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Life at Depth: *Photobacterium profundum* Genome Sequence and Expression Analysis

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Deep-sea life requires adaptation to high pressure, an extreme yet common condition given that oceans cover 70% of Earth's surface and have an average depth of 3800 meters. Survival at such depths requires specific adaptation but, compared with other extreme conditions, high pressure has received little attention. Recently, *Photobacterium profundum* strain SS9 has been adopted as a model for piezophily. Here we report its genome sequence (6.4 megabase pairs) and transcriptome analysis. The results provide a first glimpse into the molecular basis for life in the largest portion of the biosphere, revealing high metabolic versatility.

Ambient pressure has influenced the evolution and distribution of species in the oceans (*1*). Piezophiles have evolved in multiple lineages of the Bacteria and Archaea domains of life (*2*), and high-pressure–adapted vertebrates and invertebrates have also been characterized. To undertake a genome-wide analysis of life at high pressure, we sequenced the genome of *Photobacterium profundum* strain SS9 (hereafter called SS9), using the shotgun approach (*3*). SS9 was previously isolated at a depth of 2500 m (*4*). It was selected because it grows over a broad (90 MPa) pressure range, is amenable to genetic manipulation, and as a member of the family Vibrionaceae, is related to a number of piezosensitive microbes for which whole-genome sequence information is available for comparison.

The genome of SS9 shows a tripartite structure (*5*): a 4.1-Mbp major circular chromosome (chr. 1), a 2.2-Mbp minor circular chromosome (chr. 2), and an 80-kbp circular plasmid (Fig. 1 and table S1).

The presence of two circular chromosomes is common to other Vibrionaceae (*6*), but the SS9 chromosomes are about 25% larger than those of *Vibrio parahaemolyticus* and *Vibrio vulnificus* YJ016, the most closely related genomes to that of SS9 so far sequenced (*7*, *8*).

SS9 has the maximal number of ribosomal RNA (rRNA) operons so far identified in a bacterial genome, 14 on chr. 1 and 1 on chr. 2; this may reflect the ability to respond rapidly to favorable changes in growth conditions (*9*). The high intragenomic variation among these operons (5.13% and 2.56%, respectively, in 16*S* and 23*S* rRNA) is also consistent with the view that the various operons could have evolved to operate under particular physiological conditions (*10*).

The number of open reading frames (ORFs) unique to SS9 is unexpectedly high despite several Vibrionaceae genomes having been sequenced. This is particularly true for chr. 2, where 38.6% of the ORFs are unique, as compared with 18.7% for chr. 1. Transposons are also found at a higher frequency on chr. 2 (table S1), supporting the idea that whereas chr. 1 is more stable, containing the most "established" genes (*11*), chr. 2 is able to act as a "genetic melting pot." Moreover, many genes that are located on chr. 1, mainly those near the origin of replication, also are present on the large chromosome in other Vibrionaceae, although this is not true for genes on chr. 2 (fig. S1).

The SS9 genes have been functionally classified according to COG (Clusters of Orthologous Groups) (*12*) (table S2), Gene Ontology (*13*), and KEGG (Kyoto Encyclopedia of Genes and Genomes) (*14*). Owing to the high number of unknown genes on chr. 2, most functional COG categories are better represented on chr. 1 (fig. S2); this is especially evident for functional classes J, D, H, M, F, and O, which are involved in essential cellular processes. In contrast, class G (carbohydrate transport and metabolism) appears to be overrepresented on chr. 2, confirming that genes implicated in adaptation to the environment and to the available carbon sources are frequently found on chr. 2 (*7*, *11*).

An overview of the genome reveals features that may be related to the deep-sea environment. A notable omission from the SS9 genome are ORFs encoding light-activated photolyase genes, which is consistent with the absence of sunlight in the deep sea. A very uncommon trait is the presence of two complete operons for F_1F_0 ATP synthase, one on each chromosome (for the locus name, see table S3). SS9 contains three complete sets of cbb3 cytochrome oxidase genes; the one on chr. 2 was possibly acquired from an α-proteobacterium, along with an unusual diheme cytochrome c gene. These findings support the idea that modified electron and proton transport are necessary for metabolic activity at high pressure (*15*), as further discussed below. The reductases include those for both assimilatory and dissimilatory nitrate reduction, as well as those for tetrathionate, dimethylsulfoxide, fumarate, sulfite, and trimethylamine-N-oxide (TMAO).

