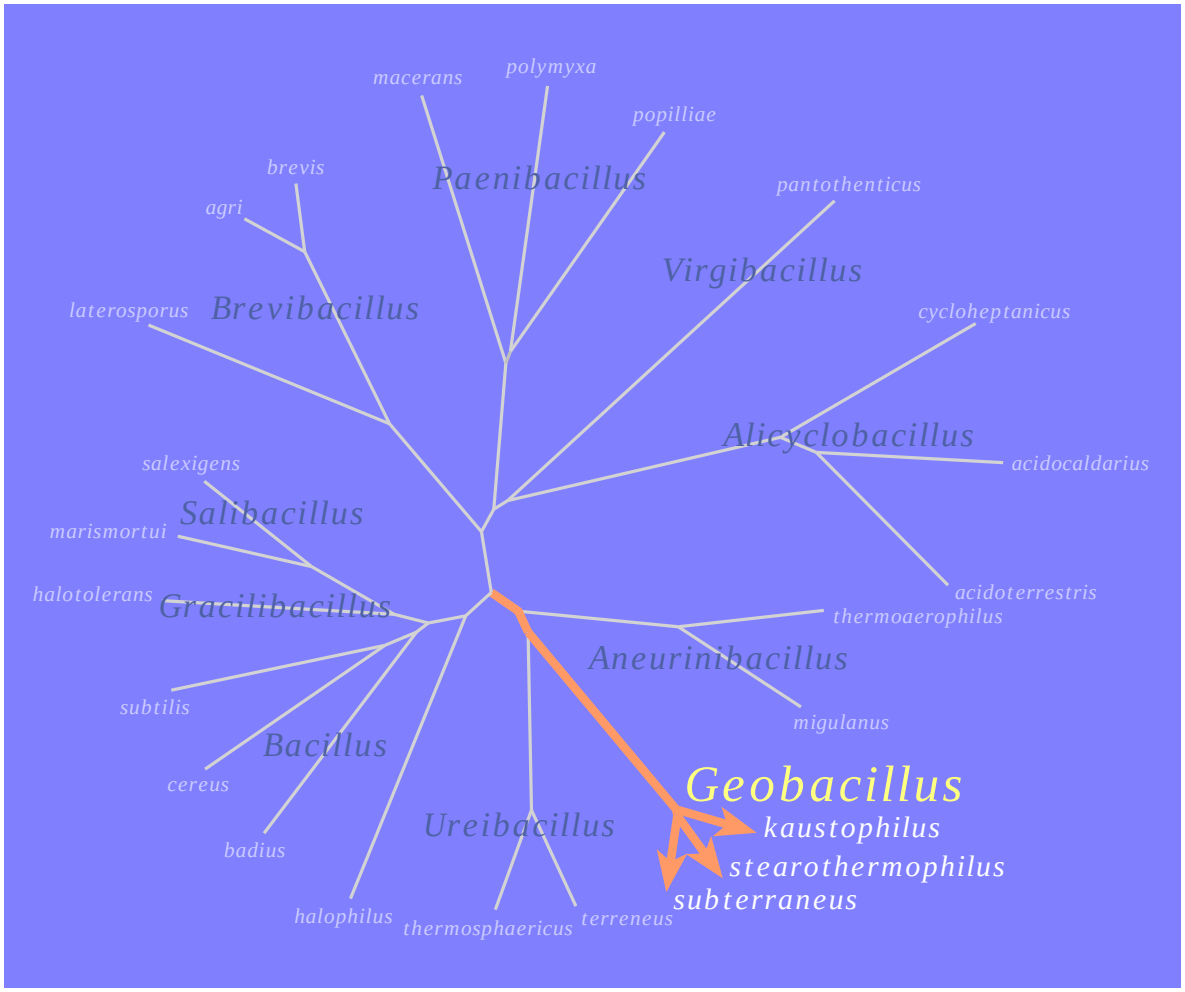
The Genus *Geobacillus*

Introduction and Strain Catalog



Bacillus Genetic Stock Center
Catalog of Strains
7th Edition, Volume 3

Bacillus Genetic Stock Center
Catalog of Strains, Seventh Edition
Volume 3: The Genus *Geobacillus*

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Disclaimer: The information in this catalog is believed to be correct. Due to the dynamic nature of the scientific process and to normal human limitations in dealing with such a large amount of data, however, some undetected errors may persist. Users bear the responsibility of verifying any important data before making a significant investment of time or other physical or financial resources.

Cover: Phylogenetic tree of the genus *Bacillus* and closely related genera, including the new genus *Geobacillus* (Nazina, 2001). 16S rRNA gene sequences were obtained from GenBank for the species represented on the tree. After they were manually trimmed to the same length, they were aligned with ClustalW to create a Phylip distance matrix. The Neighbor program from the Phylip suite was used to generate a UPGMA tree, which was visualized with TreeView32. I used MacroMedia Freehand and Microsoft Image Composer to edit the tree image. It sounds a whole lot more complicated than it was! ☺

Links: This document contains many internal hyperlinks. Clicking on a text in color opens a link to another page with more information about that strain, reference, or gene.

Table of Contents

The *Bacillus* Genetic Stock Center

Who we are • What services we offer • How to order • Pricing • Important Notice

What is the *Bacillus* Genetic Stock Center?

The primary mission of the *Bacillus* Genetic Stock Center (BGSC) is to maintain genetically characterized strains, cloning vectors, and bacteriophage for the genus *Bacillus* and related organisms and to distribute these materials without prejudice to qualified scientists and educators throughout the world. Since 1978, the National Science Foundation has funded the activities of the BGSC. The Department of Biochemistry in the College of Biological Sciences at the Ohio State University provides facilities and administrative support. The Director of the BGSC, Dr. Daniel R. Zeigler, is assisted by a technician and a data entry specialist.

What kinds of cultures are available from the BGSC?

This catalog lists only the *Geobacillus* cultures available from the BGSC, as well as any *Escherichia coli* plasmids that might be of interest to scientists working with these species. The BGSC maintains and distributes a wide range of other strains, however. Included in our collection as of July 2012 (and described in other existing and planned catalogs) are:

- The nomenclatural type strains for 34 species;
- 1291 mutant or plasmid bearing strains derived from *Bacillus subtilis* 168, including a collection of 115 genetically characterized sporulation mutants;
- 158 strains of round spore formers, comprised of 136 strains of *B. sphaericus,* 17 of *B. fusiformis,* and five of *B. pycnus*;
- 191 wild type strains of *B. thuringiensis,* along with 10 cloned *B. thuringiensis cry* genes;
- 239 genetically characterized wild-type, mutant, and plasmid-bearing strains of *B. megaterium*;
- 96 lytic or lysogenic *Bacillus* bacteriophages;
- 42 wild-type and mutant strains from the thermophilic genus *Geobacillus*
- 41 wild-type, mutant, and lysogenic strains of *Bacillus licheniformis*;
- 55 other wild-type, mutant, and plasmid-bearing *B. subtilis* isolates, including 13 from *B. subtilis* subsp. *spizizenii* and 42 from other *B. subtilis* backgrounds;
- 104 wild-type strains from the *Bacillus cereus* group, also including *B. mycoides* and *B. weihenstephanensis*;
- 18 wild-type isolates from the genus *Brevibacillus,* including *B. brevis, B. borstelensis, B. centrosporus,* and *B. laterosporus*;
- 18 wild-type and mutant strains from *B. amyloliquefaciens*;
- 30 wild-type isolates from the genus *Paenibacillus,* including *P. alvei, P. dendritiformus, P. macerans, P. polymyxa, P. popilliae, P. thiaminolyticus,* and *P. vorticalis*;
- 42 isolates from 22 other related species, including *Aneurinibacillus aneurinilyticus, A. migulanus, B. atrophaeus, B. badius, B. carboniphilus, B. circulans, B. clausii, B. coagulans, B. firmus, B. lentus, B. mojavensis, 'B. natto,' B. oleronius, B. pumilus, B. shackletonii, Marinibacillus marinus, Sporosarcina ureae*, and *Virgibacillus marismortui*
- 240 *Escherichia coli* strains bearing shuttle plasmids or cloned *Bacillus* DNA;
- Warehoused *Bacillus* strain collections of Joshua Lederberg, Eugene Nester, Bernard Reilly, Patiricia Vary, Allan Yousten, Stanley Zahler, and the late Ernst W. Freese.

Please note that the BGSC has never carried *B. anthracis* or products derived from it.
The collections maintained in the BGSC Warehouse are available to the scientific community upon request. They do not receive the same level of curation, quality control, or data publication as the strains in the main collection. However, they are maintained because of their high level of historical importance as a service to the *Bacillus* research community.

Please inquire about any of these strains that might be of interest to you.

