within the upregulated proteins from strain 5B5, three proteins were similar to copper P-type ATPase transporters and one protein was similar to the metal ion ATP-binding ABC transporter TroB. P-type ATPases, such as CtpC, in *Mycobacterium* species are responsible not only for Mn efflux, removing excess metal ion from the cells, but are also required for the metallation of proteins (Padilla-Benavides *et al.*, 2013). ABC transporters are as well responsible for Mn uptake, important in several bacterial species (Papp-Wallace & Maguire, 2006, Jensen & Jensen, 2014). In the proteome profile of strain WH15, we similarly observed a protein similar to the copper-exporting P-type ATPase and cation/multidrug efflux pump AdeG, which could be involved in maintaining optimal levels of Mn inside the bacterial cell. Furthermore, we found a protein similar to RcnB/YohN, which is an essential protein for Ni/Co homeostasis in *E. coli* (Bleriot *et al.*, 2011), and a protein similar to gene *MtrA*, which is involved in cell division control and cell wall metabolism in *M. tuberculosis* (Gorla *et al.*, 2018).

In addition to transporters, the proteomic analyses of both bacteria revealed other proteins which might be involved in the growth enhancement of both strains. The proteomic profile of strain WH15 had fewer upregulated enzymes in comparison with 5B5, demonstrating that Mn had more impact in transcription and protein expression in strain 5B5. Several enzymes that require Mn or Mg as cofactor were upregulated in the proteome of 5B5, but no enhancement of specific metabolic pathway was detected. Overall, the upregulated enzymes in strain 5B5

were involved in general metabolic pathways, which could be stimulated due to the higher metabolism necessary for a faster growth. For instance, nucleoside diphosphate kinase Ndk is a critical enzyme involved in the nucleotide metabolism of microorganisms, but is also part of posttranslational modification of proteins, as well as regulation of genes linked to quorum sensing, proteases and toxins (Yu *et al.*, 2017). Octaprenyl diphosphate synthase IspB is involved in the production of the lateral chain of ubiquinones, and is an essential enzyme for respiration and normal growth of *E. coli* (Okada et al., 1997). 3'-5' exoribonuclease YhaM was identified as a participating enzyme in mRNA turnover in *Bacillus subtilis* (Oussenko *et al.*, 2005). UDP-N-acetylmuramate--L-alanyl-gamma-D-glutamyl-meso-2,6-diaminoheptandioate ligase Mpl is a recycling enzyme that allows the constant remodeling of bacterial cell wall polymer occurring during cell growth and division (Herve *et al.*, 2007). Moreover, the categories replication, recombination and repair were only present within upregulated proteins, with proteins DNA helicase RecD2, DNA -binding protein Hup 2, protein RecA, metal-dependent hydrolase YcfH and UvrABC system protein UvrB. In addition, no specific pathway seemed to be repressed.

In the proteomic profile of strain WH15, we observed the upregulation enzymes involved in energy production, amino acid metabolism and transcription regulation. For instance, ATP synthase subunit β is part of the ATP synthase complex, which is involved in ATP synthesis and hydrolysis (Sokolov *et al.*, 1999). ATP phosphoribosyltransferase is an enzyme involved in histidine biosynthesis, a reaction that requires Mg, which can be substituted by Mn (Tebar *et al.*, 1977). Protein RsbV is a positive regulator of factor sigma β, which, in gram-positive bacteria is a key contributor to the resilience and survival of bacterial species to environmental conditions, such as variations in pH, osmotic stress or entry into stationary growth phase (Kullik & Giachino, 1997, Guldimann *et al.*, 2016). Phosphodiesterase CpdA is responsible for the hydrolysis of the second messenger cyclic AMP (cAMP), which controls cell responses to a variety of environmental conditions (Fuchs *et al.*, 2010). Similarly to the response of *Serratia marcescens* (Queiroz *et al*., 2018) to Mn stimulation, the limited number of upregulated proteins from strain WH15 could be attributed to the adaptation to the concentration of Mn used in the experiment, since it is an optimal growth condition. Furthermore, as a co-factor of several important enzymes (Crowley *et al.*, 2000, Jensen & Jensen, 2014), the presence of manganese might be improving bacterial growth by activating enzymes and enhancing metabolic activities involved in cell cycle and division, even when the enzymes were not differentially expressed. Among the repressed proteins, we found several amino acid synthases, that could be inhibited due to the ready availability of amino acids in the culture medium composition (Mader *et al.*, 2002), supplied by yeast extract.

The proteomic profiles of both strains were different, and did not exhibit the overexpression of specific pathways, indicating that Mn was more important in enhance enzymatic activity than to protein expression regulation. Finally, we did not find the most common transcriptional regulation of Mn homeostasis, implying that Mn regulation is performed by a

different gene or set of genes, but our analysis revealed candidate transporters that could be potentially involved in Mn homeostasis for *Granulicella* species. The presence of such type of transporters could facilitate the uptake of metal ions, improving the adaptability of bacteria to metal enriched environments (Campanharo *et al.*, 2016), promoting a tight regulation of metal ion homeostasis, as well as a resistance to high concentrations of metals.

Acknowledgments

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Supplementary Material

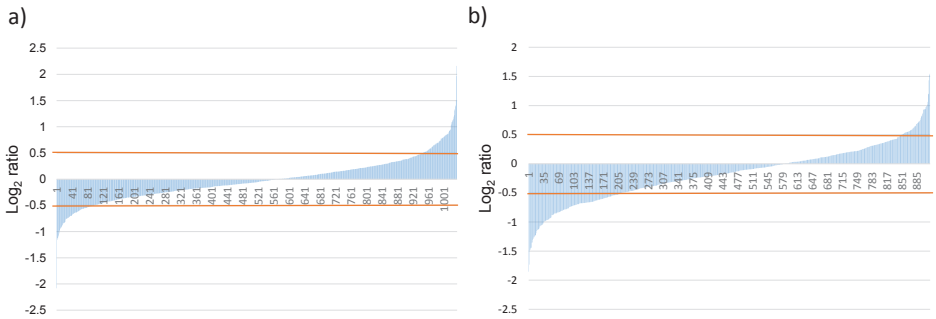

Figure S1: Expression pattern of proteins under manganese treatment of *Granulicella* sp. strain a) 5B5) and b) WH15. All proteins identified in at least two out of three replicates (excluding on/off proteins). Log_2 fold change is indicated by a red line.