To obtain a comprehensive picture of high-pressure adaptation, we used microarray technology to compare the transcriptional pro-

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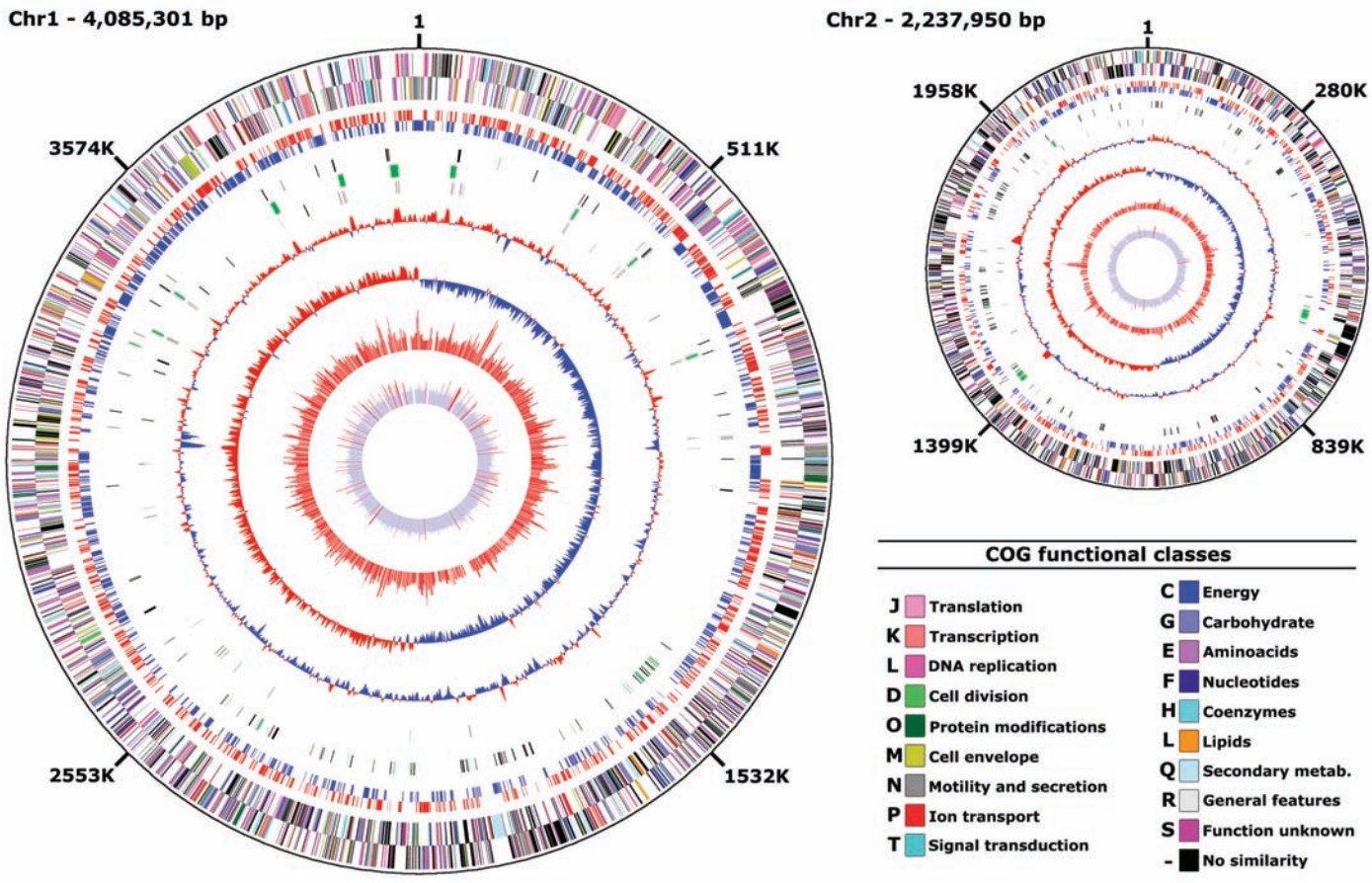

