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Complete genome sequence of *Priestia megaterium* S188, a hydrogen sulfide-degrading bacterium

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Abstract

Priestia megaterium (formerly *Bacillus megaterium*) is a gram-positive, aerobic, spore-forming bacterium found in a wide range of environmental niches. Here, we report the complete genome sequence of *P. megaterium* S188 isolated from soil, which can decrease hydrogen sulfide (H_2S) levels and help reduce malodor generation in livestock farms. Putative genes related to sulfide assimilation and conversion were found in the genome of *P. megaterium* S188; among these, one O-acetylhomoserine (O-AH) desulfhydrase, two cysteine synthases-primarily related to the biosynthesis of sulfur-containing amino acids, five rhodanese or sulfurtransferases, and one nitrogen reductase were identified. The genomic information on *P. megaterium* S188 provides insights into the possible biodegradation or conversion mechanisms of sulfur-containing substances that cause malodors, which can help reduce odor generation. Furthermore, identification of the key genes or molecules responsible for H_2S reduction would facilitate the optimization of the H_2S-degrading ability of S188.

Keywords: *Priestia*, *Bacillus megaterium*, Malodor, Hydrogen sulfide

Malodor generation during livestock production is a major problem in livestock farms, as it can negatively affect animals, humans, and the environment [1]. Particularly, hydrogen sulfide (H_2S), which is a colorless gas heavier than air with a rotten egg-like odor, can cause severe distress to livestock workers [2,3]. *Priestia megaterium* S188, originally isolated from soil, can reduce H_2S levels in manure [4]. In this study, we sequenced the complete genome of *P. megaterium* S188. Initially, S188 was grown in Nutrient Broth (Difco, Tucker, GA, USA) at 30°C for 24 h. The genomic DNA of strain S188 was extracted as described in a previous study [5], and its quality was checked using a spectrophotometer (UV-1601PC, Shimadzu, Kyoto, Japan). The genome of S188 was sequenced using the PacBio RSII platform (ver. 2.0; Pacific Biosciences) at Macrogen (Seoul, Korea). All the generated reads were *de novo* assembled using the RS HGAP Assembly (ver. 3.0) program. The assembled S188 genome was annotated using Prokka v.1.14.6, and the RAST was accessed at https://rast.nmpdr.org on May 10, 2024. BlastP (https://www.ncbi.nlm.nih.gov/blast/), UniProt (https://www.uniprot.org), ClustalOmega (https://www.ebi.ac.uk/jdispatcher/msa/clustalo), and EggNOG-mapper (http://eggnog-mapper.embl.de) were used for the annotation, alignment, and identification of proteins. KEGG (Kyoto Encyclopedia of Genes and Genomes) Mapper (https://www.kegg.jp/kegg/mapper/) was used to map

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Availability of data and material
Upon reasonable request, the datasets of this study can be available from the corresponding author.

Authors' contributions
Conceptualization: Kang DK.
Data curation: Kim SH.
Formal analysis: Kim SH, Mendoza RM.
Methodology: Kim SH, Song JH, Mendoza RM.
Validation: Kang DK.
Investigation: Kim SH, Song JH.
Writing - original draft: Kim SH, Mendoza RM, Kang DK.
Writing - review & editing: Kim SH, Song JH, Mendoza RM, Kang DK.

Ethics approval and consent to participate
This article does not require IRB/IACUC approval because there are no human and animal participants.

the genes of strain S188 to different metabolic pathways, and the DNA plotter in Artemis (v.18.2) was used to generate the genome maps of strain S188.