What you can do to help the BGSC

Our NSF grant partially subsidizes many services we offer. User fees are vitally important if we are to close the funding gap and continue operations. We greatly appreciate your understanding! Additionally, we would be grateful for the following kinds of help:

- *Strain contributions:* Although we have obtained a few cultures from other strain repositories, the vast majority of our holdings were contributed by individual researchers. Please take a moment to look over our collection and consider: are there strains, vectors, phage, or clones that you have developed or acquired that we do not have? Would these materials be of some potential use to others in the research community? If so, please take the time to deposit the material in the BGSC. There is no charge whatsoever to you. There is also no compensation--except for the knowledge that you have made the fruits of your labor more accessible for the benefit of others. Generally, all we would require would be a culture (or lysate) with appropriate reprints or other helpful information. Please contact us (see below) if you have any questions.
- *Financial Contributions:* The BGSC requires on corporate strain sales and contributions to purchase equipment and undertake special projects not covered by the NSF grant. The Ohio State University Development Fund has a separate account for the BGSC. Contributions are tax deductible to the full extent of the law. Please contact us if you wish to make such a contribution.

How to order cultures

There are several ways to place orders with or request information from the BGSC:

- E-mail: zeigler.1@osu.edu
- Internet: www.bgsc.org
- Phone: 614-292-5550
- FAX: 614-292-3206
- Mail: Daniel R. Zeigler, Ph.D.
  Department of Biochemistry
  The Ohio State University
  484 West Twelfth Avenue
  Columbus, OH 43210 / USA

All users will be invoiced for strain, plasmid, or phage requests. Payment must be in US dollars via check, bank transfer, or procurement card (Visa, MasterCard, and American Express accepted). Orders can be placed via any of the five methods above with an institutional purchase order. Credit card orders should be made via phone or fax.

Pricing information

- *Academic, Government, and Non-Profit Users*—Not-for-profit users are requested to pay a $195 yearly subscription fee. Alternatively, a user may purchase individual strains at $35 each. If you are lacking inresearch support, please contact us to inquire about a fee waiver for a particular order.

- *For-profit Corporate Users*--Users may purchase cultures as needed for a $135 per culture charge. Shipping by UPS is included for all domestic orders and for international orders of two or more items. A pre-paid service plan is available for $1950. It entitles the user to receive up to 50 cultures within the next twelve calendar months at no additional cost. UPS delivery service is included (maximum of five express deliveries per year on international shipments).

Important Notice

Please read this notice before ordering materials from this catalog!

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(2) The Materials are provided for research use only and are not to be used for commercial purposes which include, but are not limited to, the sale, lease, license, or other transfer of the Materials or modifications to a for-profit organization **without the express permission of the owners of the Materials.**

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Introduction to a New Genus

The Origins of *Bacillus* Taxonomy

The modern concept of the genus *Bacillus* can be traced largely to the work of Nathan R. Smith, Francis E. Clark, and Ruth E. Gordon in the 1930's. I would like to dedicate this catalog to these scientists and their colleagues. Although recent developments in technology allow us to see our world with new eyes, we nevertheless owe this earlier generation of *Bacillus* taxonomists a large debt of gratitude. Their tireless efforts gave us an orderly framework to build upon.

In the early years of the century, any rod-shaped organism might be described as a *Bacillus* species—witness the many early papers discussing *"B. coli."* Researchers rarely had the opportunity to compare cultures, resulting in a bewildering array of synonymous species names. Smith and his colleagues developed a working definition of the genus *Bacillus* as comprising "rod-shaped bacteria capable of aerobically forming refractile endospores that are more resistant than vegetative cells to heat, drying, and other destructive agencies" (14). Working initially with a collection of 1,134 mesophilic strains bearing 158 different species names, they assigned each isolate to one of 19 rigorously defined species (40). Considering the nearly Herculean task these workers faced in bringing order out of chaos, it is understandable that they developed something of a bias in favor of over-simplification. "Lumping" rather than "splitting" became the default mode.

Early Classification of *Bacillus* Thermophiles

In classifying thermophilic *Bacillus* strains in 1949, Gordon and Smith again followed the principle of simplification. Of 206 strains in their collection, 46 were considered to belong to the mesophilic species. The rest were divided between just two thermophilic species, *B. stearothermophilus* and *B. coagulans*. Gordon and Smith were apparently not too happy with the name *"stearothermophilus."* They wrote, "The literature was carefully searched for accounts of thermophilic aerobic spore-formers not present in this collection…With none of the cultures predating *Bacillus stearothermophilus* at hand for comparative purposes, there is no choice at present but to assign this cumbersome name to the species" (15). This "cumbersome" name had appeared in 1920 in Donk's two-page description of "spoilage samples of 'Standard Maine Style' corn" he had isolated on October 3, 1917 at the research labs of the National Canners Association (9). Despite Gordon and Smith's hope that an older culture would turn up, resulting in "the assignment of a more suitable name to the species," the designation *B. stearothermophilus* stuck. By their definition, essentially any aerobic spore-former capable of growing at 65°C would be assigned to this species, especially if it hydrolyzed starch and gelatin and showed a sensitivity to growth in lysozyme and 7% NaCl. Gordon and Smith expressed a reluctance "to accept the collection of cultures described…as representing the entire group of aerobic thermophilic sporebearing bacteria" and predicted that "when more strains are studied, more species will undoubtedly be found" (15).

A Proliferation of Species

The next three decades would see the slow but steady accumulation of evidence that thermophilic bacilli are indeed a diverse group. A number of species names were proposed but later excluded from the Approved Lists of Bacterial Names. In 1993, White *et al*. published a thorough re-examination of these organisms in which they analyzed 234 strains isolated from around the globe (53). They examined 96 phenotypic characteristics of each strain including growth characteristics, carbohydrate utilization, tolerance of inhibitors, degradation of substrates in the medium, and chromosomal DNA melting temperature. White *et al*. uncovered what they termed "enormous diversity." The thermophiles fell into 18 phenetic clusters, some of them corresponding to known species. In addition to emending existing species descriptions, White *et al*. proposed new species names to describe some of the better characterized clusters (53). The prediction of Gordon and Smith had been convincingly fulfilled.

A Revolution in Bacterial Taxonomy

"A revolution is occurring in bacterial taxonomy. What had been a dry, esoteric, and uncertain discipline—where the accepted relationships were no more than officially sanctioned speculation—is becoming a field fresh with the excitement of the experimental harvest." So wrote a multinational team of scientists, led by Carl Woese at the University of Illinois, in a landmark 1980 paper detailing new insights into bacterial phylogeny provided by the innovations of molecular biology (12). While earlier generations of taxonomists might have argued vigorously against some of the sentiments expressed by the Woese team, it is hard to dispute this basic claim: a revolution in bacterial taxonomy has occurred. Woese recognized that small subunit rRNA is a valuable molecular clock. The molecule is

extremely highly conserved among all of earth's organisms, is complex enough to provide a good statistical sample of sequence information, but is short enough to be analyzed relatively quickly and inexpensively (11). By 1977, Woese's group had looked at enough sequences to recognize that earth's life can be divided into three primary domains, two of them prokaryotic (55). Soon detailed phylogenetic groupings began to emerge, providing the basis for a radical revision in bacterial taxonomy (12).

When molecular taxonomists began to turn their attention to the genus *Bacillus,* it became readily apparent that the traditional definition of "aerobic endospore-formers," while convenient from a purely practical point of view, was misleading as to phylogenetic relationships. Many species classified as *Bacillus* seemed to be more related to non-sporulating gram-positive organisms than to each other. The classification of *Bacillus stearothermophilus* was especially in doubt (11, 42). By the early 1990's, sequencing technology had advanced to the point that large numbers of nearly full-length 16S rRNA sequences could be determined with a reasonable expenditure of time and resources. Ash *et al*. (2) analyzed 51 species of *Bacillus* and found that they fell into five distinct groupings based on 16S rRNA sequences, which they believed "clearly represent separate genera." A few of the sporulating aerobes proved to be sequence outliers, which themselves might "represent the nuclei other hitherto unrecognized genera."

A Proliferation of Genera

The publication of Ash *et al*. marked the beginning of a period of reorganization for the genus *Bacillus,* a process that is still occurring at a rapid pace today. Several new genera have been defined from organisms previously known as *Bacillus;* other genera have been created for novel isolates that previously would have been assigned to *Bacillus* based on the older morphological and physiological definition (see Figure 1 and table 1). As Ash *et al*. predicted, their phylogenetic groups have been redefined as separate genera, and their outlying species have served as the basis for novel taxa as well. As microbiologists in the 1990's explored earth's extreme environments, novel endospore-forming halophiles, acidophiles, alkaliphiles, and thermophiles turned up in large numbers. Small subunit rRNA sequencing, now routine, has often shown these extremophiles to be *Bacillus* cousins and the nuclei of still other new genera. Considering the current pace of discovery, one might expect the list of approved *Bacillus*-like genera to expand rapidly during the coming decade.

