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Transcriptome profiling provides insights into regulatory factors involved in *Trichoderma viride-Azotobacter chroococcum* biofilm formation

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ABSTRACT

Azotobacter chroococcum (*Az*) and *Trichoderma viride* (*Tv*) represent agriculturally important and beneficial plant growth promoting options which contribute towards nutrient management and biocontrol, respectively. When *Az* and *Tv* are co-cultured, they form a biofilm, which has proved promising as an inoculant in several crops; however, the basic aspects related to regulation of biofilm formation were not investigated. Therefore, whole transcriptome sequencing (Illumina NextSeq500) and gene expression analyses were undertaken, related to biofilm formation vis a vis *Tv* and *Az* growing individually. Significant changes in the transcriptome profiles of biofilm were recorded and validated through qPCR analyses. In-depth evaluation also identified several genes (*phoA*, *phoB*, *glgP*, *alg8*, *sipW*, *purB*, *pssA*, *fadD*) specifically involved in biofilm formation in *Az*, *Tv* and *Tv-Az*. Genes coding for RNA-dependent RNA polymerase, ABC transporters, translation elongation factor EF-1, molecular chaperones and double homeobox 4 were either up-regulated or down-regulated during biofilm formation. To our knowledge, this is the first report on the modulation of gene expression in an agriculturally beneficial association, as a biofilm. Our results provide insights into the regulatory factors involved during biofilm formation, which can help to improve the beneficial effects and develop more effective and promising plant- microbe associations.

1. Introduction

Microbial cells form single, dual or multispecies biofilms, which represent aggregations of cells, formed by either cohesion or adhesion, and embedded in a self-produced polymeric matrix to overcome stress in the environment (Teschler et al., 2015). Formation of biofilm provides the organism with an improved ability to survive, flourish and reproduce in a given ecological niche, despite slower growth (Davey and O'Toole, 2000). Several cell components including flagella, pili, fimbriae, cell wall are involved in biofilm formation in bacteria (Jauffred et al., 2017). Formation of biofilms requires switching of free-floating cells (planktonic mode) to biofilm mode, regulated by network of signals, based on the expression of numerous genes (Landini et al., 2010; Wolska et al., 2016).

Several genera of agriculturally important microorganisms produce single or multispecies biofilms, which enhance plant growth and nutrient transformations in the rhizosphere, besides eliciting plant defense systems in several crop plants. These include bacteria, fungi, and cyanobacteria, both as single and as multispecies biofilms (Seneviratne et al., 2008; Prasanna et al., 2013, 2014, 2015; Velmourougane et al., 2017e). Bacterial-fungal interactions also lead to changes in the metabolic networks underlying gene expression, particularly those related to biotic stress or antifungal and antibacterial defence mechanisms (Benoit et al., 2015). The Gram-negative bacterium *Azotobacter* is a popular beneficial bacterium used in agriculture for its nitrogen fixation and other plant growth promoting attributes. Similarly, *Trichoderma* is an important filamentous fungus, investigated and employed for its biocontrol potential against plant pathogens. Recently, biofilm development involving an agriculturally important bacterium (*Azotobacter chroococcum*) and fungus (*Trichoderma viride*) was reported (Triveni et al., 2013; Velmourougane et al., 2017b). Further, the qualitative aspects and modulation of exopolymeric substances (EPS) produced during biofilm development between *Trichoderma viride* and *Azotobacter chroococcum* was characterised (Velmourougane et al., 2017c; Velmourougane and Prasanna, 2017d).

Exploring natural and developed biofilms of agriculturally

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important microorganisms has become a niche area for researchers in recent years (Seneviratne et al., 2008; Velmourougane et al., 2017a; Bharti et al., 2017). However, scanty information is available regarding several fundamental questions such as the nature of metabolic regulation leading to biofilm formation in these organisms. The aim of the present study was to investigate the bacterium (*Azotobacter chroococcum-Az*) - fungus (*Trichoderma viride-Tv*) interactions involved in the development and functioning of biofilm, through transcriptome profiles and gene expression analyses.

2. Materials and methods

2.1. Experimental setup

The *Azotobacter chroococcum* (*Az*) strain (MTCC 25045) and *Trichoderma viride* (*Tv*) strain (ITCC 2211) served as the bacterial and fungal partners in the study. The biofilm development was studied through *Tv-Az* staggered inoculation (48 h grown broth culture of *T. viride* inoculated with *A. chroococcum* cells), as shown in Fig. S1 optimized earlier (Velmourougane et al., 2017c). The medium containing Jensen's broth (M973-HiMedia Laboratories Pvt. Ltd., India) and Potato dextrose broth (M403- HiMedia Laboratories Pvt. Ltd., India) in the ratio of 25:75 was used, based on earlier studies (Velmourougane et al., 2017b). Biofilm formation was quantified through crystal violet assay described by O'Toole (O'Toole, 2011).

2.2. RNA extraction, library preparation, and Illumina sequencing

For transcriptomic analyses, the total RNA was isolated from individual (*Az*, *Tv*) and their biofilm (*Tv-Az*) in duplicates (biological replicates) after 6 days of inoculation using Quick-RNA Miniprep Plus kit (ZYMO Research, CA, USA) as per the manufacture's instruction. The RNA-seq paired end illiumina library were prepared using Illumina TruSeq stranded mRNA sample preparation kit (Illumina, San Diego, CA). Quantification and mean peak size for the libraries were analysed using Qubit flourometer and 4200 Tape Station system (Agilent Technologies Inc., Santa Clara, CA) respectively using manufacturers' instructions. The library was loaded onto NextSeq500 for cluster generation and sequencing (2 × 75 bp chemistry).

2.3. De novo transcriptome assembly, and gene annotation

The sequenced raw data were processed to obtain high quality clean reads using Trimmomatic v0.35, and the high quality ($QV > 20$), paired-end reads were used for *de novo* transcriptome assembly using Trinity software (v2.2.0). The TransDecoder predicted CDS from unigenes were subjected for functional and gene ontology annotation using Blastx ($E\text{-value} \leq 1e\text{-}05$) and Blast2Go program respectively. The CDS were mapped to the reference canonical pathways in KEGG (Kyoto Encyclopedia of Genes and Genomes).

2.4. Differential gene expression (DGE) analysis

The high quality reads of *Az*, *Tv*, and biofilm (*Tv-Az*) were mapped on their respective CDS using BWA aligner (Burrows-Wheeler Aligner v0.7.15) for read count calculation. The hit accessions based on the BLAST against Nr database were identified for Differential Gene Expression (DGE) analysis. For DGE between *Az* and biofilm, high quality reads of *Az* and biofilm were mapped on *Az* annotated CDS. For DGE between *Tv* and biofilm, high quality reads of *Tv* and biofilm were mapped on *Tv* annotated CDS. The differential expression analysis was performed by employing a negative binomial distribution model (DESeq v1.8.1 package http://www-huber.embl.de/users/anders/DESeq/). Fold change (FC) and Log fold change (FC = Log2; *Az*/biofilm or *Tv*/biofilm) values for differentially expressed genes were calculated and CDS were categorised into up and down regulated genes. A *p*-value threshold of 0.05 was used to filter statistically significant results. The differentially expressed genes identified in individual (*Az* or *Tv*) and biofilm samples were analysed by hierarchical clustering. Differentially expressed genes (top 100) were used for hierarchical cluster analysis using Multiple experiment viewers (MEV v4.8.1), and the heat map (Figs. S6 and S7) was constructed with normalised value of genes based on Pearson uncentered correlation distance.

2.5. Quantitative (q) RT-PCR validation of the transcriptome data

To validate the transcriptome data, we selected six genes, three up-regulated (CDS-5300, CDS-12703, CDS-366) and 3 down-regulated (CDS-89, CDS-13443, CDS-10036) based on their significance and expression. Transcript abundance of these genes during biofilm developmental stages (3, 6 and 9 days) was quantified using qRT-PCR with single biological replicate having 3 technical replicates for each gene, using the same biofilm samples subjected for transcriptome analysis. There were no '0' day sample in the analyses, as the bacterial and fungal partner needs minimum time to develop as biofilms. Hence, the 3rd day sample was treated as control sample in the qPCR analysis. As we hypothesized that the gene expression will be different during The stages of biofilm formation between *Tv-Az*, qPCR analysis was undertaken with only the biofilm samples (*Tv-Az*). The information regarding target genes and their primers is provided in the Table S1. The total RNA was extracted from *Tv-Az* biofilm samples using RNA isolation reagent (TRIzol-Sigma) and the first strand cDNA synthesis (100 ng RNA) was carried out using RevertAid First Strand cDNA Synthesis kit (Thermo scientific, Waltham, MA, USA) as per manufactures instructions. The Real Time PCR amplification carried out in a reaction volume of 20 µl containing 2 µl of cDNA and 10 µl of SYBR Green Supermix (Bio Rad, CA, USA). The Real Time PCR reaction was carried out for 35 cycles followed by denaturation 95 °C for 30 s, annealing 52 °C–58 °C (gradient) for 30 s, extension for 72 °C for 15 s using Bio-Rad CFX96™ system (Bio Rad, CA, USA). The mRNA expression levels were normalized to the level of housekeeping gene (ITS or 16 S gene). The Ct values of the 6th and 9th day biofilm samples were calculated and the data was expressed in terms of fold change (up or down regulation) over the control sample (3rd day sample).

