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Xylella fastidiosa: An *in vivo* system to study possible survival strategies within citrus xylem vessels based on global gene expression analysis

María Teresa Federici[1,2] ✉ · Jackson A. Marcondes[2] · Simone Cristina Picchi[2,6] · Eduardo S. Stuchi[3] · André Luiz Fadel[4] · Marcelo L. Laia[5] · Manoel Victor F. Lemos[7] · Eliana Gertrudes Macedo Lemos[2]

1 Instituto Nacional de Investigación Agropecuaria (INIA), Estación Experimental Wilson Ferreira Aldunate, Unidad de Biotecnología, Rincón del Colorado, Uruguay

2 Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias, Departamento de Tecnologia, Laboratório de Bioquímica de Microrganismos e Plantas, Jaboticabal, Brazil

3 Embrapa Mandioca e Fruticultura, Núcleo Tecnológico de Recursos Genéticos e Desenvolvimento de Variedades - Nugene, Cruz das Almas, BA, Brasil and "Estação Experimental de Citricultura", Bebedouro, São Paulo, Brazil

4 Universidade de São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, São Paulo, Brazil

5 Universidade Federal dos Vales do Jequitinhonha e Mucuri, Faculdade de Ciências Agrárias, Departamento de Engenharia Florestal, Diamantina, MG, Brasil

6 Centro de Citricultura Sylvio Moreira - APTA, Cordeirópolis, SP, Brasil

7 Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Ciências Agrárias e Veterinárias. Departamento de Biologia Aplicada à Agropecuária, Jaboticabal, Brazil

✉ Corresponding author: mfederici@lb.inia.org.uy
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Abstract *Xylella fastidiosa* inhabits the plant xylem, a nutrient-poor environment, so that mechanisms to sense and respond to adverse environmental conditions are extremely important for bacterial survival in the plant host. Although the complete genome sequences of different *Xylella* strains have been determined, little is known about stress responses and gene regulation in these organisms. In this work, a DNA microarray was constructed containing 2,600 ORFs identified in the genome sequencing project of *Xylella fastidiosa* 9a5c strain, and used to check global gene expression differences in the bacteria when it is infecting a symptomatic and a tolerant citrus tree. Different patterns of expression were found in each variety, suggesting that bacteria are responding differentially according to each plant xylem environment. The global gene expression profile was determined and several genes related to bacterial survival in stressed conditions were found to be differentially expressed between varieties, suggesting the involvement of different strategies for adaptation to the environment. The expression pattern of some genes related to the heat shock response, toxin and detoxification processes, adaptation to atypical conditions, repair systems as well as some regulatory genes are discussed in this paper. DNA microarray proved to be a powerful technique for global transcriptome analyses. This is one of the first studies of *Xylella fastidiosa* gene expression *in vivo* which helped to increase insight into stress responses and possible bacterial survival mechanisms in the nutrient-poor environment of xylem vessels.

Keywords: citrus, microarray, *Xylella fastidiosa*

INTRODUCTION

Xylella fastidiosa is a gram-negative, xylem-limited bacterium, phylogenetically most closely related to *Xanthomonas* spp., and is transmitted from plant to plant by several xylem-feeding insect vectors

(Hopkins, 1989). Strains of *X. fastidiosa* cause diseases in many economically important plants, including grapevines, citrus, peach, plum, oleander, elm, sycamore, oak, maple, and coffee (Purcel, 1997; De Lima et al. 1998). The major symptoms of most *X. fastidiosa* diseases are associated with water stress, due to reduced xylem flow, which is thought to result from occlusion of the xylem vessels by bacterial aggregates that likely contain extracellular polysaccharides (EPS) (Da Silva et al. 2001), gums, and tyloses (Hopkins, 1989). Diseases caused by *Xylella fastidiosa* have attained great importance worldwide as the pathogen and its insect vectors have been disseminated.

The genome sequence determination of the pathogenic strain 9a5c, associated with citrus variegated chlorosis (CVC) was reported by Simpson et al. (2000), revealing many genes associated with pathogenicity and virulence, involving toxins, antibiotics and ion sequestration systems, as well as bacterium-bacterium and bacterium-host interactions. Also, at least 83 genes were bacteriophage-derived and included virulence-associated genes from other bacteria of the genome, providing direct evidence of phage-mediated horizontal transfer (Simpson et al. 2000).

X. fastidiosa is a member of the gamma subdivision of Proteobacteria which inhabits the plant xylem, a nutrient-poor environment. Mechanisms to sense and respond to adverse environmental conditions are certainly extremely important for bacterial survival in the plant host (Koide et al. 2006). Survival can be regarded in a broader context as the fitness of an organism to quickly adapt to particular environmental changes, and to ensure adaptation, bacteria rely on invasive DNA (transposons, integrons, plasmids and prophages) brought in by horizontal gene transfer mechanisms to generate the genetic diversity needed. Such elements can carry genes that code for a specific ecological adaptation, for new features associated with pathogenicity or bacterium-host interaction (Sullivan and Ronson, 1998; Wong and Golding, 2003; Monteiro-Vittorello et al. 2005). Also, a wide range of signal molecules, environmental stresses, and other factors trigger changes in gene expression in human, animal and plant pathogens, resulting in new phenotypes that increase the fitness of individuals (Camilli and Bassler, 2006; Shild et al. 2007). Phenotypic changes are especially important for pathogens with complex life histories, which must survive in multiple environments under different habitat-dependent stresses. Therefore, many pathogens have phenotypic plasticity that permits the exploration of multiple environments, which is likely regulated by signals or cues provided by their habitat (Killiny and Almeida, 2009). Therefore, an *in vivo* transcriptomic analysis could provide many clues to the understanding of adaptation and survival of *X. fastidiosa.*

In this work we characterized the global gene expression profile of *Xylella fastidiosa in vivo*, without culturing bacteria, in order to achieve a deeper understanding of the stress responses and adaptability of this pathogen. We focused on the transcriptional activity of some genes related to biochemical processes involved in heat shock and antioxidant responses, toxins and detoxification processes as well as other possible mechanisms involved in bacterial survival. For this purpose, we used microarray analysis to identify global changes in gene expression patterns using a DNA microarray previously standardized by the Laboratory of Biochemistry of Microorganisms and Plants, by Travensolo et al. (2008) and Travensolo et al. (2009), including all genes identified in the *X. fastidiosa* genome.

We analyzed the differential expression of genes when the bacteria infected either a susceptible or a tolerant sweet orange plant. The susceptible variety used in this study, which rapidly developed severe symptoms, was Pera (*Citrus sinensis* (L.) Osbeck) and the tolerant was the cultivar Navelina ISA 315 (commonly known as “Bahianinha”). This cultivar belongs to the navel group of sweet oranges. This cultivar was originated in Italy in 1976 as the result of a clone that was recovered *in vitro* by immature ovule culture. It was introduced in Brazil and established in the field by the year 2000 to initiate CVC resistance studies. In preliminary studies, Navelina ISA 315 was found to be an asymptomatic host for *Xylella fastidiosa* (Stuchi et al. 2007), since other sweet oranges are all susceptible to CVC. The use of resistant or tolerant varieties is one of the main options for managing the disease. Fadel et al. (2010) confirmed that Navelina ISA 315 is a tolerant cultivar using visual observation of symptoms in greenhouse and field, as well as quantification by RT qPCR analysis.

Different expression patterns in symptomatic and asymptomatic plants would be expected, due to differences in plant defenses, xylem conditions or endophytic microorganism competition. This information can provide insights into bacterial survival mechanisms within susceptible and tolerant plants, as well as into plant-pathogen interactions.

MATERIALS AND METHODS

Microarray construction

For genomic DNA extraction, *X. fastidiosa* 9a5c was cultivated in Petri dishes containing BCYE medium (Wells et al. 1981) at 28ºC for six days. Genomic DNA was extracted according to Ausubel et al. (1987) with a modification that includes RNAse treatment (200 µg/ml) for 1 hr 30 min at 37ºC. Specific primer pairs were designed using PRIMER3 software and used to amplify the 2,600 ORFs of the complete genome of *X. fastidiosa*. A melting temperature ranging from 48 to 57ºC was determined for all primers. PCR reactions were carried out in 1X PCR buffer (50 mM KCl, 20 mM Tris-HCl, pH 8.4), 2 mM $MgCl_2$, 10 mM dNTP, 2 U *Taq* DNA polymerase, 5 pmols of each primer, 60 ng of genomic DNA and pure sterile water to a total volume of 10 µl. The following reaction conditions were used: 94ºC/2 min; 35 cycles of 94ºC/1 min, 58ºC/1 min, 72ºC/1 min 30 sec; followed by a final extension of 72ºC/5 min. All products were analyzed and quantified by electrophoresis in 1.5% agarose gel in 1X TBE buffer with 0.5 µg/ml ethidium bromide. Amplified products were suspended in 50% v/v DMSO at a final concentration of 10 to 30 ng/µl and arranged in duplicate at a distance of 25 µm on glass slides treated with aminosilane (Corning). Printing of microarrays was done by a robot model GMS 417 Arrayer (Affymetrix Inc.). After printing, the DNAs were re-hydrated (42ºC for 1 sec), dried (70ºC for 1 min) and fixed by UV cross-linking (130 x 10 µJ/cm^2). The slides were kept at 70ºC for 2 hrs and then stored under vacuum at room temperature. Genetically distant negative controls were included in this array, consisting of human genes (*pHUM1* and *pHUM7*) and plant genes (707050B11-Rubisco).

Experimental design and controls

From each host, three individual plants were collected. RNA was extracted, and fluorescent labeled cDNA was prepared and used to hybridize the slides. The slides contain PCR products from the 2,600 *Xylella fastidiosa* ORFs spotted in duplicate as well as the genetically distant controls. *Xylella fastidiosa* within asymptomatic cultivar Navelina ISA 315 was considered in this analysis as the experimental condition (labeled with Cy5 fluorophore) whereas susceptible variety Pera was considered the control condition (labeled with Cy3 fluorophore). Three control hybridizations of healthy samples of the cultivar Navelina ISA 315 and healthy Pera variety were performed in order to detect non-specific hybridization with the plant genome. Both healthy varieties produced by micrografting were collected within the greenhouse which was protected from any contamination by insects.

Plant material used for total RNA extraction

Plants used in this study were collected from the Citrus Experimental Station of Bebedouro (SP-Brazil), including symptomatic leaves of the susceptible variety Pera and asymptomatic leaves from the cultivar Navelina ISA 315. Samples from three different trees of each variety were collected in order to minimize variability due to individual plants. Navelina ISA 315 plants had been grafted over symptomatic Pera to ensure a high pressure of *Xylella fastidosa* inoculum. After 10 months, bacterial concentration was evaluated by observation of symptoms in the field based on a diagrammatic scale developed by Amorim et al. (1993), and by real time PCR (RT qPCR) in two periods, January 2009 and August 2009. These plants were found to be asymptomatic in the field, however, RT qPCR revealed high concentrations of bacteria at the first evaluation, in January 2009. Bacterial concentration in these plants did not decrease between evaluations as revealed by RT qPCR, nor were symptoms observed in the field in August 2009 (Fadel et al. 2010). RT qPCR analyses were performed based on the standard curve for *Xylella fastidiosa* developed by Oliveira et al. (2002). Samples of symptomatic Pera were collected from an orchard highly affected with CVC disease, located at about 100 meters from the Navelina ISA 315 trees, and from the greenhouse in the case of the healthy plant controls.