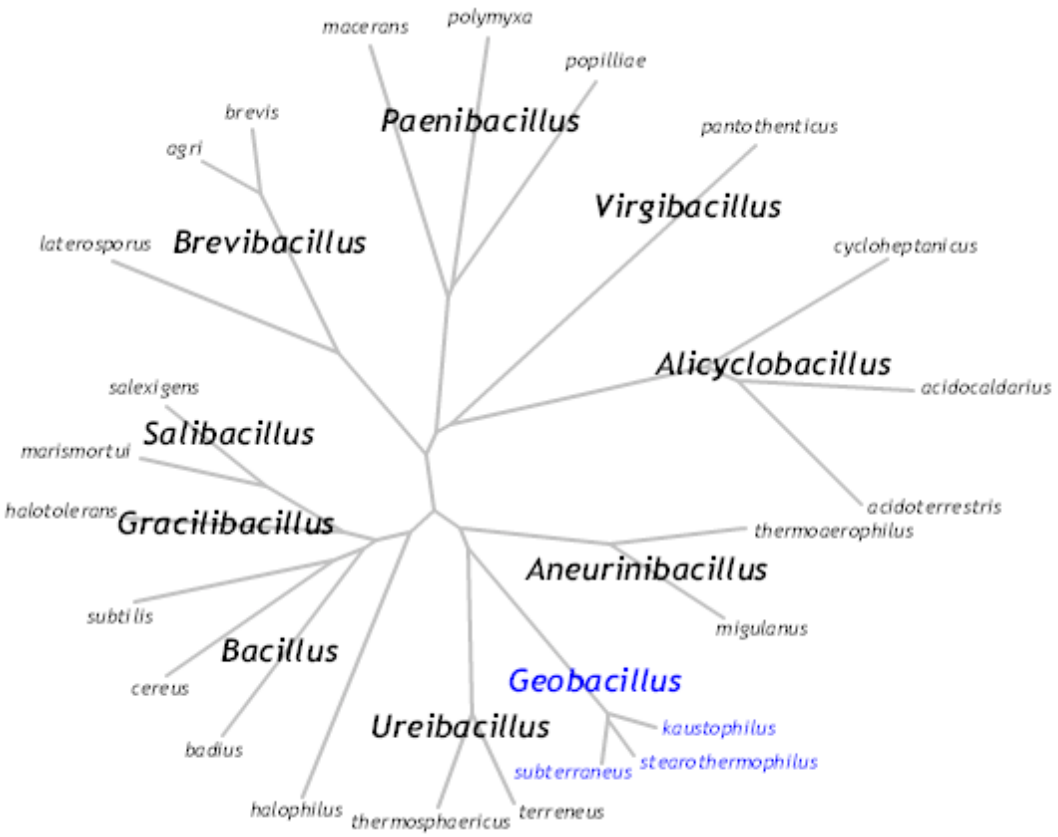


Figure 1. Phylogenetic tree based on 16S rRNA gene alignments.

Table 1. Genera of *Bacillus* sensu lato

Genus	Authors	Ref.	Meaning of Name	Brief description
Bacillus	Cohn (1872)	(39)	"small rod"	Aerobic or facultatively anaerobic, endospore-forming, rod-shaped bacteria
Amphibacillus	Niimura, *et al.* (1990)	(28)	"both ways" bacillus	Facultatively anaerobic
Alicyclobacillus	Wisotzkey, *et al.* (1992)	(54)	"cyclic fatty acid" bacillus	Acidophilic, moderately thermophilic
Paenibacillus	Ash, *et al.* (1994)	(3, 38)	"almost" a bacillus	Facultatively anaerobic, mesophilic
Aneurinibacillus	Shida, *et al.* (1996)	(37)	"thiamine" bacillus	Aerobic, mesophilic
Brevibacillus	Shida, *et al.* (1996)	(37)	"short" bacillus	Almost all aerobic, mesophilic
Halobacillus	Shida, *et al.* (1996)	(41)	"salt" bacillus	Moderately halophilic
Virgibacillus	Heyndrickx, *et al.* (1998)	(17)	"branch" of bacillus	Mesophilic, moderately halotolerant
Gracilibacillus	Wainø, *et al.* (1999)	(50)	"slender" bacillus	Mesophilic; some extremely halotolerant
Salibacillus	Wainø, *et al.* (1999)	(50)	"salt" bacillus	Moderately halophilic
Anoxybacillus	Pikuta, *et al.* (2000)	(30)	"without oxygen" bacillus	Strictly or facultatively anaerobic, alkaliphilic, moderately thermophilic
Thermobacillus	Touzel, *et al.* (2000)	(48)	"hot" bacillus	Aerobic, thermophilic
Filobacillus	Schlesner, *et al.* (2001)	(36)	"thread" bacillus	Aerobic, alkali-tolerant and halophilic
Ureibacillus	Fortina, *et al.* (2001)	(10)	"urea" bacillus	Aerobic, thermophilic
Geobacillus	**Nazina, *et al.* (2001)**	**(25)**	**"earth" bacillus**	**Aerobic or facultatively anaerobic, thermophilic**

Introducing the Genus *Geobacillus* (Nazina 2001)

Bacillus stearothermophilus and its thermophilic sister species, *B. kaustophilus* and *B. thermoglucosidasius,* belong to a distinct phylogenetic cluster that Ash *et al.* had termed "group 5" (2). A decade was to pass, however, before this new taxon was to receive a name to distinguish it from the genus *Bacillus*. At the same time Ash *et al.* were analyzing sequences, a group of Moscow scientists, led by N. A. Nazina at the Russian Academy of Sciences, began looking at the microbial ecology of high temperature oil fields in Kazakhstan. Their concern was practical. Oil production at these fields is hindered by hydrocarbon-utilizing and sulfate-reducing microbes that corrode metal equipment and reduce petroleum quality. The investigation demonstrated that injection of heated Caspian seawater to replace the original formation water in these oil fields promoted the growth of a vigorous and diverse microbial flora. The hot (70°-80°C), lower salinity, oxygenated water injected into the Uzen' field seemed especially to favor the growth of aerobic, endospore-forming thermophiles. Water samples drawn from a depth of over a kilometer at a temperature of 55-74°C were black with hydrocarbons, slime, silt and dissolved iron sulfide and rich in thermophilic bacilli (23). Subsequent studies isolated similar bacteria from oil fields in western Siberia (24) and China (26). Physiologic and genetic analysis demonstrated that these organisms fell in two groups. Two isolates were similar to *Bacillus licheniformis.* The remainder were most similar to the Ash *et al.* "group 5" organisms, including *Bacillus stearothermophilus* (26).

Nazina *et al.* undertook a thorough, polyphasic examination of their "group 5" isolates. Their results support the notion of a phylogenetically distinct, physiologically and morphologically consistent taxon, for which they have submitted the validly-described genus name of *Geobacillus* (25). The remaining group 5 species, including *B. kaustophilus, B. stearothermophilus, B. thermocatenulatus, B. thermodenitrificans, B. thermoglucosidasius,* and *B. thermoleovorans* join two new species from Nazina *et al.* in the *Geobacillus* genus. A summary of the *Geobacillus* description is given in table 2.

The thermophilic bacilli offer significant potential for biotechnological applications (see table 3). Basic research into their biology could yield significant insights into fundamental questions of microbiology in fields ranging from the ecology of extreme environments to astrobiology. The *Bacillus* Genetic Stock Center offers this new catalog of strains devoted exclusively to the genus *Geobacillus* in hopes of fostering research into this fascinating group of organisms.

Table 2. Description of the *Geobacillus* genus, adapted from Nazina *et al.* (25)

Morphology	
Vegetative cell	Rod-shaped cells, occurring either singly or in short chains and motile by means of peritrichous flagella. The cell wall structure is Gram-positive, but the Gram-stain reaction may vary between positive and negative.
Spore	One ellipsoidal or cylindrical endospore per cell, located terminally or subterminally in slightly swollen or non-swollen sporangia
Colony	Variable shape and size; pigments may be produced on certain media.
Metabolism	
Energy	Chemo-organotrophic
Oxygen	Aerobic or facultatively anaerobic. O_2 is the electron acceptor, replaceable in some species by nitrate.
Temperature	Obligately thermophilic. The growth-temperature range is 37-75°C, with an optimum at 55-65°C.
pH	Growth occurs in a pH range of 6.0 to 8.5, with an optimum at pH 6.2-7.5.
Requirements	Growth factors, vitamins, NaCl and KCl are not required by most species.
Identification tests	
Carbohydrates	Acid but no gas is produced from glucose, fructose, maltose, mannose and sucrose. Most species do not produce acid from lactose.
Enzymes	Most species form catalase. Phenylalanine is not deaminated, tyrosine is not degraded, indole is not produced, the Voges-Proskauer reaction is negative. Oxidase-positive or negative.
Biochemicals	The major cellular fatty acids are iso-15:0, iso-16:0 and iso-17:0, which make up more than 60% of the total. The main menaquinone type is MK-7.
DNA characterization	
G-C content	48.2-58 mol% (thermal denaturation method)
16S rRNA	Sequence identities higher than 96.5% among the members of this genus
Ecology	
Prevalence	Most species are widely distributed in nature.