3. Results

3.1. Biofilm formation and quantification

The data on biofilm formation in *Az*, *Tv* and their biofilm (*Tv-Az*) quantified through crystal violet assay is presented in Fig. S1. Enhanced biofilm development was observed in *Tv-Az,* as compared to the individual inoculation (*Az* or *Tv*), measured as change in the optical density (OD_{550}). Values recorded for *Tv-Az* were 2.356, 2.655 and 2.935 for 3rd, 6th, and 9th day respectively as compared to *Az* (0.920, 1.194, 1.521) and *Tv* (0.506, 0.647, 0.765).

3.2. De novo assembly and gene functional annotation

The paired-end reads were generated for *Az*, *Tv* and *Tv-Az* biofilm samples which were roughly 7, 16 and 33 million respectively (Table 1). A total of 6432 (R1) and 983 (R2) transcripts for *Az*; and 30, 419 (R1) and 43,330 (R2) for *Tv* were obtained. Based on the analyses, we predicted 538 and 322 CDS for *Az* and; 13, 078 and 16,606 CDS for *Tv* respectively, for replicates 1 and 2. Accordingly, a total of 524 and 316 CDS for *Az* and; 13, 078 and 16, 108 CDS for *Tv* were annotated against the NCBI Nr database using Blastx (Table 1). Using Gene ontology (GO) analysis, 299 and 202 CDS for *Az* (Table 2; Figs. S2 and S3; Table S2) and 9139 and 10, 947 CDS for *Tv* (Table 2; Figs. S4 and S5; Table S3) were annotated with the Gene ontology terms.

Table 1
Summary of sequencing and de novo assembly of the transcriptomes.

	Azotobacter chroococcum (Az)		*Trichoderma viride (Tv)*		*Tv-Az* biofilm	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2	Replicate 1	Replicate 2
RNASeq high quality data						
No. of reads	7,898,059	7,362,242	15,547,359	16,231,968	33,683,972	30,356,072
No. of bases	1,188,251,803	1,107,059,972	2,326,627,601	2,437,036,277	5,062,473,622	4,559,608,730
Total data (Gb)	1.18	1.10	2.32	2.43	5.06	4.55
Assembly						
No. of transcripts	6432	30419	983	43330		
Total bases	1,712,320	19,236,033	345,094	29,407,013		
Mean transcript length	266	632	351	678		
N50	248	978	333	1237		
Max. transcript length	3159	8631	3314	10057		
Min. transcript length	201	201	201	201		
Coding sequence (CDS)						
No. of CDS	538	322	13,314	16,606		
Total bases	23,037	152,703	10,020,066	15,176,673		
Mean CDS length	428	474	752	913		
Max. CDS length	1,980	2,226	7,956	9,381		
Min. CDS length	297	297	297	297		
Distribution of Blast results for CDS						
No. of CDS	538	322	13314	16606		
No. of CDS with Blast hits	524	316	13078	16108		
No. of CDS with no Blast hits	14	06	236	498		

Table 2
Gene ontology (GO) distribution of the CDS of *Azotobacter chroococcum* and *Trichoderma viride.*

Gene ontology	*Azotobacter chroococcum*		*Trichoderma viride*	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Biological processes	244	170	6519	7721
Molecular functions	247	178	7002	8390
Cellular component	186	117	5205	6121

3.3. Differential gene expression and KEGG pathway analysis

Based on the differential gene expression analyses of *Az* and *Tv-Az* biofilm samples, a total of 115 and 153 CDS were observed to be expressed in replicates 1 and 2 (Table S4 and Fig. S6). Out of the total CDS in *Az*, 59 and 76 genes were up- regulated and 56 and 77 genes were down-regulated in R1 and R2, respectively (Table 3). Based on *p*-value threshold (0.05), we have identified only one up-regulated gene in R1 and R2, while there were no down-regulated genes with significant changes in expression in both R1 and R2. In *Tv* vs. *Tv-Az* biofilm samples, a total of 8,222 and 9,166 CDS were commonly expressed in the replications 1 and 2 (Table S5 and Fig. S7). Out of the total CDS in *Tv*, 4, 114 and 4, 629 genes were up-regulated and 4, 108 and 4, 537 genes were down-regulated respectively in R1 and R2 (Table 3). Based on threshold *p*-value (0.05), we identified 72 and 92 up-regulated genes and 33 and 43 down-regulated genes respectively in R1 and R2 samples. Based on KEGG pathway analysis, we speculated the involvement of the predicted CDS ranging from 538 (*Az* – R1), 322 (*Az* – R2), 13, 314 (*Tv* – R1) and 16, 606 (*Tv* – R2) in the samples in terms of biological activity (Table 3). A total of 145 (*Az* – R1), 87 (*Az* – R2), 1638 (*Tv* – R1) and 1820 (*Tv* – R2) were classified mainly under five categories: metabolism, cellular process, genetic information processing, environmental information processing and organismal systems (Fig. 1).

Among the annotated unigenes involved in biological activities, maximum number of unigenes was involved in translation process (329), signal transduction (243) and transport and catabolism (239) in *Tv*. While, in *Az*, most of the genes were found to be involved in translation (33), carbohydrate and amino acid metabolism (15) and energy metabolism (14). Interestingly, genes involved in membrane transport were higher in *Az* (15) as compared to *Tv* (9). The genes (up and down regulated) featuring the 20 highest base mean in the transcriptomes of *Az* and *Tv* in comparison to their biofilm (*Tv-Az*) is given in the Table S6, wherein, most of the top ranked genes or gene products were hypothetical proteins reported elsewhere in bacteria, fungi and yeast. The ten top ranked up and down regulated genes in the transcriptome of *Tv-Az* biofilms, as compared to those in *Tv* and *Az* alone is presented in the Tables 4 and 5 respectively.

3.4. Up-regulated genes in the transcriptome of Tv-Az biofilm

In *Tv-Az* biofilms, significant up-regulation (662 fold change) of RNA-dependent RNA polymerase (RdRPs) and ABC transporter (55-fold change) were recorded as compared to those in *Tv* alone. While, in the transcriptome of *Tv-Az* biofilms, as compared to *Az* alone, significant up-regulation of RdRPs (7640 fold change), Translation elongation factor EF-1 alpha subunit (EF1) (2215 -fold change) and Glyceraldehyde 3-phosphate dehydrogenase (*GpdA*) (700-fold change) were documented. However, the up-regulation of RdRPs was greater in *Az* as compared to *Tv* alone.

Table 3
Differentially expressed genes of *Azotobacter chroococcum* and *Trichoderma viride,* as compared with those of biofilm.

Genes	*Azotobacter chroococcum*		*Trichoderma viride*	
	Replicate 1	Replicate 2	Replicate 1	Replicate 2
Commonly expressed	115	153	8222	9166
Up-regulated	59 (51.30 %)	76 (49.67 %)	4114 (50.03 %)	4629 (50.50 %)
Down-regulated	56 (48.69 %)	77 (50.32%)	4108 (49.96 %)	4537 (49.49 %)

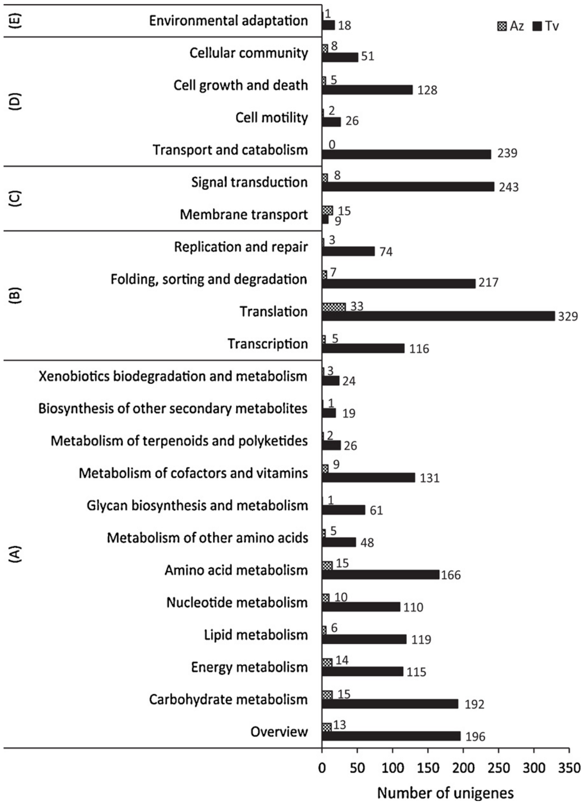

Fig. 1. KEGG function classification results of the annotated unigenes in *A. chroococcum* and *T. viride*. The y-axis lists the various KEGG pathways. The x-axis indicates the number of genes. According to unigenes participation in these KEGG pathways, the unigenes were divided into five groups: metabolism (A), genetic information processing (B), environmental information processing (C), cellular processes (D), and organismal systems (E).

3.5. Down-regulated genes in the transcriptome of Tv-Az biofilm

In terms of down-regulation, we identified a gene AF194537_1NAG13 (-64 fold change) in *Tv-Az* biofilm as compared to *Tv* alone, which encodes reverse transcriptase (non-long terminal repeat class of retrotransposons), pol-like, as reported in *Gallus gallus* (chicken). While, in *Tv-Az* biofilm as compared to *Az* alone, significant changes were recorded in Double homeobox 4 (DUX4) (-57 fold change), Urea ABC transporter substrate-binding protein (-26 fold change) and Molecular chaperone GroEL (-14 fold change).

