Journal of Biotechnology 259 (2017) 15–18

Contents lists available at ScienceDirect

Journal of Biotechnology

journal homepage: www.elsevier.com/locate/jbiotec

Short Genome Communications

Complete genome sequence of *Pseudomonas antarctica* PAMC 27494, a bacteriocin-producing psychrophile isolated from Antarctica

Jaejin Lee[a], Yong-Joon Cho[a], Jae Young Yang[b], You-Jung Jung[b], Soon Gyu Hong[b], Ok-Sun Kim[a,*]

[a] Unit of Antarctic K-route Expedition, Korea Polar Research Institute, Incheon, 21990, Republic of Korea
[b] Division of Polar Life Sciences, Korea Polar Research Institute, Incheon, 21990, Republic of Korea

ARTICLE INFO

Keywords:
Complete genome
Pseudomonas antarctica
Psychrophile
Microcin B

ABSTRACT

Antimicrobial-producing, cold-adapted microorganisms have great potential for biotechnological applications in food, pharmaceutical, and cosmetic industries. *Pseudomonas antarctica* PAMC 27494, a psychrophile exhibiting antimicrobial activity, was isolated from an Antarctic freshwater sample. Here we report the complete genome of *P. antarctica* PAMC 27494. The strain contains a gene cluster encoding microcin B which inhibits DNA regulations by targeting the DNA gyrase. PAMC 27494 may produce R-type pyocins and also contains a complete set of proteins for the biosynthesis of adenosylcobalamin and possibly induces plant growth by supplying pyrroloquinoline quionone molecules.

Introduction

Extensive explorations for new antimicrobials have made microorganisms considered as a valuable reservoir of novel antimicrobial compounds (Sánchez et al., 2009). Particularly, antimicrobial compounds such as bacteriocins can be useful to develop novel applications for natural preservatives (O'Brien et al., 2004). Up to date, antimicrobial properties of mesophiles have been primarily explored. Those antimicrobials have been used for industrial applications and applied to treat infections caused by pathogens. However, many pathogens have become resistant to pre-existing antimicrobials. The increased needs led researchers to seek for novel antimicrobials derived from biomolecules with unusual properties (Sánchez et al., 2009). Consequently, extremophiles, including psychrophiles, have been noticed as novel sources of undiscovered antimicrobial biomolecules which are adapted to unusual living conditions (Horikoshi, 1995). Compared to the antimicrobials produced by mesophiles, the antimicrobials produced by psychrophiles, which are active at low temperatures, enable the producing microorganisms to have a competitive advantage during their growth (O'Brien et al., 2004). Moreover, cold-adapted enzymes are known to have great commercial potential due to their high catalytic activity at low temperatures and low energy consumption (van den Burg, 2003). Thus, antimicrobial compounds derived from cold-loving microorganisms can be exploitable in industrial applications, especially in food, pharmaceutical, and cosmetic industries.

Pseudomonas antarctica PAMC 27494 was isolated from a freshwater sample collected at King George Island, Antarctica (62.2377° S, 58.7285° W). Members of the *Pseudomonas* genus have been found in diverse habitats, ranging from terrestrial to aquatic environments as well as from Antarctica to a desert soil. A number of pseudomonads are known to produce several secondary metabolites, including bacteriocins and antifungal antibiotics, which affect other microorganisms (Ghequire and Mot, 2014). For example, more than 90% of *Pseudomonas aeruginosa* strains are able to synthesize different types of pyocins (Michel-Briand and Baysse, 2002) and *Pseudomonas syringae* can produce microcin B-like antibacterial compounds (Metelev et al., 2013). *P. antarctica*, a Gram-negative, rod-shape, motile with a polar flagellum, and psychrophilic, was first isolated from Wright Valley, Antarctica (i.e., strain CMS 35) (Reddy et al., 2004), but its secondary metabolites, especially production of antimicrobial compounds still remain unknown.

