The impact of defense hormones on the interaction between plants and the soil microbial community

Zhang, J.

Chapter 4

Activation of the SA-associated plant defense pathway alters the functions of soil microbial communities in four sequential generations

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Abstract

Systemic acquired resistance (SAR) is an immune response of plants that regulates plant hormonal signaling pathways and strengthens the ability of the plant to withstand pathogenic microbes. Aboveground application of salicylic acid (SA) to the plant can induce SAR and we showed that it mitigates negative effects of the soil microbial community on the performance of the plant *Jacobaea vulgaris*. How SA-induced resistance affects the expression of functional genes and gene ontology in the rhizosphere and how this phenomenon extends over multiple generations is not well studied. In this study, a meta-transcriptomics approach was used to characterize gene expression profiles of microbial communities in 24 soil samples of SA-treated and control plants over 4 generations. 71.6 million reads were used for de-novo assembly of the microbial transcriptome, after which a total of 1.3 million unique contigs (genes) were identified. Multivariate analysis revealed that the SA treatment, generation and the interaction between these two affected the functional genes of the rhizosphere microbial communities of *J. vulgaris*. In general, the effect of the SA treatment on microbial gene expression was lowest in the first generation and strongest in the fourth generation. Microbes in soil samples of SA-treated and control plants showed 1663 differentially expressed genes. In the first generation only two genes differed significantly in gene expression between microbes from soils of SA treated and control plants while in the fourth generation 361 genes were differentially expressed between microbes from soils of SA treated and control plants. None of the significantly expressed SA-downregulated genes were present in all four generations, while only one SA-upregulated gene was observed in all four generations. Gene ontology (GO) analysis showed that soil microbial communities in rhizosphere soil of SA-treated plants increased the expression of thirteen GO terms in the second, third and fourth generation. These increased GO terms were mostly related to viral RNA genome replication, to interactions with host cells, to organelles of the host cells and to RNA polymerase activities. There were six GO terms of which the expression decreased in the second, third and fourth generation, and these were associated with processing nitrogen and macromolecules. Overall, our results show that aboveground activation of defenses in the plant affects the expression of functional genes in the soil microbial communities belowground. This suggests that plants may recruit functional

rhizosphere microbiomes that improve plant health and crop production in agriculture.

Keywords

Meta-transcriptomics, Soil microbial community, Functional genes, Plant-soil interactions, Induced resistance, Rhizosphere soil, Salicylic acid

Introduction

Plants can alter the microbiome of the soil in which they grow, and in turn, microorganisms can influence plant performance. The rhizosphere microbiome, defined as the microbial community established near or on plant roots, can have negative, positive and neutral effects on the growth of a host plant (Van Wees et al., 2008; Raaijmakers, et al., 2009; Berendsen et al., 2012). Microbes such as plant growth-promoting bacteria (PGPB) and arbuscular mycorrhizal fungi (AMF) are typically characterized as plant beneficial, because of their contribution to plant health and nutrient uptake (Jeffries et al., 2003; Compant et al., 2010). In contrast, pathogenic microbes typically reduce plant growth and trigger defense mechanisms in the plant (Pieterse et al., 2001). However, the overall net effect of soil microbial communities on plant growth is often negative (Nijjer et al., 2007; Wardle et al., 2011). This might be due to e.g. competition between plants and microbes for available nutrients or soil pathogens (Berendse, 1994; Callaway et al., 2004; Mazzoleni et al., 2015; Cesarano et al., 2017). In response, plants have evolved hormone-driven defensive strategies to suppress these pathogenic impacts, such as systemic acquired resistance (SAR) and induced systemic resistance (ISR) (Bruce and Pickett, 2007; Berendsen et al., 2012; Huang et al., 2014; Ökmen and Doehlemann, 2014).

Systemic acquired resistance (SAR) is a distinct transduction pathway, which is involved in the biological processes that enhance the plant's immune system and defense against microbial pathogens (Reymond and Farmer, 1998; Walters and Heil, 2007; Pieterse et al., 2014; Haney and Ausubel, 2015). An infection caused by a pathogenic microbe can induce SAR, in which plants enhance their immune system by expressing genes coding for pathogenic-proteins (PR) in infested and uninfected tissues (Kachroo and Robin, 2013; Shah and Zeier, 2013; Gao et al., 2015). Apart from local induction by pathogenic microbes, SAR can also be induced by foliar sprays of the phytohormone salicylic acid (SA) (Reymond and Farmer, 1998). Applying a low concentration of SA directly to leaf tissues results in the activation of SA signaling pathways and this has been considered an effective way to activate defense signals in many plant species (Reymond and Farmer, 1998; Pozo and Azcón-Aguilar, 2007; Vlot et al., 2009).

In Chapter 2, we showed that the application of SA mitigates the negative effects of soil microbes on the growth of *J. vulgaris* although this effect did not increase further in subsequent generations of plant growth. A number of studies have examined the expression of functional genes in soil microbial communities. For example, Xue et al. (2016) showed that changing the temperature of soil significantly altered the gene expression in soil microbial communities and these genes were related to maintaining carbon and nitrogen stability in the soil, resulting in higher plant growth. Moreover, Castro et al. (2019) recently demonstrated that plants can change the expression of functional genes (i.e., carbon metabolic genes) in the soil microbial community in response to environmental changes such as drought. Here we hypothesize that application of SA to plants can also cause changes in the expression of functional genes in the soil microbial community and we hypothesize that the altered gene expression is related to the suppression of soil microbial pathogens of plants (Maurhofer et al., 1998; Verberne et al., 2000; Tanaka et al., 2015). Moreover, we expect, that the gene expression difference in the rhizosphere microbial community of control and SA treated plants will increased over generations of plant growth.

Previously, we analyzed the changes in the composition of the microbial community in the rhizosphere soil upon foliar application with SA and showed that the composition of rhizosphere bacterial communities differed among four plant generations of *J. vulgaris* and between soils from SA treated and control plants. However, the composition differed strongly among generations (Chapter 3). Functions of the soil microbial community can be performed by different microbial taxa (Burke et al., 2011; Liu et al., 2018; Liu et al., 2020) and hence we expect that there is functional redundancy in the soil microbial community and a consistent effect of SA application on gene expression in the microbial community.

In this study we ask the following questions: (1) Does the application of SA on leaves of *J. vulgaris* significantly alter the gene expression of the microbial community in the rhizosphere? (2) Does the effect differ between generations or is there an interaction between the SA treatment and generation on the gene expression in the microbial communities? (3) Which groups of genes or gene ontology pathways in the rhizosphere microbiome are influenced by SA-application over generations?

Materials, methods and bioinformatics processing

The multi-generation growth experiment with *J. vulgaris* has been described in Chapter 3. In short, *J. vulgaris* plants were grown for four generations on soils inoculated with soil from the previous generation from the same treatment with a foliar SA application treatment and a control treatment. Each treatment had 10 replicates. For each treatment, the three successively labeled replicates (No. 1, 2, 3, No. 4, 5, 6 and No. 7, 8, 9) were mixed and used as one pooled replicate, Hence, the three pooled replicates were used for RNA extraction for each treatment in each generation and a total of 24 soil samples were used for RNA extraction (3 replicates x 2 treatments x 4 generations). RNAseq was carried out using the Illumina platform.

Processing of the data included quality control of raw reads (FastQC), data trimming (Trimmomatic 0.39), filtering out ribosomal RNAs (SortMeRNA), de novo assembly of reads (Trinity), remove duplicates (CD-HIT-EST algorithm), mapping back to the transcriptome (Bowtie2). For a detailed description see Chapter 3. Gene ontology enrichment was performed using Trinotate and Goseq against the SwissProt, NR (non-redundant) and Pfam databases (Bryant et al., 2017; Bateman, 2019; El-Gebali et al., 2019).

Statistical analyses

Prior to analysis, the raw data were normalized. TMM (trimmed mean of M-values) normalization was used for read counts among all 24 samples (Robinson and Oshlack, 2010). A principal component analysis (PCA) was employed using the normalized number of genes to examine the composition of rhizosphere soil samples of SA-treated and control plants for the four generations. A PERMANOVA test was performed using the *adonis* function (number of permutations = 999) in R within the "vegan" package to verify the effects of the SA treatment and time on the composition of all expressed genes. To compare similarities among samples of treatment SA and control over four generations, a Pearson correlation for pairwise sample comparison based on the normalized raw read counts of all replicates in the control and SA treatments was performed in R and a heatmap was produced.

Differential gene expression (DE) analysis was performed for all possible combinations of replicates of sets of 8 samples (2 treatments x 4 generations) with EdgeR with raw read counts as input. EdgeR normalizes the data to TMM before further processing. After DE analysis in EdgeR, for all differentially expressed genes of the 8 samples Volcano plots were made for the contrast between SA-treated and control samples per generation. Log2 (FC) values were used as x-variable and -log10 (FDR) for the y-variable to produce a volcano plot of differentially expressed genes between control and SA-treated soil samples per generation. Genes that were significantly differentially expressed between SA-treated and control soil samples that could be annotated were listed. A clustered heatmap based on Euclidean distances (Danielsson, 1980) of gene expression derived from EdgeR per treatment after Z-scored transformation was generated in R using the package "pheatmap" (Kolde and Kolde, 2015).

To visualize the gene expression changes among different hormonal treatments and time categories, an NMDS (nonmetric multidimensional scaling) plot using the Bray-Curtis index as a measure of dissimilarity was generated using TMM normalized read counts. To verify changes in the composition of the 1663 expressed genes due to the SA treatment and time effect, a PERMANOVA test was performed using the adonis function (number of permutations = 999) in R within the "vegan" package.

Gene ontology (GO) enrichment was performed with "GoSeq" for each generation separately. Gene functional classification was determined for three categories: biological processes, cellular components and molecular functions. GO terms affiliated to Eukaryotes (e.g. mitochondria) were removed. The rich factor was calculated as the number of differentially expressed genes in the ontology divided by the number of all genes that were used as a background gene list.

