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Complete genome sequence of butenyl-spinosyn-producing *Saccharopolyspora* strain ASAGF58

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Abstract

Purpose: This study aimed to analyze the complete genome sequence of the butenyl-spinosyn-producing strain *Saccharopolyspora* sp. ASAGF58, isolated from Zhejiang province.

Methods: PacBio RS II sequencing platform with single-molecule real-time technology was used to obtain the complete genome sequence of *Saccharopolyspora* sp. ASAGF58. Gene prediction and annotation analysis were carried out through several software and databases. The antiSMASH online server was used to evaluate the secondary metabolite potential of strain ASAGF58.

Results: The whole genome of *Saccharopolyspora* sp. ASAGF58 is 8,190,340 bp divided into one chromosome of 8,044,361 bp with a GC content of 68.1% and a plasmid of 145,979 bp with a GC content of 64.6%. A total of 7486 coding sequences, 15 rRNA genes, 61 tRNA genes, 41 miscRNA genes, and 1 tmRNA gene were predicted. The domains encoded by one of the type I polyketide synthase (T1PKS) gene clusters have 91% similarity with those encoded by a spinosad biosynthetic gene cluster from *Saccharopolyspora spinosa*. In addition, antiSMASH results predicted that the strain also contains the biosynthetic gene clusters for the synthesis of ectoine, geosmin, and erythreapeptin.

Conclusions: Our data revealed the complete genome sequence of a new isolated butenyl-spinosyn-producing strain. This work will provide some methods, from genetics to biotechnology and biochemistry, aimed at the production improvement of butenyl-spinosyns.

Keywords: Genome sequence, *Saccharopolyspora* sp., Butenyl-spinosyn, Spinosad biosynthetic gene cluster

Findings

A great percentage of natural compounds are produced by microorganisms especially actinobacteria (Katz and Baltz 2016; Genilloud 2017). There is an increased interest in the isolation of actinobacteria because of the potential to discover new compounds having novel chemical structures (Tiwari and Gupta 2012). In this study, soil samples from different ecological environments were collected throughout China. Rare actinobacteria were screened according to morphology. Briefly, 1 g of air-dried soil samples was macerated in phosphate-buffered saline (1.5 mM $NaH_2PO_4{\cdot}2H_2O$, 8.3 mM $Na_2HPO_4{\cdot}12H_2O$, 154 mM NaCl, and 1.73 mM sodium dodecyl sulfate, pH 7.4), ultrasonic shaking at 50–60 Hz and heating at 60 °C for 10 min. Then, dilutions of the resulting suspension were plated onto 1/10 ATCC 172 agar medium (50 μg/mL nystatin, 50 μg/mL cycloheximide, and 1.25 μg/mL rifampicin) and incubated at 30 °C for 14 days (Hong et al. 2009). Screened rare actinobacteria were further purified under the same conditions. Strains were incubated in 96 deep well plates containing a rich medium (glucose 50.0 g/L, cottonseed protein 20.0 g/L, NaCl 3.0 g/L, K_2HPO_4 0.2 g/L, $FeSO_4{\cdot}7H_2O$ 0.05 g/L, $CaCO_3$ 5.0 g/L, pH 7.2) at 30 °C for 7 days. The insecticidal activity test method, which was established by the lethal effect of active substances on mosquito larvae, was used for fermentation broth screening (Chen et al. 2013). A strain isolated from Zhejiang province,

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China, with the shortest fatality time to mosquito larvae was selected and named ASAGF58 (Guo et al. 2019).