Table S1: *Granulicella* strain WH15 gene annotation with BacMet databases. Annotation performed against the experimentally confirmed and predicted resistance genes databases using BacMet Scan software. Only genes with hits ≥ 40% identity in one of the databases are shown.

ORF	EXP	Compound	Identity (%)	PRE	Compound	Identity (%)
GWH15_16385	*arsB*	As	63.5	*acr3*	Arsenical-resistance protein	70.3
GWH15_11300	*mdtB*	Zn	60.5	*mdtB/yegN*	Multidrug transporter subunit MdtB	60.6
GWH15_16465	*hoxN*	Ni	60.0	*hoxN*	HoxN/HupN/NixA family Ni/Co transporter	60.0
GWH15_00760	*acn*	Fe	59.5	*acn*	Aconitate hydratase	60.1
GWH15_02935	*mdtB/yegN*	SDC, HCL	58.5	*mdtB/yegN*	Multidrug transporter subunit MdtB	58.6
GWH15_12080	*mdtB/yegN*	SDC, HCL	58.5	*mdtB*	Multidrug transporter subunit MdtB	59.2
GWH15_00470	*ruvB*	Cr, Te, Se	58.5	*ruvB*	DNA helicase RuvB	59.6
GWH15_11305	*mdtC/yegO*	SDC and other	56.5	*mdtC/yegO*	Multidrug transporter subunit MdtC	56.6
GWH15_13565	*pstB*	As	56.2	*pstB*	Phosphate ABC transporter ATP-binding protein PstB	56.6
GWH15_16395	*arsM*	As	54.4	*arsM*	Arsenite S-adenosylmethyltransferase	43.8

ORF (continued)	EXP	Compound	Identity (%)	PRE	Compound	Identity (%)
GWH15_02930	*mdtC/yegO*	SDC and other	52.9	*mdtC/yegO*	Multidrug transporter subunit MdtC	52.8
GWH15_12085	*mdtC/yegO*	SDC and other	52.5	*mdtC*	Multidrug transporter subunit MdtC	52.7
GWH15_18655	*sodA*	H_2O_2	51.3	*sodA*	Superoxide dismutase	51.3
GWH15_12800	*mdtC/yegO*	SDC and other	51.3	*mdtC*	Multidrug transporter subunit MdtC	51.2
GWH15_02405	*galE*	CTAB	50.9	*galE*	UDP-glucose 4-epimerase GalE	55.9
GWH15_16550	*pcoA*	Cu	49.8	*copA*	Cu resistance system multicopper oxidase	51.8
GWH15_04740	*arsT*	As	49.8	*arsT*	Thioredoxin-disulfide reductase	53.0
GWH15_06695	*acrR/ybaH*	Acriflavine	48.3	-	-	-
GWH15_05790	*cusA/ybdE*	Cu, Ag	48.3	*cusA/ybdE*	CusA/CzcA family heavy metal efflux RND transporter	48.3
GWH15_15485	*fabI*	Triclosan	48.2	*fabI*	Enoyl-ACP reductase FabI	50.2
GWH15_10040	*zraR/hydH*	Zn	47.7	*zraR/hydH*	Two-component system response regulator ZraR	49.2
GWH15_04095	*cpxR*	H_2O_2 and other	46.5	*cpxR*	DNA-binding response regulator	50.0
GWH15_04345	*dmeR*	Co, Ni	46.2	*dmeR*	Metal/formaldehyde-sensitive transcriptional repressor	46.2
GWH15_19170	-	-	-	*mntR*	Inner membrane protein YbiR	60.2
GWH15_14955	-	-	-	*mtrD*	Phospho-N-acetylmuramoyl-pentapeptide-transferase	58.4
GWH15_15295	-	-	-	*glpF*	Glycerol kinase	56.8
GWH15_05235	-	-	-	*merA*	Mercury(II) reductase	56.3
GWH15_15725	-	-	-	*ruvB*	DNA helicase RuvB	54.8
GWH15_14350	-	-	-	*aioE*	NAD(P)/FAD-dependent oxidoreductase	54.3
GWH15_16410	-	-	-	*arsC*	Arsenate reductase ArsC	54.0
GWH15_03545	-	-	-	*recG*	ATP-dependent DNA helicase RecG	52.8
GWH15_17515	-	-	-	*merA*	Mercury(II) reductase	52.6
GWH15_00860	-	-	-	*aioE*	NAD(P)/FAD-dependent oxidoreductase	52.3
GWH15_11710	-	-	-	*trgB*	Te resistance protein	51.6
GWH15_09530	-	-	-	*zupT/ygiE*	Zn transporter ZupT	51.1
GWH15_01540	-	-	-	*actA*	Apolipoprotein N-acyltransferase	50.0
GWH15_10285	-	-	-	*copR*	DNA-binding response regulator	50.0
GWH15_12250	-	-	-	*arsT*	Thioredoxin reductase	49.5
GWH15_16485	-	-	-	*emrBsm*	MFS transporter	48.8
GWH15_08215	-	-	-	*trgB*	Te resistance protein	48.6
GWH15_19280	-	-	-	*copG*	Uncharacterized conserved protein	48.6
GWH15_07930	-	-	-	*copC*	Cu resistance protein	48.6
GWH15_03770	-	-	-	*emhA*	Efflux RND transporter periplasmic adaptor subunit	48.6
GWH15_04310	-	-	-	*zupT/ygiE*	Zn transporter ZupT	48.6
GWH15_17565	-	-	-	*arsT*	thioredoxin-disulfide reductase	48.1
GWH15_00030	-	-	-	*modC*	ABC transporter ATP-binding protein	47.7
GWH15_07970	-	-	-	*cop-unnamed*	Cu oxidase	47.7
GWH15_10290	*copR*	Cu	44.4	*copR*	DNA-binding response regulator	47.6
GWH15_12715	-	-	-	*mdeA*	Methionine gamma-lyase	47.6
GWH15_16960	-	-	-	*fbpC*	ABC transporter ATP-binding protein	47.6
GWH15_07190	-	-	-	*gadC/xasA*	Glutamate:gamma-aminobutyrate antiporter	47.5
GWH15_00895	-	-	-	*arsT*	Thioredoxin-disulfide reductase	47.4
GWH15_08115	-	-	-	*chrA*	Chromate transporter	47.4
GWH15_13100	-	-	-	*vexD*	AcrB/AcrD/AcrF family protein	47.4
GWH15_16860	-	-	-	*copB*	Cu transporting ATPase	47.2

