# Collection associated tools: taxonomy, research, information management

Erko Stackebrandt

### DSMZ, Germany

Main reference:

Smith D, Ryan MR, Stackebrandt E. Doelle, H. W. and Da Silva, E. J. Encyclopedia of Life Support systems (EOLSS)[Biotechnology]. 2008. UNESCO, Eolss Publishers, Oxford, UK.



### Why do we need Culture Collections?

# To understand the role and potential of biodiversity, the ability to maintain and identify biological resources is crucial

Though biodiversity offers more than biotechnology, e.g., understanding the evolution of the tree of life and to educate biosystematists, biotechnology is **key to meet the needs of the 21st century**. The global taxonomic impediment has been recognised and initiatives are underway to help lead to its resolution.

The Convention on Biological Diversity has specifically included microorganisms and the importance of ex-situ collections, as well as benefit sharing.



(CBD, http://www.biodiv.org/convention/default.shtml)

#### Article 9 of the CBD: Ex-situ Conservation

Each Contracting Party shall, as far as possible and as appropriate, and predominantly for the purpose of complementing in-situ measures:

(a) Adopt measures for the ex-situ conservation of components of biological diversity, preferably in the country of origin of such components;

(b) Establish and maintain facilities for ex-situ conservation of and research on plants, animals and microorganisms, preferably in the country of origin of genetic resources;

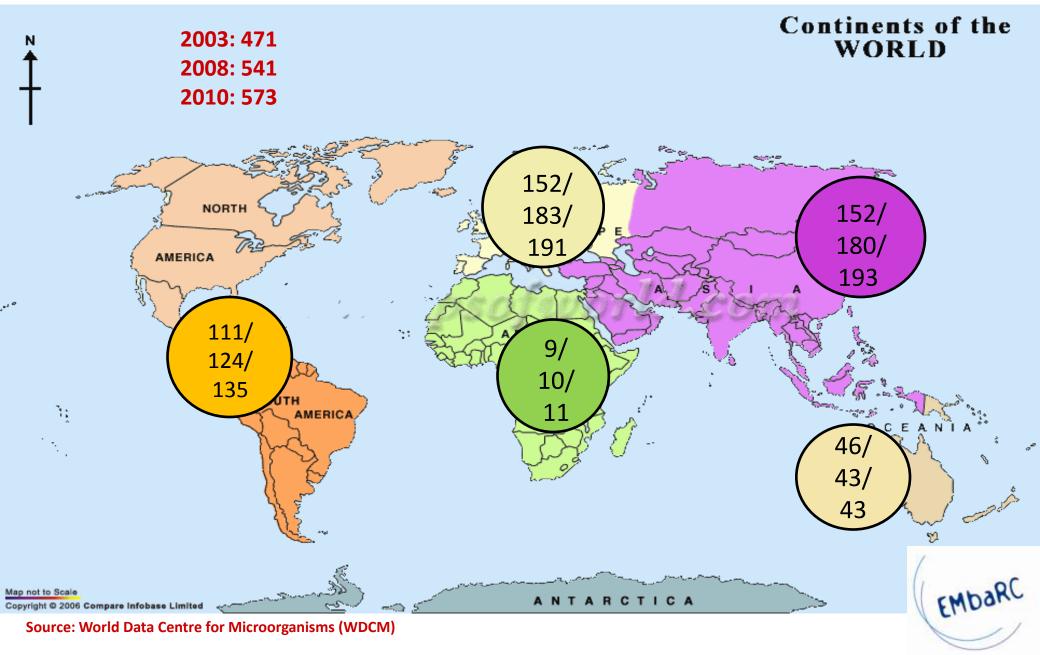
(C) Adopt measures for the recovery and rehabilitation of threatened species and for their reintroduction into their natural habitats under appropriate conditions;

(d) Regulate and manage collection of biological resources from natural habitats for ex-situ conservation purposes so as not to threaten ecosystems and in-situ populations of species, except where special temporary ex-situ measures are required under subparagraph (c) above; and

(e) Cooperate in providing financial and other support for ex-situ conservation outlined in subparagraphs (a) to (d) above and in the establishment and maintenance of ex- situ conservation facilities in developing countries.