Confirmation of active substance structure was performed by liquid chromatography mass spectrometer/mass spectrometer (LC-MS/MS). This LC-MS/MS system consisted of Agilent 1290 Infinity II and Agilent 6545 Q-TOF LC/MS (Agilent Technologies, Santa Clara, CA, USA). The mixture of fermentation broth and 2× volume of methanol was vortexed and left overnight at 4 °C. The samples were centrifuged at 4 °C, 12,000 rpm, for 10 min, and the supernatant used in HPLC analysis as described by Zhao et al. (2017). The qualitative analysis of target compounds was carried out by electrospray ionization mass spectrometry (ESI-MS) under the positive mode with multiple reaction monitoring (MRM). The ionspray voltage was 4.5 kV, and the gas temperature was 350 °C with a drying gas flow rate of 10 L/min. The result revealed that two active substance compounds were in the fermentation broth with mass-to-charge ratios the same as spinosyn α1 and δ1 which could also be produced by *Saccharopolyspora pogona* (Lewer et al. 2009). The product ion which was obtained in the secondary ion mass spectrum (MS/MS) under the condition of 60 eV of collision energy and 120 V of fragmentor was forosamine sugar fragment ion, further evidence of the two active compounds' structure (Lewer et al. 2009) (Fig. S1).

To assign taxonomy, the 16S rRNA gene sequence of strain ASAGF58 was identified via EzBioCloud (https://www.ezbiocloud.net), and the result showed that it shared a 100% similarity with *Saccharopolyspora hattusasensis* CR 3506^T and a 99.1% similarity with *Saccharopolyspora spinosa* NRRL 18395^T. A phylogenetic tree was constructed with the neighbor-joining method using the MEGA software version 5.0 (Fig. 1). It indicates that strain ASAGF58 forms a distinct cluster with members of *Saccharopolyspora* species and is most likely a strain of *S. hattusasensis* which has a close relationship with spinosyn- and butenyl-spinosyn-producing strain *S. spinosa* NRRL 18395^T and *S. pogona* NRRL 3014. *S. hattusasensis*, a new species of *Saccharopolyspora* sp., was isolated from Turkey and was found to exhibit antimicrobial activity against *Bacillus subtilis* NRRL B-209, *Citrobacter freundi* NRRL B-2643, and *Staphylococcus aureus* ATCC 29213 (Veyisoglu et al. 2017). However, no insecticidal activity has been reported.

Whole-genome sequencing was carried out for strain ASAGF58. It was cultivated in 20 mL of tryptic soy broth in 300 mL flasks at 30 °C and 240 rpm for 48 h. DNA was extracted using the Wizard® Genomic DNA Purification Kit (Promega Corporation). The extracted DNA was sequenced by Annoroad, Inc. (Beijing, China), using the PacBio RS II sequencing platform and single-molecule real-time (SMRT) technology. The raw PacBio reads were quality filtered by SMRT Pipe version 2.3, and 75,095 subreads were obtained with an N_{50} value of 12,242 and a mean value of 9453. Due to the low-quality and high randomness errors of PacBio sequencing data, de novo assembly was carried out with HGAP version 3.0 in order to obtain high-accuracy data that could meet the demand of the analysis (Chin et al. 2013). The

