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Nascent Genomic Evolution and Allopatric Speciation of *Myroides profundus* D25 in Its Transition from Land to Ocean

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ABSTRACT A large amount of bacterial biomass is transferred from land to ocean annually. Most transferred bacteria should not survive, but undoubtedly some do. It is unclear what mechanisms these bacteria use in order to survive and even thrive in a new marine environment. *Myroides profundus* D25^T, a member of the *Bacteroidetes* phylum, was isolated from deep-sea sediment of the southern Okinawa Trough near the China mainland and had high genomic sequence identity to and synteny with the human opportunistic pathogen *Myroides odoratimimus*. Phylogenetic and physiological analyses suggested that *M. profundus* recently transitioned from land to the ocean. This provided an opportunity to explore how a bacterial genome evolved to survive in a novel environment. Changes in the transcriptome were evaluated when both species were cultured under low-salinity conditions and then transferred to high-salinity conditions. Comparative genomic and transcriptomic analyses showed that *M. profundus* altered transcription regulation in the early stages of survival. In these stages, vertically inherited genes played a key role in the survival of *M. profundus*. The contribution of *M. profundus* unique genes, some possibly acquired by horizontal gene transfer (HGT), appeared relatively small, and expression levels of unique genes were diminished under the high-salinity conditions. We postulate that HGT genes might play an important role in longer-term adaptation. These results suggested that some human pathogens might have the ability to survive in and adapt to the marine environment, which may have important implications for public health control in coastal regions.

IMPORTANCE Horizontal gene transfer (HGT) is considered to be important for bacteria to adapt to a different microhabitat. However, our results showed that vertically inherited genes might play more important roles than HGT genes in the nascent adaptation to the marine environment in the bacterium *Myroides profundus*, which has recently been transferred from land to ocean. *M. profundus* unique genes had low expression levels and were less regulated under high-salinity conditions, indicating that the contribution of HGT genes to survival of this bacterium under marine high-salinity conditions was limited. In the early adaptation stages, *M. profundus* apparently survived and adapted mainly by regulating the expression of inherited core genes. These results may explain in part why human pathogens can easily be detected in marine environments.

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Bacteria have the ability to colonize almost every available niche in Earth's biosphere and can quickly adapt to ever-changing environments (1). Bacterial genomes are relatively small and can undergo rapid evolution, which aids in their survival in a variety of environments. Environmental conditions are constantly changing, and bacteria can be moved long distances by natural forces such as wind and currents. Therefore, bacteria must employ a variety of methods to survive. It can be estimated that 3×10^{28} to 4×10^{28} prokaryotic cells are annually transferred from land to ocean by river water flow (2, 3). These transported bacteria must evolve to adapt to the newly encountered conditions in order to survive. Though the significant bacterial community differences

between land and ocean suggest that a large number of these imported cells cannot survive in the ocean, terrestrial bacteria are constantly recovered from marine environments (4). For example, human pathogens have been detected in marine water (5, 6). Therefore, it is of interest to examine how the genomes of terrestrial bacteria evolved to adapt to the marine environment. This will broaden our understanding of the emergence of bacterial diversity in the ocean.

In recent years, experimental evolution and comparative genomic studies have contributed greatly to our understanding of prokaryotic adaptation and genomic evolution (7–10). For example, a population genomics study of the marine *Photobacterium*

damselae (*Vibrio cyclitrophicus*) showed that sympatric ecological differentiation emerged with high rates of gene flow within and between habitats (9). Although bacterial genomes are relatively small, their evolution is complex. Several processes can affect bacterial genomic evolution, such as vertical inheritance and gene gain, loss, and duplication (11). However, it is still unclear which genomic evolution mechanisms affect allopatric bacterial speciation and adaptation.

The bacterium *Myroides profundus* D25^T, belonging to the family *Flavobacteriaceae* of the phylum *Bacteroidetes*, was isolated from the deep-sea sediments of the southern Okinawa Trough (12). *M. profundus* D25 can secrete myroilysin, an M12 protease that has elastinolytic activity and collagen-swelling ability (13). It also can secrete myroicolsin, another subtilisin-like protease that can degrade various collagens, especially fish-insoluble collagen, suggesting an adaptation to a marine environment (14). *M. profundus* D25 shows 99% 16S rRNA gene sequence similarity to its most closely related species, the opportunistic human pathogen *Myroides odoratimimus*. However, the DNA-DNA hybridization value corresponding to hybridization between *M. profundus* D25 and *M. odoratimimus* is 49.9%, lower than the 70% threshold that delineates a bacterial species (12). These results indicate that these two strains belong to different species but have a very close genetic relationship. Our results (see Results and Discussion) suggest that the last common ancestor of both species was terrestrial and that *M. profundus* D25 has recently transitioned from the terrestrial to the marine environment. Therefore, these two species can be considered to be involved in a nascent speciation process. By sequencing and comparing the genomes and transcriptomes of the two species, we studied how the genome of *M. profundus* D25 evolved to adapt to the new marine environment and which mechanisms contributed to the allopatric speciation process.