Fig. 1. Genomic organization of *P. profundum* strain SS9 chromosome 1 (left) and chromosome 2 (right). From the outside inward: The first and second circles show predicted protein-coding regions on the plus and minus strands (colors were assigned according to the color code of the COG functional classes); the third and fourth circles show *V. vulnificus* YJ016 orthologous genes in both strands; the fifth circle shows phage- (green) and transposon- (black) related genes; the sixth circle shows rRNA operons (green); the seventh circle shows tRNA (black); the eighth circle shows percentage G+C in relation to the mean G+C for the chromosome; the ninth circle shows GC skew; the 10th circle shows the mean fluorescence value of the microarray clones at 28 MPa; and the 11th circle shows CAI (scores above 0.5 units are shown in red).

file of SS9 grown at 45, 28, and 0.1 MPa. Because the results obtained at 45 and 28 MPa are very similar, we consider only the 28- and 0.1-MPa data (*5*) (table S4). The microarray experiments led to the identification of 260 differentially expressed genes that were grouped in 14 classes according to their Gene Ontology Biological Process (*13*). Comparison of the two different pressure conditions shows that most genes for amino acid transport, ion transport, protein folding, and glycolysis are up-regulated at 0.1 MPa (fig. S3).

The absolute intensity of fluorescence from the microarray analysis indicates that transcription is much more active on chr. 1 than on chr. 2. Almost all of the highly expressed genes are on chr. 1 (Fig. 1), whereas genes on chr. 2 are very poorly expressed (Fig. 1 and fig. S4). The highest spikes of expression level often correspond to high values of the codon adaptation index (CAI) (*16*). These results give further support to the hypothesis of a differential role for the two chromosomes.

Two of the highly expressed regions of chr. 2 are up-regulated at 28 MPa: one corresponding to a cluster of unknown genes and the other to the genes responsible for the Stickland reaction. So far, the complete Stickland reaction pathway has only been found in the Clostridiales and Spirochaetales (*17*), which are anaerobic bacteria. This pathway is responsible for amino acid fermentation using an amino acid reductase containing selenocysteine. The presence of a selenocysteinyl-tRNA (SeC) synthase gene, a key enzyme for the synthesis of selenoproteins, is further evidence that SS9 may also possess this mode of fermentation. It is notable that the Stickland reaction and TMAO reductase respiratory system are both up-regulated at high pressure, even though SS9 was grown under anaerobic conditions both at 0.1 and 28 MPa. A possible explanation for this finding is that at high pressure, the membrane-based cytochrome respiratory system is not fully functional (*2*) and thus requires a supplemental contribution from the above respiratory and fermentation pathways.

Complex carbohydrates are an important carbon source in oceanic abyssal environments as polymers sink down from shallower waters. We found that the regulation of metabolic pathways for the degradation of different polymers such as chitin, pullulan, and cellulose is controlled by pressure, being activated at 28 MPa and turned off at 0.1 MPa.

Confirmation that SS9 is a true piezophile comes from the observation that several stress-response genes are activated at atmospheric pressure. Indeed, four genes up-regulated at 0.1 MPa are involved in protein folding and in response to stress conditions: *htpG*, *dnaK*, *dnaJ*, and *groEL*. In *Escherichia coli*, the abundance of the proteins encoded by these genes increases after a high-pressure shock (*18*). This indicates that the proteins of this piezophilic bacterium are optimized for high pressure and require the help of these chaperones to fold correctly at 0.1 MPa. The response of SS9 to stress at 0.1 MPa is also marked by the overexpression of genes involved in DNA repair. Furthermore, there is transcriptional induction of the glycolytic pathway (fig. S5) and trehalose phosphotransferase system, which have

been shown to be up-regulated under stress conditions (*19*).