ORF (continued)	EXP	Compound	Identity (%)	PRE	Compound	Identity (%)
GWH15_18070	-	-	-	*copS*	Two-component sensor histidine kinase	47.2
GWH15_02325	-	-	-	*vmeG*	Efflux RND transporter periplasmic adaptor subunit	47.1
GWH15_19630	-	-	-	*arsA*	As pump-driving ATPase	47.1
GWH15_10660	*corR*	Cu	43.8	*corR*	Sigma-54-dependent Fis family transcriptional regulator	46.9
GWH15_11005	-	-	-	*abeS*	Multidrug transporter	46.9
GWH15_12005	-	-	-	*soxR*	Redox-sensitive transcriptional activator SoxR	46.9
GWH15_17680	-	-	-	*arsM*	Polymerase 2 ADP-ribosyltransferase 2	46.8
GWH15_11640	-	-	-	*abeS*	Multidrug transporter	46.7
GWH15_03945	*copR*	Cu	44.9	*copR*	DNA-binding response regulator	46.6
GWH15_05310	-	-	-	*nhlF*	NHLF_RHORH RecName: Co transport protein NhlF	46.5
GWH15_02985	-	-	-	*golT*	Cu-translocating P-type ATPase	46.3
GWH15_08255	-	-	-	*pgpA/ltpgpA*	Putative ATP-binding cassette protein subfamily C, member 1	45.9
GWH15_01935	-	-	-	*ctpV*	Metal cation transporter P-type ATPase CtpV	45.8
GWH15_03140	-	-	-	*furA*	Transcriptional repressor	45.7
GWH15_17160	*comR/ycfQ*	Cu	43.9	*comR/ycfQ*	TetR/AcrR family transcriptional regulator	45.6
GWH15_02610	-	-	-	*copB*	Cu-exporting ATPase	45.5
GWH15_13575	*pstC*	As	42.2	*pstC*	Phosphate transporter permease subunit PstC	45.3
GWH15_13260	-	-	-	*copS*	Two-component sensor histidine kinase	45.3
GWH15_14490	-	-	-	*perR*	Transcriptional repressor	45.2
GWH15_00410	-	-	-	*copA*	Putative cadmium-transporting P-type ATPase	45.2
GWH15_04685	-	-	-	*fabL/ygaA*	Enoyl-acyl-carrier-protein	45.2
GWH15_07310	-	-	-	*cutO*	Cu oxidase	45.2
GWH15_09900	-	-	-	*merA*	Mercury(II) reductase	45.2
GWH15_00360	-	-	-	*mntR*	Inner membrane protein YbiR	45.0

EXP: BacMet experimentally confimed genes database. PRE: BacMet predicted genes database; SDC - Sodium Deoxycholate; HCL- Hydrochloric Acid; CTAB- Cetrimonium bromide

Table S2: *Granulicella* strain 5B5 gene annotation with BacMet databases. Annotation performed against the experimentally confirmed and predicted resistance genes databases using BacMet Scan software. Only genes with hits ≥ 40% identity in one of the databases are shown.

ORF	EXP	Compound	Identity (%)	PRE	Compound	Identity (%)
G5B5_03005	*arsB*	As, Sb	63.7	*acr3*	Arsenical-resistance protein	70.5
G5B5_06155	*hoxN*	Ni	60.3	*hoxN*	HoxN/HupN/NixA family Ni/Co transporter	60.3
G5B5_10055	*ruvB*	Cr, Te, Se	59.6	*ruvB*	DNA helicase RuvB	59.0
G5B5_07635	*acn*	Fe	58.6	*acn*	Aconitate hydratase	59.3
G5B5_15620	*silP*	Ag	58.2	*actP*	Cu-transporting P-type ATPase	66.0
G5B5_12540	*pstB*	As	55.8	*pstB*	ABC transporter ATP-binding protein PstB	56.6
G5B5_06515	*mdtC/ yegO*	SDC	55.7	*mdtC/yegO*	Multidrug transporter subunit MdtC	55.8
G5B5_06520	*mdtB/ yegN*	SDC	55.0	*mdtB*	Multidrug transporter subunit MdtB	55.4
G5B5_16735	*merA*	Hg	52.8	*merA*	Mercury(II) reductase	36.4