A zone of inhibition test was conducted to assess the antibacterial activity of *P. antarctica* PAMC 27494. After 100 µl of pre-cultured *Bacillus idriensis* was spread on R2A agar plates (Difco Laboratories, Detroit, MI, USA), 20 µl of PAMC 27494 was applied on the plates by streaking, dropping, or using discs. The plates were incubated for 7 days at 10 °C and 20 °C, respectively. As shown in Fig. 1, the zone of inhibition were clearly appeared around PAMC 27494 for all cases, which indicate that *Bacillus idriensis* was not able to survive due to antimicrobial compounds produced by PAMC 27494. As expected, the antimicrobial activity of PAMC 27494 was higher at psychrophilic condition (i.e., 10 °C) than that of at room temperature (i.e., 20 °C). Subsequently, the genome of *P. antarctica* PAMC 27494 was sequenced to get better understandings of its antimicrobial activity and

* Corresponding author.
E-mail address: oskim@kopri.re.kr (O.-S. Kim).

http://dx.doi.org/10.1016/j.jbiotec.2017.08.013
Received 2 January 2017; Received in revised form 9 August 2017; Accepted 9 August 2017
Available online 14 August 2017

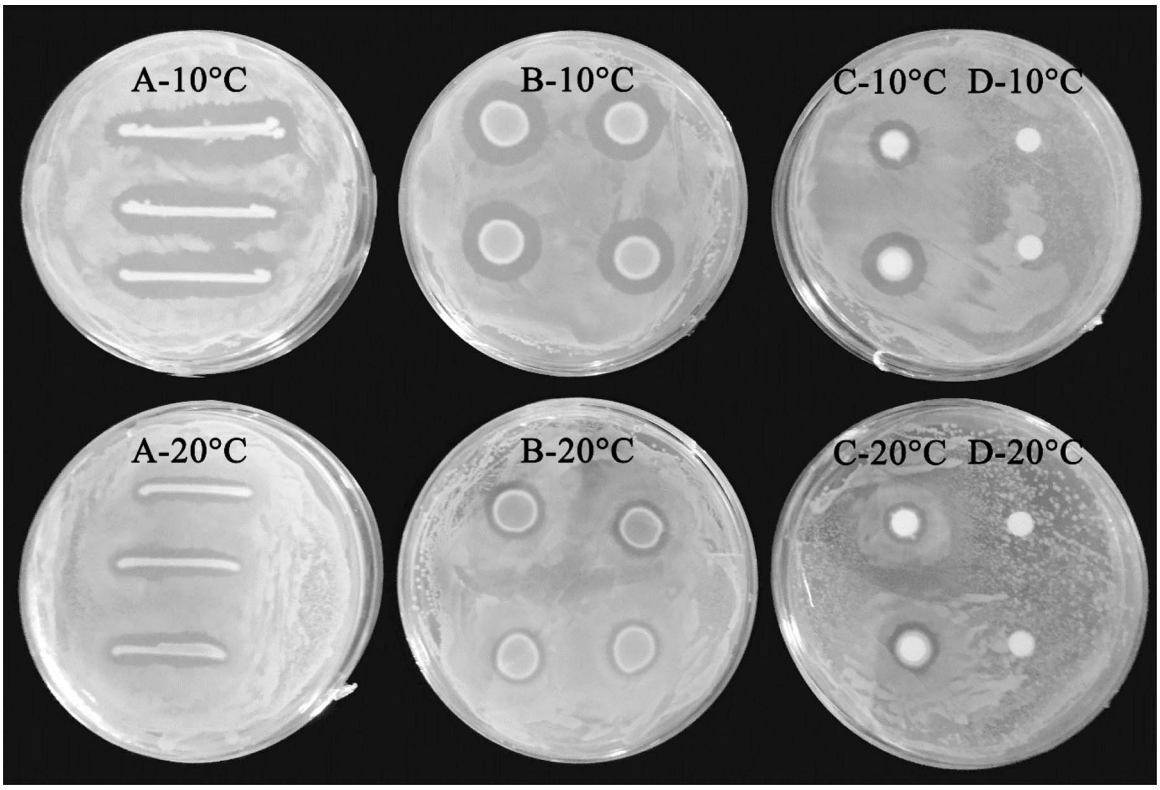

Fig. 1. A zone of inhibition test of *Pseudomonas antarctica* PAMC 27494 on *Bacillus idriensis*, a gram-positive bacterial strain. (A, streaking; B, dropping; C, on discs; D, negative control on discs).