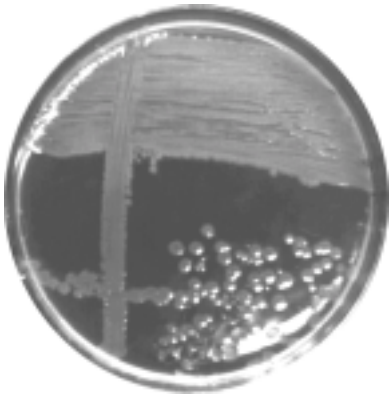
Table 3. A selection of U.S. Patents for *Geobacillus* products or processes

Product or Process	Patent No.
α-arabinofuranosidase	US05434071
acetate kinase	US05610045
alpha-amylase	US05824532, US05849549
arabino furanoside	US05491087
biological indicator for sterilization	US05073488, US05223401, US05252484, US05418167
BsrFI restriction endonuclease	US06066487
catalase	US06022721
cellobiose fermentation	US06102690
DNA polymerase	US05747298, US05830714, US05834253, US05874282, US06013451, US06066483, US06100078, US06238905
ethanol production	US05182199
glucose-6-phosphate dehydrogenase	US04331762
liquefying starch	US05756714
maleate dehydrogenase	US04331762
neutral proteases	US06103512
perillyl compounds	US05487988
polynucleotide phosphorylase	US04331762
prenyl diphosphate synthase	US06225096
pyruvate kinase	US04331762
riboflavin glucoside	US06190888
superoxide dismutase	US05772996
xylanase	US05434071
xylosidase	US05489526

Geobacillus stearothermophilus Isolates

Geobacillus stearothermophilus NRRL B-4419

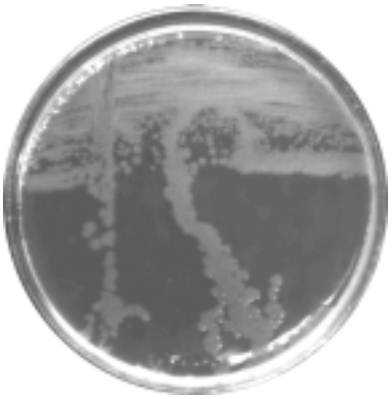
BGSC Accession No. 9A2



Species name: *stearos* (fat) + *thermo* (heat) + *philus* (loving) = fat- and heat-loving bacterium.

Recommended media: TBAB, TSA, mLB

Description: From the strain collection of John R. Gillis at the American Sterilizer Co. in Erie, PA; used as a sterilization standard. Fragments have been sequenced that correspond to a probable *valS* gene (GenBank L17321) and a *spo0A* homolog (GenBank AJ002297, CAA05307). The B-4419 predicted Spo0A amino acid sequence is 76-79% identical to homologs from various *Bacillus* species, including *B. subtilis*, *B. megaterium*, and *B. cereus*.



Geobacillus stearothermophilus XL-65-6

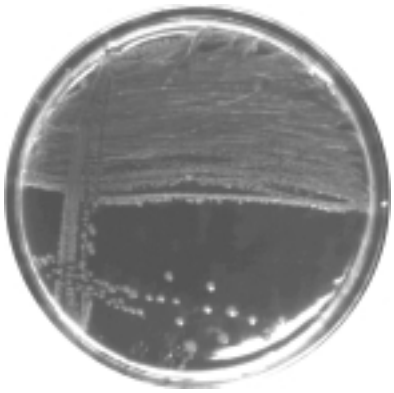
BGSC Accession No. 9A19

Recommended media: TBAB, LB, TSA, mLB

Description: Isolated from rotting wood in Florida, USA, on the basis of its ability grow at 65°C and produce a halo on powdered cellulose plates (18). The authors identified it as *B. stearothermophilus* based on an unspecified set of morphological and physiological tests from Bergey's manual. This assignment should be regarded as tentative in the absence of confirming 16S rRNA gene sequencing or DNA hybridization data. The authors went on to analyze the *ptsHI* operon (19) and the specific *cel* genes (18) that enable this strain to uptake the cellulose breakdown product, cellobiose.

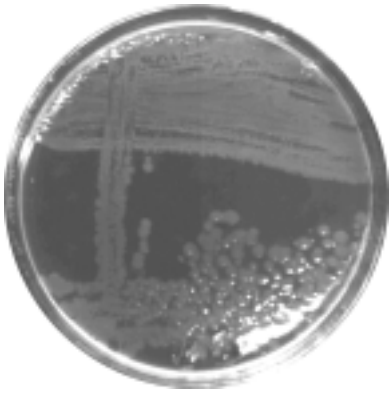
Geobacillus stearothermophilus ATCC 12980^{T}

BGSC Accession No. 9A20



Recommended media: TBAB, TSA

Description: Type strain of *Geobacillus stearothermophilus*, strain 26 from the collection of the National Canning Association. Cells are motile, 0.6-1.0 µM long by 2-3.5 µM wide, with ellipsoidal endospores. Growth requires temperatures of 37-65°C and pH 6.0-8.0 (6). G-C value is 59.1%. ATCC 12980 is the source of restriction endonuclease BstPI (33). It is used in paper strips as a biological indicator for steam sterilization (1, 29). It is also a source of thermostable enzymes for industrial applications (4).



Geobacillus stearothermophilus NUB3621

BGSC Accession No. 9A5

Recommended media: mLB, MG, TBAB, LB

Description: NUB36 has been the subject of careful genetic analysis (5, 52, 56); a preliminary genetic map is available (49). NUB36 produces restriction endonuclease *BsmI* (34). According to Chen *et al*. (5), it "was isolated from soil by classical enrichment techniques and was identified as *B. stearothermophilus* by standard physiological and biochemical criteria." A recent examination of 16S rRNA genes, however, revealed that NUB3621 was more similar to a *Geobacillus thermoglucosidasius* isolate (95% sequence identity) than to the type strain of *G. stearothermophilus* (90% identity). Until the taxonomy of NUB36 is analyzed thoroughly, its assignment as *G. stearothermophilus* should be treated as tentative.

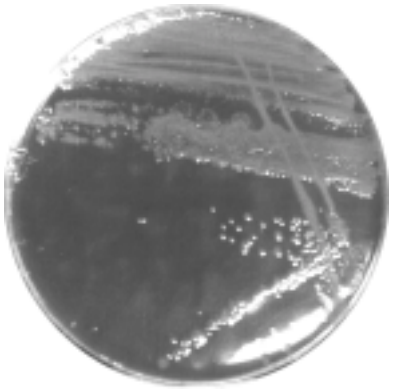
Genetically characterized mutants of *Geobacillus stearothermophilus* NUB36

Each of the following strains was derived by mutagenizing the parental strain, NUB36, with MNNG (*N*-methyl-*N'*-nitro-*N*-nitrosoguanidine). The restriction-modification system in 9A5 was inactivated by MNNG mutagenesis; the strain also bears a spontaneous mutation to rifampicin resistance. The chromosomal linkage group containing the mutant alleles in strains 9A6-9A18 is given if known.

BGSC No.	Original Code	Genotype	Linkage	Ref.
9A5	NUB3621	*hsmI hsrI rif*	-	(5)
9A6	NUB36212	*arg-9*	I	(49)
9A7	NUB3611	*asp-1*	A	(52)
9A8	NUB36230	*gly-4*	D	(49)
9A9	NUB3625	*hom-1*	C	(5)
9A10	NUB36235	*ilv-2*	G	(49)
9A11	NUB36187	*leu-2*	G	(49)
9A12	NUB36251	*met-4*	A	(49)
9A13	NUB36110	*gua-4*	B	(49)
9A14	NUB361	*thr-1*	C	(5)
9A15	NUB369	*rib-1*	-	(52)
9A16	NUB36183	*pur-5*	E	(49)
9A17	NUB36185	*his-4*	C	(49)
9A18	NUB36328	*trp-2*	A	(49)

Geobacillus stearothermophilus strain 10

BGSC Accession No. 9A21



Recommended media: TBAB, mLB

Description: Isolated from hot springs in Yellowstone National Park. The exact criteria by which strain 10 was identified as *Geobacillus stearothermophilus* are unknown. Its 16S rRNA gene sequence is about 98% identical to that of the type strain of this species, but roughly the same percent identity to the 16S genes of other *Geobacillus* species. Strain 10 was the subject of several early studies characterizing the differences between mesophilic and thermophilic *Bacillus* species in DNA base composition and DNA polymerase properties (43-45).