Isolation of total RNA

As *Xylella fastidiosa* is a xylem limited pathogen, the central veins (midribs) were cut from about 33 to 35 leaves of each plant and were macerated with liquid nitrogen. An RNA extraction methodology was used which involved a monophasic solution of phenol and guanidine isothiocyanate (TRizol®, Invitrogen). RNA integrity was verified in a 1.2% (w/v) agarose gel in 1X buffer (20 mM MOPS, 5 mM sodium acetate; 1 mM EDTA), DEPC-treated H_2O and 6.7% w/v formaldehyde. The RNA molecular marker used was “RNA ladder” (Life Technologies) with six fragments visualized from 0.24 to 9.49 kb.

The same procedure was performed for samples from both symptomatic and asymptomatic plants, as well as healthy control plants.

Synthesis of fluorescent cDNA from total RNA

Synthesis of fluorescent cDNA was carried out using 100 µg of total RNA, 2 µg of random primers (GE Healthcare, Piscataway, NJ, USA), and 4 U of RNAsin (Invitrogen). This mixture was incubated at 70ºC for 5 min and cooled to 4ºC for 5 min to allow the primers and mRNA template to anneal. Then, a solution containing 6 µl of ImProm 5X reaction buffer; 3.6 µl of $MgCl_2$ 25 mM, 3 µl of dNTPs 5 mM A/C/G, 2 mM T; 2 µl of ImPromII Reverse transcriptase (Promega), and 2 µl of dUTP-Cy3 or dUTP-Cy5 was added to the RNA/primer mixture. For the synthesis of fluorescent cDNA, the mixture was incubated at 25ºC for 5 min and 40ºC for 3 hrs in a programmable thermocycler PC-100 Programmable Thermal Controller (MJ Research Inc). Three different reverse transcription reactions were carried out for the three biological samples, which were subsequently hybridized on different slides (triplicates) to minimize variation in gene expression not related to infection. After cDNA synthesis, a step of RNA degradation was included by adding 2.5 µl of EDTA 0.5M (pH 8.0) and 5.0 µl of NaOH 1M and incubating at 37ºC for 4 min. All the reagents and primers were removed by using Microcon YM-3 columns (Ambion). The labeled cDNA was stored at 4ºC for subsequent hybridization.

Hybridization and washing

Arrays were hybridized and washed in a Gene-Tac Hybridization Station (Genomic Solutions, Ann Arbor, MI, USA). Initially, the arrays were denatured at 65ºC for 5 min. A solution containing 8 µl of blocking reagent (GE Healthcare, Piscataway, NJ, USA RPN 3601), 1 µl of SSC 2x, 5,5 µl of 2% w/v SDS, 10 pmoles of each cDNA labeled with the fluorescent dyes Cy3 or Cy5, in a final volume of 11 µl, was heated at 95ºC for 2 min. This solution was injected into the hybridization chamber to cover the arrays and the hybridization was performed for 16 hrs at 42ºC. Afterwards, the slides were washed at 25ºC with the following solutions: 0.5% (w/v) SDS in SSC of different concentrations: 2x SSC (high stringency), 0.5x SSC (medium stringency) and 0.05x SSC (low stringency). All the washing steps consisted of 1 cycle of solution flow (1s) and incubation (2s). The slides were dried for 15 min and subjected to fluorescence detection.

Image acquisition and data analyses

The slides were subjected to fluorescence reading in an Axon Gene Pix 4000B Microarray Scanner (Molecular Devices) under different wavelengths: 550 nm (Cy3) and 650 nm (Cy5). The location and identity of each gene on the slide was defined in a text file, created with the aid of the Clone-Tracker2 program (Biodiscovery) by Travensolo et al. (2008) and Travensolo et al. (2009). The signal was quantified through ImaGene software (v.4.1, BioDisco-very), in which two images from the Cy3 and Cy5 fluorescent dyes were overlapped and the spots classified according to morphology and intensity. The computer displays an electronic symbol as a false-color image where a red or green spot corresponds to expression of a gene in sample 1 or 2, respectively, while a yellow-orange spot indicates that the gene was expressed at similar levels in both samples.

Normalization and statistical analysis were carried out using the R Program (www.r-project.org) (R. Development core team, 2009) and the limma package (http// bioconductor.org/packages/2.4/bioc/html/limma.html) (Smyth, 2005). The “Array Weights” software was run to check the quality of the arrays and the background signal was discounted from the signal of each spot using the method normexp with an offset equal to 1 (Ritchie et al. 2006) and normalized by the “loess” method (Yang et al. 2001; Yang et al. 2002; Smyth and Speed, 2003; Ritchie et al. 2007). After normalization, the Students Q-Q test was applied and 400 genes were identified as differentially expressed (Smyth, 2004; Smyth et al. 2005). Genes above a threshold value of LogFC=1 were considered differentially expressed in Navelina, while those below LogFC=-1 were considered differentially expressed in Pera (Figure 1).

Microarray validation

The genes used for microchip validation by RT-qPCR were all related to pathogenicity functions with high and medium values of LogFC (in Pera or Navelina ISA 315).

Fasta sequences were downloaded from the Gene Bank of the *Xylella fastidiosa* Genome Project (http://aeg.lbi.ic.unicamp.br/xf/) and primer sequences were designed using Primer Express Software for Real Time PCR 3.0, provided by the real time PCR equipment of Applied Biosystems, model 7500. A total of fifty candidate sequences were analyzed for each gene, in order to exclude secondary structures like “hairpins” and possible primer dimer formation. Table 1 presents the sequences of the primers used in the RT-qPCR analysis. All primers were validated for amplification efficiency and specificity following the “MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments” (Bustin et al. 2009).

Reverse transcription (RT) was carried out with 2 µg of *X. fastidiosa* total RNA from the three biological replicates primed with 1.5 µl. of random hexamers pdN_6 (Invitrogen) using the reverse transcription enzyme Improm II (Promega), following kit instructions. Total RNA used in reverse transcription was composed of an equal amount of the RNA from each individual plant used in microarray analysis. Two hundred ng of the resulting cDNA was subjected to quantitative relative expression PCR (RT qPCR) on the *Applied Biosystems* model 7500 System, using 400 nM (each) of the forward and reverse primers and 6.25 µl of SYBR Green PCR Master Mix (Applied Biosystems) in a final volume of 25 µl. The default thermocycler program was used for all genes and qrPCR assays were performed in triplicate for each primer pair. Threshold values were normalized according to the threshold cycle of ORF XF0305 (*NuoA* gene) which is expressed at similar levels under all conditions tested according to our microarray data. The change in the expression of each gene was calculated using the $2^{-\Delta\Delta Ct}$ method. The ORF used as the endogenous control, *NuoA*, codes for the NADH-ubiquinone oxidoreductase (subunit NQ07) which belongs to an operon with many subunits of NADH dehydrogenase I, a multimeric protein. This enzyme participates in aerobic respiration, and would therefore be expressed in a constant and invariable way in bacteria. For this reason it is used as a standard to normalize variations in the gene expression of other genes. It had no significant variation under the experimental conditions of this study in the microarray (LogFC:-0.2)

RESULTS AND DISCUSSION

Global expression profile

Among the genes analyzed, approximately 15.4% (400 genes) were detected as differentially expressed within the plants studied. From these, 24 genes (6%) showed higher expression in susceptible Pera, and 151 genes (37.75%) in asymptomatic Navelina. Differentially expressed genes were distributed among *Xylella´s* functional categories (http://unicamp.lbi.ic.unicamp.br/xf) (Figure 2). According to the results of the microarray analysis, bacteria infecting the susceptible variety Pera expressed a higher number of genes than in the asymptomatic plant, the cultivar Navelina ISA315. We found differentially expressed genes within the eight *Xylella fastidiosa* gene categories in both hosts. Just one bacterial gene differentially expressed in Pera was included in the ninth category, “ORFs with undefined category”, but, as it is described as an integral membrane protein, we included it in category IV “Cell Structure” for a better graphic visualization. The highest percentage of differentially expressed genes in both varieties was found for hypothetical proteins (47.5%) corresponding to 190 genes (Figure 3). All genes described in “Results” are described in Table 2, including gene ID, name of gene, category, gene product and P number. Table 3 shows the abbreviations corresponding to all gene categories presented in Table 2.

Many genes distributed among the above mentioned categories are involved in bacterial survival, and influence pathogenicity. A complex network of signals between plant, bacteria and the environment, activates or represses different genes. As can be observed in Figure 2 and Figure 3, hypothetical and conserved hypothetical proteins represented 47% of the total of genes found differentially expressed in both varieties, including a percentage of 29.25% in Pera and a percentage of 18.25% in Navelina ISA 315 (corresponding to 117 and 73 genes respectively). It is important to mention that a significant number of hypothetical proteins differentially expressed in Pera presented a very high value of LogFC. This fact suggests a possible role, still unexplored, for many of them in pathogenicity. One of these proteins, ORF XFa0032, still classified as a conserved hypothetical protein at the *Xylella fastidiosa* Genome Project site (http://www.lbi.ic.unicamp.br/xf/), was identified as a hydoxynitrile lyase related to the process of cyanogenesis by Caruso et al. (2009). Its possible role in Navelina ISA 315 cultivar is further discussed below.

Differentially expressed genes involved in the response to stress conditions

Heat shock response. Three genes coding chaperons were induced in Navelina ISA 315, DNAK, DNA J and HTPG (with LogFC values of 3.20, 1.32 and 1.71 respectively) whereas only one was induced in Pera, the gene HTRA (LogFC -1.72). The complete genome sequences of different *Xylella* strains have been determined (Simpson et al. 2000; Bhattacharyya et al. 2002; Weiner III et al. 2003), revealing the presence of genes encoding different HSPs as well as the heat shock sigma factor σ^{32}. However, little is known about stress responses and gene regulation in these organisms (Koide et al. 2006) and a more direct assignment of gene function and evaluation of its association with pathogenesis require the analysis of mutants but only a few were obtained for CVC strains by homologous recombination (Da Silva Neto et al. 2002; Da Silva Neto et al. 2006; Gaurivaud et al. 2002). Koide et al. (2006) carried out a global analysis of heat shock response *in vitro* and determined genes involved in the citrus strain 9a5c, after exposure to 40ºC. In addition to the induction of classical Hsp genes, they observed the up-regulation of several genes related to pathogenesis and adaptation, as well as the repression of fimbria-related genes and genes involved in energy metabolism, revealing a complex network of genes that work together in response to heat stress in *Xylella fastidiosa*.

DNAK, the *Escherichia coli* Hsp70 homologue, is an ATP-dependent molecular chaperone that acts in conjunction with the co-chaperones, DNAJ and GrpE, to mediate protein folding and remodeling reactions in the cell. The DNAK and Hsp70 chaperone systems participate in a wide variety of cellular processes in both normal and stressed cells, including nascent protein folding, protein trafficking across intracellular membranes, proteolysis, assembly of multiprotein structures, disassembly of protein aggregates, cell division, DNA replication of several phages and plasmids, and regulation of the heat shock response (Bukau and Horwich, 1998; Bukau et al. 2000; Frydman, 2001; Kim et al. 2002). The *Xylella fastidiosa* HTPG gene*,* also diferentially expressed in Navelina ISA 315*,* has the strongest similarity with *Escherichia coli* HTPG high temperature protein G, which is a molecular chaperone, has ATPase activity and belongs to the heat shock protein 90 family. The gene HTRA, differentially expressed in Pera, codes for a protein very similar to the *E. coli* heat shock protein *htrA,* a periplasmic serine protease, which is required at high temperatures and is involved in degradation of damaged proteins. This protein is essential for bacterial survival at temperatures higher than 42ºC (*Xylella fastidiosa* Genome Project http://www.lbi.ic.unicamp.br/xf/). It is possible that this activity helps the bacteria to survive at high temperatures within Pera degrading damaged polypeptides.