### Numbers of collections and percentage of holdings



#### Of the 573 culture collections in 68 countries\*

227 of them are supported by government.

54 of them are semi-governmental.

211 of them are supported by university.

14 of them are supported by industry.

21 of them are private.

#### Of these

245 provide storage services.

265 provide distribution services.

284 provide identification services.

237 provide training services.

247 provide consultation services

EMDARC

\*, <u>http://wdcm.nig.ac.jp/statistics.html</u>; date: Feb 24, 2010

#### 573 culture collections\*

In Europe	191	633,444
Armenia	1	7,575
Belarus	1	1,175
Bulgaria	3	13,234
Czech	1	38,497
Estonia	3	6,300
Hungary	6	8,717
Kazakhstan	2	199
Latvia	1	692
Poland	9	8,464
Romania	2	760
<b>Russian Federation</b>	14	40,874
Slovak	3	4,916
Slovenia	2	4,160
Ukraine	3	3,286
Uzbekistan	3	1,456
Yugoslavia (?)	2	897
East Europe	56 (29%)	141,203 (22.3%)



\*, http://wdcm.nig.ac.jp/statistics.html; date: Feb 24, 2010

- Phenotype: morphology, physiology, cultural properties, key properties..
- Genotype: G+C content, MLSA sequences, DNA pattern, ribopattern..
- Chemotaxonomy: peptidoglycan, polar lipid, fatty acid, isoprenoid quinone protein pattern, MALDI-TOF..



- Phenotype: morphology, physiology, cultural properties, key properties..
- Genotype: G+C content, MLSA sequences, DNA pattern, ribopattern..
- Chemotaxonomy: peptidoglycan, polar lipid, fatty acid, isoprenoid quinone protein pattern, MALDI-TOF..

#### 2. Research: publications = reputation, capacity building, collaboration

- New descriptions
- Phylogeny: MLSA..
- New methods: genome sequences, ANI..
- Ecology, clone libraries, metagenomics, proteomics..
- Culture conditions..
- Long-term preservation..



- Phenotype: morphology, physiology, cultural properties, key properties..
- Genotype: G+C content, MLSA sequences, DNA pattern, ribopattern..
- Chemotaxonomy: peptidoglycan, polar lipid, fatty acid, isoprenoid quinone protein pattern, MALDI-TOF..

#### 2. Research: publications = reputation, capacity building, collaboration

- New descriptions
- Phylogeny: MLSA..
- New methods: genome sequences, ANI..
- Ecology, clone libraries, metagenomics, proteomics..
- Culture conditions..
- Long-term preservation..

#### 3. Information management: outreach, visibility, improved efficiency

- Online catalogues: what, where, when, how, by whom, which methods, references..
- In-house network: linking access, dispatch and research with online catalogue
- National and international networks: linking online catalogues
- Added value entries: separate databases on basically anything
- Improved capacity building for bioinformation scientists

- Phenotype: morphology, physiology, cultural properties, key properties..
- Genotype: G+C content, MLSA sequences, DNA pattern, ribopattern..
- Chemotaxonomy: peptidoglycan, polar lipid, fatty acid, isoprenoid quinone protein pattern, MALDI-TOF..

#### 2. Research: publications = reputation, capacity building, collaboration

- New descriptions
- Phylogeny: MLSA..
- New methods: genome sequences, ANI..
- Ecology, clone libraries, metagenomics, proteomics..
- Culture conditions..
- Long-term preservation..

#### 3. Information management: outreach, visibility, improved efficiency

• Online catalogues: what, where, when, how, by whom, which methods, references..

EWDO

- In-house network: linking access, dispatch and research with online catalogue
- National and international networks: linking online catalogues
- Added value entries: separate databases on basically anything
- Improved capacity building for bioinformation scientists

- Phenotype: morphology, physiology, cultural properties, key properties..
- Genotype: G+C content, MLSA sequences, DNA pattern, ribopattern..
- Chemotaxonomy: peptidoglycan, polar lipid, fatty acid, isoprenoid quinone protein pattern, MALDI-TOF..