3.6. Other key genes identified in the transcriptome of Tv-Az biofilm involved in biofilm formation

In addition to the significantly modulated genes identified in the transcriptome of *Tv*-Az biofilms, a few key genes mostly from *Tv*, were reported, which are known to be associated with biofilm formation in other microorganisms (Table 6). Among them, biofilm forming genes, *alg8* (alpha-1,3-glucosyltransferase), *sipW* (Signal peptidases), *pssA* (CDP-diacylglycerol-serine O-phosphatidyltransferase (CHO1)) were found to be up-regulated in *Tv-Az* biofilm, while, *pho A, B* (alkaline phosphatase), *glgP* (glycogen phosphorylase), *purB* (adenylosuccinate lyase) and *fadD* (Long-chain acyl-CoA synthetase) were down-regulated in the transcriptome of *Tv-Az* biofilm.

3.7. qRT-PCR validation of the target genes

qPCR validation of expression data for the six selected genes (up-regulation: CDS-366, CDS-5300, CDS-12703; down-regulation: CDS-89, CDS-10036, CDS-13443) resulted in conformity of expression in 4 genes [3 up-regulated:CDS-366 (RNA-dependent RNA polymerase), CDS-5300 (*alg8*), CDS-12703 (Putative Transposase-like protein) and 1 down-regulated: CDS-89 (ABC transporter substrate-binding protein)]. However, the two genes selected for down-regulation [CDS-10036 (*glgP*) and CDS-13443 (4-aminobutyrate aminotransferase (*gabT*)] based on the sequence data, showed up-regulation in qPCR over the control sample. In the up-regulated genes, the increase was by 1 and 4, 4 and 26, 19 and 33, 3 and 17, 10 and 23 fold for CDS-5300, CDS-10036, CDS-12703, CDS-13443 and CDS-366 respectively, in the 6th and 9th day samples, over the control (Figs. S8 a, b). While, in the down-regulated gene (CDS-89) the expression was 8 and 175 fold, over the control sample on 6th and 9th day in the biofilm respectively (Figs. S8 c, d).

Table 4
Selected up and down regulated genes in the transcriptome of *Tv-Az* biofilm, as compared to *Trichoderma viride.*

Gene product/Gene annotation	Common hit accession	*Tv* CDS ID	*Tv* base mean	*Tv*-Az biofilm base mean	Fold change	Log_2 Fold change
Up-regulated genes						
RNA-dependent RNA polymerase	ALD89139	CDS_1953_Transcript_6926	41	27375	662	9.37
Hypothetical protein	XP_013945445	CDS_13139_Transcript_29274	9	1090	125	6.97
Hypothetical protein	XP_013942561	CDS_10677_Transcript_22197	8	469	56	5.80
ABC transporter	AAX68676	CDS_2356_Transcript_8130	13	730	55	5.77
Hypothetical protein	KUE97908	CDS_2635_Transcript_8831	0.6	32	52	5.69
Hypothetical protein	KUE95886	CDS_2423_Transcript_8294	9	444	49	5.62
Putative Transposase-like protein	CEJ84208	CDS_12703_Transcript_26817	10	495	47	5.56
Adhesin protein Mad1	KUE94739	CDS_1783_Transcript_6456	26	1225	46	5.53
ATP-binding cassette, subfamily G	KUE99061	CDS_3599_Transcript_11050	10	444	46	5.52
Ca2+ transporting ATPase	KKP03232	CDS_7067_Transcript_16891	7	318	44	5.48
Down-regulated genes						
AF194537_1NAG13	AAG27485	CDS_12376_Transcript_24863	204	3	−64	−5.99
Hypothetical protein	XP_013939336	CDS_8918_Transcript_21402	189	3	−64	−6.00
Hypothetical protein	XP_013938074	CDS_6095_Transcript_15367	307	6	−48	−5.58
Hypothetical protein	KUE95647	CDS_3129_Transcript_9924	133	3	−41	−5.37
Hypothetical protein	KUF05252	CDS_93_Transcript_244	112	3	−38	−5.24
Hypothetical protein	KKP02862	CDS_11946_Transcript_25735	180	6	−30	−4.93
Hypothetical protein	XP_013943926	CDS_1100_Transcript_5243	175	6	−30	−4.88
Hypothetical protein	XP_013947288	CDS_2140_Transcript_8716	853	30	−29	−4.85
Hypothetical protein	XP_001486229	CDS_12616_Transcript_26215	183	6	−28	−4.83
Conserved hypothetical protein	XP_001486674	CDS_929_Transcript_3611	181	6	−28	−4.82

Table 5
Selected up and down regulated genes in the transcriptome of *Tv-Az* biofilm, as compared to *Azotobacter chroococcum.*

Gene product/Gene annotation	Common hit accession	Az CDS ID	Az base mean	*Tv*-Az biofilm base mean	Fold change	Log$_2$ Fold change
Up-regulated genes						
RNA-dependent RNA polymerase	ALD89139	CDS_366_Transcript_3224	6	43664	7640	12.90
Translation elongation factor EF-1 alpha subunit	KMK57125	CDS_378_Transcript_3445	14	31192	2215	11.11
Conserved hypothetical protein	XP_751596	CDS_283_Transcript_2180	5	4448	908	9.83
Glyceraldehyde 3-phosphate dehydrogenase GpdA	XP_748145	CDS_217_Transcript_551	11	7405	700	9.45
Translation elongation factor EF-1 alpha subunit	XP_750388	CDS_148_Transcript_336	25	16660	668	9.38
Heat shock protein 70	ACZ95776	CDS_167_Transcript_372	20	12942	634	9.31
Ubi4p	EGA61425	CDS_122_Transcript_287	7	4175	571	9.16
40S ribosomal protein S18	XP_001268409	CDS_271_Transcript_777	5	2349	466	8.86
G-protein complex beta subunit CpcB	XP_751514	CDS_121_Transcript_824	8	3557	447	8.80
ER Hsp70 chaperone BiP	XP_001260092	CDS_329_Transcript_2660	2	794	353	8.47
Down-regulated genes						
Double homeobox 4 (DUX4)	ADK24699	CDS_235_Transcript_1278	281	5	−57	−5.84
Urea ABC transporter substrate-binding protein	WP_004443324	CDS_89_Transcript_591	251	10	−26	−4.68
Molecular chaperone GroEL	WP_003522947	CDS_95_Transcript_242	57	4	−14	−3.84
Hypothetical protein	XP_728586	CDS_140_Transcript_320	300	24	−13	−3.65
Hypothetical protein C241_11001	EKJ95971	CDS_20_Transcript_89	102	10	−10	−3.38
Elongation factor EF-G	KJX88087	CDS_167_Transcript_983	39	5	−8	−3.00
Regulator of biofilm formation, Fnr family protein	CDI09359	CDS_18_Transcript_52	53	8	−7	−2.73
DNA-directed RNA polymerase subunit beta	WP_037093951	CDS_146_Transcript_328	20	4	−5	−2.34
Chaperone protein dnaK (Heat shock protein 70)	WP_004439158	CDS_110_Transcript_735	97	20	−5	−2.31
Peptide methionine sulfoxide reductase msrA	WP_022555365	CDS_10_Transcript_36	19	4	−5	−2.23

Table 6
Overview of the modulated (up/down regulated) genes identified in the transcriptome of *Tv-Az* biofilm.

KO ID/ Gene name	Sequence ID	Hit Accession	Enzyme name	*Tv* base mean	*Tv*-Az biofilm base mean	Fold change	Log$_2$ Fold change	Regulation
K03849 *alg8*	CDS_5300_Transcript_14124	XP_013946745	Alpha-1,3-glucosyltransferase	10	32	3	1.69	Up
K13280 SEC11, *sipW*	CDS_4120_Transcript_12056	XP_013947399	Signal peptidase, endoplasmic reticulum-type	166	225	1	0.44	Up
K13280 SEC11, *sipW*	CDS_5773_Transcript_16642	XP_013947399	Signal peptidase, endoplasmic reticulum-type	235	453	2	0.95	Up
K17103 CHO1, *pssA*	CDS_9796_Transcript_20940	XP_013941993	CDP-diacylglycerol—serine O-phosphatidyltransferase	101	141	1	0.48	Up
K01897 ACSL, *fadD*	CDS_8010_Transcript_18308	XP_013944675	Long-chain acyl-CoA synthetase	832	395	−2	−1.07	Down
K01897 ACSL, *fadD*	CDS_13029_Transcript_27259	XP_013944675	Long-chain acyl-CoA synthetase	1965	823	−2	−1.25	Down
K01897 ACSL, *fadD*	CDS_13470_Transcript_27869	XP_013942569	Long-chain acyl-CoA synthetase	396	305	−1	−0.38	Down
K01756 ADSL, *purB*	CDS_13706_Transcript_28212	KUE97943	Adenylosuccinate lyase	971	601	−2	−0.69	Down
K01077 *phoA, phoB*	CDS_13320_Transcript_27652	KUE98063	Alkaline phosphatase	319	290	−1	−0.14	Down
K00688 PYG, *glgP*	CDS_10036_Transcript_21277	XP_013941594	Glycogen phosphorylase	3060	987	−3	−1.63	Down
K00688 PYG, *glgP*	CDS_14948_Transcript_30168	XP_013941594	Glycogen phosphorylase	1786	1155	−2	−0.63	Down

4. Discussion

Bacterial-fungal interactions influence microbial fitness and improved functioning of ecosystems, which may be accomplished through several mechanisms including hormone-induced growth stimulation, antibiosis, signalling, chemotaxis, and gene transfer (Frey-Klett et al., 2011). This is mainly mediated by the modulation of transcription of antibacterial and antifungal molecules, resulting in synergy in action (Benoit et al., 2015). Most of the published literature on bacterial-fungal interactions in biofilms relates to industrial and clinical samples (Donlan and Costerton, 2002), with no reports on the transcriptome of agriculturally important fungal-bacterial interactions. This study aimed to identify the genes (up and down regulated) expressed during the biofilm development between agriculturally important microbes – *Trichoderma viride* and *Azotobacter chroococcum*, as a prelude to improving the functioning of biofilms as beneficial inoculants.