Genome Sequencing Project

Currently, the genomic sequence of *Geobacillus* strain 10 is being determined at the University of Oklahoma's Advanced Center for Genome Technology in Norman, Oklahoma. At the time this catalog was written, 3.4 Mb of sequence, contained in 645 contigs, had been determined, specifying 3,310 ORF's (see Table # below). Comparison of this genome with the finished genomes of *Bacillus subtilis, Bacillus halodurans, Bacillus anthracis,* and *Bacillus cereus* should provide key insights into the molecular biology of thermophily in the gram-positive bacteria. Sequence files are available from the Oklahoma server at http://www.genome.ou.edu/bstearo.html and from Integrated Genomics at http://wit.integratedgenomics.com/ERGO/CGI/stats.cgi?org_group_ids=BE.

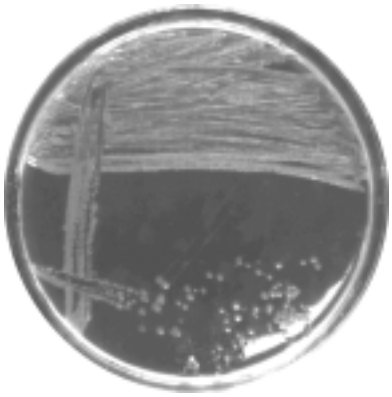
Table #. Functional Overview of the *G. stearothermophilus* 10 Genome.

Category	Number	% Total
DNA total sequenced, bases	3,392,712	100.00
DNA coding sequence, bases	2,768,565	81.60
DNA contigs	645	
DNA G+C percentage	1,787,499	52.69
ORFs total	3,310	100.00
ORFs with assigned function	2,073	62.63
ORFs with function but no similarities	1	0.03
ORFs without assigned function	1,237	37.37
ORFs without function or similarity	180	5.44
ORFs without function, with similarity	1,057	31.93
ORFs in asserted pathways	882	26.65
ORFs in asserted metabolic pathways	563	17.01
ORFs in asserted non-metabolic pathways	398	12.02
ORFs not in asserted pathways	2,428	73.35
ORFs with assigned function but no pathway	1,191	35.98
ORFs in ortholog clusters	1,936	58.49
ORFs in paralog clusters	1,018	30.76
ORFs in COGs	2,114	63.87
ORFs with Pfam matches	1,575	47.58
ORFs in chromosomal clusters	1,426	43.08
Functions assigned	1,537	123.45
Functions assigned, hypothetical	13	1.04
Functions connected to asserted pathways	683	54.86
Functions missing from asserted pathways	562	45.14
Functions with no sequence	36	2.89
Pathways asserted total	328	100.00
Pathways asserted, metabolic	276	84.15
Pathways asserted, non-metabolic	52	15.85
Clusters, orthologous groups (COGs)	1,072	

Other *Geobacillus* Species

Geobacillus kaustophilus DSM 7263^T

BGSC Accession No. 90A1

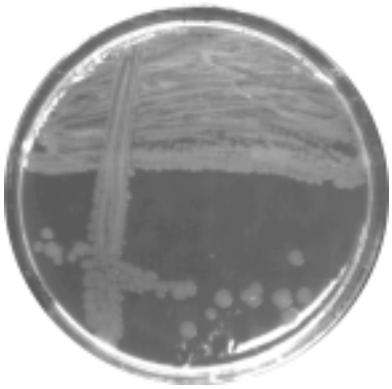


Species name: *kaustos* (heat) + *philus* (loving) = heat-loving bacterium.

Recommended media: TBAB, NB

Description: Originally isolated from pasteurized milk (31), this species was at one time identified as *Bacillus stearothermophilus* due to phenotypic similarities (15). Thorough taxonomic studies led to a proposal to re-establish the taxon (32), an idea supported by 16S rRNA gene sequencing studies (25, 46). Colonies are convex and transparent. Cells are non-motile, 1.5 µM × 3.5 µM, producing spores that are oval to cylindrical and may swell the sporangium. G-C content is 51-55%. Acid is produced from adonitol, cellobiose, inositol, maltose, mannose, salicin, sucrose, trehalose, and xylose, but not from arabinose, glycerol or ribose. Growth requires pH 6.2-7.5, 37-68°C, and 0-2% NaCl.

Geobacillus subterraneus DSM 13552^T



BGSC Accession No. 91A1

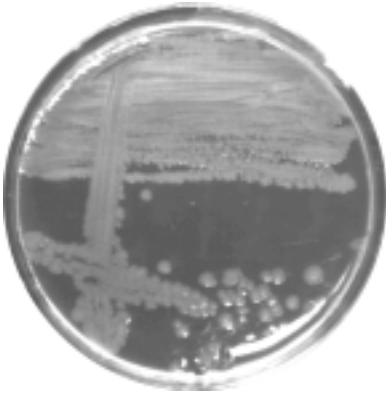
Species name: *subterraneus* = subterranean, below the Earth's surface

Recommended media: TBAB, LB, TSA, 2×SG, TS

Description: Isolated from high-temperature formation waters of the Liaohe oilfield in China. Cells are rod-shaped, motile by means of peritrichous flagella and produce subterminally or terminally located ellipsoidal spores in non-swollen sporangia. Gram staining is positive. Colonies are round, mucoid and colorless. G-C content is 49.7-52.3 mol%. Utilizes as carbon and energy sources hydrocarbons (C_{10}-C_{16}), methane-naphthenic and naphthenic-aromatic oil, phenylacetate, formate, acetate, butyrate, pyruvate, benzoate, fumarate, succinate, peptone, tryptone, nutrient broth, potato agar, yeast extract, phenol, ethanol, butanol and lactate (25).

Geobacillus uzenensis DSM 13551^T

BGSC Accession No. 92A1



Species name: *uzenensis* = of Uzen', referring to the Uzen' oilfield, Kazakhstan

Recommended media: TBAB, NB, LB, TSA, TS, mLB, MG

Description: Isolated from high temperature formation waters of the Uzen' oilfield, Kazakhstan. Cells are rod-shaped with peritrichous flagella and produce terminal ellipsoidal spores that may swell the sporangium. Gram staining is variable. Colonies are round, mucous, small and colorless. G-C content is 50.4-51.5%. Utilizes as carbon and energy sources hydrocarbons (C_{10}-C_{16}), methane-naphthenic and naphthenic-aromatic oil, acetate, propionate, butyrate, pyruvate, benzoate, phenylacetate, phenol, ethanol, butanol, malate, lactate, fumarate, succinate, peptone, tryptone, nutrient broth, potato agar and yeast extract (25).

Geobacillus thermocatenulatus DSM 730^T

BGSC Accession No. 93A1

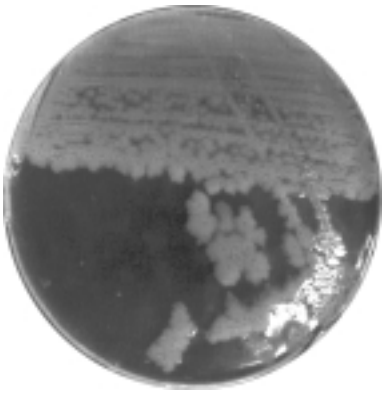
Species name: *therme* (heat) + *catenulatus* (chain-like) = heat-loving bacterium that grows in chains.

Recommended media: TBAB, mLB

Description: Isolated from a coating inside thermal bore-hole pipe in the Southern Urals. Long, thin rods (0.9 × 6-8 µM) in unbranched chains that clump in liquid media. Forms granules of poly-β-hydroxybutyric acid. Cylindrical spores swell the sporangium slightly. Growth requires temperatures of 35-78°C and 0-4% NaCl. Colonies are yellowish, round, raised, even, and pasty. Able to grow anaerobically on nitrate. Acid from glucose, laevulose, galactose, sucrose, cellobiose, mannitol, glycerol and dextrin. G-C content reported to be 69% (13).

Geobacillus thermodenitrificans DSM 465^T

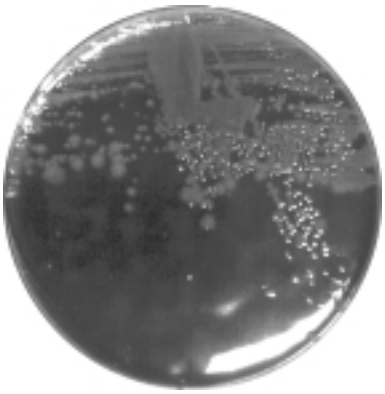
BGSC Accession No. 94A1



Species name: *therme* (heat) + *denitrificans* (denitrifying) = heat-loving bacterium able to reduce nitrate to nitrogen

Recommended media: TBAB, BST, mLB

Description: Isolated from sugar beet juice from an extraction plant in Austria. Colonies are flat, lobate, and off-white in color. Cells are Gram-positive rods 0.5-1.0 µM wide by 1.5-2.5 µM long with oval subterminal or terminal spores and grow at 45-70°C at pH 6-8 in 0-3% NaCl. Forms flat colonies. Reduces nitrate to gas; under anaerobic conditions, uses nitrate as electron acceptor. It is able to utilize glucose, fructose, maltose, trehalose, mannose, lactose, cellobiose, galactose, xylose, ribose, and arabinose as sole carbon sources. Hydrolyzes starch, pullulan, and tributyrin. G-C content is 50-53% (20, 53).



