Genomics update

Genomics against flatulence

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In the past several months, eukaryotic genome sequencing has brought us the complete genome of the tiny green alga *Ostreococcus lucimarinus* (Palenik et al., 2007) and a draft genome of the mosquito *Aedes aegypti* (Nene et al., 2007). Given that the genome sequence of another *Ostreococcus* species, *Ostreococcus tauri*, has been sequenced less than a year ago (Derelle et al., 2006), the availability of the *O. lucimarinus* genome opens a possibility to study the evolution of this unicellular planktonic organism and its adaptation to the life in the surface layer of the sea. There has been also exciting news from archaeal and bacterial genome sequencing. The list of recently sequenced genomes (Table 1) includes bacteria that inhabit a variety of ecological niches and degrade numerous environmental contaminants, as well as two \(\gamma\)-proteobacteria with near-minimal gene sets.

Archaea (archaebacteria) are generally viewed as freeliving organisms capable of surviving at extremely high temperatures, salt concentrations and extreme pH values. The discoveries, primarily in the past several years, of various mesophilic archaea have done little to shatter the perception of archaea as exotic organisms with little relevance to everyday human life. Indeed, there are no known human pathogens among the *Archaea*. Nevertheless, archaea play a key role in human gut, accounting for one of its activities that usually goes unmentioned, namely, production of gas. Some of this gas, consisting largely of methane and hydrogen, makes it all the way back the gastrointestinal tract and shows up in human breath. Most of the gas, however, is released from the large intestine of the human body. Taking into account the contribution of methanogenic archaea to the global warming (see our previous column, Galperin, 2007), a search for antimethanogen

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| Species name | Taxonomy | GenBank accession | Genome size (bp) | Proteins (total) | Sequencing centre* | Reference |
|--------------|----------|------------------|------------------|------------------|--------------------|-----------|
| *Ostreococcus lucimarinus* | Eukaryota, Viridiplantae | CP000581–CP000601 | 13 200 000 (Total) | 7651 | JGI | Palenik et al. (2007) |
| *Methanobrevibacter smithii* | Euryarchaeota | CP000678 | 1 853 160 | 1795 | Washington U. | Samuel et al. (2007) |
| *Metallosphaera sedula* | Euryarchaeota | CP000682 | 2 191 517 | 2256 | JGI | Unpublished |
| *Pyrobaculum arsenaticum* | Euryarchaeota | CP000660 | 2 121 076 | 2298 | JGI | Unpublished |
| *Clavibacter michiganensis* | Actinobacteria | AM711867 | 3 297 891 | 69 989 | 27 357 | 3029 University of Bielefeld, Germany |
| *Mycobacterium gilvum* | Actinobacteria | CP000656–CP000659 | 5 982 829 (Total) | 5579 | JGI | Unpublished |
| *Salinispora tropica* | Actinobacteria | CP000667 | 5 183 331 | 4536 | JGI | Unpublished |
| *Flavobacterium johnsoniae* | Bacteroidetes | CP000685 | 6 096 872 | 5017 | JGI | Unpublished |
| *Prosthecochloris vibrioformis* | Chlorobi | CP000607 | 1 966 858 | 1753 | JGI | Unpublished |
| *Dehalococcoides sp. BAV1* | Chloroflexi | CP000688 | 1 341 892 | 1371 | JGI | Unpublished |
| *Roseiflexus sp. RS-1* | Chloroflexi | CT978603 | 2 224 914 | 4517 | JGI | Unpublished |
| *Synechococcus sp. RCC307* | Cyanobacteria | CT971583 | 2 366 980 | 2533 | JGI | Unpublished |
| *Caldicellulosiruptor saccharolyticus* | Firmicutes | CP000679 | 2 970 275 | 2679 | JGI | Unpublished |
| *Bradyrhizobium sp. BTAi1* | α-Proteobacteria | CP000494–CP000495 | 8 264 687 | 228 826 | JGI | Unpublished |
| *Bradyrhizobium sp. ORS278* | α-Proteobacteria | CP000694 | 5 801 498 | 4517 | JGI | Unpublished |
| *Streptococcus suis 05ZYH33* | α-Proteobacteria | CP000407 | 2 970 275 | 2679 | JGI | Unpublished |
| *Streptococcus suis 98HAH33* | α-Proteobacteria | CP000408 | 2 970 275 | 2679 | JGI | Unpublished |
| *Acidiphilium cryptum* | α-Proteobacteria | CP000689–CP000697 | 3 963 080 (Total) | 3559 | JGI | Unpublished |
| *Polynucleobacter sp. QLW-P1DMWA-1* | β-Proteobacteria | CP000655 | 2 159 490 | 2077 | JGI | Unpublished |
| *Aeromonas salmonicida* | γ-Proteobacteria | CP000664 | 4 702 402 | 166 749 | 155 098 | 4413 NRC – Halifax | Unpublished |
| *Enterobacter sp. 638* | γ-Proteobacteria | CP000653 | 4 518 712 | 157 749 | 4240 | JGI | Unpublished |
| *Psychrobacter sp. PRwf-1* | γ-Proteobacteria | CP000713 | 2 978 987 | 2385 | JGI | Unpublished |

