Genomics update

The dawn of synthetic genomics

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The first month of 2008 was unusually quiet in terms of microbial genome sequencing. Still, even the relatively short list of newly released genomes includes several interesting environmental microorganisms, such as the anoxygenic phototroph *Chloroflexus aurantiacus*, the toxic bloom-forming cyanobacterium *Microcystis aeruginosa*, and the methylotroph *Methylobacterium extorquens* (Table 1). However, arguably the biggest news was the announcement by J. Craig Venter and colleagues that they ‘have synthesized a 582 970 bp *Mycoplasma genitalium* genome’ (Gibson et al., 2008). The authors used chemically synthesized oligonucleotides ~50 nucleotides in length to assemble ‘cassettes’ 5–7 kb in length, then to join them by in vitro recombination to produce intermediate assemblies, gradually increasing in size. Finally, four 144 kb pieces were cloned in *Escherichia coli* as bacterial artificial chromosomes, transferred into yeast and assembled into a full-length genome. The genome of the resulting strain, named *M. genitalium* JCVI-1.0, was virtually identical to the genome of the original strain *M. genitalium* G37. It was not immediately clear whether this technically very challenging and truly monumental work had any purpose beyond just serving as a proof of principle. However, J. C. Venter and colleagues have a record of overcoming enormous technical challenges and launching entirely new areas of biotechnology. It might be simply too early right now to ask them to explain the future of this work. In any case, the era of synthetic biology has officially begun and who knows what kind of molecules people will be synthesizing 10 or 20 years from now.

Returning to the present-day problems, scientists at the Northwest Fisheries Center have sequenced the genome of *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease (kidney granulomatosis) in salmonid fish. *Renibacterium salmoninarum* is a moderately psychrophilic actinobacterium that grows optimally at 15–18°C (Sanders and Fryer, 1980; Fryer and Sanders, 1981). It can survive for several days in fresh or sea water before infecting new fish. In addition, *R. salmoninarum* populates ovarian liquid of infected female fish and can be vertically transmitted to their eggs. The disease was first detected in 1930s in salmon from the river Dee and was initially referred to as ‘Dee disease’. It took more than 20 years to cultivate the organism and recognize its similarity to corynebacteria. The disease still remains poorly understood owing largely to the extremely slow growth of *R. salmoninarum* pure culture ( Hirvelä-Koski, 2008). The genome data are expected to help identify *R. salmoninarum* virulence factors, vaccine candidates and design improved diagnostic tests.

*Chloroflexus aurantiacus* is a facultatively aerobic phototrophic gliding filamentous bacterium, first isolated from the Hakone hot spring area west of Tokyo, Japan (Pierson and Castenholz, 1974). It is the best-studied representative of the phylum *Chloroflexi*, also referred to as Green non-sulfur bacteria, and a popular model organism for studying anoxygenic photosynthesis and autotrophic CO₂ assimilation pathways (Stackebrandt et al., 1996; Herter et al., 2001). *Chloroflexi* are an early branching bacterial phylum that has retained certain unique properties, including very unusual cell walls (Meissner et al., 1998). These organisms are particularly important for understanding the evolution of photosynthesis. In contrast to anoxygenic phototrophs that belong to green sulfur bacteria (*Chlorobium*, *C. aurantiacus* encodes photosynthetic reaction centre of type II, similar to the Photosystem II found in cyanobacteria and green plants, as well as in phototrophic proteobacteria. Accordingly, *Chloroflexi* have been proposed to be the original phototrophs (Oyaizu et al., 1987). An alternative, less exciting variant simply implied that *Chloroflexi* have acquired their photosynthetic machinery via lateral gene transfer from ancestral cyanobacteria (Mulikdijanian et al., 2006). The sequencing of *C. aurantiacus* genome has had a long history. The first version, consisting of 1142 contigs, was deposited in GenBank back in 2002. In 2005, the number of contigs was reduced to 77, and remained at that stage for more
than 2 years. Meanwhile, complete genomes of six members of *Chloroflexi* (three *Dehalococcoides* sp., two *Roseiflexus* sp., and *Herpetosiphon aurantiacus*) had been released and released to the public. Still the complete genome of *C. aurantiacus* is an important milestone, which will allow many new uses of this model organism.

*Microcystis aeruginosa* is a freshwater planktonic unicellular cyanobacterium with small coccolid cells that form gas vesicles. It is widespread in lakes and ponds around the world and is a common cause of toxic water blooms (Otsuka et al., 2001). Its toxicity is due to the production of two major types of toxins, microcystins and cyanopeptolins. Both are cyclic peptides that contain unusual amino acid residues. Microcystin (see PubChem http://pubchem.ncbi.nlm.nih.gov/summary/summary.cgi?cid=445434 or ChEBI http://www.ebi.ac.uk/chebi/searchld.do?chebiId=CHEBI:6925 web sites for representative formulas) is an inhibitor of cellular protein serine phosphatases. It is hepatotoxic and can kill fish, birds and small animals. Cyanopeptolin, also referred to as micropeptin or microcystilide, is a heptapeptide that contains a six-amino-acid-membered ring with a lactone structure between the C-terminus and the hydroxy group of threonine. It is a strong inhibitor of chymotrypsin and related proteases. The genome sequence revealed three non-ribosomal peptide synthetase gene clusters (Kaneko et al., 2007). Two of them were responsible for the synthesis of microystin and cyanopeptolin respectively. The third non-ribosomal peptide synthetase gene cluster and a putative polyketide synthase gene cluster could be involved in production of novel, still unidentified, compounds. Similarly to other cyanobacteria, *M. aeruginosa* carries the full set of photosynthetic genes; however, it encodes a relatively simple two-component signal transduction machinery.