Geobacillus thermoglucosidasius DSM 2542^T

BGSC Accession No. 95A1

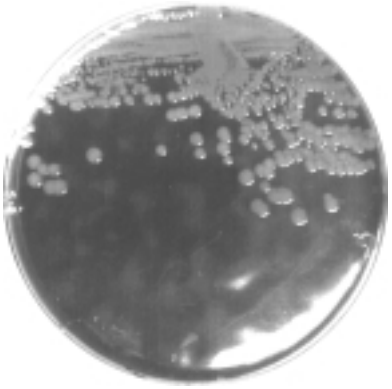
Species name: *therme* (heat) + *glucosidasius* (glucosidase producing) = heat-requiring bacterium with starch-hydrolyzing glucosidase activity

Recommended media: TBAB, mLB

Description: Isolated from soil in Japan. Growth requires 37-68°C and pH 6.0-8.0. G-C content is 43%. Most strains hydrolyze gelatin, pullulan, and starch and produce acid from adonitol, cellobiose, inositol, and D-xylitol (32, 47, 53). Colonies are off-white and mucoid.

Other *Geobacillus* species (cont.)

Geobacillus thermoleovorans DSM 5366^T



BGSC Accession No. 96A1

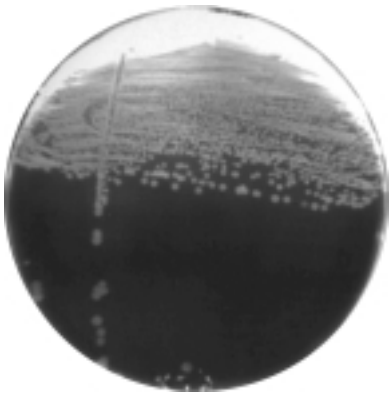
Species name: *therme* (heat) + *oleum* (oil) + *vorare* (to devour)= heat-requiring bacterium capable of utilizing hydrocarbons

Recommended media: TBAB, TS, mLB

Description: Isolated from soil near Bethlehem, PA near hot water effluent by selecting for thermophiles able to utilize *n*-alkanes as a carbon source (57). Cells are 1.5 × 3.5 µM, nonmotile, and form terminal endospores with swollen sporangia. Growth requires temperatures of 42-75°C and pH of 6.2-7.5. G-C content is 58%. Utilizable carbon sources include *n*-alkanes (C_{13}-C_{20}), acetate, butyrate, pyruvate, cellobiose, galactose, glucose, maltose, mannose, ribose, sucrose, trehalose, mannitol, casein, nutrient broth, peptone, tryptone, and yeast extract (57).

Bacillus thermantarcticus M1

BGSC Accession No. 20A1



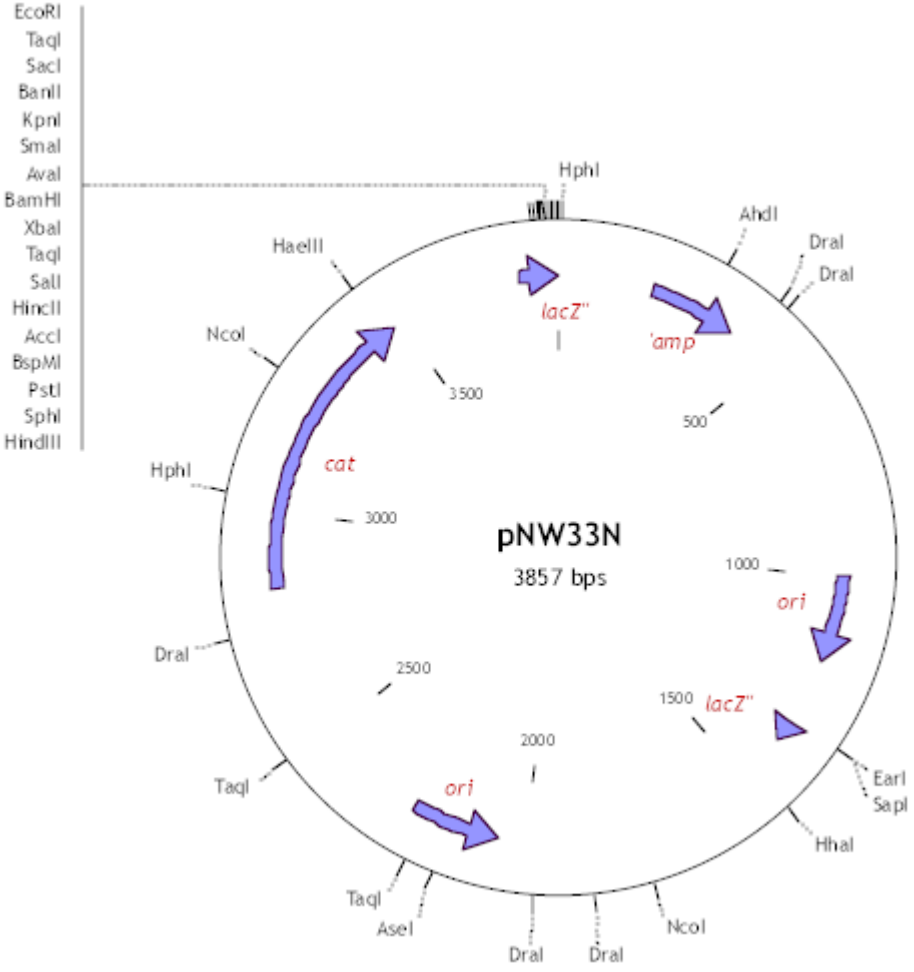
Species name: *therme* (heat) + *antarcticus* (from Antarctica) = heat-requiring bacterium from Antarctica. *Note: name formerly 'Bacillus thermoantarcticus'*

Recommended media: TBAB, LB, TS, mLB

Description: Isolated from geothermal soil from Mt. Melbourne, an active volcano in Antarctica. Growth requires 37-65°C and pH 5.5-9.0. Strain M1 secretes xylanase and α-glucosidase and utilizes glucose, trehalose, and xylose. The 16S rRNA gene sequence from M1 is 99% identical to that of *G. thermoglucosidasius* and over 97% identical to sequences from other *Geobacillus* species. Nicolaus *et al.* considered strain M1 to be a novel species since the G-C content they reported (54%) is significantly higher than published values from *G. thermoglucosidasius* (27).

Cloning Vector for *Geobacillus*

Geobacillus vectors have had a history of cloning problems—most importantly, serious rearrangements and deletions when cloning heterologous genes (51). Plasmid pNW33N, a fifth generation vector that stably replicates in *Bacillus subtilis*, *Geobacillus stearothermophilus*, and *Escherichia coli*, is available in BGSC *E. coli* strain ECE136. It features a large multiple cloning site and encodes a chloramphenicol acetyltransferase that is expressed in both gram-positives and gram-negatives. The vector is based on cryptic plasmid replicon pBC1 (1.6 kb) isolated from *Bacillus coagulans* and *Staphylococcus aureus* plasmid pC194 (7, 8). For *E. coli*, *G. stearothermophilus*, and *B. subtilis*, use 25, 7, and 5 µg/ml of chloramphenicol.



BGSC No.	Original Code	Description	Ref.
ECE136	JM109(pNW33N)	3857 bp *Geobacillus-Bacillus-E. coli* shuttle vector in JM109; Cm^R	(51)

Transformation of *Geobacillus stearothermophilus* NUB36 Protoplasts with Plasmid DNA

Protoplast transformation of *Geobacillus stearothermophilus* is slow and somewhat labor intensive, but rather efficient by *Bacillus* standards—up to 10^7-10^8 transformants per microgram of plasmid DNA. The following protocol is adapted from Neil Welker's published work (56). I do not know how well this protocol will work for other *Geobacillus* strains in the BGSC collection.

1. Protoplast formation

1.1 Streak an mLB plate with NUB36-derived strain (BGSC Accession No. 9A5-9A18) and incubate it at 60°C for 10-13 h.

1.2 Inoculate 20 ml of mLB in a 300-ml Erlenmeyer flask with a loopful of cells from the fresh plate. Shake the flask vigorously at 60°C for 2 h.