ORF (continued)	EXP	Compound	Identity (%)	PRE	Compound	Identity (%)
					NAD(P)/FAD-dependent	
G5B5_10440	*aioE*	As	51.3	*aioE*	oxidoreductase	51.3
G5B5_07160	*sodA*	Se, H_2O_2	51.2	*sodA*	Superoxide dismutase	54.0
G5B5_13030	*galE*	CTAB	50.5	*galE*	UDP-glucose 4-epimerase GalE	52.3
					Metal sensitive transcriptional	
G5B5_01815	*dmeR*	Co, Ni	50.0	*dmeR*	repressor	52.0
G5B5_04025	*smdA*	DAPI	50.0	-	-	-
G5B5_06995	*copR*	Cu	50.0	*copR*	DNA-binding response regulator	52.7
G5B5_07595	*mtrD*	Triton X-100	50.0	*chrC*	hypothetical protein AYO46_00620	32.9
G5B5_08045	*arsB*	As, Sb	50.0	-	-	-
G5B5_11195	*pitA*	Zn, Te	50.0	-	-	-
G5B5_14705	*copR*	Co	49.3	*copR*	DNA-binding response regulator	51.1
G5B5_14745	*frnE*	Cd, H_2O_2	48.7	-	-	-
G5B5_01735	*pbrD*	Pb	48.6	-	-	-
G5B5_01970	*dmeF*	Co, Cd, Ni	48.5	-	-	-
G5B5_13805	*mntA/ ytgA*	Mn, Cd	48.5	-	-	-
		Sodium				
G5B5_04635	*iclR*	acetate	48.4	-	-	-
G5B5_04715	*mtrE*	Triton X-100	48.4	-	-	-
G5B5_08485	*copA*	Cu, Ag	48.4	-	-	-
G5B5_15860	*pgpA/ ltpgpA*	As, Sb	48.4	-	-	-
G5B5_15165	*sh-fabI*	Triclosan	48.0	sh-*fabI*	Enoyl-acyl-carrier-protein	50.8
	cusA/				CusA/CzcA family heavy metal efflux	
G5B5_16145	*ybdE*	Cu, Ag	47.9	*cusA/ybdE*	RND transporter	48.0
G5B5_01825	*copS*	Cu	47.5	-	-	-
G5B5_15680	*mtrD*	Triton X-100	47.5	-	-	-
G5B5_16090	*mexY*	EtBr	47.4	-	-	-
G5B5_12645	*soxS*	Zn	47.2	-	-	-
G5B5_12930	*arsT*	As	46.9	*arsT*	Thioredoxin-disulfide reductase	51.9
G5B5_06355	*modA*	W, Mo	46.9	-	-	-
G5B5_06715	*oprN*	Triclosan	46.9	-	-	-
		Sodium				
		Glycocholate			MexC family multidrug efflux RND	
G5B5_07405	*vmeC*	and others	46.9	*mexC*	transporter subunit	31.3
					Phosphate transporter permease	
G5B5_12535	*pstA*	As	46.7	*pstA*	subunit PtsA	47.2
G5B5_04020	*phoR*	BAC	46.7	-	-	-
G5B5_04280	*oqxA*	BAC	46.7	-	-	-
G5B5_08775	*ctpV*	Cu	46.5	-	-	-
G5B5_03175	*tbtB*	TBT	46.2	-	-	-
G5B5_00865	*ctpV*	Cu	45.9	-	-	-
G5B5_07315	*copA*	Cu, Ag	45.9	-	-	-
G5B5_16340	*ttgB*		45.9	-	-	-
G5B5_16800	*copB*	Cu	45.9	-	-	-
G5B5_00800	*arsH*	AS	45.7	-	-	-
G5B5_05205	*bepE*	SDC	45.7	-	-	-
					NAD(P)/FAD-dependent	
G5B5_08125	*aioE*	As	45.7	*aioE*	oxidoreductase	42.5
G5B5_14315	*czcS*	Cd, Zn, Co	45.7	-	-	-
G5B5_14920	*czrA*	Zn, Cd	45.7	-	-	-
G5B5_10760	*vmeB*	BAC	45.7	-	-	-
G5B5_11005	*cpxR*	H_2O_2	45.7	*cpxR*	DNA-binding response regulator	46.3
					Ni ABC transporter permease subunit	
G5B5_00945	*nikC*	Ni	45.6	*nikC*	NikC	46.1
G5B5_00440	*chrR*	Cr, Fe, H_2O_2	45.5	*yieF*	NAD(P)H-dependent oxidoreductase	30.5
G5B5_03750	*mtrR*	Triton X-100	45.5	-	-	-

