Draft genome sequence of *Streptomyces hyaluromycini* MB-PO13<sup>T</sup>, a hyaluromycin producer

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**Abstract**

*Streptomyces hyaluromycini* MB-PO13<sup>T</sup> (=NBRC 110483<sup>T</sup> = DSM 100105<sup>T</sup>) is type strain of the species, which produces a hyaluronidase inhibitor, hyaluromycin. Here, we report the draft genome sequence of this strain together with features of the organism and generation, annotation and analysis of the genome sequence. The 11.5 Mb genome of *Streptomyces hyaluromycini* MB-PO13<sup>T</sup> encoded 10,098 putative ORFs, of which 5317 were assigned with COG categories. The genome harbored at least six type I PKS clusters, three type II PKS gene clusters, two type III PKS gene clusters, six NRPS gene clusters, and one hybrid PKS/NRPS gene cluster. The type II PKS gene cluster including 2-amino-3-hydroxycyclopent-2-enone synthetic genes was identified to be responsible for hyaluromycin synthesis. We propose the biosynthetic pathway based on bioinformatic analysis.

**Keywords:** Biosynthesis, C<sub>3</sub>N, Polyketide synthase, Rubromycin, *Streptomyces*

**Introduction**

Hyaluromycin is a hyaluronidase inhibitor isolated from the culture broth of an actinomycete strain MB-PO13<sup>T</sup> of the genus *Streptomyces* [1]. The structure consists of a γ-rubromycin core possessing a C<sub>3</sub>N unit as an amide substituent of the carboxyl functionality. Rubromycin's have inhibitory activities against human telomerase and the reverse transcriptase of human immunodeficiency virus-1 [2]. The core structure possesses a hexacyclic ring system and a 5,6-bisbenzannelated spiroketal structure. The most intriguing part of hyaluromycin is the C<sub>3</sub>N moiety, which is present only in a limited range of secondary metabolites of actinomycetes [3]. As for the rubromycin family biosynthesis, putative biosynthetic genes for griseorhodin A were reported [4], but there is no report on the rubromycins. Hence, the biosynthesis of rubromycin family remains unclear. In this study, we performed whole genome shotgun sequencing of the strain MB-PO13<sup>T</sup> to elucidate the biosynthetic mechanism of hyaluromycin. We herein present the draft genome sequence of *Streptomyces hyaluromycini* MB-PO13<sup>T</sup>, together with the taxonomical identification of the strain, description of its genome properties and annotation of the gene cluster for hyaluromycin synthesis. The biosynthetic pathway of hyaluromycin is also proposed on the basis of the bioinformatic prediction.

**Organism information**

**Classification and features**

During the course of screening for hyaluronidase inhibitors from actinomycetes, *Streptomyces hyaluromycini* MB-PO13<sup>T</sup> was isolated from a tunicate (*Molgula manhattensis*) collected in Tokyo Bay, Japan and found to produce hyaluromycin [1]. Colony appearance was examined after incubation at 28 °C for 14 days on an agar plate of ISP 4. Morphological features were observed under a light microscope (model BX-51; Olympus) and a scanning electron microscope (model JSM-6060; JEOL). The temperature range and optimum temperature for growth were determined by incubating the strain at 5, 10, 15, 20, 28, 37, 42, and 50 °C on ISP 2 agar plates for 14 days. The pH range for growth was determined at 28 °C in ISP 2 broth, of which pH was adjusted to 3 to 12 by 1 N HCl or 1 M Na<sub>2</sub>CO<sub>3</sub>. Tolerance to NaCl was