RESULTS AND DISCUSSION

***M. profundus* D25 has recently been transferred from land to ocean.** *M. profundus* D25 was isolated from sediment in the southern Okinawa Trough, an important depocenter of organic matter from the East China shelf, which itself is adjacent to the Changjiang River mouth. Every year, large amounts of organic materials, including bacterial biomass, are transported from mainland China to the East China shelf by Changjiang River runoff and finally deposited in the Okinawa Trough (15). The species *M. xuanwuensis*, the second-closest relative of *M. profundus* D25, was isolated from forest soil near the Changjiang River (16), showing that *Myroides* species can be found in the mainland China. Therefore, it can be postulated that *M. profundus* D25 was translocated from mainland China by river current and settled in the sediment of the Okinawa Trough.

The genus *Myroides* currently has 8 established species that have been isolated from various terrestrial and marine environments (16). The fact that *Myroides* species are consistently isolated from marine environments indicated that some species of the genus *Myroides* are genuine dwellers in the ocean. As the genus name *Myroides* (Latin, “resembling perfume”) indicates, cultures of *M. profundus* D25 can diffuse a fragrant smell. On the basis of 16S rRNA gene phylogenetic analysis, the closest characterized relative of *M. profundus* D25 is *M. odoratimimus*, isolated from human specimens (Fig. 1A) (17). The species *M. xuanwuensis*, isolated from forest soil, is also closely related to *M. profundus* D25 and *M. odoratimimus*. Two close relatives of *M. profundus* D25 are from

the terrestrial environment; thus, according to the parsimony principle, the common ancestor of species *M. profundus*, *M. odoratimimus*, and *M. xuanwuensis* should live under terrestrial conditions and *M. profundus* diverged from land to ocean. Considering the high 16S rRNA gene similarity, *M. profundus* should have diverged not long ago. This provides a unique opportunity to study bacterial early genomic evolution events during a transition from a terrestrial environment to a marine environment.

Several physiological traits support the conclusion that *M. profundus* D25 may have been recently transferred from a terrestrial to a marine environment. Salinity is an important environmental factor discriminating terrestrial and marine conditions (4). All the established species of genus *Myroides* can grow without NaCl, implying that the ancestor of this genus lived under low-salinity conditions. The optimal salinity condition for growth of *M. profundus* D25 was approximately 0.9% (1/4× sea water), showing that the strain has not totally adapted to high-salinity conditions (Fig. 1B). However, *M. profundus* D25 grew well in medium prepared with seawater (salinity, 3.5%). *M. profundus* D25 can tolerate a higher NaCl concentration than *M. odoratimimus* (18). Thus, *M. profundus* D25 is better adapted to high salinity than *M. odoratimimus*. This high-salinity-tolerance ability was also observed in other *Myroides* species isolated from the marine environment. For example, the species *M. pelagicus* isolated from seawater can tolerate up to 9% NaCl (18). *M. profundus* D25 was originally isolated from a cold deep-sea environment. *M. profundus* D25 can grow, although slowly, at 4°C (the optical density at 600 nm [OD₆₀₀] reached ~0.5 after 1 month of growth), while *M. odoratimimus* did not grow at 4°C even after 50 days of culture. The high-salinity tolerance and ability to grow at low temperature suggest that *M. profundus* D25 can indeed live in the marine environment.

Whole-genome structure comparison. We sequenced the complete genome of *M. profundus* D25. The genome size was 4.1 Mbp, and the G+C content was 33.8% (Table 1 and Fig. 2). The type strain *M. odoratimimus* CCUG 39352^T was selected for comparison in this study, and its draft genome was sequenced. The two genomes have the same G+C content and very similar genome sizes (Table 1). The average nucleotide identity (ANI) between the two genomes was 94%, indicating high genetic relatedness between these two species. This is consistent with the 16S rRNA gene analysis (Fig. 1A). These two strains showed high genomic synteny except for one inversion of 60 kbp in the *M. profundus* D25 genome (see Fig. S1 in the supplemental material). Overall, the two genomes showed no other substantial genomic rearrangements, indicating that the two species diverged recently.

Functions of quickly and slowly evolving orthologous proteins. *M. profundus* D25 and *M. odoratimimus* CCUG 39352 had 2,991 orthologous proteins, representing 84% and 81% of all the proteins of the two genomes, respectively. Of the 2,991 orthologous protein pairs, 81% (2,428 protein pairs) had sequence identity above 95%, further supporting the idea of the close relatedness of these two strains. The protein sequence identity and the rate of nonsynonymous substitutions per site (K_a) between two protein-encoding genes displayed a good linear relationship (Fig. 3A). The 95% protein sequence identity roughly corresponded to a K_a value of 0.02, which was the average calculated K_a value. We then grouped the orthologous proteins of both strains into two categories. One category contained orthologous proteins with sequence identity of >95% and K_a of ≤0.02. This category, containing 2,300 proteins, can be considered to represent slowly evolving