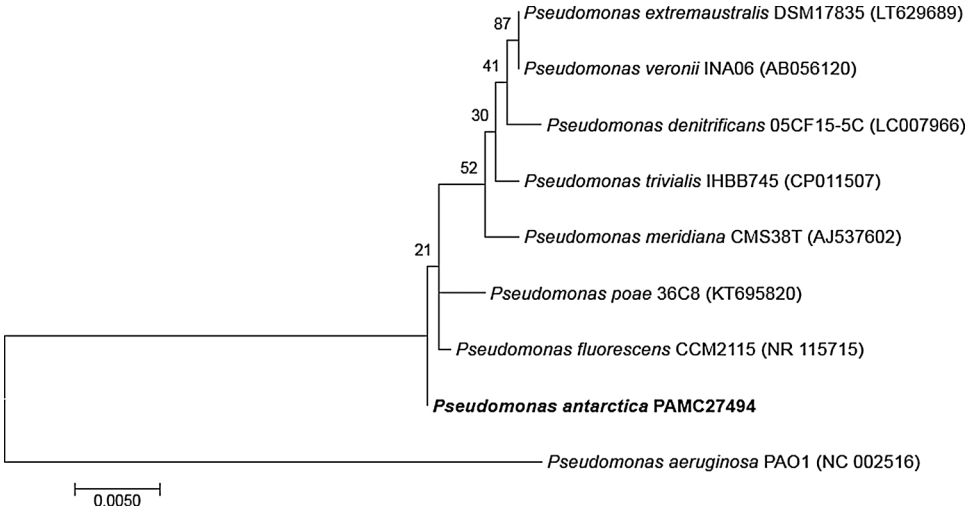

Fig. 2. A phylogenetic placement of *Pseudomonas antarctica* PAMC 27494.

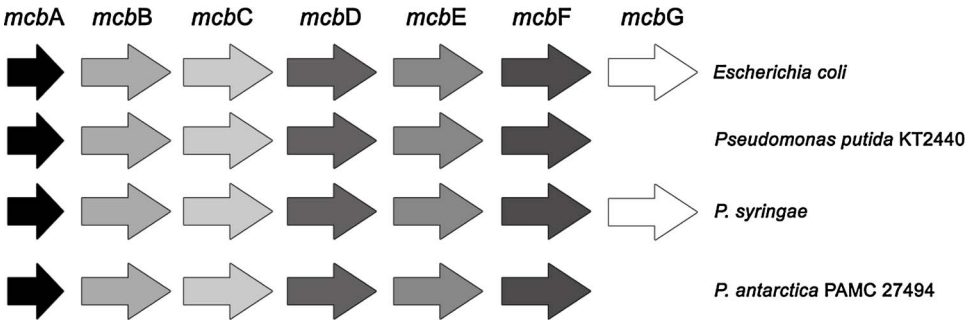

Fig. 3. Cluster of microcin B producing genes in *Escherichia coli*, *Pseudomonas putida*, *P. syringae*, and *P. antarctica* PAMC 27494. Each gene of the cluster is indicated by an arrow. Considering that the *mcb*G gene, encoding a self-immunity protein, is often missing in *Pseudomonas* strains, it may not be essential to show antimicrobial activities.

Table 1
Pseudomonas antarctica PAMC 27494 genome features.

Feature	Chromosome	pP27494_1	pP27494_2
Size [bp]	6,441,449	135,475	30,116
GC content (%)	59.9%	54.8%	49.4%
Total number of genes	5853	133	35
Protein coding genes (CDSs)	5736	119	35
Genes with a predicted function	4788	75	20
rRNAs (5S, 16S, 23S)	19	0	0
tRNAs	66	0	0

biotechnological potential.