ORF (continued)	EXP	Compound	Identity (%)	PRE	Compound	Identity (%)
	actP/	Sodium				
G5B5_05605	*yjcG*	Glycocholate	45.5	-	-	-
G5B5_05665	*fecE*	Ni, Co	45.5	-	-	-
G5B5_05745	*mexX*	EtBr	45.5	-	-	-
G5B5_06575	*fbpC*	Fe, Ga	45.5	-	-	-
G5B5_07095	*nixA*	Ni	45.5	*nrsS*	Hypothetical protein	30.4
G5B5_11940	*tbtB*	TBT	45.2	-	-	-
G5B5_09275	*cnrH*	Co, Ni	45.2	*copB*	Cu-translocating P-type ATPase	31.5
G5B5_09475	*smeE*	Triclosan	45.2	-	-	-
G5B5_12380	*wtpB*	W, Mo	45.2	-	-	-
G5B5_02995	-	-	-	*arsM*	Arsenite S-adenosylmethyltransferase	60.0
G5B5_05125	-	-	-	*mtrD*	Phospho-N-acetylmuramoyl-pentapeptide-transferase	57.6
G5B5_10215	-	-	-	*mntR*	Inner membrane protein YbiR	57.2
G5B5_09640	-	-	-	*glpF*	Glycerol kinase	56.9
G5B5_03300	*fbpC*	Fe, Ga	32.6	*ruvB*	DNA helicase RuvB	56.7
G5B5_04040	-	-	-	*wtpA*	W ABC transporter substrate-binding protein WtpA	53.3
G5B5_01300	*tbtA*	TBT	33.8	*zupT/ygiE*	Zn transporter ZupT	51.1
G5B5_16830	-	-	-	*cmtR*	ArsR family transcriptional regulator	50.0
G5B5_08815	*acrD*	Cu, Zn	32.8	*copA*	Cu-translocating P-type ATPase	50.0
G5B5_01765	*nczA*	Ni, Co, Zn	42.2	*modC*	ABC-type spermidine/putrescine transport system	50.0
G5B5_08510	*mreA*	Ni, Zn	43.8	*mreA*	Metal resistance protein	50.0
G5B5_16220	-	-	-	*zupT/ygiE*	Zn transporter ZupT	50.0
G5B5_00905	*hsmR gadW/*	EtBr	36.4	*arsT*	Thioredoxin-disulfide reductase	49.0
G5B5_11375	*yhiW*	HCl	42.4	*mtrA*	AraC family transcriptional regulator	48.8
G5B5_08230	*arsT*	As	43.8	*merA*	Mercury (II) reductase	48.6
G5B5_09395	-	-	-	*vceA*	Efflux transporter periplasmic adaptor subunit	48.4
G5B5_11185	-	-	-	*copS*	Sensor histidine kinase	47.7
G5B5_12325	*ybtP*	Fe	30.4	*ziaA*	Ca-translocating P-type ATPase	47.1
G5B5_05120	-	-	-	*aioE*	NAD(P)/FAD-dependent oxidoreductase	46.2
G5B5_04590	*irlR*	Cd, Zn	44.6	*irlR*	DNA-binding response regulator	46.2
G5B5_03145	*recG*	Cr, Te, Se	33.3	*mdtI/ydgE*	Multidrug/spermidine transporter subunit MdtI	45.9
G5B5_13015	*adeB*	Pyronin Y	40.0	*adeB*	Multidrug efflux RND transporter permease subunit	45.7
G5B5_12855	*bepE*	SDC	44.4	*bepE*	Multidrug efflux RND transporter permease subunit	45.7
G5B5_13845	*vmeV*	SDS	33.3	*trgB*	Hypothetical protein	45.6
G5B5_12165	*gadB*	HCl	36.1	*ctpV*	Metal cation transporter P-type ATPase CtpV	45.6
G5B5_04210	*comR/ ycfQ*	Cu	43.2	*comR/ycfQ*	TetR/AcrR family transcriptional regulator	45.5
G5B5_13340	-	-	-	*copD*	Hypothetical protein	45.5
G5B5_14950	-	-	-	*pbrT*	Fe permease	45.5
G5B5_15200	-	-	-	*pcm*	Protein-L-isoaspartate O-methyltransferase	45.5
G5B5_12960	*mexF*	Triclosan	31.7	*fecD*	Fe-dicitrate transporter subunit FecD	45.2

EXP: BacMet experimentally confimed genes database. PRE: BacMet predicted genes database; SDC - Sodium Deoxycholate; HCL- Hydrochloric Acid; CTAB- Cetrimonium bromide; DAPI- 4',6-diamidino-2-phenylindole; BAC- Benzylkonium Chloride; TBT-Tributyltin

Table S3: Significantly upregulated proteins in the proteomic profile of *Granulicella* sp. 5B5 with the addition of Mn. Only statistically significant differentially expressed proteins with a fold change ≥1.5 and proteins expressed only with the addition of Mn (on Mn) are shown.

ORF	Proteins	log_2 FC
G5B5_02010	D-hydantoinase	4.48
G5B5_04470	3'-5' exoribonuclease YhaM	3.91
G5B5_02385	Protein YciF	2.82
G5B5_13570	Blue-light-activated protein	2.65
G5B5_10245	Hypothetical protein	2.62
G5B5_09185	Hypothetical protein	2.52
G5B5_06075	Alpha-1,4-glucan:maltose-1-phosphate maltosyltransferase 1	2.48
G5B5_06575	Oxalate decarboxylase OxdD	2.33
G5B5_06165	Phosphoenolpyruvate carboxykinase [GTP]	2.29
G5B5_01965	Hypothetical protein	2.23
G5B5_12545	Phosphate-specific transport system accessory protein PhoU	2.17
G5B5_14775	Protein RecA	2.14
G5B5_08980	DNA-binding protein HU	2.13
G5B5_15595	Glucose-6-phosphate 1-dehydrogenase 1	2.09
G5B5_00055	ATP-dependent recd-like DNA helicase	2.04
G5B5_15800	Ribonucleoside-diphosphate reductase 1 subunit alpha	1.93
G5B5_15655	Hypothetical protein	1.92
G5B5_11545	DNA-directed RNA polymerase subunit alpha	1.91
G5B5_02760	Alpha-1,4-glucan:maltose-1-phosphate maltosyltransferase	1.90
G5B5_03225	Hypothetical protein	1.89
G5B5_03345	Hypothetical protein	1.86
G5B5_02590	Glutamate synthase [NADPH] large chain	1.82
G5B5_10000	GTP 3',8-cyclase	1.82
G5B5_11930	Hypothetical protein	1.80
G5B5_03160	Octaprenyl diphosphate synthase	1.78
G5B5_13250	Nucleoside diphosphate kinase	1.78
G5B5_02655	Uvrabc system protein B	1.75
G5B5_02610	Acetylornithine aminotransferase	1.75
G5B5_02365	UDP-N-acetylmuramate--L-alanyl-gamma-D-glutamyl-meso-2,6-diaminoheptandioate ligase	1.72
G5B5_03020	HTH-type transcriptional regulator Hpr	1.72
G5B5_03150	Putative metal-dependent hydrolase YcfH	1.72
G5B5_03725	Hypothetical protein	1.71
G5B5_08555	Hypothetical protein	1.67
G5B5_07785	Hypothetical protein	1.65
G5B5_09210	Small heat shock protein IbpB	1.65
G5B5_00640	Carbamoyl-phosphate synthase small chain	1.62
G5B5_11390	Imidazole glycerol phosphate synthase subunit HisF	1.62
G5B5_08025	Hypothetical protein	1.61
G5B5_10075	Adenine deaminase	1.60
G5B5_01745	Long-chain-fatty-acid--coa ligase Fadd15	1.58
G5B5_04740	Hypothetical protein	1.58
G5B5_08550	Regulator of RpoS	1.57
G5B5_02980	Glucose 1-dehydrogenase 1	1.56
G5B5_13190	Aminomethyltransferase	1.55
G5B5_04260	Hypothetical protein	1.51
G5B5_01715	Biotin carboxylase	1.50
G5B5_06710	Hypothetical protein	on Mn
G5B5_06495	D-threo-aldose 1-dehydrogenase	on Mn
G5B5_15040	Glutathione-independent formaldehyde dehydrogenase	on Mn
G5B5_04460	Hypothetical protein	on Mn
G5B5_04810	Hypothetical protein	on Mn
G5B5_05770	P-protein	on Mn
G5B5_07390	Nucleoid occlusion factor SlmA	on Mn
G5B5_14875	ABC transporter ATP-binding protein NatA	on Mn
G5B5_06295	Hypothetical protein	on Mn
G5B5_15170	Hypothetical protein	on Mn