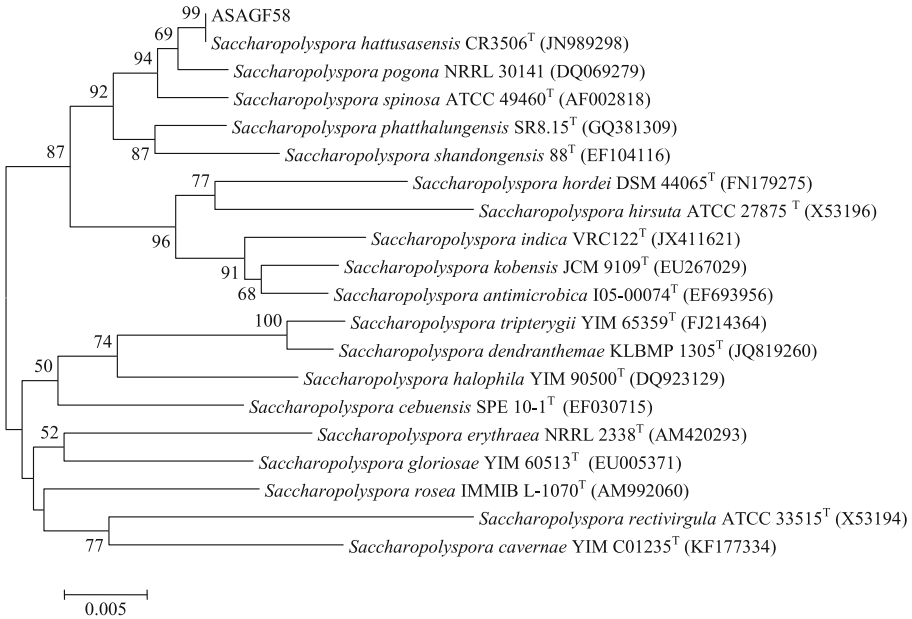

Fig. 1 Neighbor-joining tree based on 16S rRNA gene sequences showing the position of strain ASAGF58 within the *Saccharopolyspora* gene tree. The length of the 16S rRNA gene sequences used in the phylogenetic analysis was 1296 bp. Numbers at the nodes indicate levels of bootstrap support (%); only values > 50% are shown. GenBank accession numbers are given in parentheses. Bar 0.005 substitutions per site

assembled results contained 2 circle contigs, a chromosome with a base coverage of 76.15 and a plasmid with a base coverage of 52.1. After data filtering, 71,077 subreads could be mapped back to the contig. The data utilization rate was 94.69%.

We used Prodigal software version 2.6 to predict the coding sequence (Hyatt et al. 2010). Infernal version 1.1.1 (Nawrocki et al. 2009) and RNAmmer version 1.2 (Lagesen et al. 2007) were applied for the prediction of tRNA, rRNA, and ncRNA. Signal peptide, insertion sequence, phage precursor, clustered regularly interspaced short palindromic repeats (CRISPR) locus, and gene island were predicted using SignalP version 4.1 (Petersen et al. 2011), ISFinder (https://www-is.biotoul.fr/) (Siguier et al. 2006), Phage Finder version 2.0 (Fouts 2006), PILE-CR version 1.0 (Edgar 2007), and GIHunter version 1.0 (http://www5.esu.edu/cpsc/bioinfo/software/GIHunter/), respectively. The whole genome of strain ASAGF58 is 8,190,340 bp divided into one chromosome of 8,044,361 bp with a high GC content of 68.1% and a plasmid of 145,979 bp with a high GC content of 64.6% (Fig. 2). Table 1 shows that a total of 7486 coding sequences, 15 rRNA genes, 61 tRNA