*Note: JGI = Joint Genome Institute,* "Sebiahi et al. (2007)" refers to the reference "Sebiahi, et al. (2007)" and "Sirand-Pugnet et al. (2007)" refers to the reference "Sirand-Pugnet, et al. (2007)."
compounds could be quite an important undertaking. Accordingly, studies of methanogenesis and archaeal metabolism in general, which until recently appeared to be of purely academic interest, are suddenly finding unexpected applications in drug design.

Two other recently sequenced archaeal genomes belong to *Crenarchaeota*. *Metallosphaera sedula* strain DSM 5348 is an aerobic thermoacidophile related to *Sulfurovum* spp. that was first isolated from a thermal pond in the Pisciarelli Solfatara in Italy (Huber et al., 1989). It can grow at temperatures ranging from 50 to 79°C with optimal growth at 74°C and pH 2. *Metallosphaera sedula* is capable of oxidizing sulfidic ores, such as pyrite, making it an attractive organism for use in bioleaching of metals. Several of its respiratory complexes have been characterized (Kappler et al., 2005); the genome sequence should allow identification of the rest of them.

Just 2 months after completing the sequence of *Pyrobaculum calidifontis* (GenBank accession number CP000561), JGI scientists have released the genome of *Pyrobaculum arsenaticum*, the fourth member of that genus with a completely sequenced genome. *Pyrobaculum arsenaticum* is a strictly anaerobic, hyperthermophilic archaean, isolated from a hot spring in Italy. It could grow chemoaerotrophically with CO₂ as carbon source, H₂ as electron donor and arsenate, thioulate or elemental sulfur as electron acceptors (Huber et al., 2000). It could also grow organotrophically, using sulfur, selenite or arsenite as electron acceptors (Huber et al., 2000). The genome of *Pyrobaculum arsenaticum* is the second phytopathogen. The sequenced strain *C. michiganensis* ssp. *michiganensis* is only the second phytopathogen. The sequenced strain *C. michiganensis* ssp. *michiganensis* is a pathogen of tomato that causes bacterial wilt and canker. This species includes four other subspecies. One of them, *C. michiganensis* ssp. *sepedonicus*, is responsible for ring rot of potato; its genome is being sequenced at the Sanger Institute. Three other subspecies of *C. michiganensis* infect, respectively, maize, wheat and alfalfa (Jahr et al., 1999; Gartemann et al., 2003).

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in the sediment from the Grand Calumet River in Indiana as a strain capable of using pyrene as a sole source of carbon and energy. This strain was also capable of metabolizing such polycyclic aromatic hydrocarbons (PAHs) as phenanthrene and fluoranthene, but not naphthalene, chrysene, anthracene, fluorene, or benz[a]pyrene (Dean-Ross and Cerniglia, 1996). The first step of pyrene degradation is catalysed by the same two-subunit aromatic ring-hydroxylating dioxygenase NidAB as the one found in Mycobacterium vanbaalenii and other PAH-degrading mycobacteria (Brezna et al., 2003). Thus, M. gilvum probably differs from them in the downstream steps of PAH degradation, which now could be deduced through genome comparisons.