#### 2. Research: publications = reputation, capacity building, collaboration

- New descriptions
- Phylogeny: MLSA..
- New methods: genome sequences, ANI..
- Ecology, clone libraries, metagenomics, proteomics..
- Culture conditions..
- Long-term preservation..

#### 3. Information management: outreach, visibility, improved efficiency

- Online catalogues: what, where, when, how, by whom, which methods, references..
- In-house network: linking access, dispatch and research with online catalogue
- National and international networks: linking online catalogues
- Added value entries: separate databases on basically anything
- Improved capacity building for bioinformation scientists



#### ad 1. Identification and authenticity check

#### Automated identification systems, including the

- API system (Biomerieux, S.A. France) and
- Biolog plates (Biolog Inc, USA).
- MIDI FAME analysis

#### MALDI-TOF

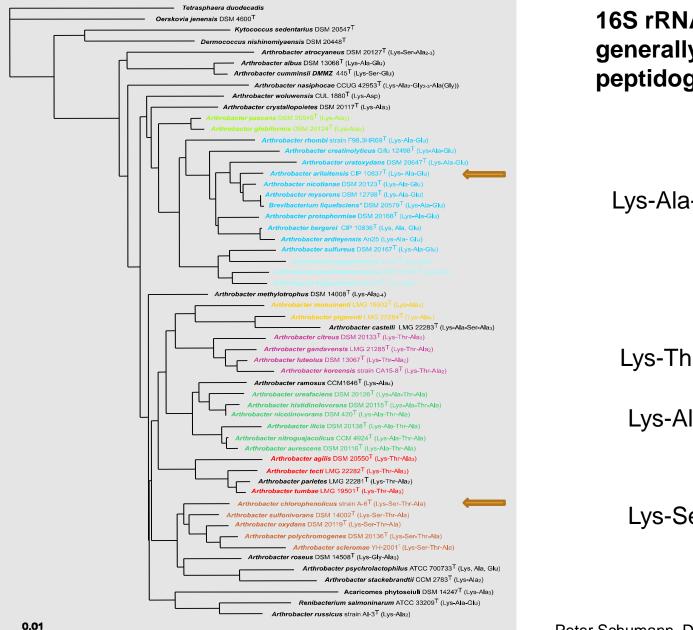
#### Analysis and characterisation of nucleic acids.

Sequencing of DNA genes, operons and spacers

Fingerprinting techniques

- Riboprinting
- RFLPs (Restriction Fragment Length Polymorphism),
- RAPDs (Random Amplified Polymorphic DNA),
- AFLP (Arbitrary Fragment Length Polymorphism),
- SSCP (Single Strand Conformation Polymorphism).
- Variable number tandem repeat (VNTR) PCR,
- Repetitive extragenic palindromic (REP) elements





16S rRNA gene phylogeny is generally consistent with peptidoglycan types

#### Lys-Ala-Glu

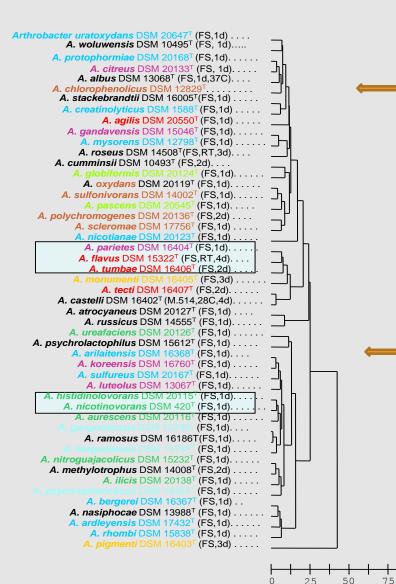
Lys-Thr-Ala

Lys-Ala-Thr-Ala

Lys-Ser-Thr-Ala



Peter Schumann, DSMZ



Euclidian Distance

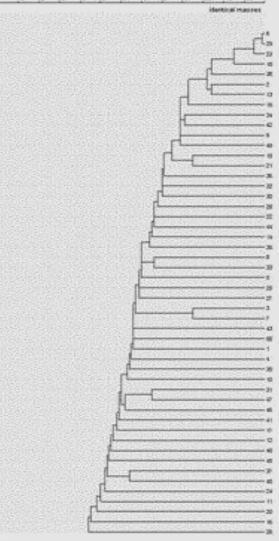
Fatty acid profiles of *Arthrobacter* type strains consist of 4-5 iso/ anteiso branched major components, **are highly similar and do not allow unambiguous differentiation**. Strain DSM 16403<sup>T</sup> contains additionally  $C_{20:0}$  and 2 unidentified late eluting components.