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tested on ISP 2 agar plates containing 2, 3, 5, 7, 9, and 12% (w/v) NaCl at 28 °C. Carbohydrate utilization was determined on ISP 9 supplemented with sterilized carbon sources [5]. The strain grow well on ISP 3, ISP 4 and yeast-starch agars but poor on ISP 2, ISP 5, ISP 6, ISP 7, glucose-asparagine, nutrient, sucrose-nitrate and skim milk agars. Soluble red pigments are produced on ISP 2, ISP 3, ISP 4, ISP 7, glucose-asparagine, nutrient and yeast-starch agars. Cells are aerobic and Gram-stain-positive. The aerial mycelia are branched and yellowish white in color, which become light grey at sporulation and the substrate mycelia are deep red on ISP 4 agar plate. Smooth surface spores (0.5–0.8 × 1.0–1.5 μm) in spiral chains are formed when cultured on nutritionally poor media. A scanning electron micrograph of the strain is shown in Fig. 1. Growth occurs at 10–37 °C (optimum 28 °C), at pH 4.0–9.0 (optimum pH 7.0) and in the presence of less than 2% NaCl (w/v). The strain utilizes L-arabinose, D-fructose, D-glucose, inositol, D-mannitol, rhamnose and D-xylose as sole carbon source for energy and growth, but not raffinose and sucrose (all at 1%, w/v). These results are summarized in Table 1. The genes encoding 16S rRNA were amplified by PCR using two universal primers, 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) [6]. GoTaq Green Master Mix (Promega) was used as described by the manufacture for the PCR. The reaction was started with denaturation at 94 °C for 5 min followed by a total 27 cycles that consisted of denaturation at 94 °C for 30 s, annealing at 57 °C for 30 s, and extension at 72 °C for 1.5 min, and extension at 72 °C for 7 min. The PCR product was purified by Wizard SV Gel and PCR Clean-Up System (Promega) and sequenced with a BigDye cycle sequencing ready reaction kit (Applied Biosystems) on an ABI PRISM 310 Genetic analyzer (Applied Biosystems). The sequence was deposited into DDBJ under the accession number AB184533. BLAST search of the sequence by the EzTaxon-e server [7] indicated the highest similarity to that of Streptomyces graminisoli JR-19T (HQ267975, 99.79%, 1440/1443). A phylogenetic tree was reconstructed on the basis of the

### Table 1 Classification and general features of *Streptomyces hyaluromycini* MB-PO13T

| MIGS ID | Property | Term | Evidence code |
|---------|----------|------|---------------|
| Classification | Domain | Bacteria | TAS [24] |
| | Phylum | Actinobacteria | TAS [25] |
| | Class | Actinobacteria | TAS [26] |
| | Order | Actinomycetales | TAS [26–29] |
| | Suborder | Streptomyces | TAS [26, 29] |
| | Family | Streptomyces | TAS [26, 28–31] |
| | Genus | Streptomyces | TAS [28, 31–33] |
| | Species | *Streptomyces hyaluromycini* | TAS [12] |
| | Strain | MB-PO13 | TAS [1] |
| Gram stain | Gram-positive | TAS [12] |
| Cell shape | Branched mycelia | TAS [12] |
| Motility | Not reported | | |
| Sporulation | Sporulating | TAS [12] |
| Temperature range | 10 °C to 37 °C | TAS [12] |
| Optimum temperature | 28 °C | TAS [12] |
| pH range; Optimum | 4 to 9, 7 | TAS [12] |
| Carbon source | Glucose, inositol, arabinose, fructose, glucose, inositol, mannitol, rhamnose, xylose | TAS [12] |

**Fig. 1** Scanning electron micrograph of *Streptomyces hyaluromycini* MB-PO13T grown on 1/10 ISP 2 agar for 14 days at 28 °C. Bar, 5 μm.
16S rRNA gene sequence together with taxonomically close *Streptomyces* type strains using CLUSTAL-W program [8] and by the neighbor-joining method [9] using the MEGA 6.0 program [10]. The resultant tree topologies were evaluated by bootstrap analysis [11] based on 1000 replicates. The phylogenetic tree is shown in Fig. 2. On the basis of these findings, strain MB-PO13T was proposed to be classified as a representative of a novel species of the genus *Streptomyces*, with the name *Streptomyces hyaluromycini* sp. nov. [12].