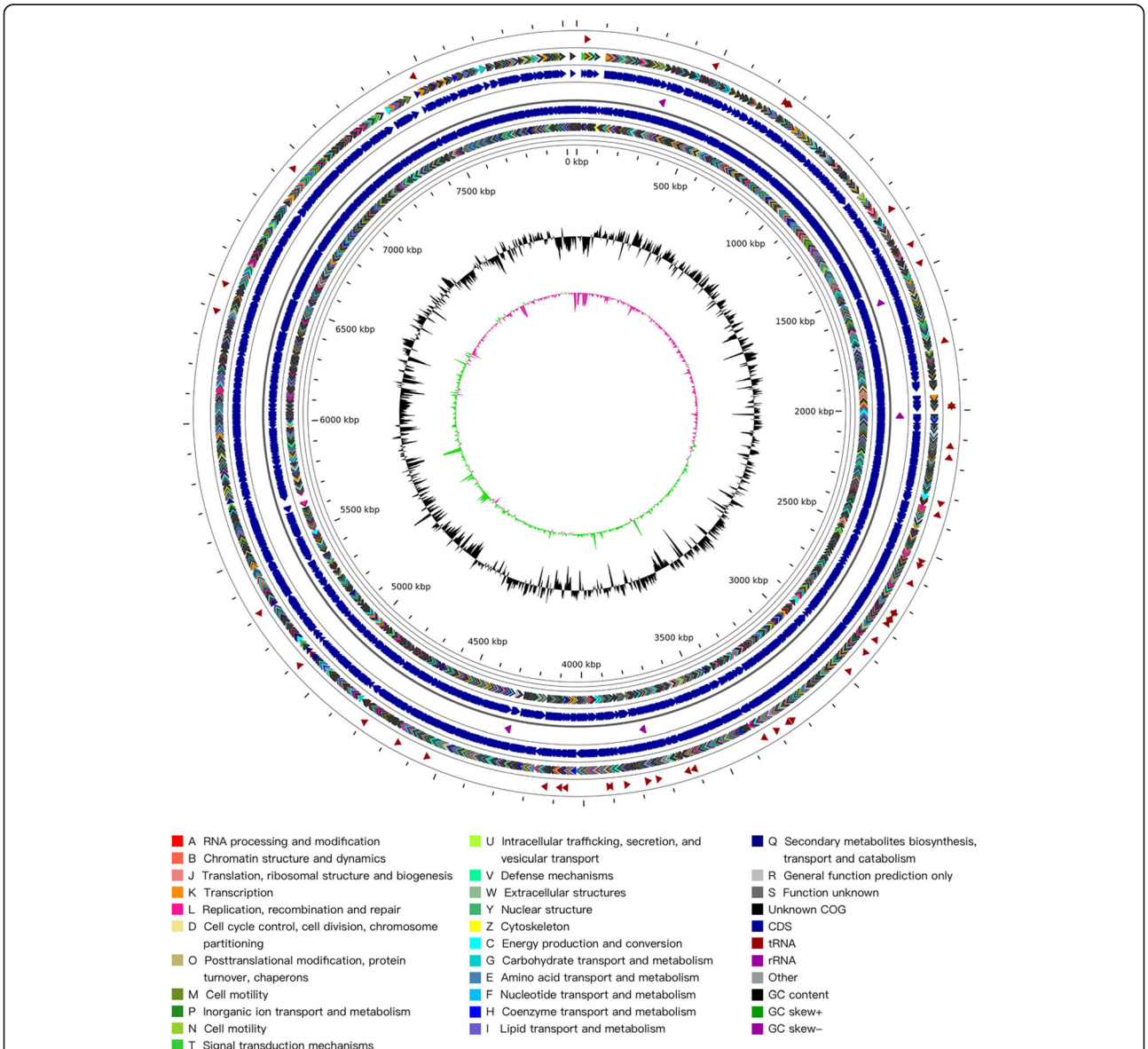

Fig. 2 Circular representation of strain ASAGF58 chromosome (from outside to inside): tRNA genes, Clusters of Orthologous Groups of proteins (COG) annotation on the forward strand, location of genes on the forward strand, rRNA genes, COG annotation on the reverse strand, location of genes on the reverse strand, GC content (taking the mean value as the baseline, the expression of the outward protrusion is higher than the mean value, and the expression of the inward protrusion is lower than the mean value), GC skew (the expression of purple means less than 0, and the expression of green means more than 0)

Table 1 Genome features of strain ASAGF58

General features	Genome of strain ASAGF58
Protein-coding gene number	7486
Gene	7604
miscRNA	41
rRNA	15
tRNA	61
tmRNA	1
Insertion sequence	0
Phage precursor	0
Gene island	0
Signal peptide	0
CRISPR unit	1
NRPS	4
T1PKS	1
T1PKS/NRPS	3
T3PKS	3

genes, 41 miscRNA genes, 1 tmRNA gene, and a 239-bp-long CRISPR unit between 1,510,973 bp and 1,511,212 bp were predicted. The genome sequence was deposited under GenBank accession number CP040605.