The S188 genome has a total length of 5,407,472 base pair (bp), with a chromosome size of 5,278,689 bp, and a putative plasmid of 128,783bp (Fig. 1). It has a guanine-cytosine (GC) content of 37.9% and comprises 5,761 genes, of which 5,494 are coding DNA sequences (CDS), 111 miscellaneous RNA, 118 transfer RNA, 37 ribosomal RNA, and one transfer-messenger RNA (Table 1). Genes related to H_2S metabolism (i.e., assimilation/conversion) were identified using KEGG Mapper and manual curation of the reported genes associated with sulfur metabolism. Putative genes related to H_2S assimilation and conversion, including one O-acetylhomoserine (O-AH) sulfhydrylase, two cysteine synthases, five rhodanese or sulfurtransferases, and one nitrogen reductase, were identified.

The amount of H_2S released by yeast into the environment depends on the levels of sulfide (S^{2-}) available [6]. Sulfide can be incorporated into sulfur-containing amino acids such as cysteine and methionine when it condenses with O-AH, a reaction catalyzed by O-AH sulfhydrylase, or with O-acetyl-L-serine, a reaction catalyzed by cysteine synthase [6,7]. Serine serves as a precursor for the biosynthesis of S-containing amino acids [6]; the expression of genes related to serine biosynthesis is lower in *Saccharomyces. cerevisiae* strains that produce H_2S than in non-H_2S

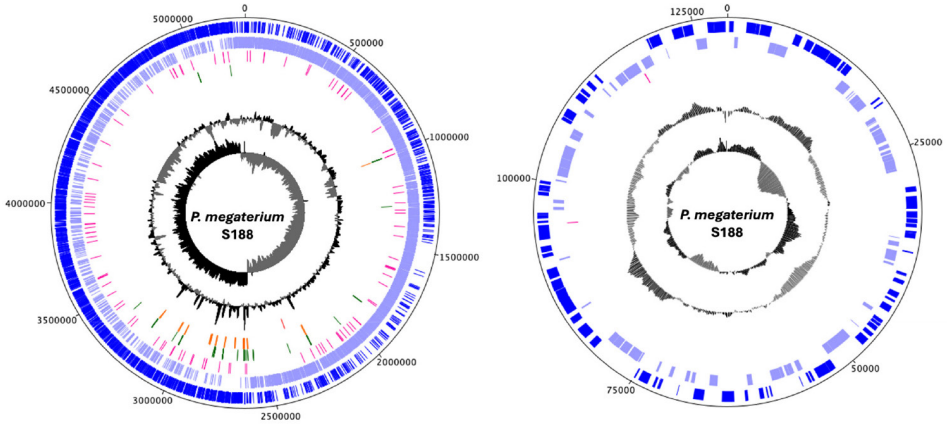

Fig. 1. Genome maps of the *Priestia megaterium* S188 chromosome (Left) and plasmid (Right). Circles illustrate the following features from the outside to the center: (1) Coding sequences on the forward strand, (2) coding sequences on the reverse strand, (3) Miscellaneous RNA (4) Transfer RNAs (tRNAs), (5) ribosomal RNAs (rRNAs), (6) G + C content, and (7) G + C skew. The figure was generated using DNA Plotter implemented in Artemis (v.18.2). G + C, guanine + cytosine.

Table 1. Genome features of *Priestia megaterium* S188

Features	Chromosome	Plasmid	Total
Genome size (bp)	5,278,689	128,783	5,407,472
G + C content (%)	38.0	34.2	37.95
Total number of genes	5,617	144	5,761
Protein-coding genes	5,352	142	5,494
Misc RNA	109	2	111
tRNA genes	118	0	118
rRNA genes	37	0	37
tmRNA	1	0	1

bp, base pair; G + C, guanine + cytosine.

producers. This suggests that the facilitation of S^{2-} incorporation into amino acids due to increased amounts of intracellular serine results in reduced H_2S production. [6]. In the S188 genome, genes for the assimilation of sulfide as well as the biosynthesis of serine, cysteine, and methionine were mapped using KEGG Mapper (Table 2).