Results

Comparing read counts between generations and treatments

A total of 898,4 million raw sequencing reads were obtained from the 24 metatranscriptomic libraries. The details of the library size and basic information

about read quality were described in Chapter 3. A principal component analysis (PCA) using log2 transformed normalized CPM showed that the read counts of contigs in the microbial community of rhizosphere soil of the *J. vulgaris* samples among generations were well separated (Fig. 1), this was in line with the permutation test (PERMANOVA $R^2 = 0.22$, F = 19.6, $df_1 = 3$, $df_2 > 999$, $p < 0.01$). In addition, the effect of SA application was significant (PERMANOVA $R^2 = 0.07$, F = 6.3, $df_1 = 1$, $df_2 > 999$, $p < 0.05$). Gene expression patterns of SA-treated *J. vulgaris* and control samples were better separated in the third and fourth generation than in the first and second generation (Fig. 1). In the correlation matrix for all sample replicates generated with PtR (a tool for comparing sample replicates in Trinity) (Fig. 1), samples within treatments were positively correlated with each other and also there was a positive correlation between samples within generations especially for the first generation. The heatmap showed clear clustering of treatments within generations except for generation 1. The separation between the SA and the control treatment became more distinct over generations (Fig. 2).

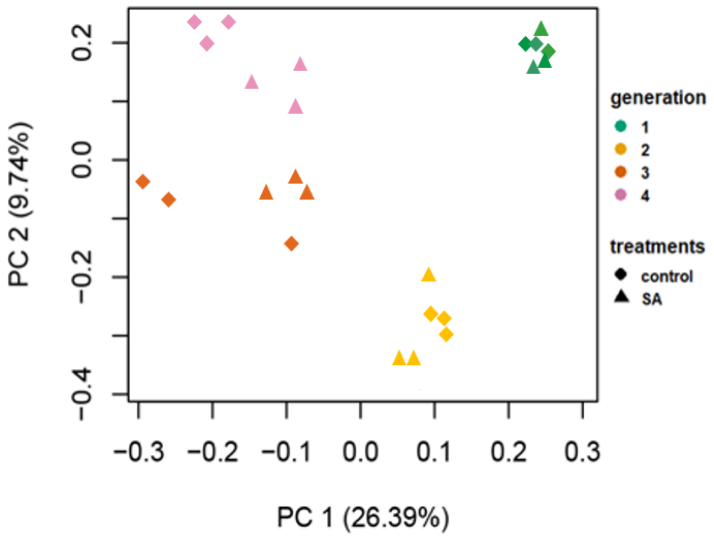

Fig. 1 Scatter plot from a principal component analysis (PCA) of TMM normalized CPM representing the overall gene expression patterns of different rhizosphere soil

samples of SA-treated and control *J. vulgaris* plants over generations. Shapes represent the treatments and colors represent generations.

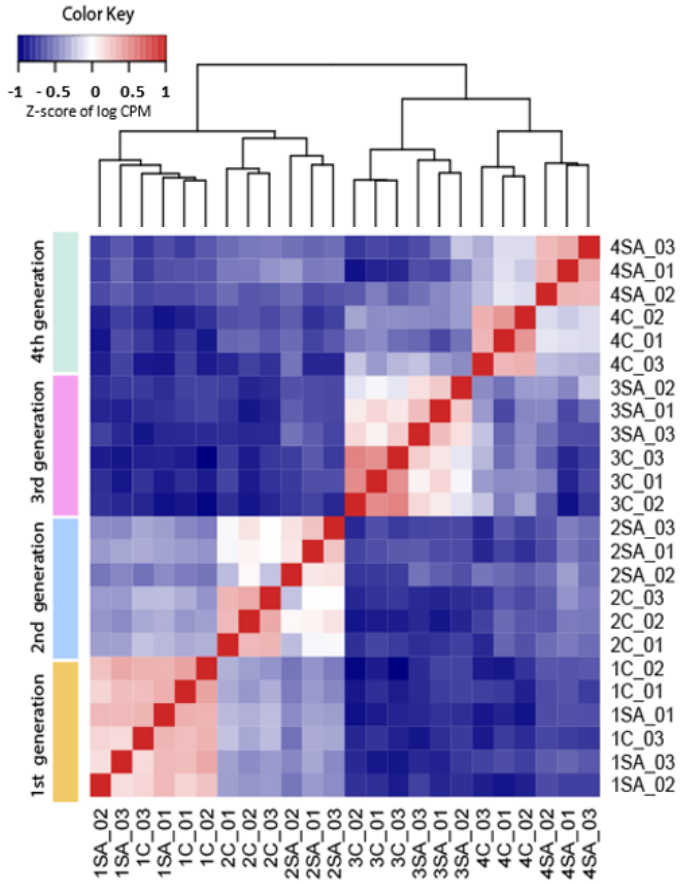

Fig. 2 Clustered heatmap visualizing the Pearson correlation matrix for pairwise sample comparisons based on TMM normalized read counts per million. The heat map shows the correlation in microbial gene expression in all paired replicates between rhizosphere soil samples of SA-treated and control *J. vulgaris* plants over four generations. The dendrogram illustrates the relationship-distance between samples and is calculated based on a Pearson correlation coefficient. The color key represents the z-score of log2 CPM. The legends on the sides represent: Generation (1-4), treatment (SA/Control) and replicate number (01-03).

Differential gene expression

In total, 0.36 million genes were detected. Of those genes, 1663 were differentially expressed between all possible combinations of replicates of sets of 8 samples (2 treatments x 4 generations). Hierarchical clustering on CPM for 1663 differentially expressed genes was performed to explore the patterns of gene expression of the microbial communities between all pairwise combinations of all the samples among SA and control treatments over four generations (Fig. 3). Except for the first generation, SA and control samples were separated from each other in different clusters (Fig. 3). However, among generations, different clusters of genes were differentially grouped. Differences were most pronounced between on the one hand, the first and second generation, and on the other hand, the third and fourth generation.

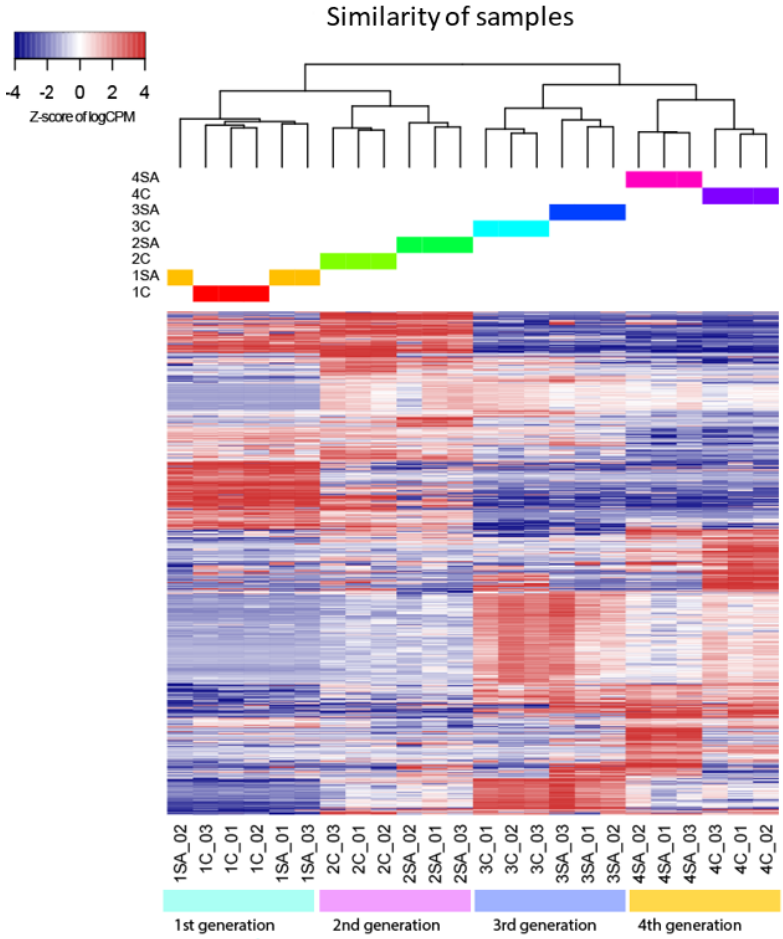

Fig. 3 Heatmap showing 1663 differentially expressed genes (FDR < 0.05 and fold change ≥ 2) between all possible combinations of replicates of rhizosphere soil samples of SA-treated and control *J. vulgaris* plants over four generations based on TMM normalized CPMs. The color key represents the z-score of log2 CPM. The dendrogram on the x-axis illustrates the hierarchal clustering of relationship-distance between replicates using TMM normalized log2-transformed CPM. The legend on the bottom represents: generation (1-4), treatment (SA/control) and replicate number (01-03).

The NMDS plot showed that the 1663 differentially expressed microbial genes detected with EdgeR were differentially expressed in the different generations

(Fig. 4, PERMANOVA $R^2 = 0.63$, F = 21.8, $df_1 = 3$, $df_2 = 1662$, $p < 0.01$) and also that genes were differently expressed between the SA treatment and the control (PERMANOVA $R^2 = 0.07$, F = 7.0, $df_1 = 1$, $df_2 = 1662$, $p < 0.01$). The effect of the SA treatment was not the same in each generation as indicated by the significant interaction (PERMANOVA $R^2 = 0.15$, F = 5.2, $df_1 = 3$, $df_2 = 1662$, $p < 0.001$).

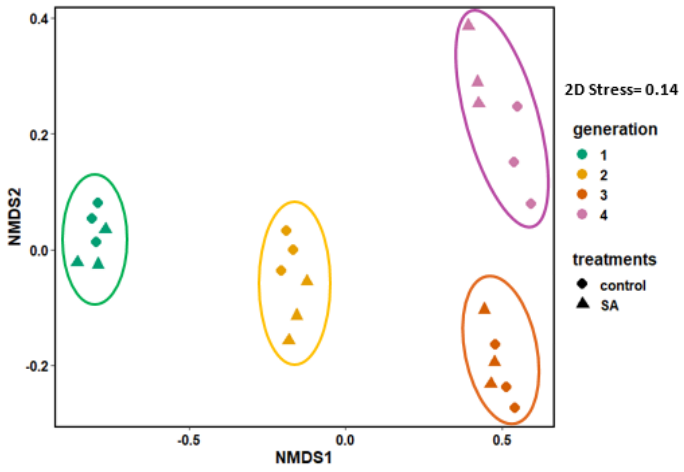

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Fig. 4 Multivariate analysis of 1663 differentially expressed microbial genes between all replicates in rhizosphere samples from SA-treated and control *J. vulgaris* plants grown in four generations. Shown are sample scores from a nonmetric multidimensional scaling (NMDS) plot.