The predicted proteins were compared with Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups (EggNOG) database version 4.0 (http://eggnog.embl.de/version_4.0.beta/), and Clusters of Orthologous Groups of proteins (COG) annotations were carried out with *E* value less than $1\times e^{-35}$ and on the mapping basis of the best hit one (Powell et al. 2013). Then, Gene Ontology (http://www.geneontology.org/) was applied for annotation of the homologous genes and their functions, location of cellular components, and biological processes. Next, all predicted genes were compared with the Kyoto Encyclopedia of Genes and Genomes (KEGG) (https://www.genome.jp/kegg/) and mapped to pathways (Kanehisa et al. 2007). The results revealed that strain ASAGF58 had complete fatty acid pathway, tricarboxylic acid cycle, glycolytic pathway, and other central metabolic pathways. The total pathways could be divided into 20 categories (Fig. S2). Finally, the genome blast search was performed against Nucleotide Sequence database (Nt) (ftp://ftp.ncbi.nih.gov/blast/db/FASTA/ns.gz), Non-Redundant Protein database (Nr) (ftp://ftp.ncbi.nih.gov/blast/db/FASTA/nr.gz), EggNOG database version 4.0 (http://eggnog.embl.de/version_4.0.beta/), KEGG database (http://www.kegg.jp/kegg/tool/annotate sequence.html), and SwissProt database (ftp://ftp.ebi.ac.uk/pub/databases/uniprot/knowledgebase/uniprot_sprot.fasta.gz) (Watanabe and Harayam 2001) for the annotation of all the functional genes (*E* value less than $1\times e^{-35}$) (Fig. S3).

The secondary metabolite-encoding gene clusters were predicted by submitting the whole-genome sequence in fasta format to the antiSMASH website (http://antismash.secondarymetabolites.org/) (Blin et al. 2013), and we found four non-ribosomal peptide synthetase (NRPS) clusters, one T1PKS cluster, three T1PKS/NRPS clusters, and three type III polyketide synthase (T3PKS) clusters (Table 1). The strain might be able to synthesize ectoine, geosmin, and erythreapeptin because of the 100% similarity with ectoine biosynthetic gene cluster (GenBank accession NO. AY524544.1) from *Streptomyces anulatus* ATCC 11523 (Prabhu et al. 2004), geosmin biosynthetic gene cluster (GenBank accession NO. AL645882.2) from *Streptomyces coelicolor* A3 (2) (Redenbach et al. 1996), and Ery-9 biosynthetic gene cluster (GenBank accession NO. AM420293.1) from *Saccharopolyspora erythraea* NRRL 2338^T (Oliynyk et al. 2007). The domains encoded by the T1PKS gene cluster in region 9 have 91% similarity with those encoded by a spinosad biosynthetic gene cluster (GenBank accession NO. AY007564.1) from *S. spinosa* (Waldron et al. 2001). The only difference between the T1PKS spinosad biosynthetic gene clusters of strain ASAGF58 and *S. spinosa* is that ctg1_4842 of strain ASAGF58 is larger than *spnA* of *S. spinosa*, because ctg1_4842 encodes an additional module (Fig. S4). The function of the additional module which contains five functional domains (ketosynthase, acetyltransferase, dehydratase, ketoreductase, and acyl carrier protein) appeared to be responsible for the biosynthesis of the butenyl side chain and unique characteristic of butenyl-spinosyns (Hahn et al. 2006).

Spinosyns are a couple of biological pesticides with high efficiency, broad spectrum, and low toxicity to birds and mammals. It includes spinosyns produced by *S. spinosa* and butenyl-spinosyns produced by *S. pogona*. The genetic relationship of the two strains is very close, and perhaps, they have a common origin of spinosyn genes (Hahn et al. 2006). The biosynthetic pathway for the butenyl-spinosyns was proposed by Hahn et al. (2006). As novel antibiotics, many efforts have been made to improve the production of butenyl-spinosyns. The mutant *S. pogona-Δfcl* was constructed and found that the yield of butenyl-spinosyns was 130% compared with that in *S. pogona*. The reason is that the GDP-fucose synthetase encoded by *fcl* gene is involved in the synthesis of GDP-fucose from GDP-mannose. The GDP-rhamnose transformed from GDP-mannose is the precursor of butenyl-spinosyns synthesis (Peng et al. 2019). Polynucleotide phosphorylase overexpression mutant of *S. pogona* also had a high production because of the improvement of biomass (Li et al. 2018). A strain of yield 1.79-fold higher than the parent strain was obtained by ribosome engineering (Luo et al. 2016). The complete genome of *Saccharopolyspora* sp. ASAGF58 will promote the research

of the synthesis mechanism of butenyl-spinosyns and also stimulate a wide range of approaches to improve butenyl-spinosyns synthesis.