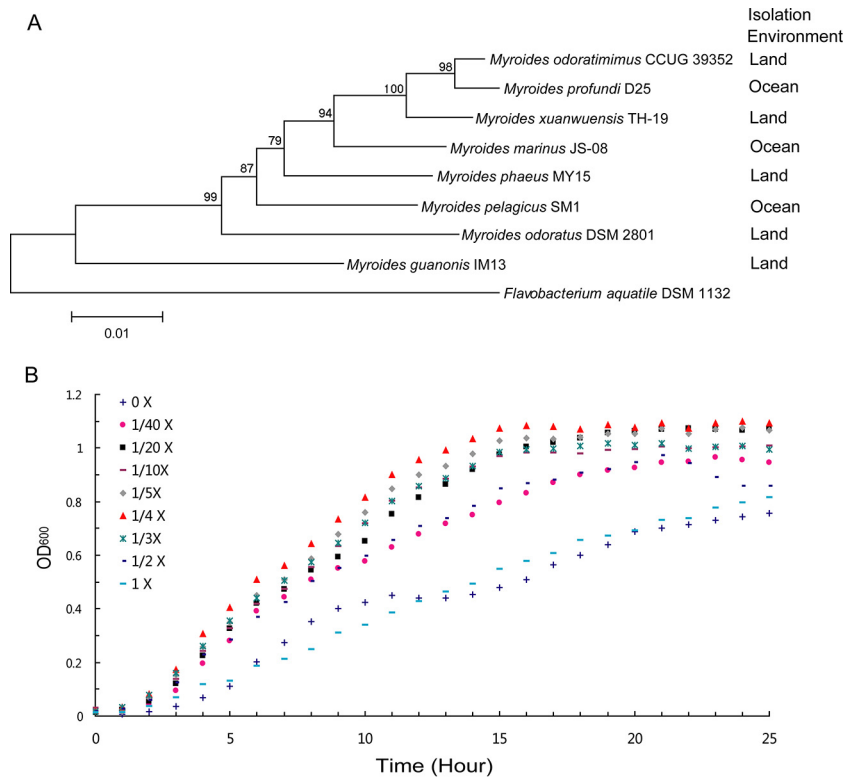


FIG 1 Phylogenetic and physiological characteristics of *Myroides profundus* (D25). (A) Relationship and isolation environment of the species of the genus *Myroides*. The tree was constructed using the 16S rRNA gene and the neighbor-joining method with 1,000 bootstrap replications (only values >50% are shown). (B) Growth curves of *M. profundus* D25 cultured in media prepared with seawater samples that were diluted to different degrees. The standard deviation for each data point was mostly lower than 0.03 (5 replicates); thus, to ensure the clarity of the figure, standard deviations are not shown.

proteins, and the functions of the proteins of this category should be conserved. The function analyses of clusters of orthologous groups (COGs) showed that the slowly evolving proteins had a higher proportion with function assigned to energy production, amino acid metabolism, and translation (Fig. 3B). This result was anticipated, as these functional groups are fundamental and are generally well conserved.

The second group, containing the remaining 691 orthologous proteins, can be considered quickly evolving proteins, as they accumulated more nonsynonymous substitutions than the slowly evolving proteins did during the same evolution time period. The

common ancestor of *M. profundus* D25 and *M. odoratimimus* CCUG 39352 was supposed to be from land, and *M. profundus* D25 was transferred to ocean recently. We then assumed that the genome of *M. profundus* D25 has acquired more changes under the new conditions. Therefore, these quickly evolving proteins of *M. profundus* D25 may confer to the strain some traits that are important for survival in the marine environment.

The quickly evolving proteins had a larger proportion of proteins with function assigned to transcription, signal transduction, and DNA recombination and repair. Horizontal gene transfer (HGT) is prevalent in nature and can contribute greatly to bacterial niche expansion. Successful HGT requires foreign DNA to be integrated into the genome, a process in which DNA recombination and repair systems play important roles (19). In *M. profundus* D25, more proteins involved in DNA recombination and repair systems were grouped with the quickly evolving proteins which have led to the increased ability of *M. profundus* D25 to acquire new genes via HGT. This will be useful for *M. profundus* D25 to acquire new physiological traits to adapt to the new environment. When bacteria encounter a new environment, phenotypes should quickly change because it is the phenotype that is really under selection. Mutations in a few regulatory genes, especially those encoding global regulators, could be responsible for important phenotypic differences (7). Many proteins related to transcription regulation changed quickly in the *M. profundus* D25 genome, showing that the strain tended to remodel the gene regulation network to adapt to the new environment in the early adaptation stage. The

TABLE 1 General features of the genomes of *M. profundus* D25 and *M. odoratimimus* CCUG 39352

Genome parameter	Value	
	<i>M. profundus</i> D25	<i>M. odoratimimus</i> CCUG 39352
Size (Mbp)	4.1	4.2
Scaffold no.	1	65
G+C content (%)	33.8	33.8
ORF ^a no.	3,548	3,715
Average size of ORFs (bp)	972	969
No. of rRNA operons (16S-23S-5S)	5	1
No. of tRNAs	100	74
ANI to <i>M. profundus</i> D25 (%)	100	94

^a ORF, open reading frame.