Frame indicates strains of high 16S rRNA sequence similarity

Peter Schumann, DSMZ



50 60 70 80 50

100.

Arthrobacter aurescens DSM 20116 <sup>T</sup>			
A. nitroguajacolicus DSM 15232 <sup>™</sup>			
A. methylotrophus DSM 14008 <sup>⊤</sup>			
A. histidinolovorans DSM 20115 <sup>™</sup>			
A. ramosus DSM 16186 <sup>T</sup>			
A. albus DSM 13068 <sup>™</sup>			
A. cumminsii DSM 10493 <sup>T</sup>			
<b>A. ilicis</b> DSM 20138 <sup>™</sup>			
A. polychromogenes DSM 20136 <sup>™</sup>			
A. scleromae DSM 17756 <sup>T</sup>			
A. chlorophenolicus DSM 12829 <sup>™</sup>			
A. ureafaciens DSM 20126 <sup>T</sup>			
A. gandavensis DSM 15046 <sup>⊤</sup>			
<i>A. koreensis</i> DSM 16760 <sup>⊤</sup>			
A. psychrolactophilus DSM 15612 <sup>T</sup>			
<b>A. pascens</b> DSM 20545 <sup>⊤</sup>			
<b>A. oxydans</b> DSM 20119 <sup>™</sup>			
A. nicotinovorans DSM 420 <sup>⊤</sup>			
<i>A. luteolus</i> DSM 13067 <sup>⊤</sup>			
A. sulfonivorans DSM 14002 <sup>⊤</sup>			
<b>A. flavus</b> DSM 15322 <sup>™</sup>			
<b>A. rhombi</b> DSM 15838 <sup>™</sup>			
A. castelli DSM 16402 <sup>™</sup>			
A. pigmenti DSM 16403 <sup>T</sup>			
A. atrocyaneus DSM 20127 <sup>⊤</sup>			
A. mysorens DSM 12798 <sup>™</sup>			
A. nicotianae DSM 20123 <sup>™</sup>			
A. ardleyensis DSM 17432 <sup>™</sup>			
<i>A. bergerei</i> DSM 16367 <sup>⊤</sup>			
A. stackebrandtii DSM 16005 <sup>⊤</sup>			
A. woluwensis DSM 10495 <sup>⊤</sup>			
A. agilis DSM 20550 <sup>⊤</sup>			
A. arilaitensis DSM 16368 <sup>⊤</sup>			
A. protophormiae DSM 20168 <sup>⊤</sup>			
A. citreus DSM 20133 <sup>T</sup>			
<i>A. parietes</i> DSM 16404 <sup>⊤</sup>			
<i>A. tumbae</i> DSM 16406 <sup>™</sup>			
A. tecti DSM 16407 <sup>⊤</sup>			
A. russicus DSM 14555 <sup>™</sup>			
A. globiformis DSM 20124 <sup>⊤</sup>			
A. crystallopoietes DSM 20117 <sup>T</sup>			
A. roseus DSM 14508 <sup>T</sup>			
A. uratoxydans DSM 20647 <sup>⊤</sup>			
A. sulfureus DSM 20167 <sup>⊤</sup>			
<b>A. monumenti</b> DSM 16405 <sup>⊤</sup>			
A. creatinolyticus DSM 15881 <sup>⊤</sup>			

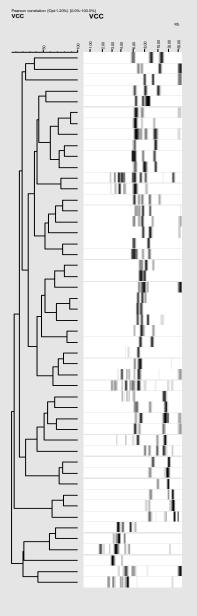
A. nasiphocae DSM 13988<sup>T</sup>

Frame indicates strains of high 16S rRNA sequence similarity

MALDI-TOF mass spectra differentiate Arthrobacter type strains (except of DSM 20116<sup>T</sup> and DSM 15232<sup>T</sup>) and allow their identification. Six pairs of type strains with similar 16S rRNA gene sequences show also similar mass spectra, demonstrating that MALDI-TOF reflects the phylogenetic relationship of highly related Arthrobacter species.



