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Draft genomes of three Antarctic *Psychrobacter* strains producing antimicrobial compounds against *Burkholderia cepacia* complex, opportunistic human pathogens

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ABSTRACT

Herein we present the draft genomes of three *Psychrobacter* strains isolated from Antarctic sponges and able to inhibit the growth of bacteria belonging to the *Burkholderia cepacia* complex, responsible for infections of the respiratory system in patients affected by Cystic Fibrosis. The comparative analysis of the annotated genomes of these *Psychrobacter* strains highlighted their differences in terms of overall genomic content (e.g. shared gene sets) and allowed the identification of gene clusters hypothetically involved in the biosynthesis of antimicrobial compounds.

1. Introduction

Bacteria inhabiting extreme and isolated environments represent potential sources of novel bioactive molecules. In particular, Antarctic bacteria have been shown to be capable of synthesizing compounds with antimicrobial activity (Papaleo et al., 2012, 2013), particularly active against bacteria belonging to the *Burkholderia cepacia* complex (Bcc).

In this work, we report the genome sequences of three strains belonging to the *Psychrobacter* genus isolated from different Antarctic sponges. Two of them (*Psychrobacter* sp. TB2 and TB15) were isolated from samples of the Antarctic sponge *Lissodendoryx nobilis*, whereas the remaining one (*Psychrobacter* sp. AC24) was isolated from *Haliclonissa verrucosa*. Strains TB2, TB15 and AC24 belong to the Italian Collection of Antarctic Bacteria of the National Antarctic Museum (CIBAN-MNA, Italy).

Inhibitory activity against representatives of the Bcc was assessed as described in Papaleo et al. (2012). Results of these tests revealed the capability of *Psychrobacter* sp. AC24 to efficiently inhibit the growth of almost all the Bcc strains tested in this work, regardless of the growth medium. Conversely, TB2 and TB15 displayed a reduced inhibitory ability compared to AC24 and, in some cases, the effect on the growth of Bcc strains was influenced by the corresponding growth medium (Supporting Information, Table S1).

Genome sequencing (using Illumina HiSeq2000) was performed in order to provide a genomic and taxonomic background able to guide future research on these strains. Obtained reads were trimmed with SolexaQA DynamicTrim (Cox et al., 2010). The resulting reads (28,229,244 for AC24, 26,667,670 for TB15 and 17,211,784 for TB2) were assembled using ABySS 1.3.6 (Simpson et al., 2009). The optimal parameters for the assemblies were determined after carrying out several trials, automatically performed with an ad hoc developed software (available at http://www.dbefcb.unifi.it/CMpro-v-p-8.html). Among obtained assemblies, we chose those for which the highest average contig lengths were obtained. After filtering out the contigs with a length <500 bp, we obtained an assembly size of 3,574,524 bp, 3,066,842 bp and 3,033,234 bp for AC24, TB15 and TB2, respectively, distributed into 88, 43 and 47 contigs. Further details for genome assemblies are shown in Table 1.

Contigs were submitted to RAST annotation server (Aziz et al., 2008), allowing the identification of 3,076, 2,627 and 2587 ORFs for AC24, TB15 and TB2, respectively. A total of 2300 (75%) ORFs of AC24, 2064 (79%) of TB15 and 2040 (79%) of TB2 were assigned to at least one of the Clusters of Orthologous Groups (COG) (Tatusov et al., 2000).

Particular attention was devoted to the search of genes involved in the biosynthesis of secondary metabolites, known to often possess

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Table 1
General features of *Psychrobacter* draft genomes.

Strain	Origin	Assembly size (bp)	N. contigs	GC content %	Predicted ORFs	tRNA	rRNA
AC24	*H. verrucosa*	3,574,524	88	43.01	3076	52	12
TB15	*L. nobilis*	3,066,842	43	43.89	2627	44	6
TB2		3,033,234	47	43.76	2587	44	6

antimicrobial activity. A search for secondary metabolites related genes was thus carried out with antiSMASH (Blin et al., 2013), revealing a variable number of clusters putatively involved in such biosynthesis; 12, 8 and 7 clusters were retrieved for AC24, TB15 and TB2 strains, respectively (Supporting Information, File S1). From a structural viewpoint, all these gene clusters showed GC% content values in the range of the ones possessed by the corresponding genome (i.e. from 39% to 43%). Unfortunately, on the basis of performed sequence-similarity searches, no hints could be derived concerning the product(s) synthesized by those clusters. This, in turn, suggests that the metabolic strategies exploited by the three *Psychrobacter* strains to inhibit the growth of *Burkholderia* representatives fall outside the range of already characterized biochemical systems and that more experimental effort will be necessary to fully elucidate them.

