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Abstract	<p>The results of the compilation of Tables 1-3, and Annex Tables 1 and 2 allow the following conclusions:</p> <ol style="list-style-type: none"> 1. The European collections included in this study cover a very broad range of prokaryotic biodiversity at all taxonomic levels and individual collections offer in-depth diversity at the species level. This concentration of diversity in terms of strain, species and genus numbers is unparalleled in the world. 2. The range of gaps at the generic level is rather small. Besides Cyanobacteria and Mollicutes, of all genera described, 12% are not covered by any of the five collections. These are those either recently described, containing obligate endosymbionts, or fastidious pathogens. 3. Collection emphasis are the range of pathogens and reference material (human, animal, plants), relevant strains to biotechnology (food, agriculture, pharmacy) and strains covering diversity in terms of habitat, metabolism and ecology (academia, industry) 4. The size of individual holdings and the expertise of curators are determined by the history of the collections. This history also explains the strength in methods use in-house for authentication and characterization but also offering the skills to the public by providing identification service and offering training courses. 5. Collections differ in the breadth and depth of phylogenetic and metabolic diversity, resulting in different average numbers of holdings per curator. It appears that any expansion of diversity, both in terms of phylogenetic diversity and in depth coverage of genus and species, will require increase of expertise and number of curators and technical staff. 6. All collections face an increasing demand for new accessions as the number of novel species is steadily increasing. 7. In terms of mycological holdings the CBS holds a dominating position world-wide. The other European collections maintain holding in specific areas, either for research or to satisfy the requests of national users
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Objectives

To assess partner collections for emphasis on holdings and develop mechanisms to specifically enrich these with strains and genetic elements (e.g. plasmids, DNA libraries) shown to be of scientific and economic value: involve editors of journals, authors/scientists, funding bodies and Resource Centres in the development of a strategic plan to facilitate deposition of microbial resources for the mutual benefits of science and users. (Task 3.1)

1 Description of work and role of participants

1.1 Approach

Only a minute fraction of microbial strains, restricted mainly to type and reference material, and of genetic elements like plasmids and DNA libraries are deposited in public Resource Centres. This gap in available microbial diversity is especially highlighted by the unavailability of many strains for which the complete genome has been analysed. However, the problem is much older and orders of magnitude greater in terms of numbers of isolates of medical, biotechnological and scientific interest, as hardly any strain included in broad scientific studies, is available to the scientific community. This stands in contrast to the publication policy of the majority of journals which expressively states in the *Instruction to Authors* that biological resources included in scientific articles need to be available to the user. Once published, most resources are either no longer maintained or are not publicly available. Despite this obligation several obstacles exist that prevents putting this obligation of authors into action. The only known example for obligate deposition includes the deposition of prokaryotic type strains as laid down in the Code of Nomenclature of Prokaryotes. This single example shows that a strategy could be successful if all partners involved are guided by the necessity to change the present practice. The work will involve measuring and discussing the present strength and weakness (bottlenecks of policy implementation) of practise, possible incentives and correcting actions, resulting in a consensus strategy involving all partners and actions for the common good.

1.2 Work breakdown

Task NA3.1 Gap Analysis and Strategic Plan to Increase Holdings of Biological Resources included in the Scientific Literature.

Lead partner: DSMZ

Contributors: INRA, CIP, CABI, UGent, UVEG-CECT, UMinho-MUM, KNAW-CBS,

2 Inventorize European Resource Centre holdings and determine the degree of overlap and uniqueness in each holding (D.NA3.1.1). Compare quality and quantity of catalogue entries, using the established CABRI network

2.1 Status quo PROKARYOTES July 2009

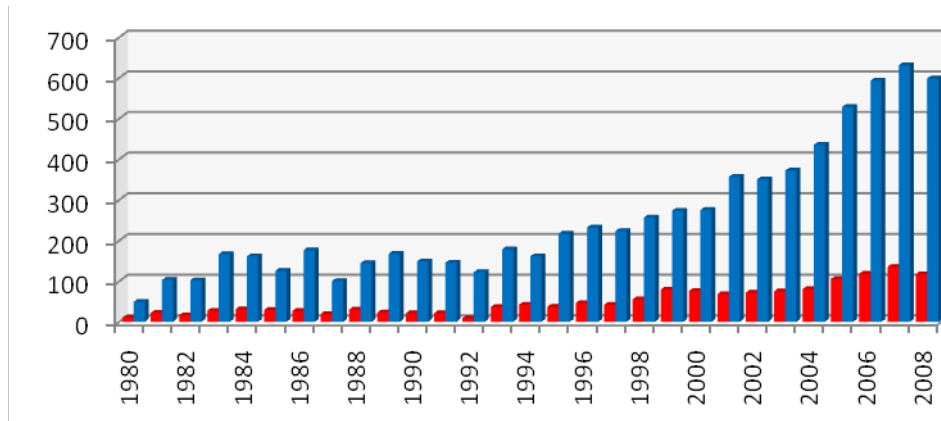
This report will not enter into the discussion about the delineation of Culture Collections Biological from Resource Centres. Elements of this evolution will be covered in NA1, considering the tasks, quality control and international recognition of collections. All repositories will be named collections in the following, but it is likely that all of them will reach the status of a BRC, once the evaluation criteria will be laid down.