### Chemotaxonomic data

The isomer of diaminopimelic acid in the whole-cell hydrolysate was analyzed according to the method described by Hasegawa et al. [13]. Isoprenoid quinones and cellular fatty acids were analyzed as described previously [14]. The whole-cell hydrolysate of strain MB-PO13T contained LL-A2pm, glucose and mannose. The detected menaquinones were identified as MK-9(H8), MK-9(H6), MK-9(H4) and MK-9(H10) (5:37:57:1). The principal polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol. Six unidentified phospholipids were also detected. The major cellular

### Table 2 Project information

| MIGS ID | Property      | Term                  |
|---------|---------------|-----------------------|
| MIGS 31 | Finishing quality | High-Quality Draft |
| MIGS 28 | Libraries used | 454 shotgun library, Illumina paired-end library |
| MIGS 29 | Sequencing platforms | 454 GS FLX+, Illumina HiSeq1000 |
| MIGS 31.2 | Fold coverage | 77x |
| MIGS 30 | Assemblers | Newbler v2.6, GenoFinisher |
| MIGS 32 | Gene calling method | Prodigal |
| Locus Tag | MB-PO13 |
| Genbank ID | BCFL01000001-BCFL01000052 |
| GenBank Date of Release | July 1, 2017 |
| GOLD ID | Not registered |
| BIOPROJECT | PRJDB4283 |
| MIGS 13 | Source Material Identifier | NBRC 110483 |
| Project relevance | Industrial |

### Table 3 Genome statistics

| Attribute                  | Value         | % of Total |
|----------------------------|---------------|------------|
| Genome size (bp)           | 11,525,033    | 100.0      |
| DNA coding (bp)            | 10,176,135    | 88.3       |
| DNA G+C (bp)               | 8,184,694     | 71.0       |
| DNA scaffolds              | 52            | –          |
| Total genes                | 10,201        | 100.0      |
| Protein coding genes       | 10,098        | 99.0       |
| RNA genes                  | 103           | 1.0        |
| Pseudo genes               | –             | –          |
| Genes in internal clusters | 4827          | 47.3       |
| Genes with function prediction | 7049        | 69.1       |
| Genes assigned to COGs     | 5317          | 52.1       |
| Genes with Pfam domains    | 7836          | 77.6       |
| Genes with signal peptides | 1003          | 9.9        |
| Genes with transmembrane helices | 2326    | 23.0       |
| CRISPR repeats             | 2             | 0          |
fatty acids (>10%) were anteiso-C\textsubscript{15}:0 (24.9%), iso-C\textsubscript{16}:0 (23.4%), iso-C\textsubscript{14}:0 (15.0%) and C\textsubscript{16}:0 (10.7%). These chemotaxonomic features corresponded to those of the genus \textit{Streptomyces}.

Genome sequencing information
Genome project history
In collaboration between Toyama Prefectural University and NBRC, the organism was selected for genome sequencing to elucidate the hyaluromycin biosynthetic pathway. We successfully accomplished the genome project of \textit{Streptomyces hyaluromycin} MB-PO13\textsuperscript{T} as reported in this paper. The draft genome sequences have been deposited in the INSDC database under the accession number BCFL01000001-BCFL01000052. The project information and its association with MIGS version 2.0 compliance are summarized in Table 2 [15].

Growth conditions and genomic DNA preparation
\textit{Streptomyces hyaluromycin} MB-PO13\textsuperscript{T} was deposited in the NBRC culture collection with the registration number of NBRC 110483\textsuperscript{T}. Its monoisolate was grown on polycarbonate membrane filter (Advantec) on 1/2 ISP 2 agar medium (0.2% yeast extract, 0.5% malt extract, 0.2% glucose, 2% agar, pH 7.3) at 28 °C. High quality genomic DNA for sequencing was isolated from the mycelia with an EZ1 DNA Tissue Kit and a Bio Robot EZ1 (Qiagen) according to the protocol for extraction of nucleic acid from Gram-positive bacteria. The size, purity, and double-strand DNA concentration of the genomic DNA were measured by pulsed-field gel electrophoresis, ratio of absorbance values at 260 nm and 280 nm, and Quant-iT PicoGreen dsDNA Assay Kit (Life Technologies), respectively, to assess the quality of genomic DNA.

Genome sequencing and assembly
Shotgun and paired-end libraries were prepared and subsequently sequenced using 454 pyrosequencing technology and HiSeq1000 (Illumina) paired-end technology, respectively (Table 2). The 77 Mb shotgun sequences and 881 Mb paired-end sequences were assembled using Newbler v2.8 and subsequently finished using GenoFinisher [16] to yield 52 scaffolds larger than 500 bp.