Salinispora tropica strain CNB-440 is a marine actinomycete, the first sequenced representative of the unique genus within the phylum Actinobacteria that is widespread in tropical and subtropical marine sediments (Jensen and Mafnas, 2006). Its cultivation has been achieved by including seawater into the growth medium (Mincer et al., 2002), which is why Salinispora spp. are now considered obligate marine bacteria. Accordingly, comparing the genome of S. tropica to other actinobacterial genomes offers a chance to examine how members of this genus have adapted to the marine life. JGI scientists are currently sequencing the genome of Salinispora arenicola, the second cultured representative of this genus (Maldonado et al., 2005). In addition to its ecological significance, S. tropica is remarkable as a producer of the various halogenated macrolides, including the anticancer agent salinosporamide A, a potent inhibitor of the 20S proteasome (Williams et al., 2005). A detailed analysis of S. tropica genome is expected to clarify the macrolide biosynthesis pathway(s) and allow genetic manipulation leading to an improved production of salinosporamide A and other secondary metabolites (Udwary et al., 2007).

Flavobacterium johnsoniae (formerly Cytophaga johnsonae), first described by Roger Stanier (1947), is an aerobic bacterium that is commonly found in soil and freshwater. It is a member of the phylum Bacteroidetes, also known as the Cytophaga-Flavobacterium-Bacteroides group. Flavobacterium johnsoniae has attracted significant attention in the recent past, both as an effective chitin degrader and as a model organism for studying gliding motility (McBride, 2001; 2004). Remarkably, these two activities seem to be linked, as defects in gliding motility also affect chitin utilization (Braun et al., 2005). The reason for that is degradation of chitin and other insoluble biopolymers by F. johnsoniae apparently requires direct contact of cell with the substrate. This resembles cellulose degradation by the closely related Cytophaga hutchinsonii, whose genome has been sequenced at the JGI a year ago (Xie et al., 2007).

Prosthecochloris vibrioformis (a synonym of Chlorobium phaeovibrioides, Imhoff, 2003) is a green sulfur phototrophic bacterium, a member of the phylum Chlorobi, which already has four members with sequenced genomes. Like other Chlorobi, P. vibrioformis gains energy by anoxygenic photosynthesis using thiosulfate as an electron acceptor and reducing it to elemental sulfur, which accumulates as extracellular globules. The cells of P. vibrioformis have brownish color owing to the large amounts of bacteriochlorophyll e and carotenoids in their chlorosomes.

Roseiflexus sp. strain RS-1, also an anoxygenic photosynthetic bacterium, is the first phototrophic representative of the phylum Chloroflexi to have a completely sequenced genome. The 5.2-Mb genome of another phototrophic member of the Chloroflexi, Chloroflexus aurantiacus strain J-10-fl has been available in GenBank for the past 5 years (accession no. AAAH0000000) but still remains at the stage of 77 contigs. Three more representatives of this phylum are members of the genus Dehalococcoides which have lost the photosynthetic genes in the process of genome contraction during their adaptation to reductive dehalogenation (see below). In contrast to P. vibrioformis, Roseiflexus sp. does not contain chlorosomes and its major photosynthetic pigment is bacteriochlorophyll a. Comparative analysis of the genomes of members of Chlorobi and Chloroflexi could provide valuable information about mechanisms of anoxygenic photosynthesis.

Dehalococcoides sp. strain BAV1 is also a member of Chloroflexi, the third representative of the genus Dehalococcoides to have a completely sequenced genome. All three Dehalococcoides spp. are capable of metabolizing chlorinated hydrocarbons, including tetrachloroethene (PCE) and trichloroethene (TCE), which are commonly used as solvents and are major contaminants of soil and groundwater. Although Dehalococcoides ethenogenes strain 195 was originally reported to be capable of metabolizing PCE and TCE all the way to ethene, its cultures were found to accumulate 1,1-dichloroethene and vinyl chloride, which is a known human carcinogen (Maymo-Gatell et al., 1997; 2001). Dehalococcoides sp. CBDB1 can dechlorinate a variety of chlorobenzens but is also incapable of using dichloroethene or vinyl chloride (Kube et al., 2005). In contrast, the newly sequenced Dehalococcoides sp. strain BAV1 could grow using vinyl chloride and all dichloroethene isomers as electron acceptors. In addition, it could cometabolize PCE and TCE. Thus, strain BAV1 can be used for complete detoxification of PCE and TCE, that is, degradation of these compounds to environmentally benign ethene and inorganic chloride (He et al., 2003a,b). Comparative analysis of all three strains could provide clues to the mechanisms of reductive dehalogenation and the substrate specificities of the corresponding enzymes.
*Clostridium botulinum* is a well-known agent of food poisoning. Its spores are resistant to heat and survive exposure to air. In anaerobic conditions, which often exist in poorly prepared canned foods, *C. botulinum* spores germinate into vegetative cells. Growing vegetative cells secrete a variety of proteases and one or more toxic neurotoxins, known collectively as the botulinum toxin. Consumption of food contaminated with nanogram quantities of the botulinum toxin can be fatal for humans, as the toxin causes paralysis of chest muscles, which leads to asphyxiation. In addition, *C. botulinum* occasionally infects open wounds. The genome description (Sebaihia *et al*., 2007) includes a detailed comparison of five *Clostridium* spp., particularly with respect to the structure of the cell surface, extracellular enzymes and the regulation of toxin production. One cannot help noting that this deadly toxin has become a popular tool in cosmetology and has been suggested for a number of other applications, such as treatment of chronic migraines.