In eukaryotic cells, rhodanese, a mitochondrial sulfur transferase, is part of the mitochondrial sulfide oxidation pathway involving sulfide quinone oxidoreductase, persulfide dioxygenase (PDO), and sulfite oxidase. This pathway ultimately oxidizes H_2S to thiosulfate and sulfate [8]. In some bacteria, particularly *Staphylococcus aureus*, naturally occurring PDO-rhodanese fusion proteins (i.e., CstB) are involved in H_2S detoxification [8,9]. Five putative sulfur transferases or rhodanese-like domain-containing proteins, which showed 18%—27% similarity to the CstB of *S. aureus*, were also identified in the genome of S188 (Table 2).

The presence of nitrate reductase (Table 2) in the S188 genome indicates another possible mechanism whereby S188 can remove or reduce H_2S. H_2S removal using nitrate-reducing and sulfide-oxidizing bacteria has also been explored; herein, H_2S serves as the electron donor for the reduction of nitrate to nitrogen gas [10].

P. megaterium S188 isolated from the soil can reduce the levels of H_2S in manure and has the potential to reduce malodors in livestock farms. The involvement of the identified putative genes related to this phenotype, such as O-AH sulfhydrylase, cysteine synthase, putative sulfur transferases, rhodanese-like domain-containing proteins, and nitrate reductase, warrants further experimentation and validation. Nonetheless, the identification of these genes offers insights into

Table 2. Genes related to sulfide assimilation and conversion identified in the genome of *Priestia megaterium* S188

Gene group	Locus Tag	Gene product
Sulfide assimilation	S188_ch_03715	O-acetyl-L-homoserine sulfhydrylase
	S188_ch_05019	Homoserine O-acetyltransferase
	S188_ch_02391; S188_ch_02939	Cysteine synthase
Serine biosynthesis	S188_01905	D-3-phosphoglycerate dehydrogenase
	S188_ch_03473	Phosphoserine aminotransferase
	S188_ch_03080; S188_ch_03084	Phosphoserine phosphatase
	S188_ch_03845	Putative phosphoserine phosphatase 2
Cysteine biosynthesis	S188_ch_02414	S-adenosylmethionine synthase
	S188_ch_03749	Homocysteine S-methyltransferase
	S188_ch_02138	5'-methylthioadenosine/S-adenosylhomocysteine nucleosidase
	S188_ch_02426	S-ribosylhomocysteine lyase
	S188_ch_02137	O-acetylserine-dependent cystathionine beta-synthase
	S188_ch_02136; S188_ch_04308	Cystathionine gamma-lyase
Methionine biosynthesis	S188_ch_01671; S188_ch_02273; S188_ch_05176	Aspartokinase
	S188_ch_01672	Aspartate-semialdehyde dehydrogenase
	S188_ch_02530	Homoserine dehydrogenase
	S188_ch_03476	O-acetyltransferase
	S188_ch_02505; S188_ch_043091	Cystathionine beta-lyase
	S188_ch_04180	Methionine synthase
Rhodanese/ sulfurtransferases	S188_ch_00937	Putative rhodanese-like domain-containing protein
	S188_ch_02024	Thiosulfate sulfurtransferase
	S188_ch_02398	Sulfurtransferase
	S188_ch_05007	Putative thiosulfate sulfurtransferase
	S188_ch_05516	Rhodanese-related sulfurtransferase
Nitrogen reduction	S188_ch_03676	Nitrate reductase

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the possible mechanisms by which S188, whether alone or in synergy with other nitrate-reducing, sulfur-oxidizing bacteria, reduces the levels of H_2S and would facilitate the optimization of S188 activity to achieve more efficient H_2S removal. Finally, complete cobalamin biosynthetic (cob) operon was also deteced in the genome of *P. megaterium* S188 (data not shown), indicating that S188 is able to synthesize vitamin B_{12}, which needs to be investigated in the future.

NUCLEOTIDE SEQUENCE ACCESSION NUMBER

The genome sequences of *P. megaterium* S188 are available at GenBank with the accession number NZ_CP049296.1.

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