The fact that more genes coding for chaperones were induced in Navelina ISA 315 suggested that bacteria could be under more stress within asymptomatic plants, maybe because of plant defenses or competition from endophytic microorganisms. Experiments had shown that *Xylella fastidiosa* growth was stimulated *in vitro* by *Methylobacterium extorquens* and inhibited by *Curtobacterium flaccumaciens,* which demonstrated that real interaction occurs between endophytic microorganisms and the pathogen (Lacava et al. 2004). The DNAK/DNAJ/GrpE system could be more active in the function of re-assembling disaggregated polypeptides in Navelina ISA 315. Defense mechanisms in the Pera variety could be less effective, generating fewer damaged polypeptides, and the DNAK/DNAJ/GrpE system may therefore not be essential for bacterial survival. In contrast, HTRA chaperone is more active in *Xylella fastidiosa* within Navelina ISA 315. A different heat shock response could be activated in each variety depending on environmental conditions within the plant, specific plant defenses or endophytic competition.

Antioxidant response, toxins and detoxification proteins. During *X. fastidiosa* infection, elicitors of plant defense responses synthesized by the bacterium and/or released from partial plant cell wall degradation may induce the generation of reactive oxygen species (ROS), such as superoxide and hydrogen peroxide which may inhibit bacterial growth and disease development (Wojtaszec, 1997). Several genes involved in ROS detoxification have been identified in *X. fastidiosa* including genes that code for catalase, superoxide dismutase, glutathione peroxidase, and genes oxyR and Ohr, suggesting that *X. fastidiosa* can respond to oxidative stresses (Simpson et al. 2000).

Four genes were differentially expressed in the cultivar Navelina ISA 315, within subcategory “Toxin production and detoxification” of the category “Pathogenicity, virulence and adaptation”: the genes KSGA, cpeB, st3F7.11 and pbp, coding for a dimethyladenosine transferase, catalase/peroxidase, peptide synthase and the protein beta-lactamase, respectively (Log FC values: 1.3; 1.5; 1.5 and 2.2). In Pera, the genes FRPC, AHPC, cutC, czcA and TolC were differentially expressed with LogFC values of -3.4, -2.0 and -1.4, -1.49 and -1.48. The last 3 mentioned genes, cutC, czcA and TolC, involved in copper resistance were also differentially expressed in Pera. Rodrigues et al. (2008) also found a

higher expression of these genes in cells forming biofilms, which also suggests a higher resistance to copper related to biofilm formation in the symptomatic variety.

The gene coding for catalase/peroxidase was differentially expressed in Navelina ISA 315 whereas subunit C of alkyl-hydroperoxide reductase was differentially expressed in Pera (-2.0 LogFC). Alkyl hydroperoxide reductase subunit C (AhpC) is the catalytic subunit responsible for alkyl-peroxide metabolism in *Xanthomonas.* The increase in catalase activity was a compensatory response to lack of AhpC, through a complex regulation response which differs from other bacteria (Mongkolsuk et al. 2000). Higher expression of catalase/peroxidase in Navelina ISA 315 could compensate for lower expression of AhpC, whereas in Pera AhpC is more expressed. Probably, *Xylella fastidiosa* is reacting in a different way within symptomatic and asymptomatic plants according to the ROS plant response which is reflected by differentially expressed antioxidant proteins.

Among the toxin-encoding genes identified in *X. fastidiosa*, four are hemolysin-like genes belonging to the RTX family (Simpson et al. 2000). Related to this family, the *FRPC* gene, which codes for a hemolysin-type calcium binding protein, is induced in Pera with a high LogFC value (-3.47). This gene's product is similar to other hemolysins of the RTX family, being located in the outer membrane and secreted. It has a GLY-rich region probably involved in binding calcium, which is required for target cell binding or cytolytic activity. This type of toxins forms pores that interact with the plasma membrane of host cells, causing leakage of cellular substances (Lally et al. 1999). Interestingly, one conserved hypothetical protein (XFa0032) of 27.1 kDa recently characterized as a α-hydroxynitrile lyase, was differentially expressed in cultivar Navelina ISA 315 with a high logFC value of 2.4. Alpha-hydroxynitrile lyases (HNLs) are plant defense enzymes that protect plant cells from hervibores or microbial attack, producing HCN through a process called cyanogenesis (Caruso et al. 2009). *Xylella fastidiosa* could be producing this enzyme in Navelina ISA 315, responding to plant defenses. This plant, or maybe an endophyte, could be secreting more HCN, which is a toxic compound to defend itself from *Xylella fastidiosa*, or from other microorganisms within the plant. *Xylella fastidiosa* in turn, codes for more HNLs to detoxify the environment and survive.

Differentially expressed genes involved in survival in atypical conditions and related mechanisms

The genes MDOH (XF1623) and MDOG (XF2682) included in subcategory "Adaptation to atypical conditions" of the category "Pathogenicity, Virulence and Adaptation" are responsible for the synthesis of osmoregulated periplasmic glycan proteins (OPGs). These genes were differentially expressed in cultivar Navelina ISA 315 and Pera with LogFC values of 1.55 and -1.55, respectively. Osmoregulated periplasmic glycan proteins (OPGs) are important membrane components of Gram negative cells which are also involved in survival at low nutrient concentrations as well as inadequate osmotic conditions, especially during the process of infection of eukaryotic hosts. Normally they are associated with phytopathogenic processes (Bohin, 2000). There is probably an opposite regulation at the transcriptional level of those genes within the varieties Pera and Navelina ISA 315, leading to different concentrations of the proteins, which then interfere in host pathogen interactions and determine the degree of pathogenicity and symptom development.

The gene SspB, highly induced in Pera variety (-3.69 LogFC) encodes the stringent starvation protein B. It was recently demonstrated that in *Escherichia coli,* the SspA gene has an important function in stress response (including acid tolerance) through negative regulation of the global regulator H-NS, which regulates multiple defense systems (Cowtan, 1994; Vassylyev et al. 2002). Williams et al. (2006) reported the starvation-induced expression of SspA and SspB in *Escherichia coli* and their functions for survival during growth and prolonged starvation. The inactivation of the SspA gene blocked SspB expression. In our microarray experiment, high expression of SspB in Pera could indicate that the bacterium is surviving in the absence of nutrients in the stationary phase and inducing acid tolerance within the xylem vessels of the symptomatic plant.

Many regulatory genes, involved in many metabolic cascades, were differentially expressed in this experiment. The gene rpfC was differentially expressed in Pera variety with a value of -1.59 LogFC. The RpfC gene codes for Rpf C protein, the sensor component of the two-component system RpfF/RpfC, which is part of the Rpf (regulation of pathogenicity factors) cluster. Chatterjee et al. (2008) proposed a model in which the same sensor molecule (rpfC) is required both to develop virulence and insect transmission. The induction of the RpfC gene in the susceptible variety is related to a gene

regulation cascade which involves biofilm formation. Perhaps different biofilm maturation or denser composition could occur within the Pera variety, which would be consistent with the severe symptoms observed, and probably with insect transmission. However, this hypothesis should be confirmed with further studies.

As shown in Table 2, other regulatory genes like *ALGR*, *colR*, *algZ* and *VapD* were differentially expressed in variety Pera, while *colR* and *ColS* were induced in Navelina ISA 315. This fact suggests changes in gene expression of sensor and regulatory molecules related to the different conditions within each plant.

Differentially expressed genes involved in iron homeostasis

Among some putative ORFs identified as possibly related to iron transport (Silva-Stenico et al. 2005), only the ORFs XF0599 (YBIL gene) and XF1038 (St3F7.11 gene) were differentially expressed in this experiment in Pera (-1.7 LogFC) and Navelina ISA315 (1.5 LogFC), respectively. The gene YBIL codes for a TonB dependent receptor and St3F7.11 for a peptide synthase enzyme. The TonB gene product is involved in iron-siderophore transport across the membrane.

When pathogenic bacteria are challenged with limiting iron concentrations, they can mantain homeostasis of this metal by releasing iron from intracellular reservoirs and enhancing the expression of the systems involved in iron uptake (Ratledge and Dover, 2000). This system allows bacterial survival in nutrient-poor environments, as iron is an essential cofactor for many proteins involved in electron transfer and redox reactions. There may be differences in iron availability between the varieties we studied which could influence bacterial response related to gene expression. Levels of expression of genes related to iron uptake may be induced depending on limiting iron conditions within the xylem vessels. As the YBIL gene was induced in Pera, probably iron is a more limited factor within this variety than in Navelina ISA 315. That would be consistent with the symptoms observed in Pera. Simpson et al. (2000) proposed that the absorption of iron and probably other metallic ions like manganese causes the depletion of essential micronutrients in the xylem, leading to the typical symptoms of variegated clorosis.

Zaini et al. (2009) reported a constant level of expression of *Xylella fastidiosa* transcripts in limiting iron conditions using microarray analysis. They observed the modulation of many CDS related to regulatory functions, pathogenicity and cellular structure, toxins and pili/ fimbriae. We found that many genes involved in pathogenicity were also induced in Pera. The complex TonB-ExB-ExbD is also likely to be involved in greater uptake of iron.

Genes related to mobile genetic elements: phages, prophages and plasmids

The genes VapD, differentially expressed in Pera and SCJ2116, in Navelina ISA 315, are related to virulence and detoxification, respectively, and are both included in mobile genetic elements. The presence of virulence genes within prophage sequences is strong evidence of the important role of horizontal transfer of genes via bacteriophages in the definition of bacterial phenotype (Hendrix et al. 1999). Many of the differentially expressed ORFs of this experiment were classified as hypothetical proteins, suggesting a high transcriptional activity in this region, perhaps related to pathogenicity or adaptability, that is still unexplored (Table 2). Figure 2 shows that more genes related to mobile genetic elements are differentially expressed in Pera than in Navelina ISA 315. Nunes et al. (2003) showed that *Xylella fastidiosa* has the biggest “pool” of laterally transmitted elements characterized until then (like prophages, plasmids and genomic islands) which contributed up to 18% of the total genome. Transcriptome analysis confirmed that these elements are transcriptionally active, possibly responding to environmental signals. The differentially expressed ORFs within these elements, found in this experiment, also confirmed this activity. A deeper study of laterally transferred elements would help to explain the adaptability and capacity of the bacteria to infect such a wide host range.

DNA repair, DNA restriction and modification

More genes related to DNA repair, restriction and modification systems were expressed at higher levels in Pera variety (Table 1 and Table 3), which could suggest a higher response to DNA damage caused by plant defense systems. *Xylella fastidiosa* could be more actively replicating in the

susceptible variety, therefore overcoming the stress generated by plant defense mechanisms within this variety. Different survival strategies involving different processes may be adopted by the bacteria depending on plant environment.

Validation of the microarray

Primers for RT-qPCR were all validated for amplification efficiency and specificity following the "MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments" (Bustin et al. 2009). Efficiencies of about 100% ± 20% were found for all of them. The RT-qPCR analysis of the genes ChpA, ColS, DNAJ, HTPG, chpA, Pil U, Vap D confirmed the results obtained by the microarray analysis in the system studied. Figure 4 shows relative levels of increased gene expression, given as the multiplication factor (number of times) in Navelina ISA 315 compared to Pera, for each gene, with both microarray and RT-qPCR analysis.

The inherent variation of both microarray and RT-qrPCR due to processing of material and normalization methods can influence the correlation between results obtained. It is more common to find in the literature the simple statement that results were validated, often with no, or extremely low, reported correlations (Freeman et al. 1999; Bustin, 2002; Chyaqui et al. 2002; Wurumbach et al. 2003). For that reason, when the same direction of expression in the same variety was found with both methods, this was considered validation.