The transport of amino acids such as Trp, Lys, His, and Leu is reduced at high pressure, owing to the volume change of activation of the transport process (*20*). The finding that SS9 overexpresses amino acid transporters at 0.1 MPa is noteworthy, because the efficiency of these transporters should be higher at low pressure. These transporters may have evolved a particular protein structure to adapt to elevated pressure; therefore, their up-regulation at 0.1 MPa could compensate the reduction of functionality.

Although the deep sea represents the most common environment in the biosphere, adaptation to its prevailing conditions is still not well understood. Here we have shown that SS9 has specific metabolic, regulatory, and structural adaptations to deep-sea conditions. For example, biodegradation of relatively recalcitrant carbon sources is turned on at high pressure. In addition, the sensitivity of SS9 to low pressure is evident by the activation of different chaperones and DNA repair proteins at atmospheric pressure (*21*).

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A Functional Dosage Compensation Complex Required for Male Killing in *Drosophila*

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Bacteria that selectively kill males ("male-killers") were first characterized more than 50 years ago in *Drosophila* and have proved to be common in insects. However, the mechanism by which sex specificity of virulence is achieved has remained unknown. We tested the ability of *Spiroplasma poulsonii* to kill *Drosophila melanogaster* males carrying mutations in genes that encode the dosage compensation complex. The bacterium failed to kill males lacking any of the five protein components of the complex.

Certain isofemale lines of *Drosophila* only give rise to daughters following the death of male embryos (*1*). Male death is due to the presence of intracellular bacteria that pass from a female to her progeny and that selectively kill males during embryogenesis (*2*). These male-killing bacteria are found in a wide range of other insect species, and many different bacteria have evolved male-killing phenotypes (*3*). In some host species, male-killers drive the host population sex ratio to levels as high as 100 females per male (*4*) and alter the pattern of mate competition (*5*). However, the underlying processes that produce male-limited mortality are unclear (*6*). Here we examine the interaction between the male-killing bacterium *Spiroplasma poulsonii* and the sex determination pathway of *D. melanogaster* (*7*).

The primary signal of sex in *Drosophila* is the X-to-autosome ratio. This signal is permanently established in expression of *Sex-lethal* (*Sxl*) in females and its absence in males (*8*, *9*). This, in turn, effects three processes: germline sexual identity, somatic sexual differentiation, and dosage compensation, the process by which the gene expression titer on the X chromosome is equalized between two sexes despite their difference in X chromosome number. Mutations in the gene *tra* that convert XX individuals to male somatic sex do not induce female death (*7*). Our observations indicate germline formation and migration happen correctly in male embryos and that dying male embryos do not express *Sxl*. We, therefore, examined the requirement of the *Spiroplasma* for genes within the system of dosage compensation.

In *Drosophila*, the single X of males is hypertranscribed. This process of hypertranscription requires the formation of the dosage compensation complex (DCC) and its binding to (and modification of) the X chromosome (*10*). SXL in female *Drosophila* inhibits the production of MSL-2 protein, which is thus only present in male *Drosophila*. MSL-2 forms a complex with four other proteins, MSL-1, MSL-3, MLE, and MOF, which collectively form the DCC. MSL-1, MSL-3, MLE, and MOF are constitutively present in both males and females and are also supplied maternally. The complete DCC binds, with JIL-1, to the male X chromosome at various entry points, and, with the products of two noncoding RNAs, *RoX1* and *RoX2*, it affects the modification of the single X chromosome and its hypertranscription.

We examined the effect of mutations within the host DCC on the ability of the male-killer to function (*11*). The survival of male progeny beyond embryogenesis to L2/L3 (and in one case adult) was scored in the presence of different loss-of-function mutations within the dosage compensation system (normal male-killing occurs during embryogenesis) (*2*), in the presence and absence of infection. Because many genes within this group additionally show strong maternal effects (*12*), the effect of mutations was in each case tested by using both mothers that were heterozygous for the loss-

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