4.1. Up-regulated genes in the transcriptome of Tv-Az biofilm

The RNA-dependent RNA polymerase (RdRPs) was found to be significantly up-regulated in *Tv-Az* biofilms as compared to *Tv* or *Az*. The RdRPs are mainly involved in synthesis of small RNAs (sRNA); single-stranded RNAs, which are used as templates by the RdRPs to generate dsRNAs. These are then processed into siRNAs by the Dicer. Since, RdRPs plays a key role in RNA silencing to generate secondary siRNA synthesis; we hypothesize that sRNA mediated gene regulation may be involved during biofilm development between *Tv* and *Az*, as a large number of sRNAs are known to directly or indirectly modulate motility and/or biofilm formation (Mika and Hengge, 2013). It has been shown that the sRNAs regulates biofilm formation through base pairing with target RNAs and protein binding (Chambers and Sauer, 2013). The key components of RNA silencing viz., Dicers, Argonautes, and RNA-dependent RNA polymerases are reported in the filamentous fungus

Aspergillus nidulans (Hammond et al., 2008); while several miRNA-like sRNAs (milRNAs) were recorded in the filamentous fungi, such as *Trichoderma reesei* (Kang et al., 2013), *Neurospora crassa* (Lee et al., 2010), *Sclerotinia sclerotiorum* (Zhou et al., 2012a) and *Metarhizium anisopliae* (Zhou et al., 2012b). The small non-coding RNAs (ncRNAs) are also reported to be involved in processes such as stress response, motility, biofilm formation, control of metabolic fluxes, virulence etc. (Wagner and Romby, 2015). The ncRNAs were associated with biofilm physiology in *Escherichia coli* and *Pseudomonas* spp. (Petrova and Sauer, 2010), and link cell surface appendage (flagella and curli) production and biofilm formation. In *E. coli*, the sRNA (MicA) in association with σE repressed various porins, including *omp*A during biofilm formation (Kint et al., 2010). The signal transduction (Gac/Rsm) pathway, comprising small non-coding RNAs (RsmZ1-7 and RsmY) and a translation repressor protein RsmA are known to be involved in the synthesis of structural components such as alginate, polyhydroxybutyrate and alkylresorcinols in the soil bacterium *Azotobacter vinelandii* (Castañeda et al., 2016). Further, in *A. vinelandii*, RgsA (Small ribosomal subunit biogenesis GTPase) controlled by RNA polymerase sigma factor (RpoS) and a response regulator/global activator (GacA), play a key role in biofilm formation and overcoming oxidative stress (Huerta et al., 2016).

In the present study, the gene coding for the ATP binding cassette (ABC) transporter was significantly up-regulated in the transcriptome of *Tv-Az* biofilms. ABC transporters majorly comprise of an ATP-binding permease and substrate-binding lipoprotein components, which play a significant role in several cellular processes, facilitating the active translocation of substrates (nutrients, ions, amino acids, antibiotics etc.) across the inner and outer membrane by the hydrolysis of ATP, in response to environmental stimuli (Chung et al., 2009). When *Tv* and *Az* are co-cultured, nutrients are perhaps shared synergistically through the up-regulation of ABC transporters, without affecting their growth and biofilm formation. ABC transporters are known to be involved in diverse functions in the cell including DNA replication, protein degradation, membrane fusion, antibiotic efflux, signal transduction, chemotaxis (Mai-Prochnow et al., 2015) and biofilm formation (Iwai et al., 2010). Several transporters are responsible in the movement of signaling molecules (N-Acyl homoserine lactones (AHL), the furanone derivative AI-2, and signal peptides) that regulate bacterial biofilm formation (Pearson et al., 1999). Through a proteomic approach, Marra et al. (2006) reported the up-regulation of ABC transporter genes in *Trichoderma atroviride* during the three-way interaction with various plants and fungal pathogens, indicating the possible role of ABC transporters in biocontrol activity and root colonization, both of which includes biofilm formation as a key process.

Under oxygen-depleted environment, an ABC transporter (BPSL1039-1040) was shown to help in nitrate acquisition in *Burkholderia pseudomallei* favouring biofilm formation and pathogenesis (Pinweha et al., 2018). The role of ABC transporter (DppBCDF) in enhancement of biofilm formation through inhibition of Pf5 prophage lysis was demonstrated in *Pseudomonas aeruginosa* (Lee et al., 2018). Zhu et al. (2008) reported the ABC transporter dependent negative regulation of biofilm formation in *Listeria monocytogenes.* ABC transporter was also found to enhance desiccation tolerance through the modulation of exopolysaccharide accumulation in *Rhizobium leguminosarum* (Vanderlinde et al., 2010). Elongation factors (EF) play a major role in protein synthesis. EF facilitates translational elongation through the formation of peptide bonds in ribosomes (Andersen and Nyborg, 2001). The role of proteins in biofilm development between *Trichoderma viride* and *Azotobacter chroococcum* has been already well established (Velmourougane et al., 2017c). Our transcriptome data confirms the findings that protein synthesis gets significantly up-regulated by encoding EFs during biofilm development. In the human gut microflora member- *Enterococcus faecalis*, translation elongation factors assisted in the transition of planktonic cells to biofilm cells causing nosocomial infection (Qayyum et al., 2016).

The glyceraldehyde 3-phosphate dehydrogenase (GAPDH) is a glycolytic protein, involved in the phosphorylation of glyceraldehyde-3-phosphate, generating 1,3-bisphosphoglycerate. Among housekeeping genes, *gapdh* is considered important as it is well conserved in eubacteria, besides being present in eukaryotes (Figge et al., 1999). Earlier reports showed that the surface GAPDH possesses the ability to adhere to various biological materials including microorganisms (Glenting et al., 2013). In *Lactobacillus reuteri* ZJ617, GAPDH helps the bacterial cells to bind to the intestinal epithelial cell through adhesion (Zhang et al., 2015). In oral biofilm, the protein coding for major fimbriae (FimA) of *Porphyromonas gingivalis* was reported to bind to the GAPDH present on the *Streptococcus oralis* surface, facilitating *P. gingivalis* colonization and biofilm formation in the oral cavity (Nagata et al., 2009). Since, the *GpdA* gene, which encodes GAPDH (adhesion properties), was up-regulated in the*Tv-Az* biofilm, we predict that the *GpdA* gene may have a role in biofilm development through facilitation of attachment of *Az* cells to the *Tv* hyphae.

4.2. Down-regulated genes in transcriptome of Tv-Az biofilm

Double homeobox, 4 (DUX4) is a protein, encoded by the *DUX4* gene in humans (Gabriëls et al., 1999). The encoded protein functions as a transcriptional activator of paired-like homeodomain transcription factor (TF) 1. The homeobox TF genes code for highly conserved DNA binding motifs composed of nearly 60 amino acids, referred to as the homeodomain (Burglin, 2011). DNA-binding TFs respond to physiological or environmental stimuli and are essentially involved in the transcriptional regulation of specific target genes (Shelest, 2008). Several genes of the homeobox TFs in eukaryotes, including fungi, are involved in mating and sexual differentiation (Holland, 2013), morphology, pattern formation, filamentation and sporulation (Kim et al., 2012). Deletion of the transcription factor *ste12* gene in *Trichoderma atroviride* reduced mycoparasitism, conidial anastomosis tube formation and vegetative hyphal fusion events (Gruber and Zeilinger, 2014). GRF10 codes for a homeodomain containing transcription factor in *Candida albicans*, associated with not only biofilm formation, but also regulating adenylate biosynthesis and virulence (Nobile et al., 2010). Deletion of homeobox gene GRF10 resulted in defective chlamydospores and biofilm formation, thereby reducing hyphal growth and virulence in *C. albicans* (Ghosh et al., 2015). Zhang et al. (2007) reported the involvement of regulator (*ybgS*) encoding putative homeobox protein, which is over expressed in *E.coli* during biofilm formation. In *Podospora anserina*, a gene coding for homeodomain protein (Arnaise et al., 2001) was observed to modulate the morphology and conidiogenesis. In *Ustilago maydis*, homeobox TFs regulated the hyphal growth and sexual cycle, besides pathogenicity (Schulz et al., 1990). In a number of *Fusarium* species, including *F. graminearum*, *F. verticillioides* and *F. oxysporum*, phialide development and conidiogenesis was regulated by a homeobox transcription factor (Htf1) (Zheng et al., 2012). In our study, a significant down regulation of homeobox domain containing transcription factor was observed, which illustrates the potential of *T. viride* to remain in the vegetative state during biofilm development and support the attachment of *A. chroococcum* cells to its hyphae; as a result, sporulation/conidiogenesis is reduced.