Supplementary information

Supplementary information accompanies this paper at https://doi.org/10.1186/s13213-020-01587-4.

Additional file 1: Fig. S1. Product ion with mass-to-charge ratios of 142.1 which is the forosamine sugar fragment ion that was obtained in the secondary ion mass spectrum from parent ion 758.5 (a) and 772.5 (b). Description of data: Ions with mass-to-charge ratios 758.5 and 772.5 were the same as spinosyn α1 and δ1 which were produced by '*Saccharopolyspora pogona*'. Product ion with mass-to-charge ratios of 142.1 which is the forosamine sugar fragment ion was obtained in the secondary ion mass spectrum (MS/MS) from parent ions 758.5 and 772.5.

Additional file 2: Fig. S2. Kyoto Encyclopedia of Genes and Genomes database (KEGG) pathway classification of the predicted genes deduced from strain ASAGF58. All predicted genes of strain ASAGF58 were compared with KEGG and mapped to pathways. The total pathways could be divided into 20 categories.

Additional file 3: Fig. S3. The comparison between the quantity of hits in each database. Abbreviations of databases: Nt Nucleotide Sequence database, Nr Non-Redundant Protein database, eggNOG Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups database, KEGG Kyoto Encyclopedia of Genes and Genomes database, Swiss SwissProt database. The genome of strain ASAGF58 blast search was performed against Nt, Nr, EggNOG, KEGG and SwissProt for the annotation of all the functional genes (*E* value less than $1*e^{-35}$). The figure shows the quantity of hits in the five databases and the comparison between the quantity of hits in each database.

Additional file 4: Fig. S4. Domains of the type I polyketide synthase (T1PKS) encoded by region 9 in the genome of strain ASAGF58 and those encoded by a spinosad biosynthetic gene cluster (GenBank accession NO. AY007564.1) from *Saccharopolyspora spinosa*. Abbreviations of domains: oMT O-methyltransferase, TE thioesterase, KS ketosynthase, AT acetyltransferase, DH dehydratase, KR ketoreductase, ER enoylreductase, ACP acyl carrier protein, AmT Aminotran. The only difference between the T1PKS spinosad biosynthetic gene clusters of strain ASAGF58 and *S. spinosa* is that ctg1_4842 of strain ASAGF58 is larger than *spnA* of *S. spinosa*, because ctg1_4842 encodes an additional module which contains five functional domains (KS, AT, DH, KR and ACP).

Abbreviations

COG: Clusters of Orthologous Groups of proteins; CRISPR: Clustered regularly interspaced short palindromic repeats; EggNOG: Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups; ESI-MS: Electrospray ionization mass spectrometry; HPLC: High-performance liquid chromatography; KEGG: Kyoto Encyclopedia of Genes and Genomes; LC-MS/MS: Liquid chromatography mass spectrometer/mass spectrometer; MRM: Multiple reaction monitoring; Nr: Non-Redundant Protein database; NRPS: Non-ribosomal peptide synthetase; Nt: Nucleotide Sequence database; SMRT: Single-molecule real-time; T1PKS: Type I polyketide synthase; T3PKS: Type III polyketide synthase

Acknowledgements

Not applicable

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

Authors' contributions

CW and CG designed the study and wrote the manuscript. WG performed the MS/MS analysis. YL made the experiments of the strain identification. CG, CW, WG, and YL were involved in the analysis of the sequence data. All authors have read and approved the final manuscript.

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Availability of data and materials

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent for publication

Not applicable

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