To identify the numbers of significantly down- or up-regulated genes in the SA treatment in each generation in the rhizosphere microbial community, volcano plots were made (Fig. 5). In the first generation, no downregulated genes were observed and only two upregulated genes were detected (Fig. 5a). This increased to 59 and 76 in the second, 89 and 26 in the third, and 187 and 174 in the fourth generation, respectively (Fig. 5b, c, d). Among all the significant differentially expressed genes, no genes were found that were downregulated after SA application in all four generations, while only one gene was observed that was upregulated in SA in all four generations (Fig. 6). Circa 90% of the genes that were significantly altered by the SA

treatment could not be annotated. Among all the annotated genes, only two of the significant differentially expressed microbial genes were detected in three generations and eight genes were detected in two generations (Fig. 6). Not all the genes could be matched with a function in the database. Detailed information of successfully annotated genes was listed in Table S1.

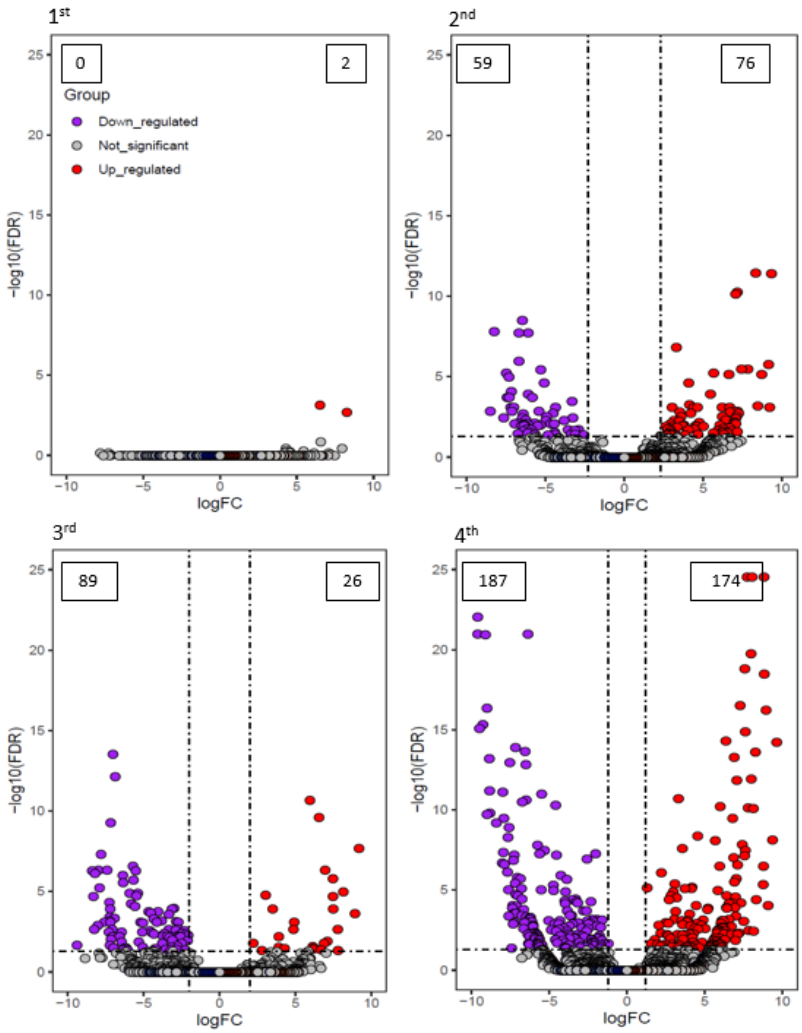


Fig. 5 Volcano plots of 1663 differentially expressed genes of the microbial community in rhizosphere samples of SA-treated and control *J. vulgaris* plants per generation. The x-axes show log2 fold changes of read counts of the genes of the SA treatment compared to thecontrol, and the y-axes show the -log10 adjusted for FDR

values. SA upregulated genes are presented in purple, and SA downregulated genes are displayed in red, while non-significant genes are shown as light grey dots. 1st, 2nd, 3rd and 4th represent the different generations. The numbers inside each box represent the number of significantly up/down-regulated genes. The two vertical dashed lines represent the positive or negative log2 fold changes in the number of readcounts in the SA treatment compared to the control in the generation when -log10(FDR) is 2 as presented by the horizontal dashed lines.

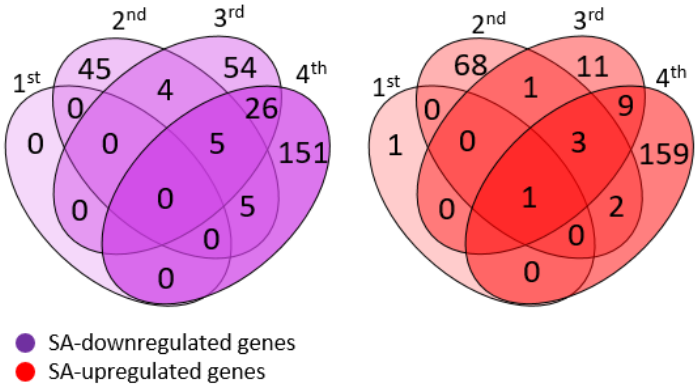


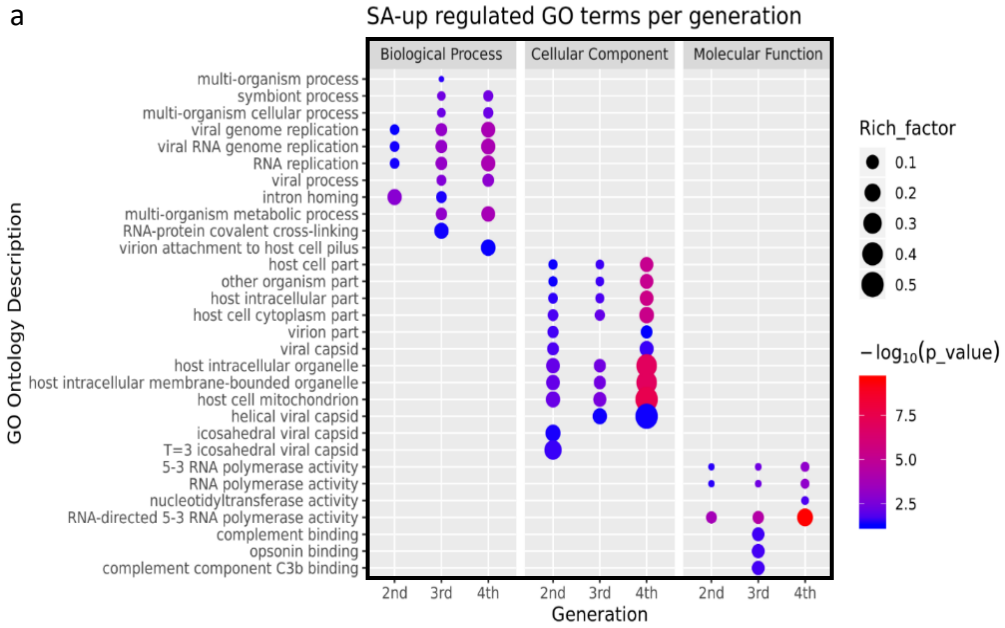
Fig. 6 Venn diagrams showing the number of shared and unique up and down-regulated microbial genes over generations in the rhizosphere of *J. vulgaris*. The numbers represent the significantly differently expressed genes from the volcano plot (Fig. 5). 1st, 2nd, 3rd and 4th represent the different generations.

Gene ontology (GO) analysis

To profile differentially expressed pathways, we performed a gene ontology (GO) analysis for the soil samples of SA-treated and control plants for each generation (Fig. 7, Table S2). No significantly upregulated or downregulated GO terms were observed in the first generation (Fig. 7a, 7b). In the second, third and fourth generations, genes from classes of the GO categories "biological processes", "cellular components" and "molecular functions" were differentially expressed (Fig. 7a). 13 GO terms were upregulated in the SA treatment in three generations, while 18 GO terms were upregulated in one or two generations (Fig. 7a). Of the 13 GO terms upregulated in

three generations three belonged to the GO category “biological processes”, and these GO terms were all related to viral RNA genome replication, seven belonged to the GO category “cellular components” and these GO terms were related to interactions with host cells and to organelles of the host cells and finally three belonged to the GO category “molecular function” and these GO terms were all related to RNA polymerase activity.

Only six GO terms were downregulated in the second, third and fourth generation in the rhizosphere of SA treated plants, while 58 GO terms were downregulated in one or two generations only (Fig. 7b). The six GO terms downregulated in three generations fell all in the GO category “biological processes” and the GO terms were related the localization of processes, to nitrogen processing and to processes involving macromolecules. None of GO terms involved in cellular components and molecular functions were present in these three generations.