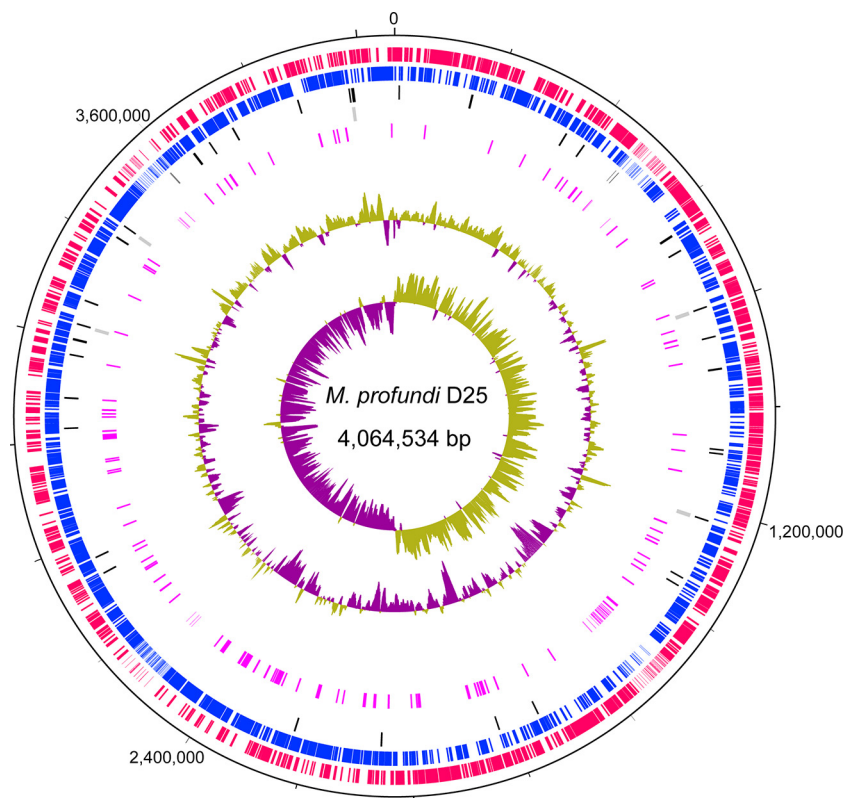


FIG 2 Circular representation of the *M. profundus* D25 genome. From the outside inward, the first and second circles show predicted coding regions transcribed in clockwise and counterclockwise directions, respectively. The third and fourth circles show the tRNA and rRNA genes, respectively. The fifth circle shows unique genes gained via HGT. The sixth and seventh circles show G+C content and a GC skew plot, respectively.

signal transduction-related proteins also changed rapidly. This showed that in the marine environment, *M. profundus* D25 adapted to change the signal transduction system to respond to different signal molecules. Evolution of signal transduction proteins and transcriptional regulators can confer to *M. profundus* D25 the potential to change the phenotype quickly to live under changing surrounding conditions.

Source and function of unique genes in *M. profundus* D25.

M. profundus D25 and *M. odoratimimus* CCUG 39352 had 557 and 723 species-unique genes, respectively. We further filtered the unique genes of these two strains by searching against all available genomes of the genus *Myroides*. After filtration of the genes that had hits in other *Myroides* genomes, the numbers of unique genes in *M. profundus* D25 and *M. odoratimimus* CCUG 39352 were 227 and 133, respectively. The number of unique genes was much lower than that reported in other studies (20, 21). These data also supported the notion of a recent divergence of these two species because only a few foreign genes have accumulated in the genome.

More unique genes in *M. profundus* D25 supported the assumption that *M. profundus* D25 faced new conditions after divergence. These 227 unique genes in *M. profundus* D25 had no orthologs in the genus *Myroides*; thus, these unique genes can be considered HGT genes acquired from organisms outside the genus *Myroides*. For 92 of the 227 unique genes in *M. profundus* D25, the source of the genes was detected and a putative donor species was identified. The donor species encompassed 6 bacterial phyla (Fig. 4A). The primary sources of the unique genes were members of the bacterial phylum *Bacteroidetes*. Gene transfer from the *Bacteroidetes*

represented ~59% of the total number of unique genes that had putative donor species. Many of the putative donor species are associated with marine and animal habitats. Interestingly, three genes had donors from freshwater species of *Cyanobacteria*. This implied that a direct ancestor of *M. profundus* D25 might have lived for some time in freshwater, which is consistent with the prediction that this strain has been transferred to the ocean by river flow. There were 135 unique genes that had no identifiable donors, indicating that many of these genes are of unknown origin and that their functions are as yet unknown.

Overall, the unique genes were distributed randomly in the genome (Fig. 2), which is consistent with other studies (22–24). Most of the unique genes were annotated as hypothetical proteins and had no putative functions. However, 11 unique genes, located in the genomic region containing genes from MPR_2125 to MPR_2141, were predicted to play a role in exopolysaccharide synthesis (Fig. 4B; see also Table S1 in the supplemental material). This genomic region was flanked by transposases, a common characteristic of HGT genes. The ability of *M. profundus* D25 to produce exopolysaccharide was analyzed and resulted in the production of 0.8 ± 0.03 g/liter exopolysaccharide, confirming the activity of this gene cluster. The role of exopolysaccharide in the survival of marine bacteria has been previously described (25). This indicates that some of the unique genes that may be acquired by HGT are functional in *M. profundus* D25 and perhaps played a role in the adaptation to marine conditions.