The evolutionary distance among these strains was assessed through a phylogenetic analysis based on their 16S rDNA sequences. The obtained phylogenetic tree (grouping TB2 and TB15 strains away from AC24) is available in Supporting Information, File S2. However, to gain a deeper knowledge of the genomic background of the isolated *Psychrobacter* strains, comparative genomics analyses were performed. A custom made Perl script (available at http://www.dbefcb.unifi.it/CMpro-v-p-8.html) that iteratively uses InParanoid (O'Brien et al., 2005) and MultiParanoid (Alexeyenko et al., 2006) to make multiple comparisons between pairs of proteins sets, was run to identify which protein sequences are shared among all the strains (core genome), by only two of them (accessory genome) or are genome specific (unique genomes). Results of this analysis are reported in Fig. 1. This analysis is in overall agreement with the relative phylogenetic position of the *Psychrobacter* representatives analyzed in this work. Moreover, it allows reducing the search space of genes related to their antimicrobial activity. Indeed, the different inhibitory activity of the three strains (higher in AC24 with respect to TB2 and TB15, see Table 1) suggests the presence of specific metabolic circuits in *Psychrobacter* sp. AC24 strain which, in turn, are likely to be encoded by its unique genome. A BLAST search confirmed this hypothesis since clusters from TB2 and TB15 all belong to core and accessory genomes whereas four of those from AC24 are encoded by its unique genome.

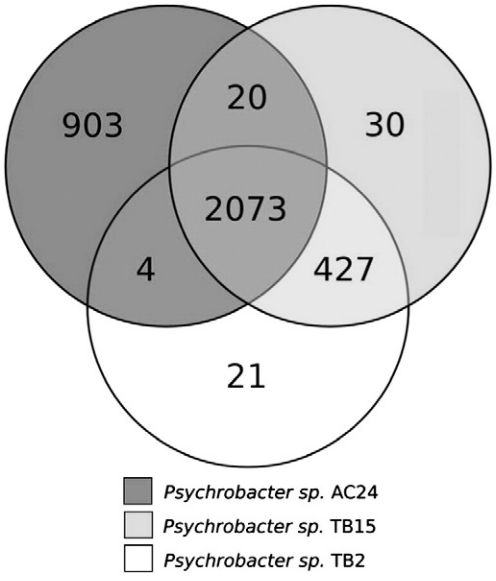

Fig. 1. Pangenome structure of the three *Psychrobacter* strains.

In conclusion, the analysis of the annotated genomes of *Psychrobacter* strains AC24, TB2 and TB15 (Genbank accessions AYXM01000000, AYUI01000000 and AYXN01000000, respectively) revealed the presence of several (still uncharacterized) gene clusters involved in secondary metabolites production that may be the object of further investigation and major differences in terms of shared gene sets. These data represent a solid platform for further characterization/exploitation of the metabolic features linked to bioactive compound biosynthesis.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.margen.2013.12.009.

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References

Papaleo, M.C., Fondi, M., Maida, I., Perrin, E., Lo Giudice, A., Michaud, L., Mangano, S., Bartolucci, G., Romoli, R., Fani, R., 2012. Sponge-associated microbial Antarctic communities exhibiting antimicrobial activity against *Burkholderia cepacia* complex bacteria. Biotechnol. Adv. 30, 272–293.

Papaleo, M.C., Romoli, R., Bartolucci, G., Maida, I., Perrin, E., Fondi, M., Orlandini, V., Mengoni, A., Emiliani, G., Tutino, M.L., Parrilli, E., de Pascale, D., Michaud, L., Lo Giudice, A., Fani, R., 2013. Bioactive volatile organic compounds from Antarctic (sponges) bacteria N. Biotechnol. 30, 824–838.

Cox, M.P., Peterson, D.A., Biggs, P.J., 2010. SolexaQA: at-a-glance quality assessment of Illumina second-generation sequencing data. BMC Bioinforma. 11, 485.

Simpson, J.T., Wong, K., Jackman, S.D., Schein, J.E., Jones, S.J., Birol, I., 2009. ABySS: a parallel assembler for short read sequence data. Genome Res. 19, 1117–1123.

Aziz, R.K., Bartels, D., Best, A.A., DeJongh, M., Disz, T., Edwards, R.A., Formsma, K., Gerdes, S., Glass, E.M., Kubal, M., Meyer, F., Olsen, G.J., Olson, R., Osterman, A.L., Overbeek, R.A., McNeil, L.K., Paarmann, D., Paczian, T., Parrello, B., Pusch, G.D., Reich, C., Stevens, R., Vassieva, O., Vonstein, V., Wilke, A., Zagnitko, O., 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9, 75.

Tatusov, R.L., Galperin, M.Y., Natale, D.A., Koonin, E.V., 2000. The COG database: a tool for genome-scale analysis of protein functions and evolution. Nucleic Acids Res. 28, 33–36.

Blin, K., Medema, M.H., Kazempour, D., Fischbach, M.A., Breitling, R., Takano, E., Weber, T., 2013. antiSMASH 2.0 — a versatile platform for genome mining of secondary metabolite producers. Nucleic Acids Res. 41, W204–W212.

O'Brien, K.P., Remm, M., Sonnhammer, E.L., 2005. InParanoid: a comprehensive database of eukaryotic orthologs. Nucleic Acids Res. 33, D476–D480.

Alexeyenko, A., Tamas, I., Liu, G., Sonnhammer, E.L., 2006. Automatic clustering of orthologs and inparalogs shared by multiple proteomes. Bioinformatics 22, e9–e15.