High quality draft genome sequence of *Streptomyces sp.* strain AW19M42 isolated from a sea squirt in Northern Norway

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Here we report the 8 Mb high quality draft genome of *Streptomyces sp.* strain AW19M42, together with specific properties of the organism and the generation, annotation and analysis of its genome sequence. The genome encodes 7,727 putative open reading frames, of which 6,400 could be assigned with COG categories. Also, 62 tRNA genes and 8 rRNA operons were identified. The genome harbors several gene clusters involved in the production of secondary metabolites. Functional screening of the isolate was positive for several enzymatic activities, and some candidate genes coding for those activities are listed in this report. We find that this isolate shows biotechnological potential and is an interesting target for bioprospecting.

Introduction

The filamentous and Gram-positive genus *Streptomyces*, belonging to the phylum *Actinobacteria* [1], are attractive organisms for bioprospecting being the largest antibiotic-producing genus discovered in the microbial world so far [2]. These species have also been exploited for heterologous expression of a variety of secondary metabolites [3]. Additionally, these species harbor genes coding for enzymes that can be applicable in industry and biotechnology [4,5].

Since the first, complete *Streptomyces* genome was published [6], a number of strains isolated from terrestrial environments have been reported [7-11]. Genomic investigations on *Streptomyces* from marine sources have, however, just recently begun [12-16].

Here, we present the draft genome sequence of *Streptomyces sp.* strain AW19M42 isolated from a marine source, together with the description of genome properties and annotation. Results from functional enzyme screening of the bacterium are also reported.

Classification and features

The *Streptomyces sp.* strain AW19M42 was identified in a biota sample collected from the internal organs of a sea squirt (class *Ascidacea*, subphylum *Tunicate*, phylum *Chordata*). The tunicate was isolated using an Agassiz trawl at a depth of 77m in Helmolofjorden, in the sub-Arctic region of Norway (Table 1). The trawling was done during a research cruise with R/V Jan Mayen in April 2010.

The bacterium was isolated during four weeks of incubation at 4-15°C on humic acid containing agar media that is selective for growth of actinomycetes [29,30]. For isolation and nucleic acid extraction the bacterium was cultivated in autoclaved media containing 0.1% (w/v) malt extract, 0.1% (v/v) glycerol, 0.1% (w/v) peptone, 0.1% (w/v) yeast extract, 2% (w/v) agar in 50% (v/v) natural sea water and 50% (v/v) distilled water, pH 8.2 [29]. The gene encoding 16S rRNA was amplified by using two universal primers, 27F (5′-AGAGTTTGATCCTGGCTCAG) and 1492R (5′-GGTTACCTTGTGACGACTT) [31], in a standard Taq polymerase driven PCR (VWR) on crude genomic DNA prepared by using InstaGene Matrix (BioRad). Following PCR purification by PureLink PCR Purification (Invitrogen), sequencing was
carried out with the BigDye terminator kit version 3.1 (Applied Biosystems) and a universal 515F primer (5′-GTGCCAGCMGCCGCGGTAA) [32]. Using the 16S rRNA sequence data in a homology search by BLAST [33] indicated that the isolate belonged to the *Streptomyces* genus, among the *Streptomycetaceae* family of Actinobacteria. A phylogenetic tree was reconstructed from the 16S rRNA gene sequence together with other *Streptomyces* homologues (Figure 1) using the MEGA 5.10 software suit [34]. The evolutionary history was inferred using the UPGMA method [35] and the evolutionary distances were computed using the Maximum Composite Likelihood method [36]. The phylogenetic analysis confirmed that the isolate AW19M42 belongs to the genus *Streptomyces*. The closest neighbor with a reported, complete genome sequence is *Streptomyces griseus subsp. griseus* [7], however, the phylogenetic tree indicates that the *Streptomyces sp.* strain AW19M42 isolate belongs to a closely related but separate clade. Draft genomes have not been reported for this clade previously.

