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Running Title (within 10 words)	Genome sequence of *Priestia megaterium* S188
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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

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24 Malodor generation during livestock production is a major problem in livestock farms, as it can
25 negatively affect animals, humans, and the environment (1). Particularly, hydrogen sulfide (H_2S), which
26 is a colorless gas heavier than air with a rotten egg-like odor, can cause severe distress to livestock
27 workers (2,3). *P. megaterium* S188, originally isolated from soil, can reduce H_2S levels in manure (4).
28 In this study, we sequenced the complete genome of *P. megaterium* S188. Initially, S188 was grown in
29 Nutrient Broth (Difco, USA) at 30 °C for 24 h. The genomic DNA of strain S188 was extracted as
30 described in a previous study (5), and its quality was checked using a spectrophotometer (UV-1601PC;
31 Shimadzu, Japan). The genome of S188 was sequenced using the PacBio RSII platform (ver. 2.0; Pacific
32 Biosciences) at Macrogen Inc. (Korea). All the generated reads were *de novo* assembled using the RS
33 HGAP Assembly (ver. 3.0) program. The assembled S188 genome was annotated using Prokka v.1.14.6,
34 and the RAST was accessed at https://rast.nmpdr.org on May 10, 2024. BlastP
35 (https://www.ncbi.nlm.nih.gov/blast/), UniProt (https://www.uniprot.org), ClustalOmega
36 (https://www.ebi.ac.uk/jdispatcher/msa/clustalo), and EggNOG-mapper (http://eggnog-
37 mapper.embl.de) were used for the annotation, alignment, and identification of proteins. KEGG Mapper
38 (https://www.kegg.jp/kegg/mapper/) was used to map the genes of strain S188 to different metabolic
39 pathways, and the DNA plotter in Artemis (v.18.2) was used to generate the genome maps of strain
40 S188.

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

49 The amount of H_2S released by yeast into the environment depends on the levels of sulfide (S^{2-})
50 available (6). Sulfide can be incorporated into sulfur-containing amino acids such as cysteine and
51 methionine when it condenses with O-acetylhomoserine (O-AH), a reaction catalyzed by *O-AH*

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52 sulfhydrylase, or with O-acetyl-L-serine, a reaction catalyzed by cysteine synthase (6,7). Serine serves
53 as a precursor for the biosynthesis of S-containing amino acids (6); the expression of genes related to
54 serine biosynthesis is lower in *Saccharomyces. cerevisiae* strains that produce H_2S than in non-H_2S
55 producers. This suggests that the facilitation of S^{2-} incorporation into amino acids due to increased
56 amounts of intracellular serine results in reduced H_2S production. (6). In the S188 genome, genes for
57 the assimilation of sulfide as well as the biosynthesis of serine, cysteine, and methionine were mapped
58 using KEGG Mapper (Table 2).

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

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

70 *P. megaterium* S188 isolated from the soil can reduce the levels of H_2S in manure and has the potential
71 to reduce malodors in livestock farms. The involvement of the identified putative genes related to this
72 phenotype, such as O-AH sulfhydrylase, cysteine synthase, putative sulfur transferases, rhodanese-like
73 domain-containing proteins, and nitrate reductase, warrants further experimentation and validation.
74 Nonetheless, the identification of these genes offers insights into the possible mechanisms by which
75 S188, whether alone or in synergy with other nitrate-reducing, sulfur-oxidizing bacteria, reduces the
76 levels of H_2S and would facilitate the optimization of S188 activity to achieve more efficient H_2S
77 removal. Finally, complete cobalamin biosynthetic (cob) operon was also deteced in the genome of *P.*
78 *megaterium* S188 (data not shown), indicating that S188 is able to synthesize vitamin B_{12}, which needs
79 to be investigated in the future.

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Data Availability

82 The genome sequences of *P. megaterium* S188 are available at GenBank with the accession number
83 NZ_CP049296.1.
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92 the tools that we used in analyzing the genome of *P. megaterium* S188 which we failed to cite in the
93 paper due to the limitation in the number of citations.
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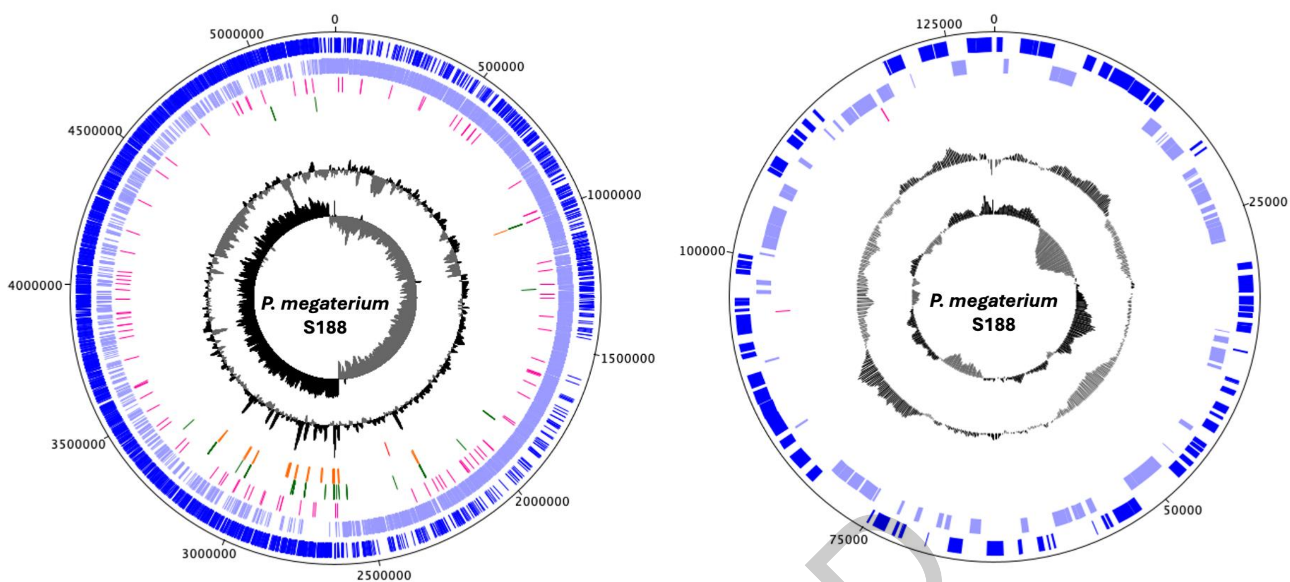

132

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

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147 **Table 2. Genes related to sulfide assimilation and conversion identified in the genome of *P. megaterium***
148 **S188**
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Gene group	Locus Tag	Gene name
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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