Healthy Pera and Navelina plants did not reveal any fluorescence due to possible non-specific hybridization with plant genome. Also, one slide without hybridization was scanned in order to detect any non-specific hybridization signal, but similar background fluorescence to the healthy control plants was obtained. These results, together with microarray internal controls and qrtPCR results, confirmed that the hybridization signal from bacteria infecting both Navelina ISA 315 and Pera, is the result of *X. fastidiosa* genes hybridization in the system studied.

CONCLUDING REMARKS

The finding that more bacterial genes were differentially expressed in the susceptible variety was to be expected since the bacteria are probably more actively migrating through vessels, blocking water and nutrient flow and degrading plant tissues while colonizing different parts of the susceptible variety. Even though bacterial concentration was found to be high in cultivar Navelina ISA315, it was probably not blocking the xylem flow in vessels as it was in Pera, since it did not cause any symptoms. Plant defenses or competition from other microorganisms may be more efficient in this variety, thus generating higher stress in the bacteria, suggested by differentially expressed "heat shock" genes, as well as the above-mentioned detoxification systems. Perhaps in Navelina ISA 315, cells are not so embedded in biofilms, and move more freely through vessels, therefore having more plankton-like characteristics and probably being exposed to much more stress without biofilm protection. Also, biofilms could have different compositions (Martins et al. 2010) in each variety.

Different bacterial gene expression patterns probably reflect different bacterial survival strategies within each variety involving differences in biofilm composition, sensing systems and migrating patterns, as well as different responses to stress conditions like detoxification proteins and chaperones. Different levels of gene expression could be the key to understanding the ability of *X. fastidiosa* to colonize different hosts and survive in such adverse conditions as xylem vessels. Moreover, bacterial non-coding small RNAs (sRNA) have attracted considerable attention due to their ubiquitous nature and roles in controlling numerous cellular processes including survival, adaptation and pathogenesis. However, little is known about small RNA in this bacterium, in spite of the fact that several whole genome sequences of *X. fastidiosa* have been published. Results from BLAST analysis showed that 34 small RNA genes were shared by all four *X. fastidiosa* strains. Species-, subspecies- and pathotype-specific small RNAs (size from 40 to 350 pb) were also identified (Chen and Huang, 2011). A clearer idea of regulation processes and survival strategies could be gained by including these promising newly identified small RNA sequences in future transcriptome analyses.

It is important to consider the adaptation potential of *X. fastidiosa* in CVC control strategies and breeding programs when using the cultivar Navelina ISA 315 because, even without symptoms, it could be a source of infection due to the concentration of bacteria inside the plant.

Spotted microrrays proved to be a powerful technique to analyze changes in gene expression for *Xylella fastidiosa* infecting citrus plants (*in vivo* system). The technique could also be used to monitor gene expression changes in time course experiments in natural habitats. Still more research is needed to understand the disease process and elucidate the environmental signals which could coordinate bacterial behaviour in terms of gene expression and regulation of cell density, as well as plant-pathogen interactions. Nevertheless, transcriptomic analysis could give many clues about the different processes involved in pathogenicity, like the heat shock response and some of the survival mechanisms presented in this paper. Many other pathogenicity and regulatory genes were differentially expressed in this experiment within each variety which has not been discussed in this article.

Other techniques and studies could be applied in order to achieve a better and deeper understanding of this system, like gene complementation analyses, assessing the diversity of other microorganisms within the plant, as well as electron microscopy. In any case, transcriptomic analysis gave a general view of different biochemical processes activated or repressed in natural conditions, considering the inherent variables of an *in vivo* system. On the other hand, it would be interesting to complement this study with transcriptomic analysis of the plant hosts studied here, which could reveal much more information about plant-pathogen interactions.

To our knowledge, this is one of the first gene expression analyses of *X. fastidiosa in vivo* within the plant, which gave a more accurate image of this pathogen in nature and its ability to live in different environments (hosts), without the usual bias introduced by *in vitro* culture conditions.

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FIGURES

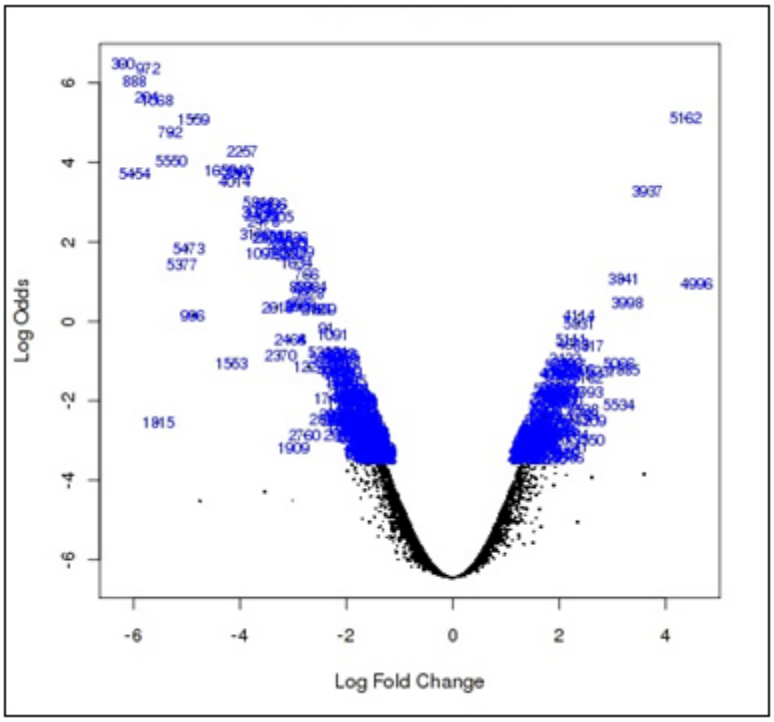

Fig. 1 Volcano plot showing differentially expressed genes (Logarithm of probability vs logarithm of LogFC). Genes above a threshold value of LogFC = 1 were considered differentially expressed in Navelina ISA 315 (Bahianinha), while those below LogFC = -1 were considered differentially expressed in Pera.

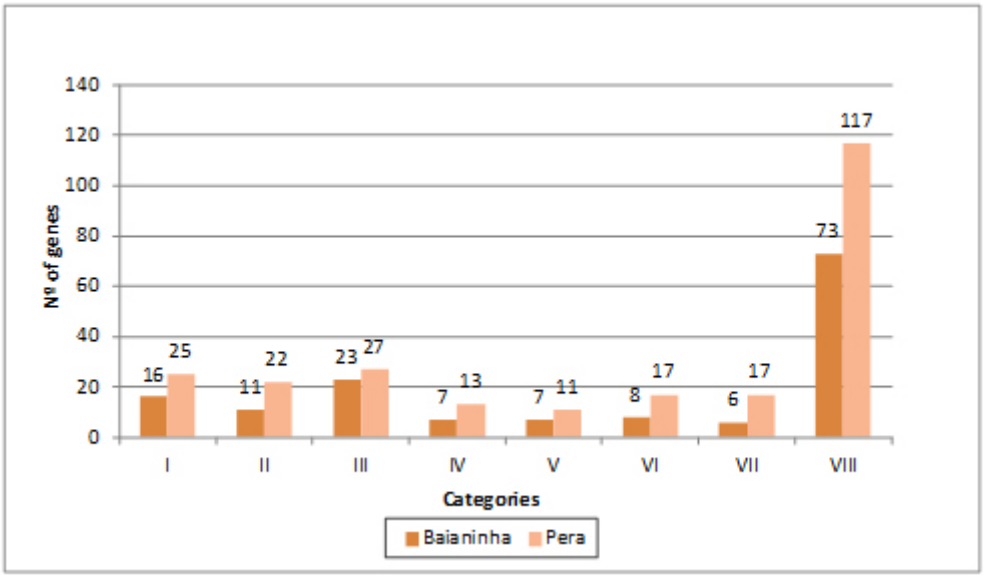

Fig. 2 Number of differentially expressed genes in each functional category: I: Intermediary metabolism; II: Biosynthesis of small molecules; III: Macromolecule metabolism; IV: Cell structure; cellular processes; V: Cellular processes; VI: Mobile genetic elements; VII: Pathogenicity, virulence and adaptation; VIII: Genes of hypothetical proteins. Varieties Navelina ISA 315 (Bahianinha) and Pera are shown.

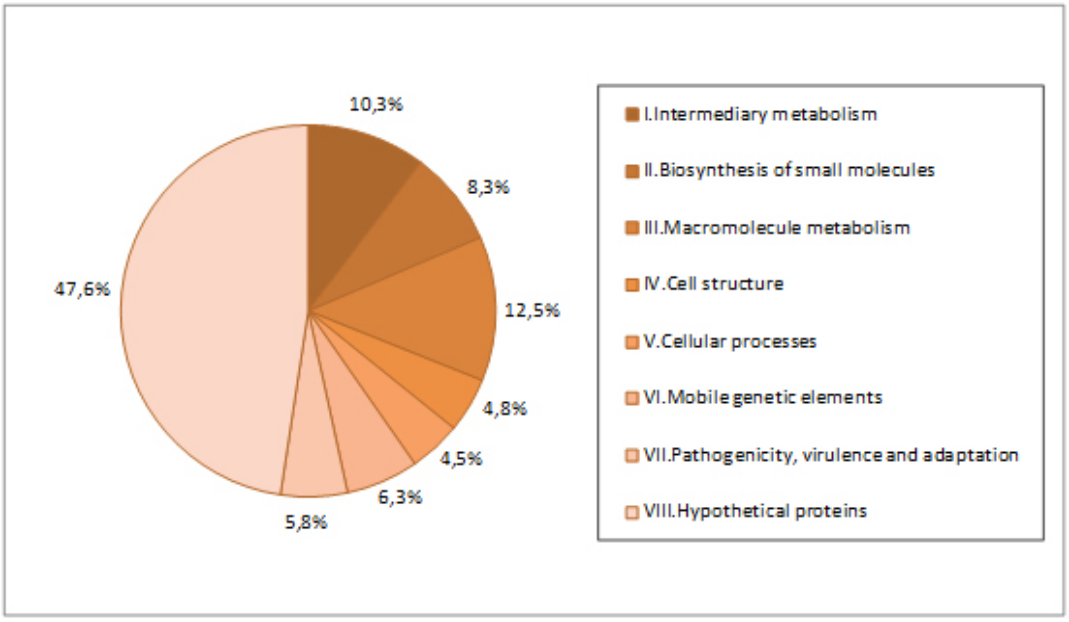

Fig. 3 Percentage of differentially expressed *X. fastidiosa* genes (total = 400) in each category, for both Pera and Bahianinha varieties together.

