Characterization of Bacterial Strains for Taxonomic Purposes

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Sep 2014

The tenet for novel taxa characterization: 

as comprehensively as possible.
Strain

Genetic-based characterization

- 16S rRNA gene
- Whole genome
- Phylogenetic tree
- DNA G+C content
- DNA-DNA hybridization
- Multilocus sequence analysis

Phenotypic characterization

- Morphology, physiology, biochemistry
  - Antibiotics resistance
  - Substrate utilization
  - Growth tolerance
  - Enzyme activity
  - Cell shape
  - Staining

Chemical characterization

- Fatty acids
- Polar lipids
- Polyamines
- Techoic acids
- Mycolic acids
- Peptidoglycan
- Lipopolysaccharides
- Respiratory lipoquinones
- Hydrophobic side chains of lipids
- Isoprenoid-based ether-linked lipids
I. Strains

• Isolation method: Spread Plate Method

• location (eg. GPS, latitude/ longitude)

• Environment (eg. pH, salinity, temperature, chemical composition)

• Designations (eg. CGMCC 1.9159\textsuperscript{T}= DSM 22955\textsuperscript{T})
I. Strains

*Bacillus* Cohn 1872, *genus*. (Type genus of the order *Bacillales* Prévet 1953 [Approved Lists 1980]; type genus of the family *Bacillaceae* Fischer 1895 [Approved Lists 1980]).

**Type species:** *Bacillus subtilis* (Ehrenberg 1835) Cohn 1872 (Approved Lists 1980).

Etymology: L. masc. n. *bacillus*, a small staff, a wand, a rod.


*Bacillus subtilis* (Ehrenberg 1835) Cohn 1872, *species*. (Type species of the genus.)

**Type strain:** (see also StrainInfo.net) strain ATCC 6051 = ATCC 6051-U = CCM 2216 = BCRC (formerly CCRC) 10255 = CCUG 163 B = CFBP 4228 = CIP 52.65 = DSM 10 = IAM 12118 = IFO (now NBRC) 13719 = IFO (now NBRC) 16412 = IMET 10758 = JCM 1465 = LMG 7135 = NCAIM B.01095 = NCCB 70064 = NCCB 32009 = NCCB 53016 = NCIMB 3610 (formerly NCDO 1769) = NCTC 3610 = NRRL B-4219 = NRRL NRS-1315 = NRRL NRS-744 = VKM B-501.

Sequence accession no. (16S rRNA gene) for the type strain: AJ276351.

Synonym: *"Vibrio subtilis"* Ehrenberg 1835.

II. Genetic-based characterization

• 16S rRNA gene

• High quality (almost complete, no ambiguities)
II. Genetic-based characterization

• 16S rRNA gene
  • Multiple alignment (use expert-maintained seed alignments)
    ARB: www.arb-home.de
    RDP: http://rdp.cme.msu.edu
    SILVA: www.arb-silva.de
  
  Alternative:
  Robust multiple alignment programs (CLUSTAL_X, W..., MEGA, T-COFFEE, MUSCLE) + by manual editing.

• Pairwise nucleotide sequence similarity
  Calculation programs: eg. ARB, PHYDIT, jPHYDIT, EzTaxon (www.eztaxon.org)
  Don’t use local alignment programs (eg. BLAST and FASTA).
II. Genetic-based characterization

• Assignment to defined taxa
  • The first indication of novel species
    16S rRNA gene sequence similarity $< 97%$
    $> 97%$
    DNA–DNA hybridization
  • The first indication of separate genera
    16S rRNA gene sequence similarity above $\sim 95%$
    To be tested by other methods to establish whether separate genera are present.
II. Genetic-based characterization

• Assignment to defined taxa
  • DNA–DNA hybridization
    • DDH value equal to or higher than 70 % as a suitable threshold for the definition of members of a species
  • Provided DDH data:
    type strain of novel species
    all other strains of novel species
    type strains of the closest related species
  • Standard deviations of at least three analyses must be given.
II. Genetic-based characterization

- Assignment to defined taxa
- DNA–DNA hybridization
II. Genetic-based characterization

• Phylogenetic tree

  • Use high quality sequences. Do not mix full and partial sequences.
  • Use high quality alignments.
  • Apply alternative treeing methods (eg. Neighbor-joining, maximum-parsimony, maximum-likelihood methods).
  • Never use sequences from single distantly related organisms as outgroup.
  • Only bootstrap proportions of 70 or higher presented in the dendrogram.
II. Genetic-based characterization