1.3 Inoculate a second 300-ml Erlenmeyer flask containing 20 ml mLB with an aliquot of the 2-h culture to obtain a cell density of 2-3 × 10^7 cfu/ml. Incubate at 60°C with vigorous shaking until late exponential phase, about 6-8 × 10^8 cfu/ml.

1.4 Pellet the culture by centrifugation at 1800-1900 × *g* at room temperature for 5 min.

1.5 Suspend the pellet in 4 ml of protoplasting medium (mLB supplemented with 10% (w/v) lactose and 10 mM $MgCl_2 \cdot 7H_2O$). Add lysozyme to 10 µg/ml (by adding 40 µl of a 1 mg/ml lysozyme suspension in protoplasting medium). Incubate in a shaking water bath at 50°C, 130 rpm, for 10 min.

1.6 Dilute the protoplasts by adding 5 ml protoplasting medium, then pellet them **gently** by centrifuging them at 700-800 × *g* for 7 min at room temperature.

1.7 Suspend the pellet **gently** in 4 ml of protoplasting medium.

2. PEG-induced transformation

2.1 Mix 5-20 µl of plasmid DNA with 0.1 ml of protoplast suspension. Add 0.9 ml of freshly prepared 40% PEG 6000 (w/v in protoplasting medium).

2.2 Incubate in a shaking water bath at 50°C, 130 rpm, for 2 min.

2.3 Dilute transformation mix with 2.5 ml of protoplasting medium, then pellet the protoplasts **gently** by centrifuging them at 700-800 × g for 7 min at room temperature.

2.4 Suspend the pellet **gently** in 0.1 ml of protoplasting medium.

2.5 Allow for plasmid gene expression by incubating the suspension in a water bath at 50°C, 130 rpm, for 1 h.

3. Regeneration and selection

3.1 Dilute protoplasts in protoplasting medium according to the expected transformation frequency.

3.2 Plate 0.1 ml aliquots of the suspension on regeneration plates (protoplasting medium supplemented with 20 mM $CaCl_2 \cdot 2H_2O$ and solidified with 0.8% agar) containing selective antibiotics. (For pNW33N, use 7 µg/ml chloramphenicol.)

3.3 Incubate plates at 50°C for 12 h and then at 60°C until colonies are formed, about 24-48 h.

3.4 Immediately transfer the colonies to mLB plates containing the appropriate antibiotic. Incubate at 60°C for 16-18 h. **Note**: Cell viability decreases rapidly on stored regeneration medium plates, so transfer to mLB is important.

Electroporation of *Geobacillus stearothermophilus* with Plasmid DNA

An alternative technique for transforming *Geobacillus* cells with plasmid DNA is electroporation. For a Japanese soil isolate identified as *B. stearothermophilus* K1041 (not available from the BGSC), frequencies of up to 5.8×10^5 transformants per µg of pUB110 DNA or 2.8×10^6 transformants per µg of pSTE33 DNA were obtained (21, 22). There are apparently limitations to this protocol. First, this strain showed the highest transformation frequency among a large collection of *Geobacillus* strains tested (22). It is likely that individual *Geobacillus* isolates will require somewhat different parameters for optimized transformation frequency. Secondly, this strain demonstrated a restriction-modification system that nearly eliminated transformants when plasmids were isolated from *E. coli* (21), but not from *Bacillus subtilis* RM125 (22) or *G. stearothermophilus* (21). Restriction-modification systems are likely to vary from strain to strain (see reference (34)), a factor that will need to be considered when designing experiments making use of shuttle vectors. Growth and recovery media may need to be adjusted for the strain of choice as well (see page 22 below). There is also evidence, from work using a different *G. stearothermophilus* strain, that a lower incubation temperature may improve recovery of transformants (16). It is hoped, however, that the protocol below can at least serve as a useful starting point for determining appropriate electroporation parameters and experimental design for other *Geobacillus* isolates.

Competent Cell Preparation

1. Grow a *Geobacillus stearothermophilus* culture overnight at 55°C in LB broth with shaking.
2. Dilute inoculum 1:100 in fresh LB; incubate at 55°C to OD_{600} = 1.42.
3. Pellet cells by centrifugation. Wash twice in ice cold distilled water.
4. Wash twice more in ice cold 10% glycerol.
5. Suspend final pellet in 1/100 of the original volume of ice cold 10% glycerol.
6. Dispense in 40µl aliquots and store at –80°C until use.

Electroporation

1. Thaw the cell suspension at room temperature and mix with 1 µl of plasmid DNA (5-50 ng of plasmid).
2. Transfer mix to cold electroporation cuvette (0.2 cm gap) and chill on ice 5 min.
3. For a BioRad Gene Pulser, set the capacitance to 25 µF and the voltage to 2000 V. Set the pulse controller to 200 Ω. Apply a single pulse (10.0 kV/cm).
4. Immediately mix cell suspension with 2 ml of cold 2×LBG broth (2% tryptone, 1% yeast extract, 1% NaCl, 0.5% glucose, and 0.01% bovine serum albumin).
5. Transfer diluted cells to a glass tube and incubate at 48°C for 90 min.
6. Spread cells on LB agar plates containing the appropriate antibiotics (10 µg/ml for chloramphenicol and 5 µg/ml for tetracycline). Incubate at 48°C for 18 h.

Genetic Analysis of *Geobacillus stearothermophilus* NUB36 by Protoplast Fusion

The Welker lab at Northwestern University has developed protoplast fusion as a means of genetic exchange for *Geobacillus stearothermophilus* NUB36 (5, 49). Although a natural transformation system has not been discovered in this organism, protoplast fusion has provided a means of genetic exchange for the purposes of genetic mapping and strain construction.

1. Protoplast formation

1.1 Streak an mLB plate with NUB36-derived strain (BGSC Accession No. 9A5-9A18) and incubate it at 60°C for 10-13 h.

1.2 Inoculate 20 ml of mLB in a 300-ml Erlenmeyer flask with a loopful of cells from the fresh plate. Shake the flask vigorously at 60°C for 2 h.

1.3 Inoculate a second 300-ml Erlenmeyer flask containing 20 ml mLB with an aliquot of the 2-h culture to obtain a cell density of 2-3 × 10^7 cfu/ml. Incubate at 60°C with vigorous shaking until late exponential phase, about 6-8 × 10^8 cfu/ml.

1.4 Pellet the culture by centrifugation at 1800-1900 × *g* at room temperature for 5 min.

1.5 Suspend the pellet in 4 ml of protoplasting medium (mLB supplemented with 10% (w/v) lactose, 10 mM $MgCl_2 \cdot 7H_2O$, and 20 mM $CaCl_2 \cdot 2H_2O$.). Add lysozyme to 10 µg/ml (by adding 40 µl of a 1 mg/ml lysozyme suspension in protoplasting medium). Incubate in a shaking water bath at 50°C, 130 rpm, for 5 min. **Note:** You may follow protoplast formation visually with phase contrast microscopy.

2. Protoplast fusion and regeneration

2.1 Mix equal volumes of a protoplast suspension of each parent(3-5×10^9 cfu/ml).

2.2 Add 0.1 ml of the protoplast mix to a 0.9 ml aliquot of freshly prepared 40% PEG 6000 (w/v in protoplasting medium). Mix gently and incubate 10 min at 60°C.

2.3 Collect the fused protoplasts by centrifuging them at 1800-2000 × g for 5 min at room temperature.

2.4 Suspend the pellet **gently** in 1 ml of protoplasting medium.

2.5 Plate 0.1 ml aliquots of the suspension on regeneration plates (protoplasting medium solidified with 0.8% agar).

Chromosomal DNA Isolation from *Geobacillus*

The following DNA isolation protocols were developed by Ronimus *et al*. (35) in developing RAPD methods for identifying *Bacillus* contaminants in industrial facilities. The "Long Method" gives purified DNA for a variety of molecular biology protocols, but the "Short Method" gives an adequate template for PCR amplifications.

Long Method

1. Inoculate 10 ml of an appropriate growth medium in a 250 ml flask with a fresh colony of *Geobacillus stearothermophilus* or other strain. Incubate at 60°C overnight with aeration.
2. Harvest by centrifugation at 4000 × *g* for 10 min at 4°C. Wash once in 10.0 ml of lysis buffer (50 mM Tris-HCl, pH 8.0, 20 mM EDTA, 50 mM glucose).
3. Suspend pellet in 10 ml lysis buffer with 0.02% lysozyme and incubate at 37°C for 30 min.
4. Add RNaseA (preboiled to inactivate DNases) to 200 µg/ml and SDS to 0.5% and incubate at 37°C for 30 min.
5. Add Proteinase K to a concentration of 250 µg/ml and incubate at 50°C for 1 h.
6. Extract sample with Tris-buffered phenol, then with phenol-chloroform, then chloroform according to standard methods.
7. Ethanol precipitate the DNA by standard methods and dissolve it in TE buffer (10 mM Tris-HCl, pH 8.0, 1 mM EDTA).