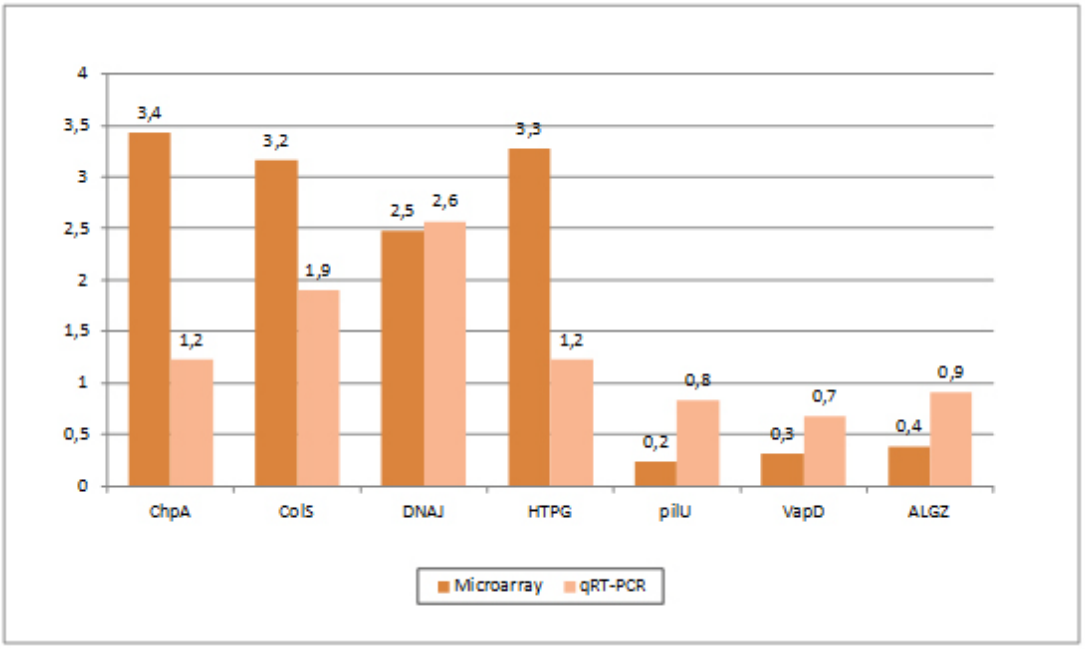

Fig. 4 Relative levels of gene expression in Navelina ISA 315 compared to Pera, given as the multiplication factor (number of times) for each indicated gene, assessed by RT-qPCR and microarray experiment.

TABLES

Table 1. Primer sequences used for real time PCR analysis.

ID/ Gene Name	Sequence (5'-3')
XF0305 *NUOA* forward	CATTGATATTGATTGGCAGGTTTC
XF0305 *NUOA* reverse	GAGGACAGCTTTTCGGAATCAG
XFa 0052 *Vap D* forward	CGTCAGACAAGCACATGGAACT
XFa 0052 *Vap D* reverse	TCGAACCATTGGAAGCGTATG
XF1632 *Pil U* forward	TCGAGAAAGTCCATGAATGCAA
XF1632 *Pil U* reverse	GCGGAAGCGACCAATGTT
XF1625 *AlgZ* forward	CAGCATTGCGCATCGTCTT
XF1625 *AlgZ* reverse	CCAAAACCCCGTCATTCG
XF2535 *ColS* forward	GCCTCGGTCATGTCGTAAGC
XF2535 *ColS* reverse	ACTCGCGGTACGCAAAGC
XF1952 *chpA* forward	GACCCAGGCAGTATTCATTCG
XF1952 *chpA* reverse	AAGCCACCGGAACTGCAA
XF2339 *DNA J*forward	GCGGACGAGGCGTTATTATTC
XF2339 *DNA J* reverse	AACACGCCCAGCACCATT
XF0978 *HTPG* forward	CCCAAGCCACCCACTCATC
XF0978 HTPG reverse	CGCAAAACGGTCCATGTCT

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Table 2. Differentially expressed genes in the microarray experiment.

Gene ID	Gene_Name	logFC	Category	Gene product
XF0846	*TM1624*	-4,16	I.A.2	beta-mannosidase precursor (100.9 kDa)
XF1472	*BEDB*	-3,02	I.A.2	benzene 1,2-dioxygenase, ferredoxin protein (12.3 kDa)
XF1234	*PRPB*	-2,86	I.A.2	carboxyphosphonoenolpyruvate phosphonomutase (32.4 kDa
XF0781	*estA*	-1,63	I.A.2	lipase/esterase (64.3 kDa)
XF1743	*est*	-1,58	I.A.2	esterase (37.8 kDa)
XF0840	*BGA*	1,82	I.A.2	beta-galactosidase (67.9 kDa)
XF2677	*AAO*	-1,39	I.A.2, VII.G	L-ascorbate oxidase (80.9 kDa)
XF2268	*GLPK*	-2,92	I.B.10	glycerol kinase (57.1 kDa)
XF1889	*FPR*	2,06	I.B.10	ferredoxin-NADP reductase (29.5 kDa)
XF2095	*None*	2,36	I.B.10	carbonic anhydrase (22.8 kDa)
XF1468	*MRSA*	-3,05	I.B.11	phosphomannomutase (47.4 kDa)
XF0609	*GMD*	-1,93	I.B.11	GDP-mannose 4,6 dehydratase (39.0 kDa)
XF0259	*XANB*	-1,58	I.B.11,III.D.1	phosphomannose isomerase-GDP-mannose pyrophosphorylase (53.0 kDa)
XF0904	*YBEZ*	2,12	I.B.9	ATP-binding protein (36.2 kDa)
XF0311	*nuoG*	-5,73	I.C.1	NADH-ubiquinone oxidoreductase, NQO3 subunit (79.5 kDa)
XF0307	*NUOC*	-3,30	I.C.1	NADH-ubiquinone oxidoreductase, NQO5 subunit (28.1 kDa)
XF2459	*CYCJ*	-1,60	I.C.3	c-type cytochrome biogenesis protein (17.3 kDa)
XF0557	*az1*	1,46	I.C.3	electron transfer protein azurin I (16.3 kDa)
XF1387	*CYOD*	1,62	I.C.3	cytochrome O ubiquinol oxidase, subunit IV (12.6 kDa)
XF0908	*PETA*	1,93	I.C.3	ubiquinol cytochrome C oxidoreductase, iron-sulfur subunit (22.9 kDa)
XF0868	*lpdA or lpd*	-1,60	I.C.6, I.C.7	dihydrolipoamide dehydrogenase (63.9 kDa)
XF1073	*SDHB*	-1,71	I.C.7	succinate dehydrogenase iron-sulfur protein (29.5 kDa)
XF1535	*GLTA*	1,83	I.C.7	citrate synthase (47.9 kDa)
XF1144	*ATPG OR UNCG OR PAPC*	1,50	I.C.8	ATP synthase, gamma chain (32.3 kDa)
XF1145	*ATPA OR UNCA*	2,11	I.C.8	ATP synthase, alpha chain (56.0 kDa)
XF1626	*ALGR*	-5,55	I.D	two-component system, regulatory protein (28.7 kDa)
XF0912	*SSPB*	-3,69	I.D	stringent starvation protein B (15.7 kDa)
XF1350	*rpoD*	-2,86	I.D	RNA polymerase sigma-70 factor (69.9 kDa)
XF1749	*OPDE*	-1,69	I.D	transcriptional regulator (42.4 kDa)
XF0833	*CYSB OR HI1200*	-1,63	I.D	transcriptional regulator (LysR family) (37.7 kDa)
XF2534	*colR*	-1,62	I.D	two-component system, regulatory protein (25.0 kDa)
XF1730	*YAFC*	-1,53	I.D	transcriptional regulator (LysR family) (31.9 kDa)
XF2476	*SUHB OR SSYA*	-1,51	I.D	extragenic supressor (30.2 kDa)

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XF1275	*phaF*	-1,40	I.D	poly(hydroxyalcanoate) granule associated protein (20.5 kDa)
XF1625	*algZ*	-1,39	I.D	two-component system, sensor protein (38.6 kDa)
XF1133	*AF0343*	1,39	I.D	tryptophan repressor binding protein (20.5 kDa)
XF1996	*C2*	1,51	I.D	transcriptional regulator (PbsX family) (14.1 kDa)
XF2336	*colR*	1,65	I.D	two-component system, regulatory protein (27.3 kDa)
XF2535	*colS*	1,66	I.D	two-component system, sensor protein (41.6 kDa)
XF0833	*CYSB OR HI1200*	1,69	I.D	transcriptional regulator (LysR family) (37.7 kDa)
XF0125	*csrA*	1,99	I.D	carbon storage regulator (8.3 kDa)
XF1000	*ARGE*	-1,78	II.A.1	acetylornithine deacetylase (38.9 kDa)
XF1001	*argB*	-1,72	II.A.1	acetylglutamate kinase (48.9 kDa)
XF1005	*PROA*	-1,68	II.A.1	gamma-glutamyl phosphate reductase (52.2 kDa)
XF1003	*ASL*	1,56	II.A.1	argininosuccinate lyase (48.9 kDa)
XF1004	*DR1827*	3,26	II.A.1	glutamate 5-kinase (40.9 kDa)
XF1121	*METF OR AQ_1429*	-5,54	II.A.2	5,10-methylenetetrahydrofolate reductase (30.5 kDa)
XF1121	*METF OR AQ_1429*	-2,12	II.A.2	5,10-methylenetetrahydrofolate reductase (30.5 kDa)
XF0118	*ASNB*	-1,80	II.A.2	asparagine synthase B (62.9 kDa)
XF1481	*dapF*	-1,71	II.A.2	diaminopimelate epimerase (30.1 kDa)
XF0863	*MET2*	1,73	II.A.2	homoserine O-acetyltransferase (37.7 kDa)
XF1375	*TRPB*	-1,55	II.A.4	tryptophan synthase beta chain (48.0 kDa)
XF0212	*TRPD*	2,43	II.A.4	anthranilate phosphoribosyltransferase (36.7 kDa)
XF2217	*HISB*	-1,50	II.A.5	hyphypoimidazoleglycerolphosphate dehydratase/histidinol-phosphate phosphatase bifunctional enzyme (41.4 kDa)thetical protein (33.8 kDa)othetical protein (33.8 kDa)
XF2213	*HISI OR HISIE*	1,51	II.A.5	phosphoribosyl-AMP cyclohydrolase/phosphoribosyl-ATP pyrophosphatase bifunctional enzyme (22.6 kDa)
XF2644	*PRSA OR PRS*	-2,99	II.B.1	phosphoribosyl pyrophosphate synthetase (33.8 kDa)
XF2571	*PYRD*	2,52	II.B.2	dihydroorotate dehydrogenase (38.7 kDa)
XF2332	*THYA*	-2,19	II.B.4	thymidylate synthase (30.1 kDa)
XF1356	*BIOH OR BIOB*	-1,76	II.D.1	biotin biosynthesis protein (27.6 kDa)
XF1199	*TRXA*	-1,47	II.D.10 ,I.C.3	thioredoxin (11.9 kDa)
XF0661	*ISPA*	-4,01	II.D.11	geranyltranstransferase (farnesyl-diphosphate synthase) (31.6 kDa)
XF1391	*ISPB OR CEL*	-2,03	II.D.11	octaprenyl-diphosphate synthase (36.2 kDa)
XF1797	*HEMY*	-3,61	II.D.12	porphyrin biosynthesis protein (44.4 kDa)
XF1512	*HEMK*	-1,37	II.D.12	protoporphyrinogen oxidase (29.5 kDa)
XF0436	*FOLB*	-1,83	II.D.2	dihydroneopterin aldolase (13.5 kDa)
XF0228	*FOLK OR HI0064*	-1,56	II.D.2	2-amino-4-hydroxy-6-hydroxymethyldihydropteridine pyrophosphokinase (18.9 kDa)
XF0230	*PANC*	1,92	II.D.5	pantoate--beta-alanine ligase (31.2 kDa)