Salinity adaptation of *M. profundus* D25. Salinity is the primary environmental factor that can affect bacterial community

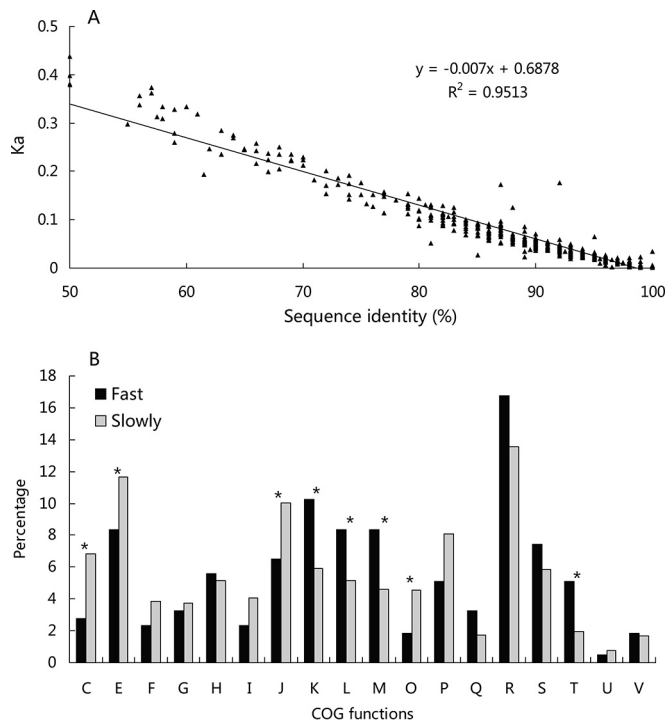


FIG 3 Analyses of the orthologous proteins between *M. profundus* D25 and *M. odoratimimus* CCUG 39352. (A) Relationship between the protein sequence identity and the rate of nonsynonymous substitutions per site (K_a) for protein-encoding genes. (B) Comparison of the identified COG functions of quickly and slowly evolving orthologous proteins. A star indicates that the percentage of the function category is statistically significantly different ($P < 0.05$, 2-sample proportions test). COG functional categories: energy production and conversion (C); amino acid transport and metabolism (E); nucleotide transport and metabolism (F); carbohydrate transport and metabolism (G); coenzyme transport and metabolism (H); lipid transport and metabolism (I); translation, ribosomal structure functions, and biogenesis (J); transcription (K); replication, recombination, and repair (L); cell wall/membrane/envelope biogenesis (M); posttranslational modification, protein turnover, or chaperone functions (O); inorganic ion transport and metabolism (P); secondary metabolite functions (Q); general function prediction only (R); unknown function (S); signal transduction mechanism functions (T); intracellular trafficking, secretion, and vesicular transport (U); defense mechanism functions (V).

structure (4, 26). As both strains can grow at the salinity of $1\times$ seawater, transcriptomes of *M. profundus* D25 and *M. odoratimimus* CCUG 39352 grown in media prepared with $1\times$ and $1/4\times$ seawater were sequenced using transcriptome sequencing (RNA-seq) technology to investigate which genes were differentially expressed and to compare the gene regulation characteristics of both strains in a high-salinity environment. The two strains had very different gene expression patterns when cultured under high-salinity compared to low-salinity conditions (see Fig. S2 in the supplemental material), indicating that each strain had its own gene regulation responses to the high-salinity stress. The numbers of genes that were up- and downregulated under high-salinity conditions were also different. There were 298 and 148 genes that were up- and downregulated more than 2-fold, respectively, in *M. profundus* D25, while there were 399 and 635 genes that were up- and downregulated more than 2-fold, respectively, in *M. odoratimimus* CCUG 39352. This comparative transcriptomic result was consistent with the genomic analysis results indicating that