Molecular chaperones are induced by nutritional stress including carbon, nitrogen, or phosphate. Proteins with chaperone activity were initially termed as heat shock proteins (Hsp) because of their overexpression during the heat shock (Ansari and Mande, 2018). Secreted chaperones are reported to recognize and bind ligands contributing to cell adherence, biofilm formation and pathogenesis in several bacteria (Ojha et al., 2005). The DnaK and GroEL are some of the common molecular chaperones found abundantly as extracellular proteins in certain bacteria (Fujiwara et al., 2010). Molecular chaperones are proteins, which play a key role in the stabilization of proteins (cellular homeostasis) under stress conditions and or changing environmental conditions (Bernal et al., 2014; Grudniak et al., 2015). However, our transcriptome data indicates a down-regulation of molecular chaperones in the *Tv-Az* biofilm. This illustrates a nutritional synergism and networking between *Tv* and *Az* during biofilm development, which prevents nutrient depletion, despite the medium having to sustain two organisms. This may also be facilitated through the up-regulation of the ABC transporters, as supported by our findings in this study.

4.3. Other key genes identified in the transcriptome of Tv-Az biofilm involved in biofilm formation

Apart from the commonly expressed, up, and down regulated genes, a few biofilm-forming genes (*alg8, sipW, pssA, fadD, purB, phoB, glgP*) in the transcriptome of *Tv-Az* biofilms were also identified, which are reported to play a key role in the regulation of biofilm formation in other microorganisms (Gaona et al., 2004; Lerner et al., 2009; Pratt et al., 2009; Chauhan and Nautiyal, 2010; Nogales et al., 2010; Terra et al., 2012; Janczarek and Rachwal, 2013). The gene *alg8* encodes alpha-1,3-glucosyltransferase enzyme, which plays a role in the synthesis of alginate. Genes involved in alginate biosynthesis and regulation (*algD,8,44,K,E,G,X,L,I,F,A,algC*) were observed in the whole genome transcriptome of *Azotobacter chroococcum* (Robson et al., 2015). The *alg*C gene involved in alginate and lipopolysaccharide synthesis was identified from *Azotobacter vinelandii* (Gaona et al., 2004) and characterized. Glucosyltransferases play an important role in biofilm formation between a bacterium (*Streptococcus gordonii)* and fungus (*Candida albicans*), as they mediate protein adhesin–ligand interactions on cell surfaces of the partners in oral mucosal surfaces (Ricker et al., 2014). Ghafoor et al. (2011) showed that at least three exopolysaccharides (alginate, Psl, and Pel) contribute towards the formation of biofilms in *Pseudomonas aeruginosa*, while alginate was found to entrap the live cells in the cap of the mushroom-like structure in the biofilms. Glucosyltransferases promoted biofilm formation in *Streptococcus mutans* through the synthesis of adhesive extracellular polysaccharides (Ren et al., 2016).

Signal peptidases represent ubiquitous enzymes encoded by *sipW* gene, which are pivotal for the processing of proteins and their transport across a membrane. Terra et al. (2012) investigated the role of the *Bacillus subtilis* signal peptidase (SipW), which has a unique role in forming biofilms on a solid surface, but is not required at an air-liquid interface. For the development of amyloid fibres in *Bacillus subtilis,* SipW is crucial, as it is involved in the processing and transport of TasA and TapA to the extracellular matrix (Romero et al., 2011). Signal peptidases were shown to be involved in motility, sporulation and nuclease activities in *Bacillus amyloliquefaciens* (Chu et al., 2002).

The *pssA* gene encodes enzyme, which initiates the synthesis of EPS repeating units, which transfers UDP-glucose to an isoprenyl phosphate lipid carrier located in the inner membrane, and the *pssA* mutant that lacks an isoprenylphosphate glycosyl transferase showed a defect in acidic-EPS biosynthesis (Pollock et al., 1998). Its crucial role was evidenced by mutational analyses which not only abolished EPS production, but also led to empty (devoid of bacteria) and non-nitrogen-fixing nodules (indeterminate-type) on the roots of clover, pea and vetch plants (Janczarek et al., 2009). A mutation or deletion of *pss* genes also affects exopolysaccharide synthesis and biofilm development in *Rhizobium leguminosarum* (Russo et al., 2006) and exopolysaccharide production and motility of *R. leguminosarum* bv. *trifolii* cells (Janczarek and Rachwal, 2013). Similar reports regarding the mutation of a gene homologous to *R. leguminosarum* bv. *trifolii* pssA, in a *R. leguminosarum* bv. *viciae* strain, led to the formation of immature biofilm on polystyrene plates, and the mutant exhibited a reduced ability to attach to the root hairs of pea plants (Williams et al., 2008).

Pho genes have diverse functions including phosphate transport, phosphate scavenging, and utilization of alternate phosphorus sources (Wanner, 1996). Phosphate and polyphosphate metabolism has been associated with biofilm development and Quorum Sensing regulatory pathway (Rashid et al., 2000). The phosphate (Pho) regulon comprises the two-component system PhoR / PhoB that is essential for bacterial adaptation to phosphate starvation and biofilm formation (Chekabab et al., 2014). The *pho* genes (*phoA* and *B*) are involved in the production of alkaline phosphatase, which plays a major role in the secretion of curli fibers, involved in cell aggregation, adhesion and biofilm formation in microbial cells (Barnhart and Chapman, 2006). The *phoB* homologues are involved in the stimulation and transcription of genes crucial for biofilm formation in several microorganisms (Yuan et al., 2005). PhoB regulates the concentration of c-di-GMP metabolic enzymes, thus affecting the motility and biofilm formation in *Vibrio cholerae* (Pratt et al., 2009). The Pho regulon was implicated to play a role in the regulation of biofilm formation in *P. fluorescens* Pf0-1 by controlling the production of the adhesion protein LapA through modulation of c-di-GMP levels via RapA, which has a c-di-GMP phosphodiesterase activity (Monds et al., 2007). The PhoR/PhoB TCS was demonstrated to induce the production of biofilms under phosphate-depleted conditions in the plant pathogen *Agrobacterium tumefaciens* (Xu et al., 2012). The Pho pathway was also reported to upregulate the acquisition of nutrients including phosphate, and to regulate carbohydrate and lipid metabolism in fungi (Lev and Djordjevic, 2018).

The CDS-13443 (4-aminobutyrate aminotransferase (*gabT*)) and CDS-10036 (glycogen phosphorylase (*glgP*)) which were selected based on their transcriptome data (6 days of inoculation), showed up-regulation in the qPCR analyses (during biofilm developmental stages viz., 3, 6 and 9 days of inoculation). This indicates that these genes were activated significantly during biofilm formation between *Tv* and *Az*. The gabT was reported to encode the enzyme 4-aminobutyrate:2-ketoglutarate aminotransferase involved in 4-aminobutyrate (GABA) catabolism (Dover and Halpern, 1974). Further, mutation of gabT gene was reported to prevent the utilization of GABA as a sole nitrogen source in *Bacillus subtilis* (Belitsky and Sonenshein, 2002). In *Bacillus subtilis*, mutation of gabT (4-aminobutyrate aminotransferase) which encodes the norspermidine biosynthetic enzymes L-2, 4-diaminobutyrate: 2-ketoglutarate 4-aminotransferase (DABA AT) was reported to prevent norspermidine biosynthesis, which subsequently prevented biofilm disassembly (Hobley et al., 2014). The authors also proposed that norspermidine inhibited biofilm formation by binding to the exopolysaccharide. Similarly, the up-regulation of glycogen phosphorylase (*glgP*) indicated the activation of glycogen phosphorylase and subsequent accumulation of glycogen. This may illustrate nutrient limited/ osmotic stress condition during biofilm formation between *Tv* and *Az* during the later part of biofilm formation. Glycogen is a reserve glucose polysaccharide present in many bacteria, which undergoes degradation when cells are in stationary phase and nutrients depleted state. Glycogen is reported to be catabolised by the enzymes GlgX and GlgP (glycogen phosphorylase) encoded by the gene *glgP* (Ballicora et al., 2003). Comparative transcriptome analyses revealed the up-regulation of genes involved in glycogen accumulation during hyperosmotic stress in *Sinorhizobium meliloti* (Domínguez-Ferreras et al., 2006). GlgS plays a negative role in *E. coli* motility and biofilm formation, by regulating propulsion, adhesion and synthesis of biofilm exopolysaccharides (Rahimpour et al., 2013). Complementation studies with *glg* genes proved the role of intracellular glycogen biosynthesis and catabolism in biofilm formation (Jackson et al., 2002). *glgP* (glycogen phosphorylase) is involved in the production of extracellular polysaccharide and biofilm formation by facilitating glycogen accumulation, and stress endurance in *Azospirillum brasilense* Sp7 (Lerner et al., 2009).

The *purB* genes encode adenylosuccinate lyase enzyme that plays an important role in de novo purine synthesis. Purine synthesis plays a crucial role in biofilm formation in nonstreptococcal species (Mack et al., 2004). Disruption of *purB* gene affects purine synthesis, which alters many biochemical pathways, including exopolysaccharide synthesis and biofilm formation. Ge et al. (2008) observed that *in vitro* biofilm formation was regulated by *purB, purL, thrB*, and *pyr*E genes in *Streptococcus sanguinis*. Mutation in the *purB* gene was found to affect root colonization, as it was crucial for the synthesis of exopolysaccharides and biofilm formation in rhizobacterium *Pantoea agglomerans* (Chauhan and Nautiyal, 2010). Infection thread formation and nodule development in *Lotus japonicus* were influenced by the disruption of *purB,* which coded for adenylosuccinate lyase (Okazaki et al., 2007). Fatty acids and fatty acid-related signals influence surface motility and biofilm formation in different bacteria (Ryan and Dow, 2011). The *fadD* genes encode long-chain acyl-CoA synthetase and FadD-related compound are involved in the control of surface motility and biofilm formation in *Sinorhizobium meliloti* (Nogales et al., 2010). Environmental concentrations of iron (siderophore production) and genes *fadD* and *rirA* coordinate surface motility and biofilm formation by *S. meliloti* in alfalfa plants (Amaya-Gómez et al., 2015).