Two other members of the order *Clostridiales* in the current list (Table 1) are finding more traditional uses in biotechnology. *Caldicellulosiruptor saccharolyticus* is an anaerobic thermophilic bacterium that was isolated from a thermal spring in New Zealand (Sissons *et al*., 1987). Both the genus name, meaning literally ‘hot cellulose disruptor’, and the species name, which means ‘lysing sugar’, reflect the ability of the organism to metabolize a variety of mono- and polysaccharides, such as arabinose, amorphous cellulose, fructose, galactose, glucose, glycogen, lactose, laminarin, lichenin, mannose, maltose, pullulan, pectin, rhamnose, starch, sucrose, xylan and xylose (Rainey *et al*., 1994). Based on its inability to form spores, *C. saccharolyticus* was first assigned to a separate lineage within the *Bacillus/Clostridium* subphylum of the Gram-positive bacteria (the current *Firmicutes*), but later recognized as a member of the order *Clostridiales*. The ability of *C. saccharolyticus* to grow at 70°C, hydrolysing both α- and β-glucans, including cellulose and pectin, makes it an attractive candidate for conversion of plant biomass into biofuel. Comparison of the genomic sequence of *C. saccharolyticus* with those of related organisms could provide an insight in the mechanisms and regulation of cellulose degradation.

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*Pelotomaculum thermopropionicum*, also a member of *Clostridiales*, is a thermophilic propionate-oxidizing anaerobic bacterium, isolated in 2000 from anaerobic sludge blanket reactor in Niigata, Japan. It grows best in a syntrophic association with methanogenic archaea, metabolizing propionate, ethanol, lactate, butanol, pentanal, 1,3-propanediol, propanol and ethylene glycol (Imachi *et al*., 2002). Even before the completion of the genome sequencing, Kosaka and colleagues (2006) analysed the propionate fermentation pathway of *P. ther-
division genes may be linked to the fact that each host cell carries only a single cell of Cand. V. okutanii, which is vertically transferred through clam oocytes.

If Cand. V. okutanii has the smallest autotrophic genome sequenced to date, the sheep pathogen *Dichelobacter nodosus* has the smallest genome of any anaerobe. The genome of *D. nodosus*, the causative agent of ovine foot rot, is less than 1.4 Mb in size and codes for less than 1300 proteins (Myers et al., 2007). However, these genes are apparently all significant, as there are few paralogues and very few pseudogenes. Given that *D. nodosus* belongs to an early branching group of *γ*-Proteobacteria, the authors suggest that its evolutionary history differed from that of most other organisms with very small genomes, which are either obligate intracellular pathogens or symbionts. Thus, massive gene loss through pseudogenization probably played only a minor role in extensive genome reduction, if any, in the evolution of *D. nodosus*.

Of the three recently sequenced pseudomonad genomes (Table 1), *Pseudomonas putida* F1 is by far the best-studied one. This obligately aerobic bacterium was originally isolated from a polluted creek in Urbana, Illinois, by enrichment with ethylbenzene as the sole source of carbon and energy (Gibson et al., 1968). This strain can also grow on benzene, toluene and *p*-cymene, which are common contaminants of groundwater, leaching from underground gasoline storage tanks. When provided with a carbon source for growth, *P. putida* F1 can oxidize a variety of aromatic and aliphatic compounds that do not support its growth. This list includes nitrotoluenes, chlorobenzenes, chlorophenols and trichloroethylene. The mechanism of toluene degradation by *P. putida* F1 toluene dioxygenase and its regulation have been studied in much detail (Zylstra et al., 1988; Lau et al., 1997). Remarkably, *P. putida* F1 senses benzene, ethylbenzene and trichloroethylene, perceiving them as chemoattractants (Parales et al., 2000). Both strains of *P. putida* F1 and KT2240, whose genome was sequenced 5 years ago (Nelson et al., 2002; Dos Santos et al., 2004), have great potential for use in bioremediation.