M. profundus D25 tended to remodel the gene regulation network to adapt to the marine environment.

We then mainly focused on the differentially expressed genes in *M. profundus* D25 to investigate which genes were related to high-salinity adaptation. COG function comparisons showed that the upregulated genes encoded a higher proportion of proteins involved in transcription and inorganic ion transport and metabolism (Fig. 5). This may indicate that *M. profundus* D25 altered transcription to produce more ion transporters to combat the high-salinity stress. There were 49 genes that were upregulated more than 4-fold under conditions of high salinity (see Table S2 in the supplemental material). The most extensively upregulated gene was MPR_3295, encoding a trimethylamine monooxygenase that can oxidize trimethylamine to trimethylamine *N*-oxide (TMAO) (27). The orthologous MPR_3295 gene in *M. odoratimimus* CCUG 39352 was also upregulated more than 4-fold at high salinity. TMAO is a common organic compatible osmolyte in marine organisms that can aid in maintaining the cellular osmotic balance (28, 29). The fact that the MPR_3295 gene was significantly upregulated under high-salinity conditions indicates that *M. profundus* D25 may accumulate TMAO to regulate its cellular osmotic balance. There were four genes (MPR_0486, MPR_0487, MPR_0662, and MPR_2201) encoding protease or peptidase whose expression levels were upregulated more than 4-fold at high salinity. Particularly interesting is the increase in expression of the well-characterized protease myroilysin (MPR_2201) (14). Myroilysin showed high elastinolytic activity and collagen-swelling ability. An ortholog of MPR_2201 with 97% protein sequence identity was identified in the pathogen *M. odoratimimus* CCUG 39352 and may have a function in the infection process because of its collagenolytic activity. In *M. profundus* D25, myroilysin is hypothesized to play a role in degradation of deep-sea sedimentary insoluble particulate organic nitrogen. The orthologs in these different strains may have different ecological functions. Many genes that are downregulated under conditions of high salinity encoded proteins related to energy production and conversion as well as carbohydrate transport and metabolism, which was consistent with the experimental result showing that the growth of *M. profundus* D25 was impaired under high-salinity conditions.

HGT genes are thought to play an important role in new niche expansion (30, 31). In this study, the filtered unique genes in *M. profundus* D25 might have been obtained by HGT. The expression levels of the unique genes were investigated to study whether these genes played a significant role in adaptation to high salinity. Of the 298 upregulated genes, 13 (~4%) genes were identified as unique, which was slightly lower than the proportion of unique genes to total genes. Moreover, among the 49 genes whose expression levels were upregulated more than 4-fold, none were identified as unique genes or as possibly recently acquired genes. This suggests that only a small percentage of genes unique to *M. profundus* D25 were specifically involved in adaptation to high salinity. The RPKM (reads per kilobase of exon model per million mapped reads) results also supported this conclusion. The RPKM value can be viewed as a metric for comparing gene expression levels under different conditions. Under high-salinity conditions, the average and median RPKM values of unique genes were 75 and 37, respectively, while the average and median RPKM values of total detected genes were 320 and 92, respectively (Fig. 6A). The unique genes acquired by HGT were obtained from other organisms, and their DNA compositions may have been abnormal compared to

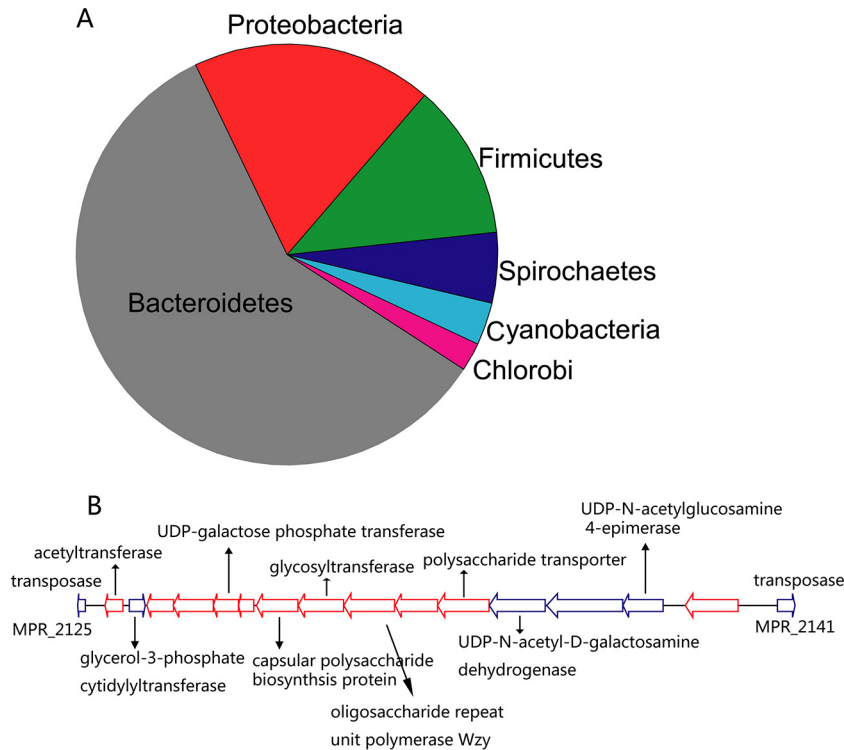


FIG 4 (A) Putative sources of unique genes at the phylum level. (B) Exopolysaccharide biosynthesis gene cluster. Unique genes are shown in red.

those of the host genes. The G+C content of unique genes in *M. profundus* D25 was significantly lower than that of other genes (Fig. 6B). This might explain why the expression levels of these genes were lower than those of *Myroides* core genes. In addition, unique genes acquired by HGT may not be incorporated in the host regulatory networks, resulting in low expression levels under certain conditions. Under high-salinity conditions, these genes showed relatively low expression levels and their expression levels were minimally regulated. This shows that the unique *M. profundus* D25 genes were less important and contributed little to the new niche adaptation in the time period of early divergence. The vertically inherited genes and remodeling of the gene expression sys-

tem were more important in the process of adaptation of *M. profundus* D25 to a high-salinity environment.