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XF0839	*PDXA*	1,65	II.D.6	pyridoxal phosphate biosynthetic protein (34.6 kDa)
XF1097	*PNCB*	1,94	II.D.7	nicotinate phosphoribosyltransferase (45.0 kDa)
XF1961	*NADE OR ADGA*	2,59	II.D.7	NH3-dependent NAD synthetase (59.3 kDa)
XF1888	*THIC OR THIA*	-1,79	II.D.8	thiamine biosynthesis protein (69.4 kDa)
XF0671	*FABG*	-2,00	II.E	3-oxoacyl-[ACP] reductase (25.5 kDa)
XF0673	*FABF*	-1,73	II.E	3-oxoacyl-[ACP] synthase II (43.3 kDa)
XF0144	*SPEA*	1,65	II.F	biosynthetic arginine decarboxylase (69.2 kDa)
XFa0061	*SSB*	-1,48	III.A.1	single-strand binding protein (15.4 kDa)
XF1353	*parC*	1,39	III.A.1	topoisomerase IV subunit (83.4 kDa)
XF0920	*TOPA OR RP326*	1,38	III.A.1, III.A.4	DNA topoisomerase I (91.8 kDa)
XF1943		-1,68	III.A.2	histone-like protein (13.9 kDa)
XF1644	*SSB*	-1,41	III.A.2	single-stranded DNA binding protein (16.9 kDa)
XF0423	*RECB OR RORA*	-2,12	III.A.3	exodeoxyribonuclease V beta chain (134.1 kDa)
XF1904	*RUVA*	-4,87	III.A.4	holliday junction binding protein, DNA helicase (20.9 kDa)
XF1905	*RUVC*	-4,06	III.A.4	holliday junction resolvase, endodeoxyribonuclease (22.0 kDa)
XF0967	*uvrB*	-3,06	III.A.4	excinuclease ABC subunit B (75.6 kDa)
XF2022	*SBCB OR XONA OR CPEA*	-2,23	III.A.4	exodeoxyribonuclease I (56.9 kDa)
XF2426	*UVRA*	-2,13	III.A.4	excinuclease ABC subunit A (106.9 kDa)
XF1299	*alkB*	-1,94	III.A.4	DNA repair system specific for alkylated DNA (22.6 kDa)
XF0755	*XSEA*	-1,87	III.A.4	exodeoxyribonuclease VII large subunit (49.9 kDa)
XF2081	*DINJ*	-1,59	III.A.4	DNA-damage-inducible protein (9.0 kDa)
XF1904	*RUVA*	-1,44	III.A.4	holliday junction binding protein, DNA helicase (20.9 kDa)
XF0164	*SCE87.25c*	1,69	III.A.4	exodeoxyribonuclease (30.8 kDa)
XF2311	*UVRC*	2,00	III.A.4	excinuclease ABC subunit C (69.8 kDa)
XF2022	*SBCB OR XONA OR CPEA*	2,09	III.A.4	exodeoxyribonuclease I (56.9 kDa)
XF2081	*DINJ*	2,20	III.A.4	DNA-damage-inducible protein (9.0 kDa)
XF2721	*hsdR1*	-1,78	III.A.5	type I restriction-modification system endonuclease (114.5 kDa)
XF0641	*sfiIM*	-1,74	III.A.5	DNA methyltransferase (41.9 kDa)
XF0641	*sfiIM*	-1,58	III.A.5	DNA methyltransferase (41.9 kDa)
XF2726	*hsds*	-1,43	III.A.5	type I restriction-modification system specificity determinant (45.8 kDa
XF0924	*SMF OR DPRA OR HI0985*	1,90	III.A.5	DNA processing chain A (41.0 kDa)
XF0935	*llaIIA*	-1,58	III.A.5,III.A.4	methyltransferase (35.9 kDa)
XF1163	*RPLX OR RP648*	-1,81	III.B.2	50S ribosomal protein L24 (11.4 kDa)
XF1177	*rplQ*	1,54	III.B.2	50S ribosomal protein L17 (14.4 kDa)

XF1173	*rpsM*	2,55	III.B.2	30S ribosomal protein S13 (13.6 kDa)
XF0107	*RPSP OR RPS16 OR HI0204*	1,95	III.B.2, III.A.5	30S ribosomal protein S16 (9.7 kDa)
XF2438	*RPSA OR SSYF*	-1,57	III.B.2, III.C.1	30S ribosomal protein S1 (62.9 kDa)
XF2201	*PRMA*	-1,67	III.B.3	ribosomal protein L11 methyltransferase (34.9 kDa)
XF2176	*LEUS*	-3,30	III.B.4	leucyl-tRNA synthetase (100.7 kDa)
XF1440	*TRMU OR ASUE*	-1,88	III.B.4	tRNA methyltransferase (43.0 kDa)
XF0169	*TYRS OR HI1610*	1,40	III.B.4	tyrosyl-tRNA synthetase (46.6 kDa)
XF0927	*FMT*	1,75	III.B.4	methionyl-tRNA formyltransferase (33.2 kDa)
XF0741	*PHES*	2,16	III.B.4	phenylalanyl-tRNA synthetase alpha chain (37.8 kDa)
XF1314	*QUEA*	2,26	III.B.4	S-adenosylmethionine: tRNA ribosyltransferase-isomerase (38.9 kDa)
XF2632	*RPOC OR TABB*	1,96	III.B.5	RNA polymerase beta' subunit (158.7 kDa)
XF0227	*PCNB*	4,58	III.B.5	polynucleotide adenyltransferase (47.6 kDa)
XF0239	*PNP*	1,33	III.B.6	polynucleotide phosphorylase (78.2 kDa)
XF0174	*PRFC OR HI1735*	-1,82	III.C.1	peptide chain release factor 3 (60.9 kDa)
XF2628	*tufA*	-1,63	III.C.1	elongation factor Tu (42.9 kDa)
XF2298	*stp1*	1,74	III.C.1	low molecular weight phosphotyrosine protein phosphatase (17.3 kDa)
XF2339	*DNAJ*	1,32	III.C.2	DnaJ protein (40.4 kDa)
XF0978	*HTPG*	1,71	III.C.2	heat shock protein G (71.8 kDa)
XF2177		1,73	III.C.2	heat shock protein G (71.8 kDa)
XF2340	*DNAK OR GRPF OR GROP OR SEG*	3,20	III.C.2	DnaK protein (68.5 kDa)
XF2330	*slpD*	-3,31	III.C.3	proteinase (57.5 kDa)
XF0220	*pepQ*	-1,59	III.C.3	proline dipeptidase (43.4 kDa)
XF2330	*slpD*	1,68	III.C.3	proteinase (57.5 kDa)
XF2230	*DACC*	-2,69	IV.A.1	penicillin-binding protein 6 precursor (43.2 kDa)
XF2334	*DGKA*	-2,08	IV.A.1	diacylglycerol kinase (16.7 kDa)
XF0764	*SCF56.03*	1,47	IV.A.1	membrane protein (27.4 kDa)
XF0851	*DADA OR DADR*	-1,58	IV.A.1,I.A.2	D-amino acid dehydrogenase subunit (47.5 kDa)
XF1363	*SLT OR SLTY*	-3,60	IV.A.2	soluble lytic murein transglycosylase precursor (80.0 kDa)
XF0321	*OPRO*	-1,90	IV.A.2	porin O precursor (45.0 kDa)
XF0384	*phuR*	-1,89	IV.A.2	outer membrane hemin receptor (74.1 kDa)
XF0872	*OMPW*	-1,64	IV.A.2	outer membrane protein (19.9 kDa)
XF1614	*pbp4*	-1,91	IV.B	penicillin binding protein (52.9 kDa)
XF0416	*VACJ*	-1,60	IV.B	lipoprotein precursor (38.9 kDa)
XF2572	*MURB*	1,54	IV.B	UDP-N-acetylpyruvoylglucosamine reductase (39.1 kDa)

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XF0276	*MPL*	1,54	IV.B	UDP-N-acetylmuramate-L-alanine ligase (50.8 kDa)
XF0542		1,55	IV.B	UDP-N-acetylmuramate-L-alanine ligase (50.8 kDa)
XF0486	*lpxD*	1,43	IV.C	UDP-3-O-[3-hydroxymyristoyl] glucosamine N-acyltransferase (21.6 kDa)
XF1637	*spsQ*	1,64	IV.C	glycosyl transferase (32.8 kDa)
XF0803	*lpxC*	-1,72	IV.C	UDP-3-O-[3-hydroxymyristoyl] N-acetylglucosamine deacetylase (33.5 kDa)
XF0369	*pilM*	-4,38	IV.D	fimbrial assembly membrane protein (42.1 kDa)
XF1632	*pilU*	-2,08	IV.D	twitching motility protein (42.5 kDa)
XF2542		1,35	IV.D	fimbrial protein (15.0 kDa)
XF1192	*SCF73.09*	-2,03	IX	integral membrane protein (37.6 kDa)
XF0500	*RACR*	-1,56	phageVI.A -related repressor protein (15.3 kDa)	phage-related repressor protein (15.3 kDa)
XF2730	*RHTC*	-1,72	V.A.1	amino acid transporter (23.6 kDa)
XF2730	*RHTC*	-1,45	V.A.1	amino acid transporter (23.6 kDa
XF1346	*CYSW OR SLR1454*	-1,95	V.A.2	ABC transporter sulfate permease (34.8 kDa)
XF2144	*PSTB OR PHOT*	1,82	V.A.2	phosphate ABC transporter ATP-binding protein (30.1 kDa)
XF1402	*PHBI*	-4,87	V.A.3	phosphotransferase system enzyme I (65.3 kDa)
XF2448	*malE*	-1,77	V.A.3	ABC transporter sugar-binding protein (49.1 kDa)
XF0599	*YBIL*	-1,72	V.A.4	TonB-dependent receptor for iron transport (85.4 kDa)
XF1401	*mgtE*	3,64	V.A.4	Mg++ transporter (31.0 kDa)
XF1223	*YADG*	-3,95	V.A.7	ABC transporter ATP-binding protein (34.2 kDa)
XF1409	*HI1148*	-1,91	V.A.7	ABC transporter ATP-binding protein (26.3 kDa)
XF2617	*UUP*	-1,39	V.A.7	ABC transporter ATP-binding protein (70.4 kDa)
XF1475	*YNHD*	1,40	V.A.7	ABC transporter ATP-binding protein (30.8 kDa)
XF1728	*f451*	1,67	V.A.7	transport protein (46.1 kDa)
XF2133	*YHES*	1,92	V.A.7	ABC transporter ATP-binding protein (71.1 kDa)
XF1322	*MINC*	-2,78	V.B	cell division inhibitor (26.2 kDa)
XF2557	*ZIPA*	2,54	V.B	cell division protein (27.3 kDa)
XF0800	*FTSQ*	-1,40	V.B,IV.A.1	cell division protein (30.8 kDa)
XF1952	*chpA*	1,86	V.C, IV.D	chemotaxis-related protein kinase (194.0 kDa)
XF0500	*RACR*	-5,98	VI.A	phage-related repressor protein (15.3 kDa)
XF0682/XF2292/XF2526		-2,27	VI.A	phage-related protein (10.5 kDa)
XF1786		-2,09	VI.A	phage-related protein (8.5 kDa)
XF2132	*INT*	-1,86	VI.A	phage-related protein (25.5 kDa)
XF2291		-1,85	VI.A	phage-related protein (28.0 kDa
XF0728	*FIIR2*	-1,59	VI.A	phage-related contractile tail tube protein (18.6 kDa