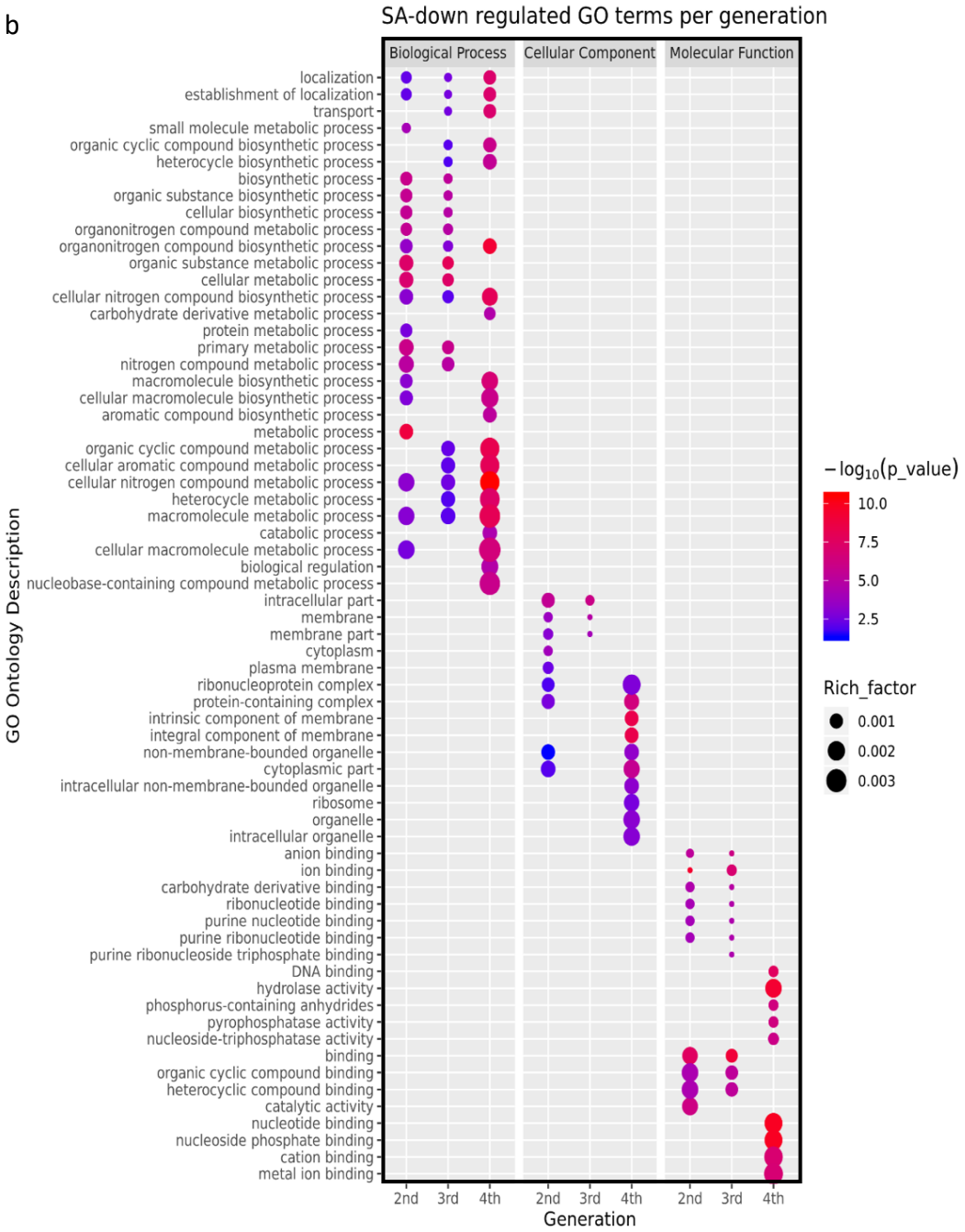


Fig. 7 Gene ontology (GO) enrichment analysis of significantly differentially expressed genes in the microbial community in the rhizosphere. A bubble chart shows

enrichment of differentially expressed GO terms. The Y-axis label lists the GO terms, the size of the bubbles represents the rich factor (= amount of differentially expressed genes enriched in the ontology/total amount of all genes in the background gene set) in different generations. Gene classification of the annotated GO terms was grouped in three categories. Colors of the bubbles represent the significance level of enrichment as calculated with Goseq. a and b represent up and down-regulated GO terms in the SA treatment, respectively. Note: in the first generation, there were no up- or down-regulated GO terms.

Discussion

In this study, a high-throughput metatranscriptomic sequencing approach was used to examine how the aboveground application of SA to the plant impacts the functional gene expression of the microbial communities in the rhizosphere over four subsequent generations of plant growth. Our study shows that the activation of the SA-associated plant defense pathways significantly affected the gene expression of the microbial communities in the rhizosphere, but this effect differed over four generations. Notably, the numbers of differentially expressed genes increased over generations, and there was almost no overlap of in the genes that were significantly expressed in the four generations. Moreover, foliar application of SA caused upregulation of genes of the microbial community related to GO terms associated to viral RNA genome replication, to interactions with host cells, to organelles of the host cells and to RNA polymerase activities, while downregulated GO terms of the microbial community were associated biosynthetic processes involving nitrogen and metabolic processes.

Our study shows that application of SA to plants changed the functional gene expression in the rhizosphere microbial community. This complements previous studies, which report that effects of different abiotic factors alter the expression of functional genes in the soil community (Xue et al., 2016; Castro et al., 2019). Interestingly, in our study, the highest number of significantly expressed genes was recorded in the fourth generation, which suggests that the effect of SA on gene expression becomes more pronounced over time. We did not find a selection-effect of SA on the rhizosphere bacterial community over multiple generations (results in Chapter 3). Hence, we cannot conclude that the increase in the number of significant

expressed genes in our study was due to a specific rhizosphere bacterial community that became increasingly active.

Our finding that the expression of functional genes differed strongly among generations is in line with the previous findings that different taxonomic groups are present in the rhizosphere of SA treated *J. vulgaris* plants in each generation (Chapter 3). However, this clearly contrasts our prediction that there will be functional redundancy in the microbial community. In the same experiment also plant biomass was measured (Chapter 2) and SA treated plants in all generations did better than the control plants showing that from the plant's perspective different microbial taxa with different gene expressions in the rhizosphere provided similar functions. Our findings are in contrast to studies (e.g. Burke et al., 2011; Liu et al., 2018; Liu et al., 2020) that mention that particular functions of the soil microbial community are often distributed across multiple microbial taxa and more closely resemble other studies that show that environmental changes can cause selection of both different taxa and functions in the soil microbial communities (Haggerty and Dinsdale, 2017). It is important to note that in our study, in each generation we placed a subset of the microbial community in a sterile background. This may have led to selection for microbes and consequently different functions in each generation.

At the gene ontology level, we mapped 13 SA-upregulated and six SA-downregulated GO terms that were expressed in the second, third and fourth generation. The proportion of significantly expressed GO terms was high, compared to the proportion of significantly expressed genes. This is because most of the functional genes in this study could not be annotated, while at the ontology level more reads were matched with a function. As the taxa significantly selected by SA differed strongly from generation to generation, it is notable that there we detected many significant GO terms that were found in multiple generations.

Our results show that activating SA resistance in the plant drives gene expression in the rhizosphere microbiome. However, whether SA application to the plant suppressed soil pathogenic microbes remains unproven in our study. SA induced resistance is often reported to play an important role in resistance to a broad range of microbial pathogens, such as bacteria, fungi and viruses (Murphy et al., 1999;

Gilliland et al., 2003; Mayers et al., 2005; Kundu et al., 2011; Li et al., 2019; Yuan et al., 2019). Interestingly, at the ontology level, we found up-regulated GO terms that were involved in viral (RNA) genome replication and viral processes, and these GO terms increased in importance over generations. These results indicate that viruses in the soil may play a role in SA-induced resistance of host plants against soil microbes. It is well known that the soil contains bacteriophages as well as virus controlling microbial pathogens (Duckworth and Gulig, 2002; Svircev et al., 2018; Jamal et al., 2019; Kortright et al., 2019; Rehman et al., 2019). However, their exact role in the rhizosphere microbiome is still poorly understood and further studies should examine these virus-microbe-plant interactions in more detail.

In conclusion, our study shows that application of SA to the plant *J. vulgaris* causes differential gene expression in the rhizosphere microbial community. However, our data also shows that these effects vary among plant generations. Plant-defense-soil microbe interactions may be regulated by viruses or viral phages.

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Supplementary data

Table S1 Log2 (FC) of 70 differentially expressed annotated genes, the expression of which are significantly altered by SA treatments in at least one generation in the rhizosphere of *J. vulgaris* plants in four generations. When Log2 (FC) is > 0, the gene is up-regulated in the SA treatment and when Log2 (FC) is < 0, the gene is down-regulated in the SA treatment. '-' indicates that the gene was not detected in the treatment; 'ns' represents the gene is not significantly altered by the SA treatment, but it is present. 1,2,3 and 4 represent the four generations.

Isoform ID	Functional gene name	1	2	3	4	SA down-/up-regulation	Go category	GO term	Homologous organisms	Function	Literature
TRINITY_DN187408_c0_g1_i1	PF05150.12^Legionella_OMP	-	-7.5	-	-	down	Biological process	adhesion of symbiont to host cell	bacteria	Major outer membrane protein; The attachment of a symbiont to a host cell via adhesion molecules, general stickiness etc., either directly or indirectly.	Jung et al., 2016; Hoppe et al., 2017; Younas et al., 2018.
TRINITY_DN237954_c0_g1_i1	PF05150.12^Legionella_OMP	-	-6.3	-	-	down	Biological process	adhesion of symbiont to host cell	bacteria	Major outer membrane protein; The attachment of a symbiont to a host cell via adhesion molecules, general stickiness etc., either directly or indirectly.	Jung et al., 2016; Hoppe et al., 2017; Younas et al., 2018.
TRINITY_DN2161_c0_g4_i1	PF00687.21^Ribosomal_L1	ns	ns	-5.1	-4.7	down	Biological process	cellular metabolic process	bacteria	Ribosomal protein L1 is the largest protein from the large ribosomal subunit, also can involve moleclar function of RNA binding and cellular component of large ribosomal subunit.	
TRINITY_DN116491_c0_g1_i1	PF00729.18^Viral_coat	-	-6.7	-	-	down	Biological process	virual process	virus	Involing with multi-organism process in which a virus is a participant. The other participant is the host. Includes infection of a host cell, replication of the viral genome, and assembly of progeny virus particles. In some cases the viral genetic material may integrate into the host genome and only subsequently, under particular circumstances, 'complete' its life cycle. Viral genome integration into host DNA.	Nandhagopal et al., 2002.
TRINITY_DN221013_c0_g1_i5	PF03863.13^Phage_mat-A	-	-7.7	-	ns	down	Biological process or cellular component	virion	bacteriophage	The process by which a virion attaches to a host cell by binding to a pilus on the host cell surface. The complete fully infectious extracellular virus particle.	Witherell et al., 1991.
TRINITY_DN342313_c0_g1_i2	PF03863.13^Phage_mat-A	ns	-	ns	-7.9	down	Biological process or cellular component	virion	bacteriophage	The process by which a virion attaches to a host cell by binding to a pilus on the host cell surface. The complete fully infectious extracellular virus particle.	Witherell et al., 1991.
TRINITY_DN497_c0_g1_i6	PF03863.13^Phage_mat-A	ns	ns	ns	-2.8	down	Biological process or cellular component	virion	bacteriophage	The process by which a virion attaches to a host cell by binding to a pilus on the host cell surface. The complete fully infectious extracellular virus particle.	Witherell et al., 1991.