ORF (continued)	Proteins	log_2 FC
G5B5_05650	Hypothetical protein	on Mn

Table S4: Significantly upregulated proteins in the proteomic profile of *Granulicella* sp. 5B5 with the addition of Mn with hits against BacMet gene databases. Annotation performed against the experimentally confirmed and predicted resistance genes databases using BacMet Scan software. Only genes with hits ≥ 40% identity in one of the databases are shown.

ORF	EXP	Compound	Identity (%)	PRE	Compound	Identity (%)
G5B5_06575	*fbpC*	Fe, Ga	45.5	-	-	-
G5B5_05650	*cueA*	Cu, Ag	43.5	*cueA*	Cu-translocating P-type ATPase	39.3
G5B5_00055	*chrA*	Cr	38.7	-	-	-
G5B5_01745	*fbpB*	Fe, Ga	38.3	-	-	-
G5B5_06295	*aioR/aoxR*	As	36.7	-	-	-
G5B5_03725	*pmrC*	Fe	36.4	*mtrA*	AraC family transcriptional regulator	32.7
G5B5_03225	*gadB*	HCl	35.9	*copA*	Cu-translocating P-type ATPase	30.9
G5B5_01965	*mexF*	Triclosan	34.7	-	-	-
G5B5_03160	*bepG*	SDC	34.3	-	-	-
G5B5_06075	*nczA*	Ni, Co, Zn	34.1	-	-	-
G5B5_08550	*actR*	Cd, Zn, HCl	33.8	*zraR/hydH*	Fis family transcriptional regulator	30.0
G5B5_06495	*tcrA*	Cu	33.3	*copA*	Cu-translocating P-type ATPase	39.6
G5B5_11930	*mexY*	EtBr	33.3	-	-	-
G5B5_07390	*bcrA*	BAC	33.0	*bcrA*	TetR/AcrR family transcriptional regulator	33.0
G5B5_13190	*adeT1*	BAC	32.6	-	-	-
G5B5_13570	*copR*	Cu	32.5	*corR*	Fis family transcriptional regulator	30.4
G5B5_09210	*ibpB*	H_2O_2	32.0	*ibpB*	Heat shock chaperone IbpB	36.7
G5B5_02980	*fabL/ygaA*	Triclosan	32.0	*fabL/ygaA*	Enoyl-acyl-carrier-protein	31.7
G5B5_02655	*copA*	Copper (Cu), Silver (Ag)	31.9	*yieF*	NAD(P)H-dependent oxidoreductase	32.1
G5B5_00640	*mtrD*	Triton X-100	31.4	-	-	-
G5B5_03020	*farR*	Palmitic acid	31.3	*farR*	Homoprotocatechuate degradation operon regulator HpaR	30.6
G5B5_15040	*mexK*	Triclosan	31.1	*arsM*	As S-adenosylmethyltransferase	33.3
G5B5_11390	*fabK*	Triclosan	30.8	*fabK*	Enoyl-acyl-carrier-protein	33.3
G5B5_04810	*copB*	Cu	30.2	-	-	-
G5B5_14875	*troB*	Zn, Mn, Fe	30.0	*troB*	Metal ABC transporter ATP-binding protein	31.2
G5B5_08555	-	-	-	*fabL/ygaA*	Enoyl-acyl-carrier-protein	36.8
G5B5_13250	-	-	-	*chrB*	Hypothetical protein	32.2
G5B5_12545	-	-	-	*pstA*	PhoU and BPD transp 1 and ABC tran domain containing protein	30.7

EXP: BacMet experimentally confimed genes database. PRE: BacMet predicted genes database; SDC - Sodium Deoxycholate; HCL- Hydrochloric Acid; BAC- Benzylkonium Chloride

Table S5: Significantly upregulated proteins in the proteomic profile of *Granulicella* sp. WH15 with the addition of Mn. Only statistically significant differentially expressed proteins with a fold change ≥1.5 are shown.

ORF	Proteins	log_2 FC
GWH15_02295	Hypothetical protein	2.92
GWH15_19690	Hypothetical protein	2.88
GWH15_18635	Putative sugar phosphate isomerase YwlF	1.92
GWH15_05130	Anti-sigma-B factor antagonist	1.91
GWH15_01045	dTDP-4-dehydrorhamnose 3,5-epimerase	1.90
GWH15_15570	ATP synthase subunit b	1.83
GWH15_13820	Hypothetical protein	1.78
GWH15_03260	3',5'-cyclic adenosine monophosphate phosphodiesterase CpdA	1.68
GWH15_00080	N5-carboxyaminoimidazole ribonucleotide mutase	1.67

ORF (continued)	Proteins	log_2 FC
GWH15_17845	Hypothetical protein	1.63
GWH15_02405	UDP-glucose 4-epimerase	1.62
GWH15_13225	ATP phosphoribosyltransferase	1.60
GWH15_19100	2-iminobutanoate/2-iminopropanoate deaminase	1.56
GWH15_13825	Sulfur carrier protein CysO	1.54
GWH15_02470	30S ribosomal protein S7	1.53
GWH15_10485	Hypothetical protein	1.50

Table S6: Significantly downregulated proteins in the proteomic profile of *Granulicella* sp. 5B5 with the addition of Mn. Only statistically significant differentially expressed proteins with a fold change ≤-1.5 and proteins expressed only in the absence of Mn (off Mn) are shown.