XF2530	*INT*	-1,56	VI.A	phage-related integrase (38.4 kDa)
XF1146	*ATPH OR UNCH*	-1,56	VI.A	ATP synthase, delta chain (18.8 kDa)
XF0684		-1,37	VI.A	phage-related protein (55.3 kDa)
XF1555	*INT*	1,37	VI.A	phage-related protein (32.1 kDa)
XF1658	*CI*	1,48	VI.A	phage-related repressor protein (25.7 kDa)
XF2530	*INT*	1,52	VI.A	phage-related integrase (38.4 kDa)
XF0719	*gpV*	1,52	VI.A	phage-related baseplate assembly protein (20.4 kDa)
XF2479		1,66	VI.A	phage-related protein (39.0 kDa)
XF0710/XF2501	*NOHA*	1,71	VI.A	phage-related protein (21.7 kDa)
XF2525	*dpoL*	-1,68	VI.A, III.A.1	phage-related DNA polymerase (79.8 kDa)
XFa0007	*traB or virB4*	-6,20	VI.B	conjugal transfer protein (91.5 kDa)
XFa0012	*traE or virB8*	-2,74	VI.B	conjugal transfer protein (30.6 kDa)
XF2052	*trbF*	-1,77	VI.B	conjugal transfer protein (26.8 kDa)
XFa0040	*trbI*	-1,60	VI.B	conjugal transfer protein (50.0 kDa)
XF2061	*TRAC*	-1,40	VI.B	DNA primase (154.5 kDa)
XF2053	*trbE*	1,70	VI.B	conjugal transfer protein (91.9 kDa)
XF1775	*IS629*	-2,04	VI.C	reverse transcriptase (64.7 kDa)
XF0325/XF0535		1,48	VI.C	transposase OrfA (11.9 kDa)
XF1530	*AHPC*	-2,06	VII.C	subunit C of alkyl hydroperoxide reductase (22.9 kDa)
XF1341	*cutC*	-1,43	VII.C	copper homeostasis protein (27.7 kDa)
XF2148	*KSGA OR RSMA*	1,35	VII.C	dimethyladenosine transferase (32.0 kDa)
XF2232	*cpeB*	1,52	VII.C	catalase/peroxidase (86.9 kDa)
XF1038	*St3F7.11*	1,55	VII.C	peptide synthase (54.9 kDa)
XF1621	*pbp*	2,26	VII.C	beta-lactamase-like protein (49.5 kDa)
XF0668	*FRPC*	-3,48	VII.C IV.A.2	hemolysin-type calcium binding protein (128.4 kDa)
XF2083	*CZCA*	-1,49	VII.C, V.A.7	cation efflux system protein (111.5 kDa)
XF2586	*TOLC OR MTCB OR MUKA OR REFI*	-1,48	VII.C,V.A.6	outer membrane export factor (49.5 kDa)
XF2366	*gumE*	-1,37	VII.E, III.D.1	GumE protein (47.7 kDa)
XF1516	*uspA1*	1,59	VII.F, IV.A.2	surface-exposed outer membrane protein (98.3 kDa)
XF2682	*MDOG*	-1,51	VII.G	periplasmic glucan biosynthesis protein (61.6 kDa)
XF1623	*MDOH*	1,55	VII.G	periplasmic glucan biosynthesis protein (67.9 kDa)
XF0285	*HTRA OR DEGP OR PTD*	-1,72	VII.G, III.C.3	heat shock protein (50.5 kDa)
XFa0052	*vapD*	-1,63	VII.H	virulence-associated protein D (17.6 kDa)
XF1114	*RPFC*	-1,59	VII.H	regulator of pathogenicity factors (73.1 kDa)

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XF1424	*chi*	-1,47	VII.H	chitinase (28.5 kDa)
XF1519	*XPSG OR PEFG*	-1,62	VII.H ,V.A.6	general secretory pathway protein G precursor (17.5 kDa)
XF1518	*xpsF*	-1,47	VII.H ,V.A.6	general secretory pathway protein F (44.0 kDa)
XF0472	*YGDH*	-2,25	VIII.A	conserved hypothetical protein (49.6 kDa)
XF1405	*YHBJ*	-2,15	VIII.A	conserved hypothetical protein (33.3 kDa)
XF1295	*YFCN*	-1,56	VIII.A	conserved hypothetical protein (20.1 kDa)
XF1012	*YFHC*	-1,55	VIII.A	conserved hypothetical protein (18.4 kDa)
XF1901	*YBGC*	-1,54	VIII.A	conserved hypothetical protein (17.2 kDa)
XF1442	*YLJA*	-1,48	VIII.A	conserved hypothetical protein (12.0 kDa)
XF0849	*YEAA*	-1,48	VIII.A	conserved hypothetical protein (17.5 kDa)
XF1469	*shf*	-1,40	VIII.A	conserved hypothetical protein (32.3 kDa)
XF1649	*b2360*	-1,34	VIII.A	conserved hypothetical protein (30.4 kDa)
XF0339	*BTUB OR BFE OR CER*	2,02	VIII.A	conserved hypothetical protein (97.9 kDa)
XF0460	*HI0366*	2,05	VIII.A	conserved hypothetical protein (27.6 kDa)
XF0115	*YFFB*	2,32	VIII.A	conserved hypothetical protein (14.0 kDa)
XFa0032	*SCJ21.16*	2,37	VIII.A	conserved hypothetical protein (27.1 kDa)
XF0593	*HI1008*	2,38	VIII.A	conserved hypothetical protein (12.0 kDa)
XF1293	*HI0672*	-5,99	VIII.A	conserved hypotetical protein
XF2575	*DR0386*	-5,76	VIII.A	conserved hypothetical protein (45.0 kDa)
XF0387	*YJBN*	-4,97	VIII.A	conserved hypothetical protein (37.9 kDa)
XF1243	*YRAM*	-3,25	VIII.A	conserved hypothetical protein (43.9 kDa)
XF2666	*YHGN*	-2,98	VIII.A	conserved hypothetical protein (21.6 kDa
XF1272	*RV1827 OR MTCY1A11.16C*	-2,84	VIII.A	conserved hypothetical protein (29.4 kDa)
XF2562	*YDIC*	-2,56	VIII.A	conserved hypothetical protein (12.2 kDa)
XF2010		-2,39	VIII.A	conserved hypothetical protein (19.6 kDa)
XF0461	*YFGA*	-2,25	VIII.A	conserved hypothetical protein (23.1 kDa)
XF0758	*YJEE*	-2,07	VIII.A	conserved hypothetical protein (17.4 kDa)
XF1047	*YAEL*	-2,05	VIII.A	conserved hypothetical protein (47.2 kDa)
XF1840	*zm10orf9*	-2,04	VIII.A	conserved hypothetical protein (25.1 kDa)
XF0167	*RP407*	-2,01	VIII.A	conserved hypothetical protein (46.6 kDa)
XF0461	*YFGA*	-1,88	VIII.A	conserved hypothetical protein (23.1 kDa)
XF0596	*DR1793*	-1,86	VIII.A	conserved hypothetical protein (19.5 kDa)
XF2551	*At2g47390*	-1,77	VIII.A	conserved hypothetical protein (98.7 kDa)
XF0233	*HI1282*	-1,71	VIII.A	onserved hypothetical protein (23.8 kDa)

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XF0433	*lporfX*	-1,66	VIII.A	conserved hypothetical protein (16.2 kDa)
XF1714	*YRFI*	-1,53	VIII.A	conserved hypothetical protein (19.7 kDa)
XF1571/XF1676	*HI1409*	-1,47	VIII.A	conserved hypothetical protein (52.4 kDa)
XF1126		1,34	VIII.A	conserved hypothetical protein (134.7 kDa)
XF1504	*YICC*	1,35	VIII.A	conserved hypothetical protein (34.1 kDa)
XF2669	*YDHD*	1,42	VIII.A	conserved hypothetical protein (11.7 kDa)
XF1384	*pqaA*	1,43	VIII.A	conserved hypothetical protein (58.2 kDa)
XF0556	*SC1A9.13*	1,45	VIII.A	conserved hypothetical protein (37.4 kDa)
XFa0050	*orfB*	1,52	VIII.A	conserved hypothetical protein (26.4 kDa)
XF1708		1,55	VIII.A	conserved hypothetical protein (42.5 kDa)
XF1454	*aq_449*	1,56	VIII.A	conserved hypothetical protein (19.8 kDa)
XF2573		1,57	VIII.A	hypothetical protein (33.8 kDa)
XF2010		1,58	VIII.A	conserved hypothetical protein (19.6 kDa)
XF0240	*SC1F2.10*	1,59	VIII.A	conserved hypothetical protein (15.9 kDa)
XF0842	*SCM11.14c*	1,84	VIII.A	conserved hypothetical protein (87.1 kDa)
XF1829	*RP471*	1,90	VIII.A	conserved hypothetical protein (24.5 kDa)
XF1895	*YBGF*	1,93	VIII.A	conserved hypothetical protein (30.2 kDa)
XF0497	*Rv2514c*	2,50	VIII.A	conserved hypothetical protein (17.8 kDa)
XF2616		-2,23	VIII.B	hypothetical protein (15.0 kDa)
XF1033		-1,85	VIII.B	hypothetical protein (6.8 kDa)
XF1969		-1,85	VIII.B	hypothetical protein (6.1 kDa)
XF2316		-1,84	VIII.B	hypothetical protein (15.6 kDa)
XF0301		-1,83	VIII.B	hypothetical protein (10.7 kDa)
XF0981		-1,75	VIII.B	hypothetical protein (16.8 kDa)
XF0540		-1,74	VIII.B	hypothetical protein (13.8 kDa)
XF0559		-1,74	VIII.B	hypothetical protein (6.7 kDa
XF0021		-1,74	VIII.B	hypothetical protein (10.8 kDa)
XF0850		-1,73	VIII.B	hypothetical protein (7.5 kDa)
XF0079		-1,70	VIII.B	hypothetical protein (9.1 kDa)
XF0330		-1,61	VIII.B	hypothetical protein (21.6 kDa)
XF2463		-1,59	VIII.B	hypothetical protein (23.7 kDa)
XF2454		-1,57	VIII.B	hypothetical protein (15.6 kDa)
XF0022		-1,57	VIII.B	hypothetical protein (8.2 kDa)
XF1986		-1,56	VIII.B	hypothetical protein (11.0 kDa)

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XF2549		-1,54	VIII.B	hypothetical protein (16.2 kDa)
XF0885		-1,54	VIII.B	hypothetical protein (49.3 kDa)
XF1712		-1,50	VIII.B	hypothetical protein (33.8 kDa)
XF2675		-1,50	VIII.B	hypothetical protein (33.8 kDa)
XF0161	*yydD*	-1,50	VIII.B	hypothetical protein (37.9 kDa)
XF1725		-1,49	VIII.B	hypothetical protein (9.0 kDa)
XF1117		-1,49	VIII.B	hypothetical protein (53.4 kDa)
XF0827		-1,48	VIII.B	hypothetical protein (28.1 kDa)
XF0561		-1,46	VIII.B	hypothetical protein (41.0 kDa)
XF0602		-1,45	VIII.B	hypothetical protein (8.7 kDa)
XF0964		-1,42	VIII.B	hypothetical protein (19.0 kDa)
XF1751		1,47	VIII.B	hypothetical protein (17.8 kDa)
XF2098		1,49	VIII.B	hypothetical protein (7.3 kDa)
XF2283		1,49	VIII.B	hypothetical protein (34.3 kDa)
XF1845		1,50	VIII.B	hypothetical protein (18.0 kDa)
XF0885		1,51	VIII.B	hypothetical protein (49.3 kDa)
XF1290		1,51	VIII.B	hypothetical protein (6.4 kDa)
XF1060		1,51	VIII.B	hypothetical protein (8.4 kDa)
XF0970		1,54	VIII.B	hypothetical protein (8.1 kDa)
XF1584/XF1689		1,61	VIII.B	hypothetical protein (15.7 kDa)
XF1218		1,65	VIII.B	hypothetical protein (10.5 kDa)
XF0606		2,02	VIII.B	hypothetical protein (6.5 kDa)
XF0027		2,03	VIII.B	hypothetical protein (7.3 kDa)
XF1036		2,03	VIII.B	hypothetical protein (111.1 kDa)
XF0770		2,18	VIII.B	hypothetical protein (14.9 kDa)
XF1779		2,21	VIII.B	hypothetical protein (19.9 kDa)
XF2445		2,23	VIII.B	hypothetical protein (118.5 kDa)
XF1592		3,13	VIII.B	hypothetical protein (12.1 kDa)
XF0137		-5,30	VIII.B	hypothetical protein (17.6 kDa)
XF1515		-5,29	VIII.B	hypothetical protein (7.1 kDa)
XF0386		-5,10	VIII.B	hypothetical protein (5.2 kDa)
XF0829		-4,09	VIII.B	hypothetical protein (8.3 kDa)
XF1922		-3,70	VIII.B	hypothetical protein (4.5 kDa)
XF0272		-3,66	VIII.B	hypothetical protein (21.1 kDa)