TRINITY_DN2233_c0_g1_i1	PF03863.13^Phage_mat-A	ns	ns	ns	-1.5	down	Biological process or cellular component	virion	bacteriophage	The process by which a virion attaches to a host cell by binding to a pilus on the host cell surface. The complete fully infectious extracellular virus particle.	Witherell et al., 1991.
TRINITY_DN418_c0_g1_i14	PF00460.20^Flg_bb_rod	ns	ns	ns	-4.6	down	Cellular component	bacterial-type flagellum basal body, rod	bacteria	The central portion of the bacterial-type flagellar basal body, which spans the periplasm and threads through the rings.	
TRINITY_DN4152_c3_g1_i1	PF05736.11^OprF	-	ns	-4.3	ns	down	Cellular component	cell outer membrane	bacteria	This domain represents the presumed membrane-spanning region of the OprF proteins. This region is involved in channel formation and is thought to form an 8-stranded beta-barrel.	De Mot et al., 1994.
TRINITY_DN5762_c0_g1_i2	PF11645.8^PDDEXK_5	ns	ns	-	-3.7	down	Cellular component	integral component of membrane	bacteria	This family of endonucleases includes a group I intron-encoded endonuclease. This family belongs to the PD (D/E)XK superfamily	Bonocora and Shub, 2001.
TRINITY_DN21112_c0_g1_i1	PF03863.13^Phage_mat-A	-	-	ns	-5.6	down	Cellular component	membrane	bacteria	Flagellins polymerise to form bacterial flagella. This family includes flagellins and hook associated protein 3. Structurally this family forms an extended helix that interacts with PF00700.	Felix et al., 1999 ; Ramos et al., 2004; Beatson et al., 2006.
TRINITY_DN1559755_c0_g1_i1	PF00564.24^PB1	-	-	-6.1	ns	down	Cellular component	membrane		Phox and Bem1 (PB1) domains contain approximately 80 amino acids and are found in a number of cytoplasmic signaling proteins. A PB1 domain may form heterodimers with a paired PB1 domain, although not all PB1 domains will associate with one another. A highly conserved internal sequence known as OPR, PC or AID motifs is necessary for PB1 domain function. Regions outside the OPR, PC and AID help confer specificity for binding.	Ito et al., 2001; Moscat et al., 2006; Sumimoto et al., 2007.
TRINITY_DN4077_c1_g2_i1	PF00573.22^Ribosomal_L4	-	-4.7	ns	ns	down	Cellular component	ribosome	eubacteria	This family includes Ribosomal L4/L1 from eukaryotes and archaebacteria and L4 from eubacteria. L4 from yeast has been shown to bind rRNA.	Yeh and Lee, 1998.
TRINITY_DN4472_c0_g1_i5	PF00281.19^Ribosomal_L5	-	ns	ns	-6.8	down	Cellular component	ribosome			

TRINITY_DN164781_c0_g2_i2	PF03725.15^RNase_PH_C	-	ns	ns	-5.8	down	Molecular function	5'-3' exoribonuclease activity	bacteria	This family includes 3'-5' exoribonucleases. Ribonuclease PH contains a single copy of this domain, and removes nucleotide residues following the -CCA terminus of tRNA. Polyribonucleotide nucleotidyltransferase (PNPase) contains two tandem copies of the domain. PNPase is involved in mRNA degradation in a 3'-5' direction. The exosome is a 3'-5' exoribonuclease complex that is required for 3' processing of the 5.8S rRNA.	Mitchell et al., 1997.
TRINITY_DN41896_c0_g1_i1	PF05221.17^AdoHcyase	-	ns	-	-5.5	down	Molecular function	adenosylhomocysteinase activity		AdoHcyase is an enzyme of the activated methyl cycle, responsible for the reversible hydration of S-adenosyl-L-homocysteine into adenosine and homocysteine.	Sganga et al., 1992.
TRINITY_DN30386_c0_g2_i1	PF12081.8^GldM_N	-	-	ns	-5.7	down	Molecular function	ATP binding	bacteria	This domain is found in bacteria at the N-terminus of the GldM protein. This domain is typically between 169 to 182 amino acids in length. This domain has two completely conserved residues (Y and N) that may be functionally important. GldM is named for the member from *Cytophaga johnsonae* (*Flavobacterium johnsoniae*), which is required for a type of rapid gliding motility found in certain members of the *Bacteriodetes*.	Braun et al., 2005.
TRINITY_DN24155_c0_g2_i1	PF01471.18^PG_binding_1	-	ns	-	-6.0	down	Molecular function	binding	bacetria	Putative peptidoglycan binding domain. It is found at the N or C terminus of a variety of enzymes involved in bacterial cell wall degradation	Krogh et al., 1998; Steen et al., 2003; Briers et al., 2009.
TRINITY_DN1498_c0_g1_i2	PF01471.18^PG_binding_1	-	-	-	-3.2	down	Molecular function	binding	bacetria	Putative peptidoglycan binding domain. It is found at the N or C terminus of a variety of enzymes involved in bacterial cell wall degradation.	Krogh et al., 1998; Steen et al., 2003; Briers et al., 2009.
TRINITY_DN332299_c0_g1_i1	PF13620.6^CarboxypepD_reg	-	ns	ns	-5.9	down	Molecular function	carboxypeptidase activity			

TRINITY_DN664942_c0_g2_i1	PF00270.29^DEAD	-	-	-	-5.4	down	Molecular function	DEAD/H-box RNA helicase binding		The DEAD/DEAH box helicases are a family of proteins whose purpose is to unwind nucleic acids. The DEAD box helicases are involved in various aspects of RNA metabolism, including nuclear transcription, pre mRNA splicing, ribosome biogenesis, nucleocytoplasmic transport, translation, RNA decay and organellar gene expression.	Johnson and McKay, 1999.
TRINITY_DN16479_c0_g1_i8	PF01541.24^GIY-YIG	ns	-7.0	-7.1	-7.1	down	Molecular function	endonuclease activity	bacteriophage	It is involved in degradation of host DNA, permitting scavenging of host-derived nucleotides for phage DNA synthesis; in enzymes involved in DNA repair and recombination.	Sharma et al., 1992; Dunin-Horkawicz et al., 2006; Andersson et al., 2010.
TRINITY_DN4160_c0_g2_i3	PF01541.24^GIY-YIG	-	-6.4	ns	ns	down	Molecular function	endonuclease activity	bacteriophage	It is involved in degradation of host DNA, permitting scavenging of host-derived nucleotides for phage DNA synthesis; in enzymes involved in DNA repair and recombination.	Sharma et al., 1992; Dunin-Horkawicz et al., 2006; Andersson et al., 2010.
TRINITY_DN2301_c0_g4_i1	PF00006.25^ATP-synt_ab	-	-5.1	-	-	down	Molecular function	hydrolase activity or RNA binding	bacteria, archaea eukaryotes	Catalysis of the hydrolysis of various bonds, e.g. C-O, C-N, C-C, phosphoric anhydride bonds, etc. Hydrolase is the systematic name for any enzyme of EC class 3 or Interacting selectively and non-covalently with an RNA molecule or a portion thereof.	Wilkens et al., 2005.
TRINITY_DN1070_c1_g1_i1	PF13609.6^Porin_4	-	-5.7	-4.6	-4.0	down	Molecular function	porin activity	bacteria	Enables the transfer of substances, sized less than 1000 Da, from one side of a membrane to the other. The transmembrane portions of porins consist exclusively of beta-strands, which form a beta-barrel. They are found in the outer membranes of Gram-negative bacteria, mitochondria, plastids and possibly acid-fast Gram-positive bacteria.	Saier, 2000.
TRINITY_DN6228_c0_g1_i3	PF00989.25^PAS	-	-	-6.5	-5.9	down	Molecular function	protein binding	bacteria	L13are positioned at the amino terminus of signaling proteins such as sensor histidine kinases, cyclic-di-GMP synthases and hydrolases, and methyl-accepting chemotaxis proteins.	Henry and Crosson, 2011; Liu et al., 2015.

TRINITY_DN135_c0_g1_i7	PF13360.6^PQQ_2	ns	ns	ns	-2.5	down	Molecular function	pyrroloquinoline quinone binding		Interacting selectively and non-covalently with pyrroloquinoline quinone, PQQ, the coenzyme or the prosthetic group of certain alcohol dehydrogenases and glucose dehydrogenases.	
TRINITY_DN20104_c0_g1_i6	PF05919.11^Mitovir_RNA_pol	ns	-7.0	ns	-6.8	down	Molecular function	RNA-directed 5'-3' RNA polymerase activity	virus	Mitovirus RNA-dependent RNA polymerase proteins. The family also contains fragment matches in the mitochondria of *Arabidopsis thaliana*.	Hong et al., 1998; Yao et al., 2020.
TRINITY_DN264317_c0_g1_i1	PF03431.13^RNA_replicase_B	-	ns	-8.0	ns	down	Molecular function	RNA-directed 5'-3' RNA polymerase activity	virus	This family is of Leviviridae RNA replicases. The replicase is also known as RNA dependent RNA polymerase.	Fernando et al., 2020.
TRINITY_DN109372_c0_g1_i2	PF05919.11^Mitovir_RNA_pol	ns	-5.5	ns	-7.2	down	Molecular function	RNA-directed 5'-3' RNA polymerase activity	virus	Mitovirus RNA-dependent RNA polymerase proteins. The family also contains fragment matches in the mitochondria of *Arabidopsis thaliana*.	Hong et al., 1998; Yao et al., 2020.
TRINITY_DN5435_c0_g1_i4	PF05919.11^Mitovir_RNA_pol	-	-5.0	-	-7.3	down	Molecular function	RNA-directed 5'-3' RNA polymerase activity	virus	Mitovirus RNA-dependent RNA polymerase proteins. The family also contains fragment matches in the mitochondria of *Arabidopsis thaliana*.	Hong et al., 1998; Yao et al., 2020.
TRINITY_DN34032_c0_g1_i1	PF05919.11^Mitovir_RNA_pol	-	ns	-7.2	-6.6	down	Molecular function	RNA-directed 5'-3' RNA polymerase activity	virus	Mitovirus RNA-dependent RNA polymerase proteins. The family also contains fragment matches in the mitochondria of *Arabidopsis thaliana*.	Hong et al., 1998; Yao et al., 2020.
TRINITY_DN19010_c0_g4_i3	PF00978.21^RdRP_2	ns	-6.3	-	-	down	Molecular function	viral replication or RNA-directed RNA polymerase activity	virus	RNA-dependent RNA polymerase or RNA replicase is an enzyme that catalyzes the replication of RNA from an RNA template. Specifically, it catalyses synthesis of the RNA strand complementary to a given RNA template.	. Hardy et al., 1979; Boonrod et al., 2004; Schwan et al., 2005.
TRINITY_DN243032_c0_g1_i2	PF05919.11^Mitovir_RNA_pol	-	-	-	-6.3	down	Biological process	RNA-directed 5'-3' RNA polymerase activity	virus	Mitovirus RNA-dependent RNA polymerase proteins. The family also contains fragment matches in the mitochondria of *Arabidopsis thaliana*.	Hong et al., 1998; Yao et al., 2020.