In summary, the data generated from this investigation led to better insights into gene regulation during biofilm formation between two agriculturally important organisms, a fungus (*Trichoderma viride*) and a bacterium (*Azotobacter chroococcum*). Attachment of *A. chroococcum* cells to the *T. viride* hyphae during biofilm development led to significant changes in the transcriptome profiles, particularly in the expression of genes related to biofilm formation in *T. viride*. Specifically, genes coding for RNA-dependent RNA polymerase, ABC transporters, Translation elongation factor EF-1 and molecular chaperones were found to be crucial for biofilm formation between *T. viride* and *A. chroococcum*. Functional validation of all the genes identified in the transcriptome data can provide a more comprehensive understanding of mechanisms involved in the progressive steps of biofilm formation.

Data accessibility

The raw Illumina sequence reads related to *Azotobacter chroococcum* (*Az*), *Trichoderma viride* (*Tv*) and their biofilm (*Tv-Az*) are deposited at Sequence Read Archive (NCBI/SRA) under the accession number SRP115830. The transcriptome data are deposited in BioProject in GenBank via Bioproject number **PRJNA398979** accessible at: https://www.ncbi.nlm.nih.gov/bioproject/PRJNA398979/.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.micres.2019.06.002.

References

Amaya-Gómez, C.V., Hirsch, A.M., Soto, M.J., 2015. Biofilm formation assessment in *Sinorhizobium meliloti* reveals interlinked control with surface motility. BMC Microbiol. 15, 58.

Andersen, G.R., Nyborg, J., 2001. Structural studies of eukaryotic elongation factors. Cold Spring Harb. Symp. Quant. Biol. 66, 425–437.

Ansari, M.Y., Mande, S.C., 2018. A glimpse into the structure and function of Atypical Type I Chaperonins. Mol. Biosci. https://doi.org/10.3389/fmolb.2018.00031.

Arnaise, S., Zickler, D., Poisier, C., Debuchy, R., 2001. pah1: a homeobox gene involved in hyphal morphology and microconidiogenesis in the filamentous ascomycete *Podospora anserina*. Mol. Microbiol. 39, 54–64.

Ballicora, M.A., Iglesias, A.A., Preiss, J., 2003. ADP-Glucose pyrophosphorylase, a regulatory enzyme for bacterial glycogen synthesis. Microbiol. Mol. Biol. Rev. 67, 213–225.

Barnhart, M.M., Chapman, M.R., 2006. Curli biogenesis and function. Ann. Rev. Microbiol. 60, 131–147.

Belitsky, B.R., Sonenshein, A.L., 2002. GabR, a member of a novel protein family, regulates the utilization of gamma-aminobutyrate in *Bacillus subtilis*. Mol. Microbiol. 45, 569–583.

Benoit, I., van den Esker, M.H., Patyshakuliyeva, A., Mattern, D.J., Blei, F., Zhou, M., Dijksterhuis, J., Brakhage, A.A., Kuipers, O.P., de Vries, R.P., Kovacs, A.T., 2015. *Bacillus subtilis* attachment to *Aspergillus niger* hyphae results in mutually altered metabolism. Environ. Microbiol. 17, 2099–2113.

Bernal, V., Castaño-Cerezo, S., Gallego-Jara, J., Écija-Conesa, A., de Diego, T., Iborra, J.L., Canovas, M., 2014. Regulation of bacterial physiology by lysine acetylation of proteins. Nat. Biotechnol. 31, 586–595.

Bharti, A., Velmourougane, K., Prasanna, R., 2017. Phototrophic biofilms: diversity, ecology and applications. J. Appl. Phycol. 29, 2729–2744.

Burglin, T.R., 2011. Homeodomain subtypes and functional diversity. Subcell. Biochem. 52, 95–122.

Castañeda, M., López-Pliego, L., Espín, G., 2016. Azotobacter vinelandii small RNAs: their roles in the formation of cysts and other processes. In: Leitão, A., Enguita, F. (Eds.), Non-Coding RNAs and Inter-Kingdom Communication. Springer, Cham, pp. 67–82.

Chambers, J.R., Sauer, K., 2013. Small RNAs and their role in biofilm formation. Trends Microbiol. 21, 39–49.

Chauhan, P.S., Nautiyal, C.S., 2010. The *purB* gene controls rhizosphere colonization by *Pantoea agglomerans*. Lett. Appl. Microbiol. 50, 205–210.

Chekabab, S.M., Harel, J., Dozois, C.M., 2014. Interplay between genetic regulation of phosphate homeostasis and bacterial virulence. Virulence 5, 786–793.

Chu, H.H., Hoang, V., Kreutzmann, P., Hofemeister, B., Melzer, M., Hofemeister, J., 2002. Identification and properties of type I-signal peptidases of *Bacillus amyloliquefaciens*. FEBS J. 269, 458–469.

Chung, C.W., You, J., Kim, K., Moon, Y., Kim, H., Ahn, J.H., 2009. Export of recombinant proteins in *Escherichia coli* using ABC transporter with an attached lipase ABC transporter recognition domain (LARD). Microb. Cell Fact. 8, 11.

Davey, M.E., O'Toole, G.A., 2000. Microbial biofilms: from ecology to molecular genetics. Microbiol. Mol. Biol. Rev. 64, 847–867.

Domínguez-Ferreras, A., Pérez-Arned, R., Becker, A., Olivares, J., Soto, A.J., Sanjuán, J., 2006. Transcriptome profiling reveals the importance of plasmid pSymB for osmoadaptation of Sinorhizobium meliloti. J. Bacteriol. 188, 7617–7625.

Donlan, R.M., Costerton, J.W., 2002. Review. Biofilms: survival mechanisms of clinically relevant microorganisms. Clin. Microbiol. Rev. 15, 167–193.

Dover, S., Halpern, Y.S., 1974. Genetic analysis of the gamma-aminobutyrate utilization pathway in *Escherichia coli* K-12. J. Bacteriol. 117, 494–501.

Figge, R.M., Schubert, M., Brinkmann, H., Cerif, R., 1999. Glyceraldehyde-3-phosphate dehydrogenase gene diversity in eubacteria and eukaryotes: evidence for intra- and inter-kingdom gene transfer. Mol. Biol. Evol. 16, 429–440.

Frey-Klett, P., Burlinson, P., Deveau, A., Barret, M., Tarkka, M., Sarniguet, A., 2011. Bacterial-fungal interactions: hyphens between agricultural, clinical, environmental, and food microbiologists. Microbiol. Mol. Biol. Rev. 75, 583–609.

Fujiwara, K., Ishihama, Y., Nakahigashi, K., Soga, T., Taguchi, H., 2010. A systematic survey of in vivo obligate chaperonin-dependent substrates. EMBO J. 29, 1552–1564.

Gabriëls, J., Beckers, M.C., Ding, H., De Vriese, A., Plaisance, S., van der Maarel, S.M., Padberg, G.W., Frants, R.R., Hewitt, J.E., Collen, D., Belayew, A., 1999. Nucleotide sequence of the partially deleted D4Z4 locus in a patient with FSHD identifies a putative gene within each 3.3 kb element. Gene 236, 25–32.

Gaona, G., Nunez, C., Goldberg, J.B., Linford, A.S., 2004. Characterization of the *Azotobacter vinelandii* algC gene involved in alginate and lipopolysaccharide production. FEMS Microbiol. Lett. 238, 199–206.

Ge, X., Kitten, T., Chen, Z., Lee, S.P., Munro, C.L., Xu, P., 2008. Identification of Streptococcus sanguinis genes required for biofilm formation and examination of their role in endocarditis virulence. Infect. Immun. 76, 2551–2559.

Ghafoor, A., Hay, I.D., Rehm, B.H.A., 2011. Role of exopolysaccharides in *Pseudomonas aeruginosa* biofilm formation and architecture. Appl. Environ. Microbiol. 77, 5238–5246.

Ghosh, A.K., Wangsanut, T., Fonzi, W.A., Rolfes, R.J., 2015. The GRF10 homeobox gene regulates filamentous growth in the human fungal pathogen *Candida albicans*. FEMS Yeast Res. 15. https://doi.org/10.1093/femsyr/fov093.

Glenting, J., Beck, H.C., Vrang, A., Riemann, H., Ravn, P., Hansen, A.M., Antonsson, M., Ahrne, S., Israelsen, H., Madsen, S., 2013. Anchorless surface associated glycolytic enzymes from *Lactobacillus plantarum* 299v bind to epithelial cells and extracellular matrix proteins. Microbiol. Res. 168, 245–253.

Gruber, S., Zeilinger, S., 2014. The Transcription Factor Ste12 mediates the regulatory role of the Tmk1 MAP kinase in mycoparasitism and vegetative hyphal fusion in the filamentous fungus *Trichoderma atroviride*. PLoS One 9, e111636.

Grudniak, A.M., Włodkowska, J., Wolska, K.I., 2015. Chaperone DnaJ influences the formation of biofilm by *Escherichia coli*. Pol. J. Microbiol. 64, 279–283.

Hammond, T.M., Bok, J.W., Andrewski, M.D., Reyes-Dominguez, Y., Scazzocchio, C., Keller, N.P., 2008. RNA silencing gene truncation in the filamentous fungus *Aspergillus nidulans*. Eukaryot. Cell 7, 339–349.