| MIGS ID | Property                        | Term                                      | Evidence code |
|---------|---------------------------------|-------------------------------------------|---------------|
|         | Domain                          | *Bacteria*                                | TAS [18]      |
|         | Phylum                           | *Actinobacteria*                          | TAS [1]       |
|         | Class                            | *Actinobacteria*                          | TAS [19]      |
|         | Subclass                         | *Actinobacteridae*                        | TAS [19,20]   |
|         | Current classification           | Order *Actinomycetales*                   | TAS [19-22]   |
|         |                                  | Suborder *Streptomycinae*                 | TAS [19,20]   |
|         |                                  | Family *Streptomycetaceae*                | TAS [19,20,22-24] |
|         |                                  | Genus *Streptomyces*                      | TAS [22,24-27] |
|         | Species                          | *Streptomyces sp.*                        | NAS           |
|         | Strain                           | AW19M42                                   | IDA           |
|         | Gram stain                       | Gram positive                             | NDA           |
|         | Cell shape                       | Branched mycelia                          | NDA           |
|         | Motility                         | Dispersion of spores                      | NDA           |
|         | Sporulation                      | Sporulating                               | NDA           |
|         | Temperature range                | Range not determined, grows at 15°C and 28°C | IDA           |
|         | Salinity                         | Not determined, but survives 50% natural sea water | IDA           |
|         | Oxygen requirements              | Aerobic                                   | NDA           |
|         | Carbon source                    | Not reported                              |               |
|         | Energy source                    | Not reported                              |               |
| MIGS-6.3| Habitat                         | Inner organs of sea squirt                | IDA           |
| MIGS-22| Biotic relationship              | Free-living                               | IDA           |
| MIGS-14| Pathogenicity                    | Non-pathogenic                            | NDA           |
|         | Biosafety level                  | 1                                         |               |
| MIGS-4  | Geographic location             | Hellmofjorden, Norway                     | IDA           |
| MIGS-5  | Sample collection time           | April 2010                                | IDA           |
| MIGS-4.1| Latitude                        | N67 49.24316                              | IDA           |
| MIGS-4.2| Longitude                       | E16 28.99465                              | IDA           |
| MIGS-4.3| Depth                           | 77.35 m                                   | IDA           |

Evidence codes - IDA: Inferred from Direct Assay (first time in publication); TAS: Traceable Author Statement (i.e., a direct report exists in the literature); NAS: Non-traceable Author Statement (i.e., not directly observed for the living, isolated sample, but based on a generally accepted property for the species, or anecdotal evidence). These evidence codes are from of the Gene Ontology project [28]. If the evidence code is IDA, then the property was directly observed for a live isolate by one of the authors or an expert or mentioned in the acknowledgements.

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![Figure 1. Phylogenetic tree indicating the phylogenetic relationship of Streptomyces sp. strain AW19M42 relative to other Streptomyces species. The phylogenetic tree was made by comparing the 16S rDNA sequence of the Streptomyces sp. strain AW19M42 to the closest related sequences from both validated type strains and unidentified isolates. S. venezuelae is used as outgroup. All positions containing gaps and missing data were eliminated. There were a total of 1,389 positions in the final dataset. The bar shows the number of base substitutions per site.](image-url)

### Genome sequencing and annotation

The organism was selected for genome sequencing on the basis of its phylogenetic position. The genome project is part of a Norwegian bioprospecting project called **Molecules for the Future** (MARZymes) which aims to search Arctic and sub-Arctic regions for marine bacterial isolates that might serve as producers of novel secondary metabolites and enzymes. High quality genomic DNA for sequencing was isolated with the GenElute Bacterial Genomic DNA Kit (Sigma) according to the protocol for extraction of nucleic acids from gram positive bacteria. A 700 bp paired-end library was prepared and sequenced using the HiSeq 2000 (Illumina) paired-end technology (Table 2). This generated 13.94 million paired-end reads that were assembled into 670 contigs larger than 500 bp using the CLC Genomics Workbench 5.0 software package [37]. Gene prediction was performed using Glimmer 3 [38] and gene functions were annotated using an in-house genome annotation pipeline.
Genome properties

The total size of the genome is 8,008,851 bp and has a GC content of 70.57% (Table 3), similar to that of other sequenced *Streptomyces* isolates. A total of 7,727 coding DNA sequences (CDSs) were predicted (Table 3). Of these, 6,400 could be assigned to a COG number (Table 4). In addition, 62 tRNAs and 8 copies of the rRNA operons were identified.