TRINITY_DN2369_c0_g1_i4	PF00669.20^Flagellin_N	ns	ns	ns	-1.7	down	Cellular component	membrane	bacteria	Flagellins polymerise to form bacterial flagella. This family includes flagellins and hook associated protein 3. Structurally this family forms an extended helix that interacts with PF00700.	Felix et al., 1999; Ramos et al., 2004; Beatson et al., 2006.
TRINITY_DN306_c0_g2_i5	COG1278^Cold shock protein	ns	3.8	ns	ns	up	Biological process	Many biological processes like regulation of transcription, translation,		Response to stress, any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a cold stimulus, a temperature stimulus below the optimal temperature for that organism. Cold shock proteins are multifunctional RNA/DNA binding proteins, characterized by the presence of one or more cold shock domains. I	D'Amico et al., 2006; Williams et al., 2010; Lindquist and Mertens, 2018.
TRINITY_DN1057_c0_g1_i4	COG1278^Cold shock protein	ns	4.4	ns	-	up	Biological process	Many biological processes like regulation of transcription, translation,		Response to stress, any process that results in a change in state or activity of a cell or an organism (in terms of movement, secretion, enzyme production, gene expression, etc.) as a result of a cold stimulus, a temperature stimulus below the optimal temperature for that organism. Cold shock proteins are multifunctional RNA/DNA binding proteins, characterized by the presence of one or more cold shock domains.	D'Amico et al., 2006; Williams et al., 2010; Lindquist and Mertens, 2018.
TRINITY_DN473649_c0_g1_i3	PF05551.11^zf-His_Me_endon	-	ns	-	5.8	up	Biological process	nucleic acid phosphodiester bond hydrolysis		Zinc-binding loop region of homing endonuclease. This domain is the short zinc-binding loops region of a number of much longer chain homing endonucleases.	Krishna et al., 2003.
TRINITY_DN3358_c0_g1_i2	PF05551.11^F25:F73n	-	-	-	5.9	up	Biological process	nucleic acid phosphodiester bond hydrolysis		Zinc-binding loop region of homing endonuclease. This domain is the short zinc-binding loops region of a number of much longer chain homing endonucleases. This domain is the short zinc-binding loops region of a number of much longer chain homing endonucleases.	Krishna et al., 2003.

TRINITY_DN422335_c0_g1_i1	PF01618.16^MotA_ExbB	-	ns	ns	3.3	up	Biological process	protein transport	bacteria	This family groups together integral membrane proteins that appear to be involved translocation of proteins across a membrane. These proteins are probably proton channels. MotA is an essential component of the flageller motor that uses a proton gradient to generate rotational motion in the flageller. ExbB is part of the TonB-dependent transduction complex. The TonB complex uses the proton gradient across the inner bacterial membrane to transport large molecules across the outer bacterial membrane	Braun et al., 1999.
TRINITY_DN1442_c0_g1_i12	PF13860.6^FlgD_ig	ns	ns	ns	2.5	up	Biological process	proteolysis	bacteria	This domain has an immunoglobulin like beta sandwich fold. It is found in the FlgD protein the flagellar hook capping protein. THe structure for this domain shows that it is inserted within a TUDOR like beta barrel domain	Kuo et al., 2008.
TRINITY_DN220624_c0_g1_i6	PF00072.24^Response_reg	-	-	ns	5.4	up	Biological Process	signal transduction		Response regulator receiver domain. This domain receives the signal from the sensor partner in bacterial two-component systems. It is usually found N-terminal to a DNA binding effector domain.	Pao and Saier, 1995.
TRINITY_DN6829_c0_g2_i1	PF03863.13^Phage_mat-A	-	ns	ns	2.2	up	Biological process or cellular component	virion	bacteriophage	The process by which a virion attaches to a host cell by binding to a pilus on the host cell surface. Or The complete fully infectious extracellular virus particle.	Witherell et al., 1991.
TRINITY_DN924_c0_g3_i1	PF00189.20^Ribosomal_S3_C	-	4.6	ns	-	up	Biological process or molecular function	cellular metabolic process or structural constituent of ribosome		The cellular metabolic process in which a protein is formed, using the sequence of a mature mRNA or circRNA molecule to specify the sequence of amino acids in a polypeptide chain. Translation is mediated by the ribosome, and begins with the formation of a ternary complex between aminoacylated initiator methionine tRNA, GTP, and initiation factor 2, which subsequently associates with the small subunit of the ribosome and an mRNA or circRNA. Translation ends with the release of a polypeptide chain from the ribosome or The action of a molecule that contributes to the structural integrity of the ribosome.	Burd and Dreyfuss, 1994.

TRINITY_DN5169_c0_g1_i4	PF12771.7^SusD-like_2	-	-	ns	2.4	up	Cellular component	cell outer membrane		SusD is a secreted starch-binding protein with an N-terminal lipid tail that allows it to associate with the outer membrane.	
TRINITY_DN520_c0_g2_i1	PF13715.6^CarbopepD_reg_2	-	ns	ns	2.7	up	Cellular component	integral component of membrane	bacteria, archaea or eukaryotes	This domain family is found in bacteria, archaea and eukaryotes, and is approximately 90 amino acids in length. The family is found in association with PF07715 and PF00593.	Cho and Salyers, 2001.
TRINITY_DN297806_c0_g2_i3	PF00669.20^Flagellin_N	ns	3.9	ns	ns	up	Cellular component	membrane	bacteria	Flagellins polymerise to form bacterial flagella. This family includes flagellins and hook associated protein 3. Structurally this family forms an extended helix that interacts with PF00700.	Felix et al., 1999; Ramos et al., 2004; Beatson et al., 2006.
TRINITY_DN429_c0_g2_i2	PF03401.14^TctC	-	-	-	2.2	up	Cellular component	membrane	bacteria	These probable extra-cytoplasmic solute receptors are strongly overrepresented in several beta-proteobacteria. This family, formerly known as Bug - Bordetella uptake gene (bug) product - is a family of bacterial tripartite tricarboxylate receptors of the extracytoplasmic solute binding receptor-dependent transporter group of families, distinct from the ABC and TRAP-T families	Antoine et al., 2003.
TRINITY_DN3530_c0_g1_i1	PF03458.13^UPF0126	-	-	ns	5.6	up	Cellular component	membrane	bacteria	This domain contains three transmembrane helices, with conserved glycine, which are suggestive of an ion channel. Proteins containing this domain have since been shown to be important in glycine utilizaion, and thus are predicted to be glycine transporters. This domain is always found as pair in such sequences.	Price et al., 2018.
TRINITY_DN1598_c0_g1_i1	PF07963.12^N_methyl	-	-	3.9	ns	up	Cellular component	membrane or integral component of membrane	prokaryotes	Prokaryotic N-terminal methylation motif. This short motif directs methylation of the conserved phenylalanine residue. It is most often found at the N-terminus of pilins and other proteins involved in secretion.	
TRINITY_DN653_c7_g1_i1	PF16320.5^Ribosomal_L12_N	-	ns	ns	2.9	up	Cellular component	ribosome		Catalysis of the hydrolysis of the terminal or penultimate peptide bond at the C-terminal end of a peptide or polypeptide.	
TRINITY_DN4687_c0_g1_i2	PF00466.20^Ribosomal_L10	-	ns	ns	4.5	up	Cellular component	ribosome		Catalysis of the hydrolysis of the terminal or penultimate peptide bond at the C-terminal end of a peptide or polypeptide.	

TRINITY_DN2568_c0_g1_i1	PF00466.20^Ribosomal_L10	-	-	-	5.4	up	Cellular component	ribosome		Catalysis of the hydrolysis of the terminal or penultimate peptide bond at the C-terminal end of a peptide or polypeptide.	
TRINITY_DN3585_c0_g1_i4	COG0402^deaminase	-	-	ns	3.8	up	Molecular function	deaminase activity		Catalysis of the removal of an amino group from a substrate, producing ammonia	
TRINITY_DN3461_c0_g3_i1	PF01471.18^PG_binding_1	-	3.5	-	5.7	up	Molecular function	binding	bacetria	Putative peptidoglycan binding domain. It is found at the N or C terminus of a variety of enzymes involved in bacterial cell wall degradation	Krogh et al., 1998; Steen et al., 2003; Briers et al., 2009.
TRINITY_DN3461_c0_g1_i1	PF01471.18^PG_binding_1	-	7.2	6.6	ns	up	Molecular function	binding	bacetria	Putative peptidoglycan binding domain. It is found at the N or C terminus of a variety of enzymes involved in bacterial cell wall degradation	Krogh et al., 1998; Steen et al., 2003; Briers et al., 2009.
TRINITY_DN64210_c0_g2_i1	PF01471.18^PG_binding_1	-	-	6.2	-	up	Molecular function	binding	bacetria	Putative peptidoglycan binding domain. It is found at the N or C terminus of a variety of enzymes involved in bacterial cell wall degradation	Krogh et al., 1998; Steen et al., 2003; Briers et al., 2009.
TRINITY_DN35_c0_g1_i1	PF01471.18^PG_binding_1	-	ns	ns	5.7	up	Molecular function	binding	bacetria	Putative peptidoglycan binding domain. It is found at the N or C terminus of a variety of enzymes involved in bacterial cell wall degradation	Krogh et al., 1998; Steen et al., 2003; Briers et al., 2009.
TRINITY_DN12161_c0_g4_i3	PF01193.24^RNA_pol_L	-	-	-	5.4	up	Molecular function	DNA-directed 5'-3' RNA polymerase activity or protein dimerization activity		The prokaryotic equivalent of the Rpb3/Rpb11 platform is the alpha-alpha dimer. The dimerisation domain of the alpha subunit/Rpb3 is interrupted by an insert domain (PF01000). Some of the alpha subunits also contain iron-sulphur binding domains	Zhang and Darst, 1998.
TRINITY_DN6187_c0_g1_i1	COG0302^GTP cyclohydrolase i	-	-	ns	3.4	up	Molecular function	GTP cyclohydrolase I activity	bacteria	This family includes GTP cyclohydrolase enzymes and a family of related bacterial proteins	Nar et al., 1995.
TRINITY_DN193314_c0_g1_i1	PF00557.24^Peptidase_M24	-	-	-	5.7	up	Molecular function	hydrolase activity	bacteria	This family contains metallopeptidases. It also contains non-peptidase homologues such as the N terminal domain of Spt16, which is a histone H3-H4 binding module.	Stuwe et al., 2008.
TRINITY_DN1632_c0_g1_i1	PF13328.6^HD_4	-	ns	ns	2.2	up	Molecular function	hydrolase activity		HD domains are metal dependent phosphohydrolases.	Aravind and Koonin, 1998.