Genome annotation
Coding sequences were predicted by Prodigal [17] and tRNA-scanSE [18]. The gene functions were annotated using an in-house genome annotation pipeline, and PKS and NRPS-related domains were searched using the SMART and PFAM domain databases. PKS and NRPS gene clusters were determined as reported previously [19]. BLASTP search against the NCBI nr databases were also used for predicting function of

| Table 4 Number of genes associated with general COG functional categories |
|-----------------------------|---|----------------------|--------------------------------|
| Code | Value | %age | Description |
| J   | 244   | 2.4  | Translation, ribosomal structure and biogenesis |
| A   | 0     | 0    | RNA processing and modification |
| K   | 948   | 9.4  | Transcription |
| L   | 129   | 1.3  | Replication, recombination and repair |
| B   | 1     | 0.1  | Chromatin structure and dynamics |
| D   | 45    | 0.4  | Cell cycle control, cell division, chromosome partitioning |
| V   | 205   | 2.0  | Defense mechanisms |
| T   | 477   | 4.7  | Signal transduction mechanisms |
| M   | 279   | 2.8  | Cell wall/membrane biogenesis |
| N   | 25    | 0.2  | Cell motility |
| U   | 24    | 0.2  | Intracellular trafficking and secretion |
| O   | 176   | 1.7  | Posttranslational modification, protein turnover, chaperones |
| C   | 397   | 3.9  | Energy production and conversion |
| G   | 563   | 5.6  | Carbohydrate transport and metabolism |
| E   | 480   | 4.8  | Amino acid transport and metabolism |
| F   | 108   | 1.1  | Nucleotide transport and metabolism |
| H   | 332   | 3.3  | Coenzyme transport and metabolism |
| I   | 497   | 4.9  | Lipid transport and metabolism |
| P   | 281   | 2.8  | Inorganic ion transport and metabolism |
| Q   | 380   | 3.8  | Secondary metabolites biosynthesis, transport and catabolism |
| R   | 708   | 7.0  | General function prediction only |
| S   | 82    | 0.8  | Function unknown |
| –   | 4781  | 47.3 | Not in COGs |

The total is based on the total number of protein coding genes in the genome.

The \textit{rub}, \textit{Orf1}- and \textit{grh} are rubromycin-, hyarulomycin- and griseorhodin-biosynthetic gene clusters, respectively. Hyarulomycin-biosynthetic genes are indicated with orf numbers as shown in Table 5.
### Table 5: Putative hyaluromycin biosynthetic gene cluster and the neighboring genes