### Table 2. Genome sequencing project information

| MIGS ID | Property                  | Term                                      |
|---------|---------------------------|-------------------------------------------|
| MIGS-31 | Finishing quality         | Improved high quality draft               |
| MIGS-28 | Libraries used            | One Illumina Paired-End library           |
| MIGS-29 | Sequencing platforms      | Illumina HiSeq2000                       |
| MIGS-31.2| Fold coverage            | 350×                                      |
| MIGS-30 | Assemblers                | CLC paired-end assembly                   |
| MIGS-32 | Gene calling method       | Glimmer 3                                 |

| Genbank ID | CBRG0000000000           |
| Genbank Date of Release | September 11, 2013         |
| GOLD ID    | Gi0070794                |

| Project relevance | Bioprospecting |

### Table 3. Genome statistics, including nucleotide content and gene count levels

| Attribute                             | Value  | % of total^a |
|---------------------------------------|--------|--------------|
| Genome size (bp)                      | 8,008,851 | 100          |
| DNA coding region (bp)                | 6,979,999 | 87.2         |
| DNA G+C content (bp)                  | 4,951,797 | 70.6         |
| Total genes                           | 7,813   | n/a          |
| rRNA operons                          | 8       | n/a          |
| tRNA genes                            | 62      | n/a          |
| Protein-coding genes                  | 7,727   | 100          |
| Genes assigned to COGs                | 6,400   | 82.8         |
| Genes with signal peptides            | 987     | 12.8         |
| Genes with transmembrane helices      | 1,660   | 21.5         |

^aThe total is based on either the size of the genome in base pairs or the total number of protein coding genes in the annotated genome.
**Table 4.** Number of genes associated with the 25 general COG functional categories

| Code | Value | %age | Description                                                      |
|------|-------|------|------------------------------------------------------------------|
| J    | 264   | 3.4  | Translation                                                      |
| A    | 1     | 0.0  | RNA processing and modification                                  |
| K    | 836   | 10.8 | Transcription                                                   |
| L    | 330   | 4.3  | Replication, recombination and repair                            |
| B    | 5     | 0.1  | Chromatin structure and dynamics                                 |
| D    | 71    | 0.9  | Cell cycle control, mitosis and meiosis                         |
| Y    | 0     | 0.0  | Nuclear structure                                               |
| V    | 159   | 2.1  | Defense mechanisms                                              |
| T    | 442   | 5.7  | Signal transduction mechanisms                                  |
| M    | 338   | 4.3  | Cell wall/membrane biogenesis                                   |
| N    | 28    | 0.4  | Cell motility                                                   |
| Z    | 6     | 0.1  | Cytoskeleton                                                    |
| W    | 0     | 0.0  | Extracellular structures                                        |
| U    | 79    | 1.0  | Intracellular trafficking and secretion                         |
| O    | 200   | 2.6  | Posttranslational modification, protein turnover, chaperones     |
| C    | 409   | 5.3  | Energy production and conversion                                |
| G    | 665   | 8.6  | Carbohydrate transport and metabolism                           |
| E    | 730   | 9.4  | Amino acid transport and metabolism                              |
| F    | 123   | 1.6  | Nucleotide transport and metabolism                              |
| H    | 262   | 3.4  | Coenzyme transport and metabolism                               |
| I    | 330   | 4.3  | Lipid transport and metabolism                                  |
| P    | 435   | 5.6  | Inorganic ion transport and metabolism                           |
| Q    | 417   | 5.4  | Secondary metabolites biosynthesis, transport and catabolism     |
| R    | 1,181 | 15.3 | General function prediction only                                 |
| S    | 465   | 6.0  | Function unknown                                                |
| -    | 1,327 | 17.2 | Not in COGs                                                     |