TRINITY_DN2812_c0_g1_i4	COG0791^NLPP60 protein	-	-	ns	3.6	up	Molecular function	hydrolase activity		The function of this domain is unknown. It is found in several lipoproteins.	Anantharaman and Aravind, 2003.
TRINITY_DN9091_c0_g1_i1	PF02874.23^ATP-synt_ab	ns	ns	3.9	ns	up	Molecular function	hydrolase activity or RNA binding	bacteria, archaea eukaryotes	Catalysis of the hydrolysis of various bonds, e.g. C-O, C-N, C-C, phosphoric anhydride bonds, etc. Hydrolase is the systematic name for any enzyme of EC class 3 or Interacting selectively and non-covalently with an RNA molecule.	Wilkens et al., 2005.
TRINITY_DN1905_c1_g2_i1	PF03431.13^RNA_replicase_B	-	ns	8.2	-	up	Molecular function	RNA-directed 5'-3' RNA polymerase activity	virus	This family is of Leviviridae RNA replicases. The replicase is also known as RNA dependent RNA polymerase.	Fernando et al., 2020.
TRINITY_DN1761_c0_g2_i1	PF01819.17^Levi_coat	-	ns	ns	3.1	up	Molecular function	viral capsid	bacteriophage	The Levivirus coat protein forms the bacteriophage coat that encapsidates the viral RNA. 180 copies of this protein form the virion shell. The MS2 bacteriophage coat protein controls two distinct processes: sequence-specific RNA encapsidation and repression of replicase translation-by binding to an RNA stem-loop structure of 19 nucleotides containing the initiation codon of the replicase gene. The binding of a coat protein dimer to this hairpin shuts off synthesis of the viral replicase, switching the viral replication cycle to virion assembly rather than continued replication	Convery et al., 1998.
TRINITY_DN17868_c1_g1_i9	PF09984.9^DUF2222	-	-	ns	3.3	up				Domain of unknown function	
TRINITY_DN8253_c0_g1_i9	PF01595.20^DUF21	-	ns	ns	3.4	up				Domain of unknown function	

Table S2 The definition of GO terms in Fig. 7.

GO ID	GO term	SA regulation	Catergory	Definition
GO:0051704	multi-organism cellular process	up	Biological process	Interaction between organisms | physiological interaction between organisms | physiological interaction with another organism
GO:0044403	symbiotic process	up	Biological process	A process carried out by gene products in an organism that enable the organism to engage in a symbiotic relationship, a more or less intimate association, with another organism. Microscopic symbionts are often referred to as endosymbionts.
GO:0044764	multi-organism cellular process	up	Biological process	Any process that is carried out at the cellular level, which involves another organism of the same or different species.
GO:0019079	viral genome replication	up	Biological process	Any process involved directly in viral genome replication, including viral nucleotide metabolism.
GO:0039694	viral RNA genome replication	up	Biological process	The replication of a viral RNA genome.
GO:0039703	RNA replication	up	Biological process	The cellular metabolic process in which a cell duplicates one or more molecules of RNA.
GO:0016032	viral process	up	Biological process	A multi-organism process in which a virus is a participant. The other participant is the host. Includes infection of a host cell, replication of the viral genome, and assembly of progeny virus particles. In some cases, the viral genetic material may integrate into the host genome and only subsequently, under particular circumstances, 'complete' its life cycle.
GO:0006314	intron homing	up	Biological process	Lateral transfer of an intron to a homologous allele that lacks the intron, mediated by a site-specific endonuclease encoded within the mobile intron. It involves with cellular macromolecule metabolic and nucleic acid metabolic process
GO:0044033	multi-organism metabolic process	up	Biological process	A metabolic process - chemical reactions and pathways, including anabolism and catabolism, by which living organisms transform chemical substances, which involves more than one organism.
GO:0018144	RNA-protein covalent cross-linking	up	Biological process	The formation of a covalent cross-link between RNA and a protein. It involves in cellular protein modification.
GO:0039666	virion attachment to host cell pilus	up	Biological process	The process by which a virion attaches to a host cell by binding to a pilus on the host cell surface. Pili are retractile filaments that protrude from gram-negative bacteria. Filamentous viruses can attach to the pilus tip, whereas icosahedral viruses can attach to the pilus side.
GO:0033643	host cell part	up	Cellular Component	Any constituent part of a host cell. The host is defined as the larger of the organisms involved in a symbiotic interaction.
GO:0044217	other organism part	up	Cellular Component	Any constituent part of a secondary organism with which the first organism is interacting.
GO:0033646	host intracellular part	up	Cellular Component	Any constituent part of the living contents of a host cell; the matter contained within (but not including) the plasma membrane, usually taken to exclude large vacuoles and masses of secretory or ingested material. In eukaryotes it includes the nucleus and cytoplasm. The host is defined as the larger of the organisms involved in a symbiotic interaction.
GO:0033655	host cell cytoplasm part	up	Cellular Component	Any constituent part of the host cell cytoplasm, all of the contents of a cell excluding the plasma membrane and nucleus, but including other subcellular structures. The host is defined as the larger of the organisms involved in a symbiotic interaction.
GO:0044423	virion part	up	Cellular Component	Any constituent part of a virion, a complete fully infectious extracellular virus particle.
GO:0019028	viral capsid	up	Cellular Component	The protein coat that surrounds the infective nucleic acid in some virus particles. It comprises numerous regularly arranged subunits, or capsomeres.
GO:0033647	host intracellular organelle	up	Cellular Component	Organized structure of distinctive morphology and function, occurring within the host cell. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton. Excludes the plasma membrane. The host is

				defined as the larger of the organisms involved in a symbiotic interaction.
GO:0033648	host intracellular membrane-bounded organelle	up	Cellular Component	Organized structure of distinctive morphology and function, as found in host cells, bounded by a single or double lipid bilayer membrane and occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, and vesicles. Excludes the plasma membrane. The host is defined as the larger of the organisms involved in a symbiotic interaction.
GO:0033650	host cell mitochondrion	up	Cellular Component	A semiautonomous, self-replicating organelle as found in host cells that occur in varying numbers, shapes, and sizes in the cell cytoplasm. The host is defined as the larger of the organisms involved in a symbiotic interaction.
GO:0019029	helical viral capsid	up	Cellular Component	The protein coat that surrounds the infective nucleic acid in some virus particles; the subunits are arranged to form a protein helix with the genetic material contained within. Tobacco mosaic virus has such a capsid structure.
GO:0019030	icosahedral viral capsid	up	Molecular function	The protein coat that surrounds the infective nucleic acid in some virus particles; the subunits are arranged to form an icosahedron, a solid with 20 faces and 12 vertices. Icosahedral capsids have 12 pentamers plus 10(T-1) hexamers, where T is the triangulation number. Tobacco satellite necrosis virus has such a capsid structure.
GO:0039617	T=3 icosahedral viral capsid	up	Molecular function	The protein coat that surrounds the infective nucleic acid in some virus particles where the subunits (capsomeres) are arranged to form an icosahedron with T=3 symmetry. The T=3 capsid is composed of 12 pentameric and 20 hexameric capsomeres.
GO:0034062	5'-3' RNA polymerase activity	up	Molecular function	Catalysis of the reaction: nucleoside triphosphate + RNA (n) = diphosphate + RNA (n+1); the synthesis of RNA from ribonucleotide triphosphates in the presence of a nucleic acid template, via extension of the 3'-end.
GO:0097747	RNA polymerase activity	up	Molecular function	Catalysis of the reaction: nucleoside triphosphate + RNA (n) = diphosphate + RNA (n+1); the synthesis of RNA from ribonucleotide triphosphates in the presence of a nucleic acid template.
GO:0016779	nucleotidyltransferase activity	up	Molecular function	Catalysis of the transfer of a nucleotidyl group to a reactant. The upper group belongs to trasnfeerase activety.
GO:0003968	RNA-directed 5'-3' RNA polymerase activity	up	Molecular function	Catalysis of the reaction: nucleoside triphosphate + RNA (n) = diphosphate + RNA (n+1); uses an RNA template, i.e., the catalysis of RNA-template-directed extension of the 3'-end of an RNA strand by one nucleotide at a time.
GO:0001848	complement binding	up	Molecular function	Interacting selectively and non-covalently with any component or product of the complement cascade.
GO:0001846	opsonin binding	up	Molecular function	Interacting selectively and non-covalently with an opsonin, such as a complement component or antibody, deposited on the surface of a bacteria, virus, immune complex, or other particulate material.
GO:0001851	complement component C3b binding	up	Molecular function	Interacting selectively and non-covalently with the C3b product of the complement cascade.
GO:0051179	localization	down	Biological process	Any process, in which a cell, a substance, or a cellular entity, such as a protein complex or organelle, is transported, tethered to or otherwise maintained in a specific location. In the case of substances, localization may also be achieved via selective degradation.
GO:0051234	establishment of localization	down	Biological process	Any process that localizes a substance or cellular component. This may occur via movement, tethering or selective degradation.
GO:0006810	transport	down	Biological process	The directed movement of substances (such as macromolecules, small molecules, ions) or cellular components (such as complexes and organelles) into, out of or within a cell, or between cells, or within a multicellular organism by means of some agent such as a transporter, pore or motor protein.
GO:0044281	small molecule metabolic process	down	Biological process	The chemical reactions and pathways involving small molecules, any low molecular weight, monomeric, non-encoded molecule.