Peter Schumann, DSMZ



obacter	albus	DSM13068T
obacter	polychromogenes	DSM20136T
obacter	russicus	DSM14555T
obacter	cumminsii	DSM10493T
obacter	creatinolyticus	DSM15881T
obacter	sulfonivorans	DSM14002T
obacter	nitroguajacolicus	DSM15232T
obacter	ureafaciens	DSM20126T
obacter	castelli	DSM16402T
obacter	aurescens	DSM20116T
obacter	ilicis	DSM20138T
robacter	bergerei	DSM16367T
obacter	ardleyensis	DSM17432T
obacter	histidinolovorans	DSM20115T
obacter	citreus	DSM20133T
obacter	chlorophenolicus	DSM12829T
obacter	rhombi	DSM15838T
obacter	nicotinovorans	DSM420T
obacter	luteolus	DSM13067T
obacter	flavus	DSM15322T
obacter	gandavensis	DSM15046T
robacter	koreensis	DSM16760T
obacter	scleromae	DSM17756T
robacter	parietes	DSM16404T
obacter	stackebrandtii	DSM16005T
obacter	agilis	DSM20550T
obacter	kerguelensis	DSM15797T
obacter	oxydans	DSM20119T
obacter	gangotriensis	DSM157961
obacter obacter	sulfureus	DSM20167T DSM13988T
	nasiphocae	
obacter	globiformis ramosus	DSM20124T DSM16186T
obacter		DSM20545T
obacter obacter	pascens crystallopoietes	DSM205451 DSM20117T
obacter	atrocyaneus	DSM201171
obacter	monumenti	DSM16405T
obacter	tecti	DSM16407T
obacter	tumbae	DSM16406T
obacter	methylotrophus	DSM14008T
obacter	psychrolactophilus	DSM15612T
obacter	mysorens	DSM12798T
obactor	nsychrophenolieus	DSM15454T
obacter	protophormiae	DSM20168T
obacter	arilaitensis	DSM16368T
obacter	nicotianae	DSM20123T
obacter	woluwensis	DSM10495T
obacter	uratoxydans	DSM20647T

Arthro Arthro Arthro Arthro

Arthro Arthro Arthro Arthro

Arthro Arthro Arthro

Arthi

Arthr Arthr Arthr Arthr

Arthro Arthro Arthro Arthro

Arthro Arthro Arthro Arthro Arthro Arthro Arthro

Arthro Arthro Arthro Arthro Arthro Arthro Arthro Arthro Arthro Arthro Arthro

Arth

Arthro Arthro Arthro Arthro Arthro Riboprints:*Pvu*II-RiboPrint patterns areuseful for differentiation of*Arthrobacter* type strainsbut do not correlate withtheir phylogenetic relationship



Frame indicates strains of high 16S rRNA sequence similarity



### ad 1. Despite recognized needs we see a reduction in numbers of taxonomists

Today, **traditional microbiology found its alliance in molecular biology** and modern non-culture tools are now available to recognize the vast diversity of microorganisms, a small fraction of which has been grown in culture. The huge numbers of microbial species yet to be discovered requires skilled taxonomists, innovative isolation strategies, automated identification and a high quality global network of bioinformation of properties of organisms already in culture.

#### **Conclusion ad 1. Improve taxonomy**

Taxonomic expertise is absolutely essential to ensure microorganisms are correctly identified and culture collections need access to such skills to ensure that the identity can be monitored during storage, handling and distribution.

Maintain the available skills: do not fully replace trained taxonomists by molecular biologist

Expand the molecular skills: apply sequence analysis and rapid and reliable DNA pattern analysis

Be involved in genome sequencing projects

#### ad 2. Storage and maintenance

The primary objective of preserving and storing an organism maintaining it in a viable state **without morphological, physiological, or genetic change** until it is required for future use.