ORF	Proteins	log_2 FC
G5B5_06260	Transcriptional regulator YqjI	-2.08
G5B5_04865	Hypothetical protein	-1.16
G5B5_12975	Hypothetical protein	-1.13
G5B5_00540	Hypothetical protein	-1.10
G5B5_03300	ATP-dependent zinc metalloprotease FtsH	-1.08
G5B5_02930	(R)-stereoselective amidase	-0.98
G5B5_07395	Putative D-xylose utilization operon transcriptional repressor	-0.95
G5B5_12740	Glutamine--fructose-6-phosphate aminotransferase	-0.93
G5B5_06120	3-phenylpropionate-dihydrodiol/cinnamic acid-dihydrodiol dehydrogenase	-0.92
G5B5_00460	3-methyl-2-oxobutanoate hydroxymethyltransferase	-0.92
G5B5_02750	Ferredoxin-NADP reductase	-0.90
G5B5_12735	N-acetylmuramic acid/N-acetylglucosamine kinase	-0.88
G5B5_02110	Hypothetical protein	-0.85
G5B5_08120	Putative aminoacrylate peracid reductase RutC	-0.84
G5B5_02460	Hypothetical protein	-0.84
G5B5_09120	Ribosome-binding ATPase YchF	-0.83
G5B5_11445	Carbonic anhydrase 1	-0.83
G5B5_00615	Hypothetical protein	-0.82
G5B5_05430	Cysteine synthase B	-0.75
G5B5_13900	Gluconate 5-dehydrogenase	-0.75
G5B5_07595	Phospho-2-dehydro-3-deoxyheptonate aldolase	-0.74
G5B5_06545	Putative nicotinate-nucleotide pyrophosphorylase	-0.74
G5B5_09865	Beta-hexosaminidase	-0.74
G5B5_12210	Hypothetical protein	-0.73
G5B5_08645	Queuine trna-ribosyltransferase	-0.72
G5B5_04975	1-deoxy-D-xylulose 5-phosphate reductoisomerase	-0.70
G5B5_15980	Hypothetical protein	-0.70
G5B5_01030	Hypoxanthine phosphoribosyltransferase	-0.70
G5B5_12065	Hypothetical protein	-0.70
G5B5_04745	Hypothetical protein	-0.68
G5B5_15830	Hypothetical protein	-0.67
G5B5_09255	Putative glucose-6-phosphate 1-epimerase	-0.66
G5B5_02600	N-acetyl-gamma-glutamyl-phosphate reductase	-0.66
G5B5_02395	Hypothetical protein	-0.66
G5B5_09685	Hypothetical protein	-0.65
G5B5_15240	O-succinylbenzoate synthase	-0.65
G5B5_12965	2-amino-5-chloromuconic acid deaminase	-0.64
G5B5_11795	L-threonine dehydratase catabolic TdcB	-0.64
G5B5_02345	Hypothetical protein	-0.63
G5B5_15805	Ribonucleoside-diphosphate reductase subunit beta	-0.63
G5B5_13090	Aspartate carbamoyltransferase catalytic subunit	-0.62
G5B5_02400	S-methyl-5'-thioadenosine phosphorylase	-0.62
G5B5_13505	Quinone oxidoreductase 1	-0.60
G5B5_07290	Hypothetical protein	off Mn
G5B5_01820	Hypothetical protein	off Mn

ORF (continued)	Proteins	log_2 FC
G5B5_04790	Hypothetical protein	off Mn

Table S7: Significantly downregulated proteins in the proteomic profile of *Granulicella* sp. WH15 with the addition of Mn. Only statistically significant differentially expressed proteins with a fold change ≤-1.5 and proteins expressed only in the absence of Mn (off Mn) are shown.

ORF	Proteins	log_2 FC
GWH15_14260	Xyloglucanase	-1.85
GWH15_04015	Putative aliphatic sulfonates-binding protein	-1.73
GWH15_15380	N-acetyl-lysine deacetylase	-1.44
GWH15_10420	ATP-dependent Clp protease ATP-binding subunit ClpX	-1.44
GWH15_13385	Hypothetical protein	-1.36
GWH15_19375	Alanine racemase	-1.35
GWH15_11185	UvrABC system protein A	-1.31
GWH15_17800	Galactose-1-phosphate uridylyltransferase	-1.29
GWH15_15990	Hypothetical protein	-1.27
GWH15_15970	Multifunctional 2-oxoglutarate metabolism enzyme	-1.26
GWH15_04000	Hypothetical protein	-1.23
GWH15_12720	UvrABC system protein A	-1.19
GWH15_03100	Acyl carrier protein	-1.16
GWH15_14335	Alpha-ketoglutaric semialdehyde dehydrogenase	-1.14
GWH15_17270	Threonine synthase	-1.13
GWH15_06265	Glutamyl-tRNA(Gln) amidotransferase subunit A	-1.13
GWH15_14265	Xyloglucanase Xgh74a	-1.11
GWH15_11400	Beta-hexosaminidase	-1.08
GWH15_12565	Fumarate reductase flavoprotein subunit	-1.07
GWH15_02575	50S ribosomal protein L18	-1.06
GWH15_00875	Phosphoribosylformylglycinamidine synthase subunit PurL	-1.06
GWH15_12285	Protein TolB	-1.06
GWH15_17475	Hypothetical protein	-1.05
GWH15_17105	Prolyl tripeptidyl peptidase	-1.05
GWH15_03220	Phosphoribosylamine--glycine ligase	-1.03
GWH15_08475	Hypothetical protein	-1.03
GWH15_12210	GDP-L-fucose synthase	-1.01
GWH15_14365	Glycine--trna ligase alpha subunit	-0.99
GWH15_13275	Hypothetical protein	-0.99
GWH15_02875	2,3-dehydroadipyl-coa hydratase	-0.98
GWH15_13930	Trehalose synthase/amylase TreS	-0.97
GWH15_12385	Ribosomal protein S12 methylthiotransferase RimO	-0.97
GWH15_13180	Soluble aldose sugar dehydrogenase YliI	-0.97
GWH15_17185	Hypothetical protein	-0.96
GWH15_13830	Putative adenylyltransferase/sulfurtransferase MoeZ	-0.95
GWH15_00705	Outer membrane protein assembly factor BamA	-0.94
GWH15_01035	Dtdp-4-dehydrorhamnose reductase	-0.93
GWH15_02690	Inosine-5'-monophosphate dehydrogenase	-0.92
GWH15_18680	Hypothetical protein	-0.88
GWH15_08290	NADH-quinone oxidoreductase subunit 4	-0.88
GWH15_14415	P-protein	-0.88
GWH15_18875	Hypothetical protein	-0.87
GWH15_01065	Glutamyl-tRNA(Gln) amidotransferase subunit A	-0.86
GWH15_14475	Methanol dehydrogenase activator	-0.86
GWH15_19145	Cysteine desulfurase IscS	-0.85
GWH15_01945	Cyanophycinase	-0.85
GWH15_19215	Hypothetical protein	-0.84
GWH15_14360	Glycine--tRNA ligase beta subunit	-0.84
GWH15_18480	Putative formaldehyde dehydrogenase AdhA	-0.84
GWH15_18350	Thymidylate synthase ThyX	-0.83
GWH15_01270	Phosphoenolpyruvate carboxylase	-0.82
GWH15_00365	UDP-N-acetylglucosamine 4-epimerase	-0.81