| Orf | Size (aa) | Proposed function | Closest homolog | Homolog (I/S, %) in grh cluster | Homolog (I/S, %) in rub cluster |
|-----|-----------|-------------------|-----------------|----------------------------------|---------------------------------|
| 768 | 656       | ABC transporter ATP-binding protein | multidrug ABC transporter ATP-binding protein, Actinopolymorpha alba, WP_020576731 | 70/83 – – | – – |
| 767 | 577       | multidrug ABC transporter ATPase | multidrug ABC transporter ATPase, Streptomyces vansovicensis, WP_030881385 | 69/81 – – | – – |
| 766 | 117       | MarR family transcriptional regulator | MarR family transcriptional regulator, Actinomadura macro, WP_067468911 | 45/63 – – | – – |
| 765 | 72        | unknown | hypothetical protein, Streptomyces aurantiacus, WP_055507532 | 56/60 – – | – – |
| 764 | 498       | transcriptional regulator | hypothetical protein, Streptomyces sp. NRRL WC-3742, WP_051836320 | 55/63 GrhR2 (34/48) | – – |
| 763 | 533       | amide synthetase | hypothetical protein, partial, Streptomyces sp. NRRL WC-3742, WP_078910860 | 60/70 – – | – – |
| 762 | 405       | 5-aminolevulinate synthase | AsuD2, Streptomyces nodosus subsp. asukaensis, AD158646 | 77/85 – – | – – |
| 761 | 515       | 5-aminolevulinate CoA ligase | AMP-dependent synthetase, Streptomyces uncialis, OKH94380 | 77/83 – – | – – |
| 760 | 183       | unknown | hypothetical protein, Streptomyces prunicolor, WP_019061819 | 50/60 – – | – – |
| 759 | 122       | unknown | hypothetical protein, Streptomyces fulvoviolaceus, WP_030615859 | 72/82 GrhI (61/73) | – – |
| 758 | 477       | oxygenase | hypothetical protein, Streptomyces yerevanensis, WP_033324694 | 72/82 GrhO1 (72/80) | Rubl (71/80) |
| 757 | 257       | 3-oxoacyl-ACP reductase | SDR family oxidoreductase, Streptomyces fulvoviolaceus, WP_030615854 | 83/92 GrhO2 (73/81) | RubJ (83/91) |
| 756 | 325       | acetyltransferase | GrhJ, Streptomyces sp. CN48+, AIE76926 | 68/74 GrhJ (67/73) | – – |
| 755 | 540       | monoxygenase | hypothetical protein, Streptomyces prunicolor, WP_026151147 | 73/80 GrhO5 (69/75) | RubL (73/80) |
| 754 | 161       | transcriptional regulator | putative transcriptional repressor GrhR3, Streptomyces sp. CN48+, AIE76928 | 76/88 GrhR3 (76/88) | RubM (74/83) |
| 753 | 501       | monoxygenase | RubN, Streptomyces collinus, AAM97364 | 80/86 GrhO6 (73/80) | RubN (80/86) |
| 752 | 325       | oxidoreductase | hypothetical protein, Streptomyces sp. TSRI0261, WP_073806081 | 86/93 GrhO7 (78/89) | – – |
| 751 | 343       | methyltransferase | hypothetical protein, Streptomyces fulvoviolaceus, WP_030615823 | 81/86 GrhL (77/83) | – – |
| 750 | 535       | monoxygenase | hypothetical protein, Streptomyces prunicolor, WP_019061807 | 74/82 GrhO8 (70/79) | RubO (63/72) |
| 749 | 534       | oxidoreductase | hypothetical protein, Streptomyces sp. TP-A0875, WP_053912978 | 74/80 GrhO9 (71/79) | RubP (74/80) |
| 748 | 161       | unknown | hypothetical protein, Streptomyces prunicolor, WP_019061805 | 81/85 GrhM (80/86) | RubQ (80/85) |
| 747 | 174       | unknown | hypothetical protein, Streptomyces fulvoviolaceus, WP_030615810 | 67/74 GrhN (56/64) | RubW (64/74) |
| 746 | 623       | asparagine synthase | RubR, Streptomyces collinus, AAM97368 | 80/86 GrhP (74/81) | RubR (80/86) |
| 745 | 569       | transcriptional regulator | RubS, Streptomyces collinus, AAM97369 | 63/75 GrhR2 (43/56) | RubS (63/75) |
| 744 | 123       | cyclase | putative cyclase, Streptomyces collinus, AAG03065 | 83/88 GrhQ (75/88) | RubE (83/88) |
| 743 | 143       | cyclase | cupin, Streptomyces sp. TSRI0261, OKU1252 | 83/90 GrhS (66/77) | RubD (79/85) |
| 742 | 424       | ketosynthase α subunit | type II polyketide synthase 4, Streptomyces sp., APD71740 | 89/95 GrhA (85/91) | RubA (89/93) |
| 741 | 420       | ketosynthase β subunit | type II polyketide synthase S, Streptomyces sp., APD71741 | 82/88 GrhB (76/83) | RubB (79/85) |
proteins encoded in the hyaluronsaminic biosynthetic gene cluster.

Genome properties
The total size of the genome of *Streptomyces hyaluromycini* MB-PO13\textsuperscript{T} is 11,525,033 bp and the GC content is 71.0% (Table 3), similar to other genome-sequenced *Streptomyces* members such as *Streptomyces violaceoniger* Tu4133, *Streptomyces bingchenggensis* BCW-1 [20] and *Streptomyces rapamycinicus* NRRL 5491 T. Of the total 10,201 genes, 10,098 are protein-coding genes and 103 are RNA genes. The classification of genes into COGs functional categories is shown in Table 4. As for secondary metabolite pathways by PKSs and NRPSs, *Streptomyces hyaluromycini* MB-PO13\textsuperscript{T} has at least six type I PKS gene clusters, three type II PKS gene clusters, two type III PKS gene clusters, six NRPS gene clusters, and one hybrid PKS/NRPS gene cluster.