*The total is based on the total number of protein coding genes in the annotated genome.*
All putative protein coding sequences were assigned KEGG orthology [39], and mapped onto pathways using the KEGG Automatic Annotation Server (KAAS) server [40]. The analysis revealed that *Streptomyces* sp. strain AW19M42 harbors several genes related to biosynthesis of secondary metabolites. We have identified genes that map to the streptomycin biosynthesis pathway (glucose-1-phosphate thymidyltransferase (EC 2.7.7.24), dTDP-glucose 4,6-dehydratase (EC 4.2.1.46) and dTDP-4-dehydrorhamnose reductase (EC 1.1.1.133)). Also, several genes map to the pathways for biosynthesis of siderophore group nonribosomal peptides, biosynthesis of type II polyketide product pathway and polyketide sugar unit biosynthesis. Interestingly, two clusters, comprising five genes, both mapped to the biosynthesis of type II polyketide backbone pathway. These genes clusters comprise genes STREP_3146-3150 and STREP_4370-4374. This suite of genes may contribute to a distinct profile of secondary metabolites production.

**Insights from the Genome Sequence**

The isolate was successfully screened for lipase, caseinase, gelatinase, chitinase, amylase and DNase activities (Figure 2), by using marine broth (Difco) agar plates incubated at 20°C [41-46]. The plates were supplemented with 1% (v/v) tributyrin, 1% (w/v) skim milk, 0.4% (w/v) gelatin, 0.5% (w/v) chitin or 2% (w/v) starch, respectively (all substrates from Sigma), whereas DNase test agar (Merck) was supplemented with 0.3M NaCl, representing sea water salt concentration, before screening for DNase activity. Putative genes coding for these activities were identified in the genome based on annotation or by homology search (Table 5).

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**Figure 2.** Degradation halos around colonies of *Streptomyces* sp. AW19M42 growing on agar plates supplemented with A, skim milk, B, gelatin, C, tributyrin, D, DNA, E, chitin and F, starch.
Table 5. Candidate genes coding for putative lipase, caseinase, gelatinase and DNase activities identified in *Streptomyces sp.* strain AW19M42 draft genome.