• Other genetic-based characterization

  • Multilocus sequence analysis (MLSA)

  • Nucleic acid fingerprinting (strain level)

  • Whole genome sequences

  • DNA G+C content
III. Phenotypic characterization
-- morphology, physiology, biochemistry

• Morphology
  • Morphological criteria
    • Cell shape and size – supported by photographs
    • Characteristic features (eg. stalks, prosthecae, budding or branching, cell aggregates)
  • Spore formation
  • Location of flagella
  • Motility (form, speed)
  • Intracellular structures
  • Colony shape and size
  • Cellular pigments
III. Phenotypic characterization
--morphology, physiology, biochemistry

• Morphology

• Staining

  • Gram stain (the reaction may alter as the cells age)
  
  • Acid-fast staining (strains containing mycolic acids)
  
  • Sudan Black staining (stains containing lipophilic cellular inclusions, eg. polyhydroxybutyric acid)
III. Phenotypic characterization
  --morphology, physiology, biochemistry

• Physiology and biochemistry
  • The growth tolerance (e.g. pH, temperature, NaCl concentration)
  • Enzyme activity, substrate utilization, antibiotics resistance, etc.

Note:
• To test with identical media and conditions or at least comparable.
• To compare with type strain of type species of appropriate genera.
• To analyze including strains of the most closely related taxa rather than using the previously published data.
III. Phenotypic characterization
-- chemical characterization

• Respiratory lipoquinonones (cell membrane)

• Three structural classes:
  
  Benzoquinones
  
  • ubiquinones (classes α, γ, β-proteobacteria)
  • rhodoquinones (some of the classes α, γ-proteobacteria)
  • plastoquinones

Naphthoquinones: * include menaquinones, demethylmenaquinones, mono-
  methylmenaquin, dimethylmenaquinones and menathioquinones.
  * the vast majority of Bacteria and Archaea known to synthesize naphthoquinone (menaquinone) derivatives.

Benzothiophene derivatives: members of the order Sulfolobales
III. Phenotypic characterization -- chemical characterization

• Polar lipids (cell membrane)

• Vast diversity in prokaryotes and have yet to be fully elucidated.

• Document the lipids by image with all visualized known and unidentified lipids.

• Good image quality for publication:
  8 bit, grey scale, 7 × 7 cm, 300 d.p.i.
III. Phenotypic characterization

-- chemical characterization

• Peptidoglycan (outer cell layers)
  • Analysis is requirement for all novel Gram-positive species description.
  • Analysis includes characteristic diamino acid in the cross-linking peptide, peptidoglycan type (A or B), mode of cross-linkage, complete amino acid composition.

• Mycolic acids (outer cell layers)
  • occur in certain high G+C Gram-positive bacteria
  • additional taxonomic markers
III. Phenotypic characterization
-- chemical characterization

• Other constituents

• Lipopolysaccharides (LPS)
• Polyamines
• Hydrophobic side chains of lipids
• Non- /Isoprenoid-based ether-linked lipids
IV. Minimal standards

• To provide detailed information on the characterization of specific organisms, and complement these guidelines.

• Aerobic, endospore-forming bacteria (Logan et al., 2009)
• Anoxygenic phototrophic bacteria (Imhoff & Caumette, 2004)
• Genus Brucella (Corbel & Brinley Morgan, 1975a, b)
• Family Campylobacteraceae (Ursing et al., 1994)
• Family Flavobacteriaceae (Bernardet et al., 2002)
• Order Halobacteriales (Oren et al., 1997)
• Family Halomonadaceae (Arahal et al., 2007; Arahal et al., 2008)
• Genus Helicobacter (Dewhirst et al., 2000)
• Methanogenic bacteria (Archaea) (Boone & Whitman, 1988)
• Suborder Micrococcineae (Schumann et al., 2009)
• Class Mollicutes (Division Tenericutes, Order Mycoplasmatales) (International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mollicutes, 1979; International Committee on Systematic Bacteriology Subcommittee on the Taxonomy of Mycoplasmatales, 1972; Brown et al., 2007)
• Genera Moraxella and Acinetobacter (Bøvre & Henriksen, 1976)
• Genus Mycobacterium (Lévy-Frédault & Portaels, 1992)
• Family Pasteurellaceae (Christensen et al., 2007)
• Root and stem nodulating bacteria (Graham et al., 1991)
• Staphylococci (Freney et al., 1999)
• Genus Streptomyces (not a minimal standard, but a standard reference work, Shirling & Gottlieb, 1966)
Thanks for your attention!