Genomic DNA of *P. antarctica* PAMC 27494 was extracted using the i-genomic BYF Mini Kit (iNtRON Biotechnology, Republic of Korea). A standard PacBio library (an average of 20 kb inserts) was prepared and sequenced using Pacific Biosciences (PacBio) RS II single-molecule real-time (SMRT) sequencing technology (Pacific Biosciences, CA) (Eid et al., 2009). *De novo* assembly using the hierarchical genome assembly process (HGAP) pipeline of the SMRT Analysis version 2.3.0 (Chin et al., 2013) was carried out for 122,086 reads with 7956 nucleotides on the average (971,322,935 bp in total), which resulted in one circular chromosome (110-fold coverage), two circular plasmids (90-fold coverage for pP27494_1 and 60-fold for pP27494_2) (Fig. 4). The protein coding sequences (CDSs) were predicted by Prokaryotic Dynamic Programming Genefinding Algorithm (Prodigal) version 2.6.1 (Hyatt et al., 2010). For functional annotation of the predicted genes, UniProt (Wu et al., 2006), Pfam (Finn et al., 2014), and COG (Tatusov et al., 2013) databases were used. Ribosomal RNA, transfer RNA, and miscellaneous features were predicted using Rfam version 12.0 (Griffiths-Jones et al., 2005). The genome of *P. antarctica* PAMC 27494 consists of one circular chromosome of 6,441,450 bp and two circular plasmids, designated as pP27494_1 (135,475 bp) and pP27494_2 (30,116 bp). The GC contents of the chromosome and the plasmids were 59.9%, 54.8%, and 49.4%, respectively. Among 5890 protein coding genes found in the genome, 4883 genes were assigned to a putative function and the remaining were annotated as hypothetical proteins. The genome possesses 19 rRNA (five operons made up of 5S, 16S and 23S, and an operon of two 5S, 16S, and 23S) and 66 tRNA genes. The general genome features of *P. antarctica* PAMC 27494 are summarized in Table 1. A phylogenetic tree was constructed for PAMC 27494 and close relatives using MEGA7 (Kumar et al., 2016). For the tree inference, a neighbor-joining algorithm with a bootstrap test (1000 replicates) was used (Fig. 2).

According to the annotation results, *P. antarctica* PAMC 27494 has a complete gene cluster to produce microcin B, which is a ribosomally synthesized antibacterial peptide that prevents DNA regulation by targeting the DNA gyrase (Severinov et al., 2007; Shkundina et al., 2014). The microcin B precursor, which is produced by *mcb*A gene (A7J50_3673), can be post-translationally converted to the mature form by McbBCD synthase (A7J50_3670 to A7J50_3672). The produced microcin B can be exported through the microcin ABC transport system coded by *mcb*EF genes (A7J50_3668 to A7J50_3669) (Fig. 3). R-type pyocin-encoding genes (A7J50_1217, A7J50_1223, A7J50_1239, A7J50_3754) were also present in two of five prophage regions. Genes for biosynthesis of a pyoverdin, a fluorescent siderphore (A7J50_3264 to A7J50_3273) and three other non-ribosomal peptide synthase (NRPS) (A7J50_2438 to A7J50_2455, A7J50_3674 to A7J50_3691, and A7J50_4055 to A7J50_4073) exist in the genome of PAMC 27494.

A complete gene set for the biosynthesis of adenosylcobalamin was found in the genome. PAMC 27494 may produce the uroporphyrinogen-III from glutamyl-tRNA using proteins involved in the tetrapyrrol biosynthesis. Then, the uroporphyrinogen-III can be transferred to cobyrinate a,c-diamide by proteins involved in the cob(II)yrinate a,c-diamide biosynthesis pathway. Finally, the adenosylcobalamin can be generated from the cob(II)yrinate a,c-diamide by proteins encoded in the adenosylcobalamin biosynthesis gene cluster (A7J50_4162 to A7J50_4170). The strain possibly supplies PQQ molecules to induce plant growth by encoding proteins involved in the pyrroloquinoline quinone biosynthesis pathway (A7J50_5328 to A7J50_5336). Aromatic compounds such as 4-hydroxyphenylacetate and benzoate can be used by PAMC 27494 as carbon sources via genes encoding proteins involved in 4-hydroxyphenylacetate *meta*-cleavage pathway (*hap*GEDFHI and