Hobley, L., Kim, S.H., Maezato, Y., Wyllie, S., Fairlamb, A.H., Stanley-Wall, N.R., Michael, A.J., 2014. Norspermidine is not a Self-Produced trigger for biofilm disassembly. Cell 156, 844–854.

Holland, P.W.H., 2013. Evolution of homeobox genes. WIREs Dev. Biol. 2, 31–45.

Huerta, J.M., Aguilar, I., López-Pliego, L., Fuentes-Ramírez, L.E., Castañeda, M., 2016. The role of the ncRNA RgsA in the oxidative stress response and biofilm formation in *Azotobacter vinelandii*. Curr. Microbiol. 72, 671–679.

Iwai, S., Doi, K., Fujino, Y., Nakazono, T., Fukuda, K., Motomura, Y., Ogata, S., 2010. Silica deposition and phenotypic changes to *Thermus thermophilus* cultivated in the presence of supersaturated silicia. ISME J. 4, 809–816.

Jackson, D.W., Suzuki, K., Oakford, K., Simecka, J.W., Hart, M.E., Romeo, T., 2002. Biofilm formation and dispersal under the influence of the global regulator CsrA of *Escherichia coli*. J. Bacteriol. 184, 290–301.

Janczarek, M., Jaroszuk-Ścisel, J., Skorupska, A., 2009. Multiple copies of rosR and pssA genes enhance exopolysaccharide production, symbiotic competitiveness and clover nodulation in *Rhizobium leguminosarum bv. trifolii*. Antonie Van Leeuwenhoek 96, 471–486.

Janczarek, M., Rachwał, K., 2013. Mutation in the pssA gene involved in exopolysaccharide synthesis leads to several physiological and symbiotic defects in *Rhizobium leguminosarum bv. trifolii*. Int. J. Mol. Sci. 14, 23711–23735.

Jauffred, L., Vejborg, R.M., Korolev, K.S., Brown, S., Oddershede, L.B., 2017. Chirality in microbial biofilms is mediated by close interactions between the cell surface and the substratum. ISME J. 11, 1688–1701.

Kang, K., Zhong, J., Jiang, L., Liu, G., Gou, C.Y., Wu, Q., Wang, Y., Luo, J., Gou, D., 2013. Identification of microRNA-Like RNAs in the filamentous fungus *Trichoderma reesei* by Solexa Sequencing. PLoS One 8, e76288.

Kim, J.Y., Kwon, E.S., Roe, J.H., 2012. A homeobox protein Phx1 regulates long-term survival and meiotic sporulation in Schizosaccharomyces pombe. BMC Microbiol. 12, 86.

Kint, G., De Coster, D., Marchal, K., Vanderleyden, J., De Keersmaecker, S.C.J., 2010. The small regulatory RNA molecule MicA is involved in *Salmonella enterica* serovar *typhimurium* biofilm formation. BMC Microbiol. 10, 276.

Landini, P., Antoniani, D., Burgess, J.G., Nijland, R., 2010. Molecular mechanisms of compounds affecting bacterial biofilm formation and dispersal. Appl. Microbiol. Biotechnol. 86, 813–823.

Lee, H.C., Li, L., Gu, W., Xue, Z., Crosthwaite, S.K., Pertsemlidis, A., 2010. Diverse pathways generate microRNA-like RNAs and Dicer-independent small interfering RNAs in fungi. Mol. Cell 38, 803–814.

Lee, Y., Song, S., Sheng, L., Zhu, L., Kim, J.-S., Wood, T.K., 2018. Substrate binding protein DppA1 of ABC transporter DppBCDF increases biofilm Formation in *Pseudomonas aeruginosa* by inhibiting Pf5 prophage lysis. Front. Microbiol. 9, 30.

Lerner, A., Castro-Sowinski, S., Lerner, H., Okon, Y., Burdman, S., 2009. Glycogen phosphorylase is involved in stress endurance and biofilm formation in *Azospirillum brasilense* Sp7. FEMS Microbiol. Lett. 300, 75–82.

Lev, S., Djordjevic, J.T., 2018. Why is a functional PHO pathway required by fungal pathogens to disseminate within a phosphate-rich host: a paradox explained by alkaline pH-simulated nutrient deprivation and expanded PHO pathway function. PLoS Pathog. 14, e1007021.

Mack, D., Becker, P., Chatterjee, I., Dobinsky, S., Knobloch, J.K., Peters, G., Rohde, H., Herrmann, M., 2004. Mechanisms of biofilm formation in *Staphylococcus epidermidis* and *Staphylococcus aureus*: functional molecules, regulatory circuits, and adaptive responses. Int. J. Med. Microbiol. 294, 203–212.

Mai-Prochnow, A., Hui, J.G.K., Kjelleberg, S., Rakonjac, J., McDougald, D., Rice, S.A., 2015. Big things in small packages: the genetics of filamentous phage and effects on fitness of their host. FEMS Microbiol. Rev. 39, 465–487.

Marra, R., Ambrosino, P., Carbone, V., Vinale, F., Woo, S.L., Ruocco, M., Ciliento, R., Lanzuise, S., Ferraioli, S., Soriente, I., Gigante, S., Turra, D., Fogliano, V., Scala, F., Lorito, M., 2006. Study of the three-way interaction between *Trichoderma atroviride*, plant and fungal pathogens by using a proteomic approach. Curr. Genet. 50, 307–321.

Mika, F., Hengge, R., 2013. Small regulatory RNAs in the control of motility and biofilm formation in *E. coli* and *Salmonella*. Int. J. Mol. Sci. 14, 4560–4579.

Monds, R.D., Newell, P.D., Gross, R.H., O'Toole, G.A., 2007. Phosphate-dependent modulation of c-di-GMP levels regulates *Pseudomonas fluorescens* Pf0-1 biofilm formation by controlling secretion of the adhesin LapA. Mol. Microbiol. 63, 656–679.

Nagata, H., Iwasaki, M., Maeda, K., Kuboniwa, M., Hashino, E., Toe, M., Minamino, N., Kuwahara, H., Shizukuishi, S., 2009. Identification of the binding domain of *Streptococcus oralis* glyceraldehyde-3-phosphate dehydrogenase for *Porphyromonas gingivalis* major fimbriae. Infect. Immun. 77, 5130–5138.

Nobile, C.J., Fox, E.P., Nett, J.E., Sorrells, T.R., Mitrovich, Q.M., Hernday, A.D., 2010. A recently evolved transcriptional network controls biofilm development in *Candida albicans*. Cell 148, 126–138.

Nogales, J., Domínguez-Ferreras, A., Amaya-Gómez, C., van Dillewijn, P., Cuéllar, V., Sanjuán, J., Olivares, J., Soto, M.J., 2010. Transcriptome profiling of a *Sinorhizobium meliloti* fadD mutant reveals the role of rhizobactin 1021 biosynthesis and regulation genes in the control of swarming. BMC Genomics 11, 157.

Ojha, A., Anand, M., Bhatt, A., Kremer, L., Jacobs, Jr W.R., Hatfull, G.F., 2005. GroEL1: a dedicated chaperone involved in mycolic acid biosynthesis during biofilm formation in mycobacteria. Cell 123, 861–873.

Okazaki, S., Hattori, Y., Saeki, K., 2007. The *Mesorhizobium loti* purB gene is involved in infection thread formation and nodule development in *Lotus japonicus*. J. Bacteriol. 189, 834–852.

O'Toole, G.A., 2011. Microtiter dish biofilm formation assay. J. Visual. Expt. 47, 1–2.

Pearson, J.P., Van Delden, C., Iglewski, B.H., 1999. Active efflux and diffusion are involved in transport of *Pseudomonas aeruginosa* cell-to-cell signals. J. Bacteriol. 181, 1203–1210.

Petrova, O.E., Sauer, K., 2010. The novel two-component regulatory system BfiSR regulates biofilm development by controlling the small RNA rsmZ through CafA. J. Bacteriol. 192, 5275–5288.

Pinweha, P., Pumirat, P., Cuccui, J., Jitprasutwit, N., Muangsombut, V., Srinon, V., Boonyuen, U., Thiennimitr, P., Vattanaviboon, P., Cia, F., Willcocks, S., Bancroft, G.J., Wren, B.W., Korbsrisate, S., 2018. Inactivation of *bpsl1039-1040* ATP-binding cassette transporter reduces intracellular survival in macrophages, biofilm formation and virulence in the murine model of *Burkholderia pseudomallei* infection. PLoS One 13, e0196202.

Pollock, T.J., van Workum, W.A., Thorne, L., Mikolajczak, M.J., Yamazaki, M., Kijne, J.W., Armentrout, R.W., 1998. Assignment of biochemical functions to glycosyl transferase genes which are essential for biosynthesis of exopolysaccharides in *Sphingomonas* strain S88 and *Rhizobium leguminosarum*. J. Bacteriol. 180, 586–593.

Prasanna, R., Hossain, F., Babu, S., Ngangom, B., Adak, A., Verma, S., Shivay, Y.S., Nain, L., 2015. Prospecting cyanobacterial formulations as plant growth-promoting agents for maize hybrids. S. Afr. J. Plant Soil 32, 199–207.

Prasanna, R., Kumar, A., Babu, S., Chawla, G., Chaudhary, V., Singh, S., Gupta, V., Nain, L., Saxena, A.K., 2013. Deciphering the biochemical spectrum of novel cyanobacterium-based biofilms for use as inoculants. Biol. Agric. Hortic. 29, 145–158.