The exopolysaccharide biosynthesis cluster was an exception, however. Six unique genes in this cluster had RPKM values of more than 100, which is consistent with the result showing that the strain produced exopolysaccharide under the high-salinity conditions. This also implies that the genes gained as a cluster could easily function in the host cell.

Implications for bacterial allopatric speciation. The speciation of *M. profundus* D25 can be considered a bacterial allopatric speciation process. In the nascent speciation stage, regulation of inherited genes played a primary role in survival in a new environment. Then, mutations of existing genes contributed to the speciation process. Our results showed that different genes had different rates of evolution. Gene gain by HGT may accelerate the allopatric speciation. In the new marine environment, *M. profundus* D25 would have had the opportunity to exchange DNA with surrounding marine bacteria. This would have increased the genetic distance between *M. profundus* D25 and its terrestrial relatives, leading to the emergence of a new species. Considering the large quantity of bacteria that are being transferred from land to ocean, it is conceivable that allopatric speciation of terrestrial bacteria can contribute greatly to the diversity of the marine bacterial community.

Conclusion and perspective. When *M. profundus* D25 was transferred from the land to the ocean, it had to face a significantly different environment. The genome had to evolve to adapt to the newly encountered environment. In the nascent adaptation process, survival was achieved mainly by regulating the expression of core ancestral genes. For bacteria, HGT plays a key role in the

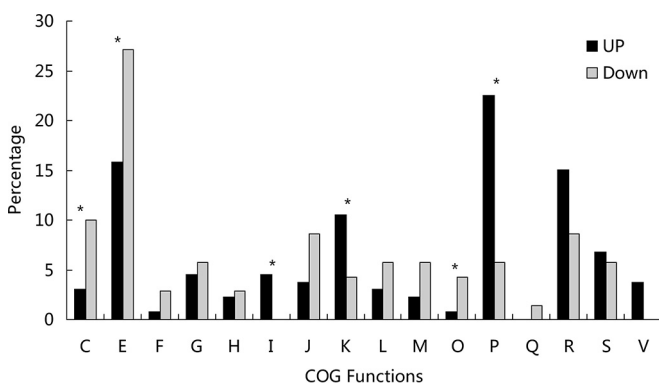


FIG 5 Comparison of COG functions of up- and downregulated genes growing under high-salinity conditions. A star indicates that the percentage of the category is statistically significantly different ($P < 0.05$, 2-sample proportions test). COG functional categories are described in the Fig. 3 legend.

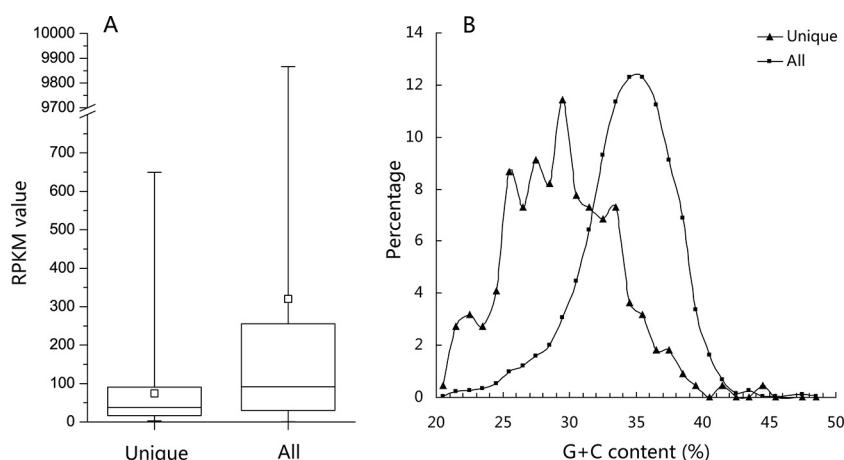


FIG 6 RPKM and G+C content analyses of unique genes and all genes of *M. profundii* (D25). (A) Box charts of RPKM values determined for growth of *M. profundii* D25 under high-salinity conditions. (B) G+C content distribution of unique genes and all genes. A dot represents the percentage of G+C content region corresponding to the x axis number ± 0.5 .

acquisition of novel abilities and the potential to adapt to a different microhabitat (9, 32). In this study, however, HGT made only a small contribution to the nascent adaptation to a novel environment. A foreign gene integrated into a bacterial genome should be beneficial for the recipient host, or it will be lost quickly (19). Recently gained genes may undergo rapid turnover in the genome (20, 33). Therefore, in the nascent adaptation process of *M. profundii* D25, many HGT genes may be under selection and have no obvious selective advantages. HGT may help *M. profundii* D25 to thrive in the new marine environment, exemplified by the acquisition of exopolysaccharide synthesis genes. These results suggested that human pathogens could survive in the marine environment using a similar mechanism, which is consistent with previous reports (5, 6). The salinity of the human environment is approximately 0.9%. Pathogens are preadapted to this salinity of the human body; thus, they can survive in the higher-salinity marine environment. Similar phenomena have been observed in other experimental evolution studies (34, 35). This has important implications for public health in marine environments, especially in coastal regions.