ORF (continued)	Proteins	log_2 FC
GWH15_01110	CTP synthase	-0.79
GWH15_02270	3-oxoacyl-[acyl-carrier-protein] synthase 3	-0.78
GWH15_10500	Pyrroline-5-carboxylate reductase	-0.78
GWH15_17030	Putative glutamate--cysteine ligase 2	-0.77
GWH15_11850	Hypothetical protein	-0.77
GWH15_11780	Hypothetical protein	-0.74
GWH15_11475	Leucine--tRNA ligase	-0.73
GWH15_02195	Putative competence-damage inducible protein	-0.72
GWH15_07040	Putative oxidoreductase YdgJ	-0.71
GWH15_10160	3-oxoacyl-[acyl-carrier-protein] reductase FabG	-0.71
GWH15_01120	Hypothetical protein	-0.71
GWH15_08275	NADH-quinone oxidoreductase subunit F	-0.69
GWH15_14850	Farnesyl diphosphate synthase	-0.69
GWH15_14470	Hypothetical protein	-0.68
GWH15_00550	2Fe-2S ferredoxin	-0.68
GWH15_02350	3-isopropylmalate dehydratase large subunit	-0.68
GWH15_08050	Hypothetical protein	-0.68
GWH15_18880	Prolyl tripeptidyl peptidase	-0.68
GWH15_12655	Hypothetical protein	-0.68
GWH15_18355	3-oxoacyl-[acyl-carrier-protein] reductase FabG	-0.67
GWH15_07045	Outer membrane protein OprM	-0.67
GWH15_10555	Cytosol aminopeptidase	-0.67
GWH15_07840	Putative zinc protease	-0.67
GWH15_11610	Thiol:disulfide interchange protein DsbD	-0.67
GWH15_15540	Shikimate dehydrogenase (NADP(+))	-0.66
GWH15_01860	D-alanine--D-alanine ligase	-0.66
GWH15_19240	Biopolymer transport protein ExbD	-0.66
GWH15_17740	Alanine--tRNA ligase	-0.66
GWH15_11500	Sulfite reductase [NADPH] flavoprotein alpha-component	-0.66
GWH15_18340	Protein RecA	-0.65
GWH15_13090	Elongation factor Ts	-0.65
GWH15_05960	Hypothetical protein	-0.65
GWH15_00690	Chaperone SurA	-0.63
GWH15_07860	3-ketoacyl-coa thiolase	-0.63
GWH15_03065	Quercetin 2,3-dioxygenase	-0.63
GWH15_02430	6,7-dimethyl-8-ribityllumazine synthase	-0.61
GWH15_07165	Aminopeptidase YpdF	-0.61
GWH15_00040	Delta-aminolevulinic acid dehydratase	-0.61
GWH15_07145	Long-chain-fatty-acid--coa ligase Fadd15	-0.61
GWH15_06995	Pyruvate dehydrogenase E1 component subunit alpha	-0.61
GWH15_07935	Malate synthase A	-0.60
GWH15_06210	Putative FAD-linked oxidoreductase	off Mn
GWH15_09885	Putative pyridine nucleotide-disulfide oxidoreductase RclA	off Mn
GWH15_18545	Tyrosine--tRNA ligase	off Mn
GWH15_19630	Chromosome partitioning protein ParA	off Mn
GWH15_04710	ATP-dependent DNA helicase PcrA	off Mn
GWH15_15115	Acetyltransferase YpeA	off Mn
GWH15_05335	N5-carboxyaminoimidazole ribonucleotide mutase	off Mn
GWH15_00245	Putative metallo-hydrolase YflN	off Mn
GWH15_02910	Hypothetical protein	off Mn
GWH15_00345	Hypothetical protein	off Mn
GWH15_16500	Hypothetical protein	off Mn
GWH15_19325	Isoaspartyl peptidase	off Mn
GWH15_14120	Xaa-Pro dipeptidase	off Mn
GWH15_01485	Hypothetical protein	off Mn
GWH15_19600	Hypothetical protein	off Mn
GWH15_10235	Cyclase-dehydratase-3-O-methyl transferase TcmN	off Mn
GWH15_14935	Hypothetical protein	off Mn
GWH15_15315	Xyloglucanase Tgh74A	off Mn
GWH15_18775	Regulatory protein BlaR1	off Mn