| Putative gene | Annotation                                                                 | Size (aa) |
|---------------|---------------------------------------------------------------------------|-----------|
| **Lipase**    |                                                                           |           |
| STREP_0737    | Lipase                                                                    | 273       |
| STREP_1671    | Triacylglycerol lipase                                                    | 266       |
| STREP_1821    | G-D-S-L family lipolytic protein                                          | 281       |
| STREP_2698    | Lipase class 2                                                            | 297       |
| STREP_2704    | Triacylglycerol lipase                                                    | 269       |
| STREP_4585    | Secreted hydrolase                                                        | 268       |
| STREP_5662    | Lipase or acylhydrolase family protein                                    | 367       |
| STREP_6665    | Esterase/lipase                                                           | 259       |
| STREP_6850    | Esterase/lipase                                                           | 429       |
| STREP_7611    | Triacylglycerol lipase                                                    | 366       |
| **Gelatinase**|                                                                           |           |
| STREP_5784    | Peptidase M4 thermolysin                                                 | 523       |
| STREP_6038    | Peptidase M4 thermolysin                                                 | 680       |
| STREP_3662    | Peptidase M4 thermolysin                                                 | 358       |
| **Caseinase** |                                                                           |           |
| STREP_0198    | Putative secreted serine protease                                         | 361       |
| STREP_0258    | Protease                                                                  | 278       |
| STREP_0974    | Protease                                                                  | 488       |
| STREP_1078    | Serine protease                                                           | 388       |
| STREP_1313    | M6 family metalloprotease domain-containing protein                       | 398       |
| STREP_1389    | M6 family metalloprotease domain protein                                  | 1,389     |
| STREP_2216    | Putative secreted subtilisin-like serine protease                         | 511       |
| STREP_2239    | Metalloprotease                                                          | 296       |
| STREP_3135    | Metalloprotease domain protein                                            | 127       |
| STREP_3964    | ATP-dependent protease La                                                 | 808       |
| STREP_3975    | ATP-dependent metalloprotease FtsH                                       | 673       |
| STREP_4000    | Streptorisin-B - Pronase enzyme B SGPB/Serine protease B                 | 299       |
| STREP_5179    | ATP-dependent Clp protease proteolytic subunit                           | 222       |
| STREP_5180    | ATP-dependent Clp protease, ATP-binding subunit ClpX                     | 432       |
| STREP_5944    | Protease                                                                  | 527       |
| STREP_5945    | Protease                                                                  | 534       |
| STREP_6196    | Protease                                                                  | 383       |
| STREP_6570    | Protease                                                                  | 701       |
| STREP_6821    | Putative protease                                                         | 352       |
| STREP_7179    | Serine protease                                                           | 635       |
| STREP_7580    | Protease                                                                  | 856       |
| **DNase**     |                                                                           |           |
| STREP_0436    | Exodeoxyribonuclease VII, large subunit                                  | 403       |
| STREP_0437    | Exodeoxyribonuclease VII small subunit                                   | 91        |
| STREP_1352    | Exodeoxyribonuclease III Xth                                            | 268       |
| STREP_1969    | TatD-related deoxyribonuclease                                           | 1,969     |
| STREP_2155    | Deoxyribonuclease V                                                       | 220       |
Table 5 (cont.). Candidate genes coding for putative lipase, caseinase, gelatinase and DNase activities identified in *Streptomyces* sp. strain AW19M42 draft genome.

| Putative gene  | Annotation                                               | Size (aa) |
|---------------|----------------------------------------------------------|-----------|
| STREP_2430    | Deoxyribonuclease/rho motif-related TRAM                | 452       |
| STREP_4206    | Deoxyribonuclease                                       | 776       |
| STREP_6678    | Probable endonuclease 4 - Endodeoxyribonuclease         | 275       |
| Chitinase     |                                                          |           |
| STREP_2729    | Chitinase, glycosyl hydrolase 18 family                 | 628       |
| STREP_5817    | Chitinase, glycosyl hydrolase 18 family                 | 424       |
| STREP_5513    | Carbohydrate-binding CenC domain protein                | 577       |
| STREP_3508    | Glycoside hydrolase family protein                      | 609       |
| STREP_4257    | Putative endochitinase                                  | 350       |
| STREP_6187    | Chitinase, glycosyl hydrolase 19 family                 | 297       |
| STREP_6188    | Chitinase, glycosyl hydrolase 19 family                 | 291       |
| Amylase       |                                                          |           |
| STREP_1696    | Glycoside hydrolase starch-binding protein              | 573       |
| STREP_5789    | Secreted alpha-amyrase                                  | 458       |
| STREP_7405    | Malto-oligosyltrehalose synthase                        | 834       |
| STREP_1697    | Alpha-1,6-glucosidase, pullulanase-type                 | 1,774     |

Conclusion

The 8 Mb draft genome belonging to *Streptomyces* sp. strain AW19M42, originally isolated from a marine sea squirt in the sub-Arctic region of Norway has been deposited at ENA/DDBJ/GenBank under accession number CBRG000000000. The isolate was successfully screened for several enzymatic activities that are applicable in biotechnology and candidate genes coding for the enzyme activities were identified in the genome. *Streptomyces* sp. strain AW19M42 will serve as a source of functional enzymes and other bioactive chemicals in future bioprospecting projects.

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