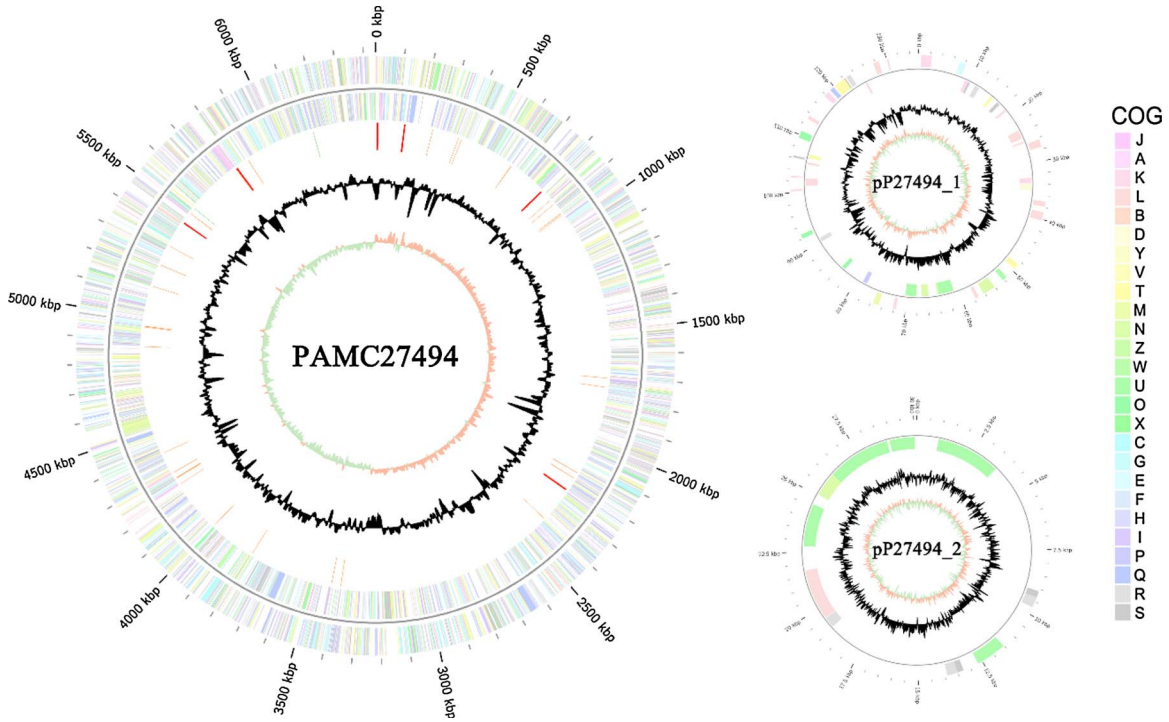

Fig. 4. Graphical map of the chromosome of *P. antarctica* PAMC 27494 and two plasmids designated as pP27494_1 and pP27494_2. From the outside to the center: Genes on forward strand (color by COG categories), genes on reverse strand (color by COG categories), RNA genes (tRNAs in orange, rRNAs in red, other RNAs in green), GC content (black), GC skew (light green/light orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

*hap*R; A7J50_3679 to A7J50_3686) and benzoate 1,2-dioxygenase (A7J50_4940 and A7J50_4941). The genome has genes encoding ABC transporter related to exporting or importing phenolic compounds, HatABCDE (A7J50_0892 to A7J50_0896), which implies that PAMC 27494 can overcome chemical stresses caused by aromatic compounds (eg. toluene) or transport homogentisic acid as a precursor/a carbon source into cytosol using this ABC transporter.

Nucleotide sequence accession number

The genome sequence of *Pseudomonas antarctica* PAMC 27494 has been deposited at GenBank under the accession number CP015600 (chromosome), CP015601 (plasmid designated as pP27494_1), and CP015602 (plasmid designated as pP27494_2). The strain PAMC 27494 is available from the Polar and Alpine Microbial Collection (Korea Polar Research Institute, Incheon, Republic of Korea).

Acknowledgements

This work was supported by Korea Polar Research Institute (Grant PE17090).

References

Chin, C.S., Alexander, D.H., Marks, P., Klammer, A.A., Drake, J., Heiner, C., Clum, A., Copeland, A., Huddleston, J., Eichler, E.E., Turner, S.W., Korlach, J., 2013. Nonhybrid, finished microbial genome assemblies from long-read SMRT sequencing data. Nat. Methods 10, 563–569.