MATERIALS AND METHODS

Bacterial strain and phenotypic characteristics. Strain *M. odoratimimus* CCUG 39352^T (JCM 7460^T; LMG 4029^T) was purchased from Japan Collection of Microorganisms (JCM). Strain *M. profundii* D25^T (CCTCC M 208030^T; DSM 19823^T) was routinely kept in our laboratory. Both strains can grow in 2216E medium containing 5 g peptone, 1 g yeast extract, and 1 liter artificial seawater (containing 35 g sea salt [Sigma, USA]) (pH 7.5). To investigate the effect of salinity on the growth of *M. profundii* D25, artificial seawater diluted 2 \times , 3 \times , 4 \times , 5 \times , 10 \times , 20 \times , and 40 \times with freshwater was used to prepare modified 2216E medium. The corresponding media were termed 1/2 \times , 1/3 \times , 1/4 \times , 1/5 \times , 1/10 \times , 1/20 \times , and 1/40 \times seawater media. “1 \times medium” refers to undiluted seawater, and “0 \times medium” means that freshwater instead of seawater was used to prepare the medium. The growth curves of *M. profundii* D25 cultured in different media were measured using a Bioscreen C MBR instrument (Oy Growth Curves Ab Ltd., Finland) at 30°C. The exopolysaccharide concentration was determined using the phenol-sulfuric acid method (36).

Genome sequencing and assembly. The complete genome of *M. profundii* D25 was sequenced using an Illumina HiSeq2000 instrument with library insertion sizes of 500 bp, 2 kbp, 5 kbp, 8 kbp, and 10 kbp. The reads

were 90 bp long, and the data were assembled with SOAPdenovo software into 14 large scaffolds. A fosmid library with an average insertion size of 35 kbp was constructed and sequenced for scaffold orientations and genome structure validation. Gaps were closed by primer walking and PCR segment sequencing. The Phred-Phrap-Consed package (37) was used for genome assembly and finishing. The draft genome of *M. odoratimimus* CCUG 39352 was sequenced using an Illumina MiSeq platform with a paired-read length of 300 bp and assembled with SOAPdenovo software.

Genomic and comparative genomic analyses. The gene prediction and annotation were performed using methods previously described (38). The whole-genome alignment was performed using MUMmer software (39). The ANI value was calculated using jSpecies software (40). The proteins that were orthologous in *M. profundii* D25 and *M. odoratimimus* CCUG 39352 were determined using the bidirectional best BLASTp hit method with an E value of less than 1e-10, sequence identity of more than 40%, and an alignable region of the query protein sequence of more than 50%. The unique genes in every species were further filtered by searching other available *Myroides* genomes using the BLASTp program with 40% sequence identity and an E value of less than 1e-10. Only the genes that had no hit to *Myroides* genomes were considered unique genes in every genome. The protein sequences of orthologs were aligned using Muscle (41). The corresponding nucleotide sequences of the aligned protein sequences were subsequently aligned using the pal2nal script (42). Rates of nonsynonymous substitutions per site (K_a) were calculated by using KaKs_Calculator (43). The 16S rRNA gene phylogenetic tree was built using the neighbor-joining method embodied in the MEGA5 program with the Tamura 3-parameter model and 1,000 bootstrap replications (44).

Transcriptome analyses. For transcriptome sequencing, strains *M. profundii* D25 and *M. odoratimimus* CCUG 39352 were cultured in 1/4 \times seawater 2216E medium to an OD₆₀₀ of 0.6, and then the cells were collected by centrifugation. The collected cells were divided into two aliquots. One aliquot was washed with 1/4 \times seawater and resuspended in 1/4 \times seawater 2216E medium. Another aliquot was washed with 1 \times seawater and resuspended in 1 \times seawater 2216E medium. After 30 min of growth at 30°C with a shaking rate of 180 rpm, the cells cultured in both media were collected by centrifugation, and total RNA was isolated from RNA-stabilized bacteria with an RNeasy Protect Bacteria minikit (Qiagen, Germany). The transcriptome sequencing of two samples was performed by the Beijing Genomics Institute (BGI) (China). The cDNA libraries were sequenced using an Illumina HiSeq2000 instrument with a read length of 90 bp. After clean sequencing data were obtained, the reads were mapped to the *M. profundii* D25 genome and the *M. odoratimimus* CCUG 39352 genome with SOAP2 (45), respectively. Gene expression

was normalized using the RPKM (reads per kilobase of exon model per million mapped reads) method, and the RPKM value of each gene was used to compare levels of expression variation under different conditions (46). A gene was considered to be differentially regulated under two sets of conditions when the gene showed a >2-fold absolute change ratio and displayed a false-discovery-rate (FDR)-adjusted *P* value of <0.01.

Accession numbers. The complete genome sequence of *M. profundus* D25 was submitted to GenBank with accession number CP010817. All the RNA-seq read data of two strains have been deposited in NCBI's sequence read archive (SRA) under project accession number PRJNA272637.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01946-15/-DCSupplemental>.

Figure S1, TIF file, 0.5 MB.

Figure S2, TIF file, 0.2 MB.

Table S1, DOC file, 0.04 MB.

Table S2, DOC file, 0.1 MB.

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