Short Method

1. Grow an overnight 60°C culture of *Geobacillus stearothermophilus* or other strain on an appropriate agar plate, inoculating with heavy streaks.
2. Harvest the cells by scraping and suspend them in 4 ml of 50 mM Tris-HCl, 100 mM NaCl, 20 mM EDTA, pH 8.0.
3. Pellet cells by centrifugation at 4000 × *g* for 10 min at room temperature. Suspend the pellet in 0.5-3.0 ml distilled, deionized water (depending on pellet size).
4. Boil cell suspension for 10 min.
5. Remove cell debris by centrifuging as above.
6. Determine A_{260} of the supernatant; dilute with sterile water to a final A_{260} of 0.3. Use 2.5 µl of boiled supernatant for PCR.

Media for Growth of *Geobacillus* Strains

Growth of *Geobacillus* Strains in Laboratory Media

The following table compares in semi-quantitative terms the growth of our *Geobacillus* stocks on solid media at 60°C after 18 h incubation (36 h for MG). Recipes for each medium follow. In our hands, the best general-purpose solid medium for *Geobacillus* is Difco® Tryptose Blood Agar Base, or TBAB. Each strain formed large, regular colonies on TBAB. Modified LB (mLB) also gave excellent results for most strains, although its preparation is somewhat laborious. Nutrient agar (NB) or Trypticase Soy Agar (TSA) performed acceptably for most strains. We prepared our media with column-purified water. We do not know the effects of trace ions in tap water on growth but do note that adding salts to NB (to make 2×SG) generally reduced growth. Neil Welker (pers. comm.), in working with the NUB series, observes that the strong chelation agent, NTA, is absolutely required for growth in some media, such as LB. Trace amounts of certain cations in laboratory water might well cause location-dependent differences in growth. If your lab fails to obtain good growth on TBAB, please consider the possibility that mLB agar might be a superior choice in your location.

		Common "Off the Shelf" Media					Specialized Media				
BGSC	Strain	TBAB	LB	NB	TSA	AB3	2×SG	BST	TS	mLB	MG[a]
9A2	B4419	+++[b]	++	++	+++	++	+	++	+	+++	+++
9A5	NUB3621	+++	++	++	+++	+	-	++	+	+++	+++
9A6	NUB36212	+++	+	++	+	+	++	++	+	++	+++
9A7	NUB3611	+++	++	++	++	+	++	++	+	+++	++
9A8	NUB36230	+++	++	++	++	++	++	++	+	++	++
9A9	NUB3625	+++	++	++	++	++	++	++	+	+++	++
9A10	NUB36235	+++	++	++	++	++	++	++	+	++	+++
9A11	NUB36187	+++	++	++	++	++	++	++	+	++	++
9A12	NUB36251	+++	++	++	++	++	++	++	+	+++	+++
9A13	NUB36110	+++	++	++	++	+	-	++	+	++	++
9A14	NUB361	+++	++	++	++	++	++	++	+	+++	++
9A15	NUB369	+++	++	++	++	++	++	++	+	+++	++
9A16	NUB36183	+++	++	++	++	++	++	++	+	+++	++
9A17	NUB36185	+++	++	+++	+++	++	++	++	+	++	++
9A18	NUB36328	+++	++	++	++	+	-	++	+	++	++
9A19	XL-56-6	+++	+++	++	+++	++	+	+	++	+++	++
9A20	ATCC 12980	+++	+	++	+++	++	++	++	++	++	-
9A21	10	+++	+	++	++	+	+	++	+	+++	++
20A1	M1	+++	+++	++	+	-	++	++	+++	+++	++
90A1	DSM 7263^T	+++	+	+++	++	+	-	+	++	+	++
91A1	DSM 13552^T	+++	+++	++	+++	++	+++	+	+++	++	+
92A1	DSM 13551^T	+++	+++	+++	+++	+	-	+	+++	+++	+++
93A1	DSM 730^T	+++	++	+	++	-	-	++	++	+++	++
94A1	DSM 465^T	+++	+	+	++	+	+	+++	++	+++	+
95A1	DSM 2542^T	+++	-	++	++	++	++	++	++	+++	++
96A1	DSM 5366^T	+++	+	+	+	+	+	+	+++	+++	++

[a]For 9A6-9A18, MG was supplemented with growth requirements as given in the strain description.

[b]Growth on test media plates: (-), no significant growth; (+), some growth in heavy portion of streak, but no isolated colonies; (++), isolated colonies, but somewhat smaller and lighter than optimal; (+++) optimal colony morphology, as defined by the species description.

Common Laboratory Media

1. TBAB (Tryptose Blood Agar Base)—Difco®; prepare according to package directions.

2. LB (Luria-Bertani Medium)—Per liter of distilled water:

Tryptone	10 g
Yeast Extract	5 g
NaCl	5 g
Agar (if desired)	15 g

3. NB (Nutrient Broth)—Difco®; prepare according to package directions, adding agar to 15 g/L prior to autoclaving.

4. TSA (Trypticase Soy Agar)—Difco®; prepare according to package directions.

5. AB3 (Antibiotic Medium No. 3; Penassay Broth)—Difco®; prepare according to package directions, adding agar to 15 g/L prior to autoclaving.

Specialized *Bacillus* Media

6. 2×SG Medium—Leighton, T. J. and R. H. Doi. 1971. J. Biol. Chem. **246:**3189-3195.

Difco® Nutrient broth	16.0 g
KCl	2.0 g
$MgSO_4 \cdot 7H_2O$	0.5 g
Agar (if desired)	17.0 g
Water	1000 ml

Adjust the pH to 7.0 with 1 M NaOH. Autoclave. Cool to 55˚C and add 1 ml each of:

1 M $Ca(NO_3)_2$	1.0 M
0.1 M $MnCl_2 \cdot H_2O$	0.1 M
1 mM $FeSO_4$	1 mM

Then add 2 ml of :

Glucose, filter-sterilized	50% (w/v)

7. BST (*Bacillus stearothermophilus* medium)—Harwood, C. R. and S. M. Cutting, eds. 1990. *Molecular Biological Methods for Bacillus,* p. 545. John Wiley & Sons: Chichester.

Antibiotic Medium #3	17.5 g
Agar (if desired)	15 g
Water	1000 ml

Autoclave. Cool to 55˚C and add 0.8 ml each of the following sterile stocks:

$CaCl_2 \cdot 2H_2O$	5% (w/v)
$FeCl_2$	5% (w/v)

Then add 10 ml of :

Glucose, filter-sterilized	20% (w/v)

8. TS Medium—N. Welker, Northwestern University, personal communication.

Tryptone	40 g
NaCl	5 g
Agar (if desired)	15 g
Water	995 ml
NaOH (10% w/v)	1.25 ml

Autoclave. Cool to 55˚C and add 1 ml each of the following sterile stocks:

Nitrilotriacetic acid[a]	1.05 M
$MgSO_4 \cdot 7H_2O$	0.59 M
$CaCl_2 \cdot 2H_2O$	0.91 M
$FeSO_4 \cdot 7H_2O$	0.04 M

9. mLB Medium (Modified L-Broth)—N. Welker, Northwestern University, personal communication.

Tryptone	10 g
Yeast Extract	5 g
NaCl	5 g
Agar (if desired)	15 g
Water	995 ml
NaOH (10% w/v)	1.25 ml

Autoclave. Cool to 55˚C and add 1 ml each of the following sterile stocks:

Nitrilotriacetic acid[a]	1.05 M
$MgSO_4 \cdot 7H_2O$	0.59 M
$CaCl_2 \cdot 2H_2O$	0.91 M
$FeSO_4 \cdot 7H_2O$	0.04 M

10. MG Medium (Minimal Glucose Medium)—N. Welker, Northwestern University, personal communication.

Minimal salts (below)	20 ml
Agar, if desired	15 g
Water	955 ml
10% KOH (w/v)	0.68 ml

After autoclaving, add 1 ml each of the following sterile stocks:

Nitrilotriacetic acid[a]	1.05 M
$MgSO_4 \cdot 7H_2O$	0.59 M
$CaCl_2 \cdot 2H_2O$	0.91 M
$FeSO_4 \cdot 7H_2O$	0.04 M

Then add 25 ml of:

Glucose, filter-sterilized	20% (w/v)

Minimal salts (do not sterilize; store refrigerated):

K_2HPO_4	26.1 g
KH_2PO_4	11.3 g
NH_4NO_3	25.0 g
Water	1000 ml

[a]**CAUTION:** Possible carcinogen. Wear gloves and face mask. Dissolve by adding NaOH pellets directly to NTA and water, then adjust the pH to 6.6-6.8 by adding 1 M NaOH. Filter sterilize and store at room temperature.

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