Eid, J., Fehr, A., Gray, J., Luong, K., Lyle, J., Otto, G., Peluso, P., Rank, D., Baybayan, P., Bettman, B., Bibillo, A., Bjornson, K., Chaudhuri, B., Christians, F., Cicero, R., Clark, S., Dalal, R., Dewinter, A., Dixon, J., Foquet, M., Gaertner, A., Hardenbol, P., Heiner, C., Hester, K., Holden, D., Kearns, G., Kong, X., Kuse, R., Lacroix, Y., Lin, S., Lundquist, P., Ma, C., Marks, P., Maxham, M., Murphy, D., Park, I., Pham, T., Phillips, M., Roy, J., Sebra, R., Shen, G., Sorenson, J., Tomaney, A., Travers, K., Trulson, M., Vieceli, J., Wegener, J., Wu, D., Yang, A., Zaccarin, D., Zhao, P., Zhong, F., Korlach, J., Turner, S., 2009. Real-time DNA sequencing from single polymerase molecules. Science 323, 133–138.

Finn, R.D., Bateman, A., Clements, J., Coggill, P., Eberhardt, R.Y., Eddy, S.R., Heger, A., Hetherington, K., Holm, L., Mistry, J., Sonnhammer, E.L.L., Tate, J., Punta, M., 2014. Pfam: the protein families database. Nucleic Acids Res. 42, D222–D230.

Ghequire, M.G.K., Mot, R.D., 2014. Ribosomally encoded antibacterial proteins and peptides from *Pseudomonas*. FEMS Microbiol. Rev. 38, 523–568.

Griffiths-Jones, S., Moxon, S., Marshall, M., Khanna, A., Eddy, S.R., Bateman, A., 2005. Rfam: annotating non-coding RNAs in complete genomes. Nucleic Acids Res. 33, D121–D124.

Horikoshi, K., 1995. Discovering novel bacteria with an eye to biotechnological applications. Curr. Opin. Biotech. 6 (292), 297.

Hyatt, D., Ghen, G.L., LoCascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J., 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinf. 11, 119.

Kumar, S., Stecher, G., Tamura, K., 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Metelev, M., Serebryakova, M., Ghilarov, D., Zhao, Y., Severinov, K., 2013. Structure of microcin B-like compounds produced by Pseudomonas syringae and species specificity of their antibacterial action. J. Bacteriol. 195, 4129–4137.

Michel-Briand, Y., Baysse, C., 2002. The pyocins of *Pseudomonas aeruginosa*. Biochimie 84, 499–501.

O'Brien, A., Sharp, R., Russell, N., Roller, S., 2004. Antarctic bacteria inhibit growth of food-borne microorganisms at low temperatures. FEMS Microbiol. Ecol. 48, 157–167.

Reddy, G.S.N., Matsumoto, G.I., Schumann, P., Stackebrandt, E., Shivaji, S., 2004. Psychrophilic pseudomonads from Antarctica: Pseudomonas antarctica sp. nov., Pseudomonas meridian sp. nov. and Pseudomonas proteolytic asp. nov. Int. J. Syst. Evol. Microbiol. 54, 713–719.

Sánchez, L.A., Gómez, F.F., Delgado, O.D., 2009. Cold-adapted microorganisms as a source of new antimicrobials. Extremophiles 13, 111–120.

Severinov, K., Semenova, E., Kazakov, A., Gelfand, M., 2007. Low-molecular-weight post-translationally modified microcins. Mol. Microbiol. 65, 1380–1394.

Shkundina, I., Serebryakova, M., Severinov, K., 2014. The C-terminal part of microcin B is crucial for DNA gyrase inhibition and antibiotic uptake by sensitive cells. J. Bacteriol. 196, 1759–1767.

Tatusov, R.L., Fedorova, N.D., Jackson, J.D., Jacobs, A.R., Kiryutin, B., Koonin, E.V., Krylov, D.M., Mazumder, R., Mekhedov, S.L., Nikolskaya, A.N., Rao, B.S., Smirnov, S., Van Den Burg, B., 2013. Extremophiles as a source for novel enzymes. Curr. Opin. Microbiol. 6, 213–218.

Van Den Burg, B., 2003. Extremophiles as a source for novel enzymes, Curr. Opin. Microbiol . 6, 213–218.

Wu, C.H., Apweiler, R., Bairoch, A., Natale, D.A., Barker, W.C., Boechmann, B., Ferro, S., Gasteiher, E., Huang, H., Lopez, R., Magrane, M., Martin, M.J., Mazumder, R., O'Donovan, C., Redaschi, N., Suzek, B., 2006. The Universal Protein Resource (UniProt): an expanding universe of protein information. Nucleic Acids Res. 34, D187–D191.