GO:1901362	organic cyclic compound biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of organic cyclic compound.
GO:0018130	heterocycle biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of heterocyclic compounds, those with a cyclic molecular structure and at least two different atoms in the ring (or rings).
GO:0009058	biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of substances; typically, the energy-requiring part of metabolism in which simpler substances are transformed into more complex ones.
GO:1901576	organic substance biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of an organic substance, any molecular entity containing carbon.
GO:0044249	cellular biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of substances, carried out by individual cells.
GO:1901564	organonitrogen compound metabolic process	down	Biological process	The chemical reactions and pathways involving organonitrogen compound.
GO:1901566	organonitrogen compound biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of organonitrogen compound.
GO:0071704	organic substance metabolic process	down	Biological process	The chemical reactions and pathways involving an organic substance, any molecular entity containing carbon.
GO:0044237	cellular metabolic process	down	Biological process	The chemical reactions and pathways by which individual cells transform chemical substances.
GO:0044271	cellular nitrogen compound biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of organic and inorganic nitrogenous compounds.
GO:1901135	carbohydrate derivative metabolic process	down	Biological process	The chemical reactions and pathways involving carbohydrate derivative.
GO:0019538	protein metabolic process	down	Biological process	The chemical reactions and pathways involving a protein. Includes protein modification.
GO:0044238	primary metabolic process	down	Biological process	The chemical reactions and pathways involving those compounds, which are formed as a part of the normal anabolic and catabolic processes. These processes take place in most, if not all, cells of the organism.
GO:0006807	nitrogen compound metabolic process	down	Biological process	The chemical reactions and pathways involving organic or inorganic compounds that contain nitrogen.
GO:0009059	macromolecule biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of a macromolecule, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetitions of units derived, actually or conceptually, from molecules of low relative molecular mass.
GO:0034645	cellular macromolecule biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of a macromolecule, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass, carried out by individual cells.
GO:0019438	aromatic compound biosynthetic process	down	Biological process	The chemical reactions and pathways resulting in the formation of aromatic compounds, any substance containing an aromatic carbon ring.
GO:0008152	metabolic process	down	Biological process	Metabolic process resulting in cell growth | metabolism | metabolism resulting in cell growth | multicellular organism metabolic process | single-organism metabolic process
GO:1901360	organic cyclic compound metabolic process	down	Biological process	The chemical reactions and pathways involving organic cyclic compound.
GO:0006725	cellular aromatic compound metabolic process	down	Biological process	The chemical reactions and pathways involving aromatic compounds, any organic compound characterized by one or more planar rings, each of which contains conjugated double bonds and delocalized pi electrons, as carried out by individual cells.

GO:0034641	cellular nitrogen compound metabolic process	down	Biological process	The chemical reactions and pathways involving various organic and inorganic nitrogenous compounds, as carried out by individual cells.
GO:0046483	heterocycle metabolic process	down	Biological process	The chemical reactions and pathways involving heterocyclic compounds, those with a cyclic molecular structure and at least two different atoms in the ring (or rings).
GO:0043170	macromolecule metabolic process	down	Biological process	The chemical reactions and pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetitions of units derived, actually or conceptually, from molecules of low relative molecular mass.
GO:0009056	catabolic process	down	Biological process	The chemical reactions and pathways resulting in the breakdown of substances, including the breakdown of carbon compounds with the liberation of energy for use by the cell or organism.
GO:0044260	cellular macromolecule metabolic process	down	Biological process	The chemical reactions and pathways involving macromolecules, any molecule of high relative molecular mass, the structure of which essentially comprises the multiple repetition of units derived, actually or conceptually, from molecules of low relative molecular mass, as carried out by individual cells.
GO:0065007	biological regulation	down	Biological process	Any process that modulates a measurable attribute of any biological process, quality or function.
GO:0006139	nucleobase-containing compound metabolic process	down	Biological process	Any cellular metabolic process involving nucleobases, nucleosides, nucleotides and nucleic acids.
GO:0033646	intracellular part	down	Cellular Component	Any constituent part of the living contents of a host cell; the matter contained within (but not including) the plasma membrane, usually taken to exclude large vacuoles and masses of secretory or ingested material. In eukaryotes it includes the nucleus and cytoplasm. The host is defined as the larger of the organisms involved in a symbiotic interaction.
GO:0016020	membrane	down	Cellular Component	A lipid bilayer along with all the proteins and protein complexes embedded in it an attached to it.
GO:0005737	cytoplasm	down	Cellular Component	All of the contents of a cell excluding the plasma membrane and nucleus, but including other subcellular structures.
GO:0005886	plasma membrane	down	Cellular Component	The membrane surrounding a cell that separates the cell from its external environment. It consists of a phospholipid bilayer and associated proteins.
GO:1990904	ribonucleoprotein complex	down	Cellular Component	A macromolecular complex containing both protein and RNA molecules.
GO:0032991	protein-containing complex	down	Cellular Component	A stable assembly of two or more macromolecules, i.e., proteins, nucleic acids, carbohydrates or lipids, in which at least one component is a protein and the constituent parts function together.
GO:0031224	intrinsic component of membrane	down	Cellular Component	The component of a membrane consisting of the gene products having some covalently attached portion, for example part of a peptide sequence or some other covalently attached group such as a GPI anchor, which spans or is embedded in one or both leaflets of the membrane.
GO:0016021	integral component of membrane	down	Cellular Component	The component of a membrane consisting of the gene products and protein complexes having at least some part of their peptide sequence embedded in the hydrophobic region of the membrane.
GO:0043228	non-membrane-bounded organelle	down	Cellular Component	Organized structure of distinctive morphology and function, not bounded by a lipid bilayer membrane. Includes ribosomes, the cytoskeleton and chromosomes.
GO:0005737	cytoplasmic part	down	Cellular Component	All of the contents of a cell excluding the plasma membrane and nucleus, but including other subcellular structures.
GO:0043228	non-membrane-bounded organelle	down	Cellular Component	Organized structure of distinctive morphology and function, not bounded by a lipid bilayer membrane. Includes ribosomes, the cytoskeleton and chromosomes.
GO:0005840	ribosome	down	Cellular Component	An intracellular organelle, about 200 A in diameter, consisting of RNA and protein. It is the site of protein biosynthesis resulting from translation of messenger RNA (mRNA). It

				consists of two subunits, one large and one small, each containing only protein and RNA.
GO:0043226	organelle	down	Cellular Component	Organized structure of distinctive morphology and function. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton, and prokaryotic structures such as anammoxosomes and pirellulosomes. Excludes the plasma membrane.
GO:0043229	intracellular organelle	down	Cellular Component	Organized structure of distinctive morphology and function, occurring within the cell. Includes the nucleus, mitochondria, plastids, vacuoles, vesicles, ribosomes and the cytoskeleton. Excludes the plasma membrane.
GO:0043168	anion binding	down	Molecular function	Interacting selectively and non-covalently with anions, charged atoms or groups of atoms with a net negative charge.
GO:0043167	ion binding	down	Molecular function	Interacting selectively and non-covalently with ions, charged atoms or groups of atoms.
GO:0097367	carbohydrate derivative binding	down	Molecular function	Interacting selectively and non-covalently with a carbohydrate derivative.
GO:0032553	ribonucleotide binding	down	Molecular function	Interacting selectively and non-covalently with a ribonucleotide, any compound consisting of a ribonucleoside that is esterified with (ortho) phosphate or an oligophosphate at any hydroxyl group on the ribose moiety.
GO:0032555	purine ribonucleotide binding	down	Molecular function	Interacting selectively and non-covalently with a purine ribonucleotide, any compound consisting of a purine ribonucleoside that is esterified with (ortho) phosphate or an oligophosphate at any hydroxyl group on the ribose moiety.
GO:0032557	pyrimidine ribonucleotide binding	down	Molecular function	Interacting selectively and non-covalently with a pyrimidine ribonucleotide, any compound consisting of a pyrimidine ribonucleoside that is esterified with (ortho) phosphate or an oligophosphate at any hydroxyl group on the ribose moiety.
GO:0035639	purine ribonucleoside triphosphate binding	down	Molecular function	Interacting selectively and non-covalently with a purine ribonucleoside triphosphate, a compound consisting of a purine base linked to a ribose sugar esterified with triphosphate on the sugar.
GO:0003677	DNA binding	down	Molecular function	Any molecular function by which a gene product interacts selectively and non-covalently with DNA (deoxyribonucleic acid).
GO:0016787	hydrolase activity	down	Molecular function	Catalysis of the hydrolysis of various bonds, e.g., C-O, C-N, C-C, phosphoric anhydride bonds, etc. Hydrolase is the systematic name for any enzyme of EC class 3.
GO:0016818	phosphorus-containing anhydrides	down	Molecular function	Catalysis of the hydrolysis of any acid anhydride, which contains phosphorus.
GO:0016462	pyrophosphatase activity	down	Molecular function	Catalysis of the hydrolysis of a pyrophosphate bond between two phosphate groups, leaving one phosphate on each of the two fragments.
GO:0017111	nucleoside-triphosphatase activity	down	Molecular function	Catalysis of the reaction: a nucleoside triphosphate + H2O = nucleoside diphosphate + phosphate.
GO:0005488	binding	down	Molecular function	The selective, non-covalent, often stoichiometric, interaction of a molecule with one or more specific sites on another molecule.
GO:0097159	organic cyclic compound binding	down	Molecular function	Interacting selectively and non-covalently with an organic cyclic compound, any molecular entity that contains carbon arranged in a cyclic molecular structure.
GO:1901363	heterocyclic compound binding	down	Molecular function	Interacting selectively and non-covalently with heterocyclic compound.
GO:0003824	catalytic activity	down	Molecular function	Catalysis of a biochemical reaction at physiological temperatures. In biologically catalyzed reactions, the reactants are known as substrates, and the catalysts are naturally occurring macromolecular substances known as enzymes. Enzymes possess specific binding sites for substrates, and are usually composed wholly or largely of protein, but RNA that has catalytic activity (ribozyme) is often also regarded as enzymatic.
GO:0000166	nucleotide binding	down	Molecular function	Interacting selectively and non-covalently with a nucleotide, any compound consisting of a nucleoside that is esterified with (ortho) phosphate or an oligophosphate at any hydroxyl group on the ribose or deoxyribose.

GO:1901265	nucleoside phosphate binding	down	Molecular function	Interacting selectively and non-covalently with nucleoside phosphate.
GO:0043169	cation binding	down	Molecular function	Interacting selectively and non-covalently with cations, charged atoms or groups of atoms with a net positive charge.
GO:0046872	metal ion binding	down	Molecular function	Interacting selectively and non-covalently with any metal ion.