*Bacillus weihenstephanensis* is a Gram-positive, facultatively anaerobic, spore-forming bacterium, a close relative of *Bacillus cereus*. It was recognized as a separate species based primarily on the ability of its strains to grow in the presence of an inhibitory agent.
at low temperatures (< 7°C) and absence of growth above 38°C (Lechner et al., 1998). Subsequent multiple-locus sequence typing (MLST) analyses revealed that most psychrophilic B. cereus-like isolates belong to B. weihenstephanensis, although some psychrophilic strains still had to be assigned to B. cereus (Stenfors and Granum, 2001; Sorokin et al., 2006). Owing to its tolerance to cold, B. weihenstephanensis was reported to be the most common representative of the B. cereus group found in frozen soil in Paris area and in Danish sandy loam (Hendriksen et al., 2006). Certain strains of B. weihenstephanensis have been shown to carry plasmid-encoded genes coding for B. cereus haemolysins and enterotoxins. Coupled with the ability of B. weihenstephanensis spores to survive heat treatment and give rise to vegetative cells that can grow at refrigeration temperatures, presence of these genes makes B. weihenstephanensis a potential food-borne pathogen. Indeed, this organism has been isolated from spoiled whole liquid egg products (Baron et al., 2007). The sequenced strain B. weihenstephanensis KBAB4 was originally isolated in December 2000 from forest soil at La Minière near Versailles, France, and subsequently identified as B. weihenstephanensis using MLST (Sorokin et al., 2006). Its genome sequence could shed light on the mechanisms of psychrophilic adaptations in bacilli.

The α-proteobacterium Gluconacetobacter diazotrophicus is a nitrogen-fixing member of family Acetobacteraceae, originally isolated from sugar cane (Gillis et al., 1989), but later found also in association with rice. It is an endophyte that colonizes plant roots, but can propagate to the xylem of the lower stem. Gluconacetobacter diazotrophicus can fix N₂ in the presence of nitrate and is being used as model organism to study the mechanisms and regulation of nitrogen fixation. Sequencing of its genome should help in understanding the physiology of nitrogen-fixing acetic acid bacteria.

Methylotrophic (or, more precisely, methanotrophic) bacteria attract a lot of attention thanks to their ability to utilize natural gas, producing biomass and synthesizing a variety of useful compounds (see Hanson and Hanson, 1996; Trotsenko et al., 2005; Hakemian and Rosenzweig, 2007 for reviews). The first methylotroph to undergo genome sequencing was the α-proteobacterium M. extorquens strain AM1, a well-characterized model organism. Although its genome sequence has not been finished, it provided a useful insight in the mechanisms of methylotrophy (Chistoserdova et al., 2003). The first methylotroph with a complete genome sequence was the γ-proteobacterium Methylococcus capsulatus (Ward et al., 2004). It was followed by complete genomes of two methylotrophic β-proteobacteria, Methylobacillus flagellatus (Chistoserdova et al., 2007) and Methylibium petroleiphilum (Kane et al., 2007). JGI scientists have just released the complete genome of M. extorquens strain PA1, which reportedly colonizes plants more efficiently than AM1 strain, and are currently sequencing genomes of four more members of the genus Methylobacterium. The availability of complete genomes from three different subdivisions of Proteobacteria opens new possibilities for methylotroph genome analysis and should allow addressing the question of Wood and colleagues (2004) about the causes of obligate methanotrophy. While all sequenced organisms are members of the Proteobacteria, methylotrophy has also been found among members of other phyla, such as Planctomycetes (Chistoserdova et al., 2004). Very recently, papers from three different groups reported isolation of extremely acidophilic methanotrophs belonging to the phylum Verrucomicrobia (Dunfield et al., 2007; Pol et al., 2007; Islam et al., 2008). These three organisms, isolated from substantially different ecological niches, were all thermophiles capable of growing aerobically at 55–60°C with methane as the sole carbon source. These findings indicate that methanotrophy is far more common in bacteria than previously believed and hold great promise for use of methylotrophs in biotechnology.

All strains of Bordetella characterized in the 20th century were animal or avian pathogens, with Bordetella pertussis and Bordetella parapertussis known as causative agents of whooping cough. In 2001, however, a sample of an anaerobic, trichlorobenzene-dechlorinating consortium taken from sediment of the River Saale near Jena, Germany, was found to contain a Bordetella-like organism (von Wintzingerode et al., 2001). Unlike other Bordetella sp., this isolate, assigned to the new species Bordetella petrii, was capable of growing anaerobically by reducing nitrate and/or selenate. Very similar strains were subsequently isolated from soil polluted with chlorinated benzenes (Wang et al., 2007), and from patients with mandibular osteomyelitis and chronic suppurative mastoiditis (Fry et al., 2005; Stark et al., 2007). Thus, B. petrii appears to be an opportunistic pathogen, after all, which might prevent its use as a bioremediation agent.

Among the genomes of new strains of previously sequenced species, it is worth noting the detailed description of Actinobacillus pleuropneumoniae JLO3 genome (Xu et al., 2008), published simultaneously with a brief description of the genome of A. pleuropneumoniae L20 (Foote et al., 2008), which had been released a year earlier. The latter paper appeared in the new ‘Genome Announcement’ section of the Journal of Bacteriology. This new section seems to be a very timely initiative, aimed at clearing the backlog of completely sequenced genomes that still remain without proper description or even a suitable citation.

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