Prasanna, R., Triveni, S., Ngangom, B., Babu, S., Yadav, K., Adak, A., Khetarpal, S., Pal, M., Shivay, Y.S., Saxena, A.K., 2014. Evaluating the efficacy of cyanobacterial formulations and biofilmed inoculants for leguminous crops. Arch. Agron. Soil Sci. 60, 349–366.

Pratt, J.T., McDonough, E., Camilli, A., 2009. PhoB regulates motility, biofilms, and cyclic di-GMP in *Vibrio cholera*. J. Bacteriol. 191, 6632–6642.

Qayyum, S., Sharma, D., Bisht, D., Khan, A.U., 2016. Protein translation machinery holds a key for transition of planktonic cells to biofilm state in *Enterococcus faecalis*: a proteomic approach. Biochem. Biophys. Res. Commun. 474, 652–659.

Rahimpour, M., Montero, M., Almagro, G., Viale, A.M., Sevilla, A., Canovas, M., 2013. GlgS, described previously as a glycogen synthesis control protein, negatively regulates motility and biofilm formation in *Escherichia coli*. J. Biochem. 452, 559–573.

Rashid, M.H., Rumbaugh, K., Passador, L., Davies, D.G., Hamood, A.N., Iglewski, B.H., Kornberg, A., 2000. Polyphosphate kinase is essential for biofilm development, quorum sensing, and virulence of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U. S. A. 97, 9636–9641.

Ren, Z., Cui, T., Zeng, J., Chen, L., Zhang, W., Xu, X., Cheng, L., Li, M., Li, J., Zhou, X., Li, Y., 2016. Molecule targeting glucosyltransferase inhibits Streptococcus mutans biofilm formation and virulence. Antimicrob. Agents Chemother. 60, 126–135.

Ricker, A., Vickerman, M., Dongari-Bagtzoglou, A., 2014. *Streptococcus gordonii* glucosyltransferase promotes biofilm interactions with *Candida albicans*. J. Oral Microbiol. 6. https://doi.org/10.3402/jom.v6.23419.

Robson, R.L., Jones, R., Robson, R.M., Schwartz, A., Richardson, T.H., 2015. *Azotobacter* Genomes: the genome of *Azotobacter chroococcum* NCIMB 8003 (ATCC 4412). PLoS One 10, e0127997.

Romero, D., Vlamakis, H., Losick, R., Kolter, R., 2011. An accessory protein required for anchoring and assembly of amyloid fibres in *B. subtilis* biofilms. Mol. Microbiol. 80, 1155–1168.

Russo, D.M., Williams, A., Edwards, A., Posadas, D.M., Finnie, C., Dankert, M., Downie, J.A., Zorreguieta, A., 2006. Proteins exported via the PrsD-PrsE Type I secretion system and the acidic exopolysaccharide are involved in biofilm formation by *Rhizobium leguminosarum*. J. Bacteriol. 188, 4474–4486.

Ryan, R.P., Dow, J.M., 2011. Communication with a growing family: diffusible signal factor (DSF) signaling in bacteria. Trends Microbiol. 19, 145–152.

Schulz, B., Banuett, F., Dahl, M., Schlesinger, R., Schafer, W., Martin, T., 1990. The b alleles of U. maydis, whose combinations program pathogenic development, code for polypeptides containing a homeodomain-related motif. Cell 60, 295–306.

Seneviratne, G., Zavahir, J.S., Bandara, W.M.M.S., Weerasekara, M.L.M.A.W., 2008. Fungal-bacterial biofilms: their development for novel biotechnological applications. World J. Microbiol. Biotechnol. 24, 739–743.

Shelest, E., 2008. Transcription factors in fungi. FEMS Microbiol. Lett. 286, 145–151.

Terra, R., Stanley-Wall, N.R., Cao, G., Lazazzera, B.A., 2012. Identification of *Bacillus subtilis* SipW as a bifunctional signal peptidase that controls surface-adhered biofilm formation. J. Bacteriol. 194, 2781–2790.

Teschler, J.K., Zamorano-Sanchez, D., Utada, A.S., Warner, C.J.A., Wong, G.C.L., Linington, R.G., Yildiz, F.H., 2015. Living in the matrix: assembly and control of *Vibrio cholera* biofilms. Nat. Rev. Microbiol. 13, 255–268.

Triveni, S., Prasanna, R., Shukla, L., Saxena, A.K., 2013. Evaluating the biochemical traits of novel *Trichoderma*-based biofilms for use as plant growth-promoting inoculants. Ann. Microbiol. 63, 1147–1156.

Vanderlinde, E.M., Harrison, J.J., Muszyński, A., Carlson, R.W., Turner, R.J., Yost, C.K., 2010. Identification of a novel ABC transporter required for desiccation tolerance, and biofilm formation in*Rhizobium leguminosarum* bv. *viciae* 3841. FEMS Microbiol. Ecol. 71, 327–340.

Velmourougane, K., Prasanna, R., Saxena, A.K., 2017a. Agriculturally important microbial biofilms: present status and future prospects. J. Basic Microbiol. 57, 548–573.

Velmourougane, K., Prasanna, R., Saxena, A.K., Singh, S.B., Chawla, G., Kaushik, R., Ramakrishnan, B., Nain, L., 2017b. Modulation of growth media influences aggregation and biofilm formation between *Azotobacter chroococcum* and *Trichoderma viride*. Appl. Biochem. Microbiol. 53, 546–556.

Velmourougane, K., Prasanna, R., Singh, S.B., Kumar, R., Saha, S., 2017c. Sequence of inoculation influences the nature of exopolymeric substances and biofilm formation in *Azotobacter chroococcum* and *Trichoderma viride*. FEMS Microbiol. Ecol. 93. https://doi.org/10.1093/femsec/fix066.

Velmourougane, K., Prasanna, R., 2017d. Influence of L-amino acids on aggregation and biofilm formation in *Azotobacter chroococcum* and *Trichoderma viride*. J. Appl. Microbiol. 123, 977–991.

Velmourougane, K., Prasanna, R., Singh, S., Chawla, G., Kumar, A., Saxena, A.K., 2017e. Modulating rhizosphere colonisation, plant growth, soil nutrient availability and plant defense enzyme activity through *Trichoderma viride-Azotobacter chroococcum* biofilm inoculation in chickpea. Plant Soil 421, 157–174.

Wagner, E.G., Romby, P., 2015. Small RNAs in bacteria and archaea: who they are, what they do, and how they do it. Adv. Genet. 90, 133–208.

Wanner, B.L., 1996. P assimilation and control of the Pho regulon, in *Escherichia coli* and *Salmonella*. In: Neidhardt, F.C., Curtiss, R., Ingraham, J.L., Lin, E.C.C.C., Low, K.B., Low, B., Magasanik, B., Reznikoff, W., Riley, M., Schaechter, M., Umbarger, H.E. (Eds.), Cellular and Molecular Biology. ASM Press, Washington, pp. 1357–1381.

Williams, A., Wilkinson, A., Krehenbrink, M., Russo, D.M., Zorreguieta, M., Downie, J.A., 2008. Glucomannan-mediated attachment of *Rhizobium leguminosarum* to pea root hairs is required for competitive nodule infection. J. Bacteriol. 190, 4706–4715.

Wolska, K.I., Grudniak, A.M., Rudnicka, Z., Markowska, K., 2016. Genetic control of bacterial biofilms. J. Appl. Genetics 57, 225–238.

Xu, J., Kim, J., Danhorn, T., Merritt, P.M., Fuqua, C., 2012. Phosphorus limitation increases attachment in *Agrobacterium tumefaciens* and reveals a conditional functional redundancy in adhesin biosynthesis. Res. Microbiol. 163, 674–684.

Yuan, Z.C., Zaheer, R., Finan, T.M., 2005. Phosphate limitation induces catalase expression in *Sinorhizobium meliloti*, *Pseudomonas aeruginosa* and *Agrobacterium tumefaciens*. Mol. Microbiol. 58, 877–894.

Zhang, W.M., Wang, H.F., Gao, K., Wang, C., Liu, L., Liu, J.X., 2015. *Lactobacillus reuteri* glyceraldehyde-3-phosphate dehydrogenase functions in adhesion to intestinal epithelial cells. Can. J. Microbiol. 61, 373–380.

Zhang, X.-S., Garcıa-Contreras, R., Wood, T.K., 2007. YcfR (BhsA) influences *Escherichia coli* biofilm formation through stress response and surface hydrophobicity. J. Bacteriol. 189, 3051–3062.

Zheng, W., Zhao, X., Xie, Q., Huang, Q., Zhang, C., Zhai, H., Xu, L., Lu, G., Shim, W.-B., Wang, Z., 2012. A conserved homeobox transcription factor Htf1 is required for phialide development and conidiogenesis in *Fusarium* species. PLoS One 7, e45432.

Zhou, J., Fu, Y., Xie, J., Li, B., Jiang, D., Li, G., Cheng, J., 2012a. Identification of microRNA-like RNAs in a plant pathogenic fungus *Sclerotinia sclerotiorum* by high-throughput sequencing. Mol. Genet. Genomics 287, 275–282.

Zhou, Q., Wang, Z., Zhang, J., Meng, H., Huang, B., 2012b. Genome-wide identification and profiling of microRNA-like RNAs from *Metarhizium anisopliae* during development. Fungal Biol. 116, 1156–1162.

Zhu, X., Long, F., Chen, Y., Knochel, S., She, Q., Shi, X., 2008. A putative ABC transporter is involved in negative regulation of biofilm formation by Listeria monocytogenes. Appl. Environ. Microbiol. 74, 7675–7683.