XF2445	-3,57	VIII.B	hypothetical protein (118.5 kDa)
XFa0035	-3,45	VIII.B	hypothetical protein (5.4 kDa)
XF1444	-3,44	VIII.B	hypothetical protein (7.9 kDa)
XF2191	-3,42	VIII.B	hypothetical protein (5.7 kDa)
XF2111	-3,32	VIII.B	hypothetical protein (10.4 kDa)
XF1104	-3,20	VIII.B	hypothetical protein (12.7 kDa)
XF0638	-3,04	VIII.B	hypothetical protein (21.3 kDa)
XF2758	-2,94	VIII.B	hypothetical protein (19.5 kDa)
XF0898	-2,88	VIII.B	hypothetical protein (13.0 kDa)
XF2583	-2,73	VIII.B	hypothetical protein (5.1 kDa)
XF1083	-2,67	VIII.B	hypothetical protein (6.6 kDa)
XF0471	-2,48	VIII.B	hypothetical protein (4.7 kDa)
XF2743	-2,44	VIII.B	hypothetical protein (5.6 kDa)
XF1757	-2,40	VIII.B	hypothetical protein (9.7 kDa)
XF0542	-2,36	VIII.B	hypothetical protein (6.7 kDa)
XF2277	-2,31	VIII.B	hypothetical protein (12.4 kDa)
XF0638	-2,25	VIII.B	hypothetical protein (21.3 kDa)
XF1324	-2,22	VIII.B	hypothetical protein (44.0 kDa)
XFa0017	-2,20	VIII.B	hypothetical protein (85.1 kDa)
XF2758	-2,18	VIII.B	hypothetical protein (19.5 kDa)
XF0410	-2,17	VIII.B	hypothetical protein (6.0 kDa)
XF2039	-2,15	VIII.B	hypothetical protein (32.4 kDa)
XF1135	-2,13	VIII.B	hypothetical protein (5.3 kDa)
XF2667	-2,09	VIII.B	hypothetical protein (8.8 kDa)
XF2065	-2,04	VIII.B	hypothetical protein (10.0 kDa)
XF2035	-2,02	VIII.B	hypothetical protein (10.9 kDa)
XF2001	-1,96	VIII.B	hypothetical protein (15.3 kDa)
XF2120	-1,94	VIII.B	hypothetical protein (41.7 kDa)
XF0394	-1,91	VIII.B	hypothetical protein (7.8 kDa)
XF0469	-1,89	VIII.B	hypothetical protein (9.8 kDa)
XF1661	-1,84	VIII.B	hypothetical protein (9.3 kDa
XF1758	-1,83	VIII.B	hypothetical protein (15.2 kDa)
XF1194	-1,82	VIII.B	hypothetical protein (8.0 kDa)
XF0897	-1,80	VIII.B	hypothetical protein (29.5 kDa)

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XF1874	-1,76	VIII.B	hypothetical protein (11.6 kDa)
XF2138	-1,73	VIII.B	hypothetical protein (6.1 kDa)
XF0992	-1,71	VIII.B	hypothetical protein (35.6 kDa)
XF2359	-1,67	VIII.B	hypothetical protein (26.1 kDa)
XF0850	-1,64	VIII.B	hypothetical protein (7.5 kDa)
XF0860	-1,64	VIII.B	hypothetical protein (27.2 kDa)
XF2271	-1,64	VIII.B	hypothetical protein (15.2 kDa
XF0588	-1,62	VIII.B	hypothetical protein (42.0 kDa)
XF1820	-1,60	VIII.B	hypothetical protein (9.1 kDa)
XF1880	-1,59	VIII.B	hypothetical protein (11.2 kDa)
XF0545	-1,59	VIII.B	hypothetical protein (6.6 kDa)
XF1075	-1,59	VIII.B	hypothetical protein (14.3 kDa)
XF2067	-1,55	VIII.B	hypothetical protein (10.1 kDa)
XF1330	-1,52	VIII.B	hypothetical protein (132.0 kDa)
XF0516	-1,51	VIII.B	hypothetical protein (11.6 kDa)
XF1989	-1,51	VIII.B	hypothetical protein (45.8 kDa)
XF2064	-1,48	VIII.B	hypothetical protein (7.9 kDa)
XF0351	-1,48	VIII.B	hypothetical protein (8.7 kDa)
XF0836	-1,47	VIII.B	hypothetical protein (9.8 kDa)
XFa0031	-1,44	VIII.B	hypothetical protein (38.0 kDa)
XF2169	-1,43	VIII.B	hypothetical protein (77.6 kDa)
XF1239	-1,40	VIII.B	hypothetical protein (15.6 kDa)
XF0473	-1,40	VIII.B	hypothetical protein (18.2 kDa)
XF2004	-1,39	VIII.B	hypothetical protein (23.5 kDa)
XF2377	-1,36	VIII.B	hypothetical protein (6.6 kDa)
XF0283	1,37	VIII.B	hypothetical protein (15.9 kDa)
XF2662	1,38	VIII.B	hypothetical protein (13.7 kDa)
XF2182	1,38	VIII.B	hypothetical protein (6.5 kDa)
XF0643	1,39	VIII.B	hypothetical protein (6.9 kDa)
XF0519	1,41	VIII.B	hypothetical protein (15.9 kDa)
XF1696	1,42	VIII.B	hypothetical protein (11.5 kDa)
XF2406	1,44	VIII.B	hypothetical protein (23.3 kDa)
XF1701	1,47	VIII.B	hypothetical protein (13.5 kDa)
XF2189	1,50	VIII.B	hypothetical protein (11.8 kDa)

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XF1579/XF1684	1,54	VIII.B	hypothetical protein (13.5 kDa)
XF1588	1,56	VIII.B	hypothetical protein (12.4 kDa)
XF1712	1,56	VIII.B	hypothetical protein (33.8 kDa)
XFa0053	1,59	VIII.B	hypothetical protein (21.6 kDa)
XF2711	1,59	VIII.B	hypothetical protein (11.3 kDa)
XF1277	1,67	VIII.B	hypothetical protein (7.2 kDa)
XF1386	1,69	VIII.B	hypothetical protein (5.3 kDa)
XF1394	1,71	VIII.B	hypothetical protein (5.3 kDa)
XF1095	1,73	VIII.B	hypothetical protein (5.9 kDa)
XF0646	1,74	VIII.B	hypothetical protein (15.9 kDa)
XF2382	1,80	VIII.B	hypothetical protein (8.3 kDa)
XF1704	1,83	VIII.B	hypothetical protein (42.5 kDa)
XF0293	1,89	VIII.B	hypothetical protein (6.2 kDa)
XF2441	1,90	VIII.B	hypothetical protein (12.0 kDa)
XF0477	1,96	VIII.B	hypothetical protein (6.3 kDa)
XF0201	2,01	VIII.B	hypothetical protein (22.7 kDa)
XF1109	2,01	VIII.B	hypothetical protein (32.2 kDa)
XFa0030	2,07	VIII.B	hypothetical protein (13.1 kDa)
XF1772	2,08	VIII.B	hypothetical protein (15.0 kDa)
XF1941	2,13	VIII.B	hypothetical protein (18.4 kDa)
XF1794	2,14	VIII.B	hypothetical protein (22.6 kDa)
XF1508	2,25	VIII.B	hypothetical protein (92.6 kDa)
XF1478	2,43	VIII.B	hypothetical protein (15.6 kDa)

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Table 3. Official gene categories summerized in the site of *Xylella fastidiosa* genome project: http://aeg.lbi.ic.unicamp.br/xf/ in the section "About the genome" in "Gene Categories".

I. Intermediary metabolism

a. Degradation

1. Degradation of polysaccharides
2. Degradation of small molecules

b. Central intermediary metabolism

1. Amino sugars
2. Entner-Douderoff
3. Gluconeogenesis
4. Glyoxylate bypass
5. Miscellaneous glucose metabolism
6. Non-oxidative branch, pentose pathway
7. Nucleotide hydrolysis
8. Nucleotide interconversions
9. Phosphorus compounds
10. Pool, multipurpose conversions
11. Sugar-nucleotide biosynthesis, conversions
12. Sulfur metabolism

c. Energy metabolism, carbon

1. Aerobic respiration
2. Anaerobic respiration and fermentation
3. Electron transport
4. Glycolysis
5. Oxidative branch, pentose pathway
6. Pyruvate dehydrogenase
7. TCA cycle
8. ATP-proton motive force interconversion

d. Regulatory functions

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II. Biosynthesis of small molecules

a. Amino acids biosynthesis

1. Glutamate family,nitrogen assimilation
2. Aspartate family, pyruvate family
3. Glycine-serine family|sulfur metabolism
4. Aromatic amino acid family
5. Histidine

b. Nucleotides biosynthesis

1. Purine ribonucleotides
2. Pyrimidine ribonucleotides
3. 2'-Deoxyribonucleotides
4. Salvage of nucleosides and nucleotides

c.Sugars and sugar nucleotides biosynthesis

d. Cofactors, prosthetic groups, carriers biosynthesis

1. Biotin
2. Folic acid
3. Lipoate
4. Molybdopterin
5. Pantothenate
6. Pyridoxine
7. Pyridine nucleotides
8. Thiamin
9. Riboflavin
10. Thioredoxin, glutaredoxin, glutathione
11. Menaquinone, ubiquinone
12. Heme, porphyrin
13. Biotin carboxyl carrier protein (BCCP)
14. Cobalamin
15. Enterochelin
16. Biopterin
17. Others

e. Fatty acid and phosphatidic acid biosynthesis

f. Polyamines biosynthesis

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III. Macromolecule metabolism

a. DNA metabolism

1.Replication

2. Structural DNA binding proteins (10) (2) (0)

3. Recombination

4. Repair

5. Restriction, modification

b. RNA metabolism

1. Ribosomal and stable RNAs

2. Ribosomal proteins

3. Ribosomes - maturation and modification

4. Aminoacyl tRNA synthetases, tRNA modification

5. RNA synthesis, modification, DNA transcription

6. RNA degradation

c. Protein metabolism

1. Translation and modification

2. Chaperones

3. Protein degradation

d. Other macromolecules metabolism

1. Polysaccharides

2. Phospholipids

IV. Cell structure

a. Membrane components

1. Inner membrane

2. Outer membrane constituents

b. Murein sacculus, peptidoglycan

c. Surface polysaccharides, lipopolysaccharides, and antigens

d. Surface structures

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V. Cellular processes

a. Transport

1. Amino acids, amines
2. Anions
3. Carbohydrates, organic acids, alcohols
4. Cations (24)
5. Nucleosides, purines, pyrimidines
6. Protein, peptide secretion
7. Other

b. Cell division

c. Chemotaxis and mobility

d. Osmotic adaptation

e. Cell killing

VI. Mobile genetic elements

a. Phage-related functions and prophages

b. Plasmid-related functions

c. Transposon- and intron-related functions

VII. Pathogenicity, virulence, and adaptation

a. Avirulence

b. Hypersensitive response and pathogenicity

c. Toxin production and detoxification

d. Host cell wall degradation

e. Exopolysaccharides

f. Surface proteins

g. Adaptation, atypical conditions

h. Other

VIII. Hypothetical

a. Conserved hypothetical proteins

b. Hypothetical proteins (includes no hits/only low score hits)

IX. ORFs with undefined category

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