The *Shewanella* genome sequencing project has released yet another complete genome, this time of the facultative anaerobe *Shewanella putrefaciens*. While some strains of *S. putrefaciens* have been isolated from clinical samples and appear to be opportunistic human pathogens (Holt et al., 2005), the sequenced strain is interesting primarily because of its capability to effectively reduce polyvalent metals and radionuclides including solid phase oxides of Fe, Mn, Cr, U(VI) and Tc (VII), using lactate as the electron donor.

The δ-proteobacterium *Geobacter uraniumreducens* is also a powerful reducer of metals. The sequenced strain *G. uraniumreducens* Rf4 has been isolated from a contaminated aquifer at the former uranium ore processing facility in Rifle, Colorado (Anderson et al., 2003). Injection of acetate (1–3 mM) into the groundwater led to an enrichment of the groundwater with *G. uraniumreducens*, which coincided with a decrease in U(VI) levels. Comparison of *G. uraniumreducens* genome sequence with that of *Geobacter metallireducens* and other metal-reducing bacteria should help in finding the best strains for bioremediation of uranium and other radionuclides.

Eight years after the publication of the genome sequence of an obligately anaerobic hyperthermophilic bacterium *Thermotoga maritima* (Nelson et al., 1999), the genome of a second member of the phylum *Thermotogae* has been released. While also an obligate anaerobe similar to *T. maritima* in its sugar utilization profile, *Thermotoga petrophila* strain RKU-1 has been isolated from a deep subterranean oil reservoir in Niigata, Japan, and could grow at a much wider range of temperatures, from 48 to 88°C, with an optimum at 80°C (Takahata et al., 2001). These features make *T. petrophila* an attractive source of thermostable enzymes, as well as a convenient model organism to study the mechanisms of thermostolerance in bacteria.

The list of the recently sequenced genomes also includes several important bacterial pathogens. The genome of *Orientia* (formerly *Rickettsia*) *tsutsugamushi*, the causative agent of scrub typhus, is remarkable primarily for the abundance of repeat elements, including numerous *tra* genes, transposases, phage integrases, reverse transcriptases and potential host–cell interaction proteins (Cho et al., 2007). Genome sequencing of two strains of *Streptococcus suis* serotype 2, which caused an outbreak of streptococcal toxic shock syndrome in China, revealed a shared pathogenicity island that appeared responsible for the high-virulence phenotype (Chen et al., 2007). In an article with an unusually impressive title, genome analysis of the ruminant pathogen *Mycoplasma agalactiae* and closely related mycoplasmas revealed traces of horizontal gene transfer, suggesting that it could have played a role in the mycoplasmal evolution (Sirand-Pugnet et al., 2007). The fifth complete genome of a *Brucella* spp. comes from the sheep pathogen *Brucella ovis*, which causes ovine contagious epididymitis in rams and premature abortion in pregnant ewes. It should be noted that in 1988, the ICSB Subcommittee on the Taxonomy of *Brucella* concluded that the *Brucella* is a monospecific genus with a single species *Brucella melitensis*. Indeed, all *Brucella* spp. have genomes consisting of two chromosomes with similar sizes, similar G + C content of 57.2% and encoding many proteins that are 99–100% identical. Thus, *B. ovis* is currently considered an accepted synonym for *Brucella melitensis* biovar Ovis.

The genome of *Aeromonas salmonicida* spp. salmonicida strain A449 was sequenced in an effort to find new
ways to control this widespread fish pathogen. The draft version of the genome has already been used to construct
A. salmonicida DNA microarray and identify potential virulence genes and candidates for vaccine development (Nash et al., 2006).

Last but not least, a recent article has uncovered the function of one of widespread ‘conserved hypothetical’ genes. This gene, which has been designated yebR in Escherichia coli, ytsP in Bacillus subtilis and YKL069w in Saccharomyces cerevisiae, has been shown to encode an enzyme that reduces free methionine sulfoxide (Lin et al., 2007). Two previously identified methionine sulfoxide reductases act predominantly on oxidized methionine residues in protein and cannot protect the cellular pool of free amino acid from oxidation. This work is yet another proof that ‘house-cleaning’ is a major cellular function that might employ a fair number of the uncharacterized ‘hypothetical’ proteins (see Galperin et al., 2006 for a review).

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