Insights from the genome sequence
Hyaluronsaminic biosynthetic pathway in *Streptomyces hyaluromycini* MB-PO13\textsuperscript{T}
Hyaluronsaminic is a derivative of γ-rubromycin, possessing a C\textsubscript{5}N unit instead of a methoxy group as a side chain. The rubromycin-biosynthetic (rub) gene cluster is published in the GenBank (accession no. AF293355.2), but the biosynthetic mechanism has not been reported yet. Among the members of rubromycin family, only the griseorhodin-biosynthetic (grh) pathway has been extensively studied: griseorhodin A is synthesized by type II PKSs and modification enzymes [4, 21]. In the genome sequence of *S. hyaluromycini* MB-PO13\textsuperscript{T}, three type II PKS gene clusters are present. Among them, the type II PKS gene cluster in scaffold000001 resembles those of rubromycin and griseorhodin as shown in Fig. 3 and Table 5. But, unlike rub and grh gene clusters, the cluster also encodes amide synthase (Orf1-763), 5-aminolevulinate synthase (Orf1-762) and AMP-dependent synthase (Orf1-761) essential for C\textsubscript{5}N unit synthesis [22]. Thus, we considered it to be the biosynthetic gene cluster for hyaluronsaminic. According to the proposed biosynthetic mechanisms of griseorhodin [4] and C\textsubscript{5}N [22, 23], we predicted the biosynthetic pathway of hyaluronsaminic as shown in Fig. 4. The polyketide chain is synthesized by the iterative condensation of an acyl-CoA starter and 12 malonyl-CoA units. This elongation cycle is catalyzed by KS\textsubscript{α}, KS\textsubscript{β} (chain length factor) and acyl carrier protein. Since almost all the homologs of Grh enzymes are present in the putative hyaluronsaminic-biosynthetic gene cluster (Table 5, Fig. 3),

(continued)

| Orf1-763 | Proposed function | Closest homolog | Homolog (I/S, %) in grh cluster | Homolog (I/S, %) in rub cluster |
|---------|------------------|-----------------|-------------------------------|-------------------------------|
| 740     | 87               | acyl carrier protein | acyl carrier protein, *Streptomyces collinus*, AAG03069 | 68/79 GrhC (34/61) | RubC (68/79) |
| 739     | 398              | cyclase/reductase  | hypothetical protein, *Streptomyces prunici*, WP_019061796 | 79/87 GrhT (67/78) | RubF (78/85) |
| 738     | 249              | ketoreductase   | SDR family oxidoreductase, *Streptomyces prunici*, WP_019061795 | 86/94 GrhO10 (79/89) | RubG (86/93) |
| 737     | 108              | monooxygenase  | hypothetical protein, *Streptomyces collinus*, AAG03072 | 88/93 GrhU (75/84) | RubH (88/93) |
| 736     | 113              | unknown        | hypothetical protein, *Streptomyces fulvoviolaceus*, WP_078659944 | 73/80 GrhV (77/67) | RubT (70/81) |
| 735     | 417              | cytochrome P450 | cytochrome P450, *Streptomyces fulvoviolaceus*, WP_030615776 | 80/86 GrhO3 (37/53) | RubU (80/86) |
| 734     | 301              | unknown        | DUF1963 domain-containing protein, *Streptacidiphilus carbonis*, WP_042397320 | 78/85 – | – |
| 733     | 155              | cupin          | cupin, *Streptomyces prunici*, WP_019056246 | 93/97 – | – |
| 732     | 322              | esterase       | alpha/beta hydrolase, *Actinobacteria* bacterium OK074, KPI24488 | 83/88 – | – |
| 731     | 313              | transcriptional regulator | transcriptional regulator, *Streptomyces hokutanesis*, WP_043260174 | 79/85 – | – |
| 730\textsuperscript{a} | 491              | unknown | dolichyl-phosphate-mannose-protein mannosyltransferase, *Micromonospora auratinigra*, SB13146 | 57/67 – | – |
| 729     | 42               | unknown        | – | – | – |
| 728\textsuperscript{a} | 333              | transcriptional regulator | LacI family transcriptional regulator, ‘*Streptomyces humi*’, WP_046734674 | 93/96 – | – |