Complete viability and stability should be achieved, especially for **important** research and industrial isolates.

Preservation techniques range from

continuous growth methods

to methods that reduce rates of metabolism

to the ideal situation where metabolism is suspended.

No preservation technique has been successfully applied to all microorganisms, although storage in liquid nitrogen appears to approach the ideal.



#### ad 3. Quality management

The global system for Biological Resource Centres needs a **common standard** that can be worked to by all its members **to ensure conformity** and therefore at the very least the system chosen must be based on a common general standard.

Several collections have already adopted ISO 9000 series certification, a system that ensures quality through critical management of processes.

The system requires that procedures and practices are documented and that auditing procedures are put in place to ensure that what is said is done is actually carried out.



#### ad 3. Information technology

There are enormous possibilities for generating information on microorganisms from descriptive text on

- morphology,
- information on isolation and geographic location,
- host and substrate etc.,
- to digital images,
- metabolic
- genomic data.

Scatterered information is available:

- World Data Centre for Microorganisms (http://wdcm.nig.ac.jp),
- Global Information Facility (<u>http://www.gbif.org</u>),
- EMBL and GENBANK

# To ensure the data provided by culture collections is authentic, of high quality and relevance they must institute quality assurance measures for recording, management and exchange.



#### ad 3.: No collection is working fully independently

Actions like handling, maintenance, storage, shipping etc must be carried out safely and compliant with the various legislation and regulations that control these matters. Not only does the legislation exist but also from time to time it is changed or added to (<u>http://wdcm.nig.ac.jp/wfcc/wfccreports.pdf</u>).

CCs/BRCs must comply with biosafety requirements.

These responsibilities are wide ranging and incorporate:

- Health and Safety requirements
- Classification of Micoorganisms on the Basis of Hazard
- Quarantine regulations
- Ownership of Intellectual Property Rights (IPR)
- Convention on Biological Diversity
- Safety information provided to the recipient of microorganisms
- Regulations governing shipping of cultures
- Control of Distribution of Dangerous Organisms



#### Summary 1:

In order to cope with the anticipated massive expansion of biological resources, including living biological materials and data on genomics, CC/BRCs need to:

- Contribute to the co-ordination of efforts to conserve biodiversity and to provide access to natural and engineered biological resources.
- Assist in the development of a co-ordinated international system for decision making to guide appropriate acquisition, maintenance and distribution of biological resources so as to avoid unnecessary duplication of effort while preserving critical levels of biodiversity.
- **Modernise** to incorporate the latest developments in web-based electronic communication, bioinformational science and informatics technologies.
- **Co-ordinate** and unify catalogues and databases to meet the requirements of science in the developing post-genomics era.
- **Develop** new systems and technologies for the long-term maintenance and distribution of large numbers of diverse biological resources.
- **Co-ordinate** curation, as well as development and networking of informatics tools for data analysis, comparison and visualisation.
- Ensure that the scientific community has access to affordable products and services.

#### **Summary 2.: Culture collection benefits**

- Recognition that they operate to international scientifically based quality criteria
- An international mark of quality
- Raised profile
- Sharing of tasks
- Common policies and procedures
- Competitive edge
- Common access to data enabling links to be made to other international initiatives without duplication of effort
- Common approach to data access, sharing and interoperability
- Improved data usage
- Collaborative research and development



## Summary 3.: Potential Income Streams Anticipated for CCs/BRCs (examples)

- cDNA libraries, genomic libraries, filter sets, clones, plates, PCR products
- Microarrays and reagents
- Accreditation/standardization-added value products and services
- Data storage and retrieval
- Software development/collaborations data mining tools
- Technology development/collaborations
- Sequence database annotation/phenotypic analysis
- Linking genomics databases to proteomics



### At the end: where are the Culture Collection benefits from increasing its performance?

- Recognition that they operate to international scientifically based quality criteria
- An international mark of quality
- Raised profile
- Sharing of tasks
- Common policies and procedures
- Competitive edge
- Common access to data enabling links to be made to other international initiatives without duplication of effort
- Common approach to data access, sharing and interoperability
- Improved data usage
- Collaborative research and development