\*encoded in complementary strand. \(\text{I/S, identity/similarity. Orf1-763 also shows 48\% sequence identity/61\% sequence similarity to AsuD1 of Streptomyces nodosus subsp. asukaensis (AD58645); Orf1-761 shows 73\% sequence identity/81\% sequence similarity to AsuD3 of S. nodosus subsp. asukaensis (AD58647).}
the resulting polyketide chain is likely cyclized and modified to the polycyclic intermediate bearing a spiroketal moiety in a similar fashion to griseorhodin biosynthesis. Unlike griseorhodin A, the epoxide functionality is not present in the spiroketal moiety of rubromycin and hyaluromycin. This can be explained by the absence of homolog of grhO4 encoding ferredoxin responsible for epoxide formation of griseorhodin A in rubromycin- and hyaluromycin-biosynthetic gene clusters. It was unable to predict a gene responsible for the removal of the hydroxyl group at the spiroketal only by this bioinformatic analysis. 5-Aminolevulinate synthase (Orf1-762), 5-aminolevulinate CoA ligase (Orf1-761) and amide synthase (Orf1-763) are involved in the formation of C$_5$N unit and its coupling with the aromatic core.

**Conclusions**

The 11.5 Mb draft genome of *Streptomyces hyaluromycinici* MB-PO13$^T$, a producer of hyaluromycin, isolated from tunicate (*Molgula manhattensis*) has been deposited at GenBank/ENA/DDBJ under the accession number BCFL00000000. We successfully identified the gene cluster for hyaluromycin synthesis and proposed the plausible biosynthetic pathway. These findings provide useful information for genetic engineering to synthesize more potential hyaluronidase inhibitors and discovering new bioactive aromatic polyketides possessing the C$_5$N unit.

**Abbreviations**

Apm: Diaminopimelic acid; ABC: ATP-binding cassette; ACP: Acyl carrier protein; C$_5$N: 2-amino-3-hydroxycyclopent-2-enone; CLF: Chain length factor; CoA: Coenzyme A; DDBJ: DNA Data Bank of Japan; Fd: Ferredoxin; ISP: International *Streptomyces* project; KR: Ketoreductase; KS: Ketosynthase; MT: Methyltransferase; NBRC: Biological Resource Center, National Institute of Technology and Evaluation; NRPS: Nonribosomal peptide synthetase; PKS: Polyketide synthase

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**Fig. 4** Putative biosynthetic pathways of hyarulomycin, rubromycin and griseorhodin. Each step is catalyzed by enzymes encoded following genes as proposed in griseorhodin biosynthesis [4]. $^1$grhA/orf1-742 (KS$_\alpha$), grhB/orf1-744, grhC/orf1-739, grhO1/orf1-750, grhO9/orf1-749 (monoxygenases), grhL/orf1-751 (MT), grhM/orf1-748 (unknown) and grhP/orf1-746 (asparagine synthase); $^2$grhO5/orf1-755 (monoxygenase) and grhJ/orf1-758 (oxyanase); $^3$grhO6/orf1-753 (monoxygenase) and grhI/orf1-756 (acyltransferase); $^4$grhO10/orf1-738 (KR) or grhT/orf1-739 (cyclase/reductase); $^5$grhO3/orf1-735 (cytochrome P450), grhO4/orf1-752 (oxidoreductase). Homologs are connected with slashes in order of rubromycin/griseorhodin/hyarulomycin. ACP, acyl carrier protein; CLF, chain length factor; Fd, ferredoxin; KS, ketosynthase; KR, ketoreductase; MT, methyltransferase; –, no homolog in the sequence.
Authors’ contributions
EH performed chemotaxonomic experiments, examined features of the strain, and drafted the manuscript. KH elucidated the hyaluronic acid-biosynthetic pathway. NI annotated the genome sequences. AH sequenced the strain, and drafted the manuscript. YI designed this study and edited the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interest.

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