The term 'gap' will be monitored in two ways: Firstly it is the gap in described and available diversity at the generic level among the five collections handling prokaryotes. This will be followed by a more intense survey at the type strain level. Secondly, the term 'gap' refers to the discrepancy of non-type strains/isolates included in the scientific literature and the fraction of these which are deposited in public collections. This assessment will be done on a number of selected journals issued 2008.

Deposition of prokaryotic type strains

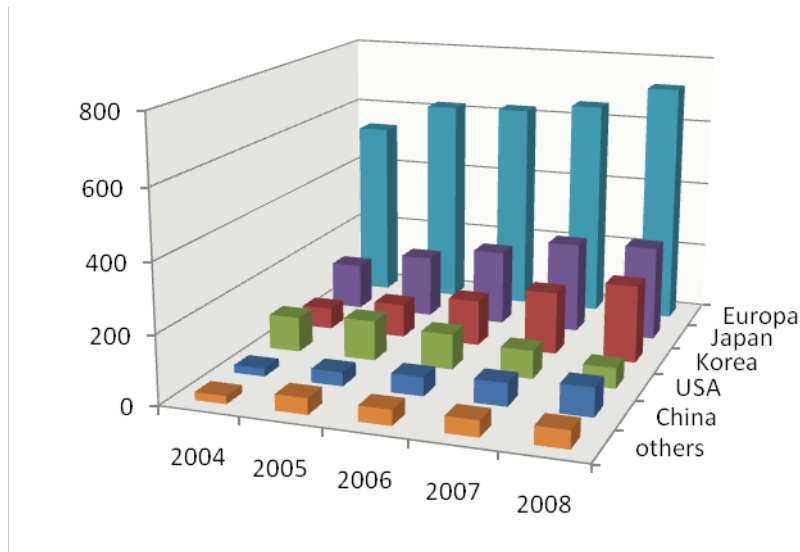
It is mandatory for scientists to deposit the type strains of new prokaryotic species in at least two public collections in at least two different countries. The increasing number of newly described species (Figure 1) will challenge collections by freezing manpower and increasing maintenance cost while deposition is free of charge. Costs include not only the administrative responsibilities according to the CBD and other rules and regulations (Smith et al, 2008), but also identification, authentication, maintenance and long term storage, as well as the generation of accompanying bio-information and regular methodological updates and training. Some revenues are generated by providing strains to users but this income does not cover the costs involved in state-of-the-art maintenance of resources. As determined for the 2007 accessions into the DSMZ, of the xx new type strains accessioned only xx were dispatched in 2008. This indicates that even with the descriptions reaching a plateau in the next years (due to the lack of systematists, not of novel organisms) the workload of maintaining and provision novel biodiversity under high quality standards remain a huge task for responsible collections.

Figure 1: Number of prokaryotic genera (red) and validly published/notified species (blue) in IJSB/IJSEM between 1980 and 2008



As shown in Figure 2, the European collections are used most frequently as repositories for new strains. The decline of the USA collections as primary source for deposition is worth noting as is the increased depositions in Korea, Japan and China, countries that today contribute most to the description of novel species.

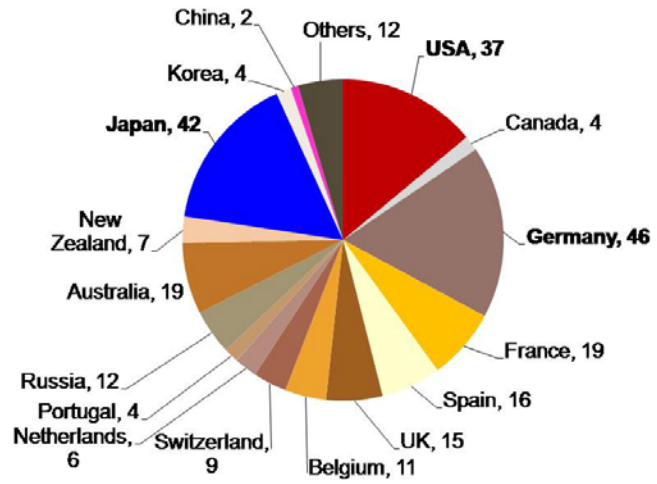
Figure 2: Deposition of new strains in culture collections per country/region



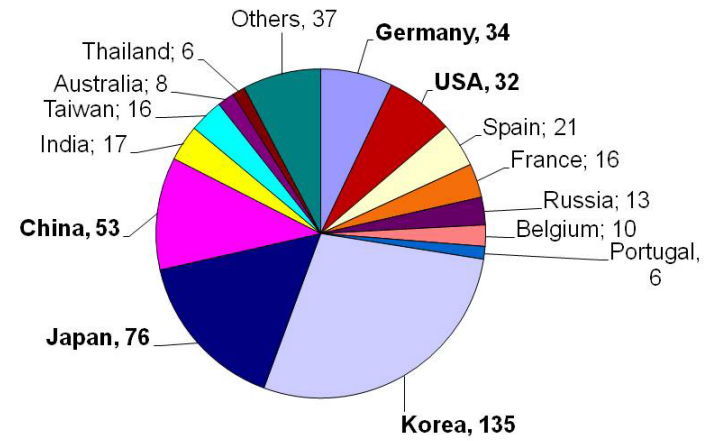
This is exemplified in Figure 3 which compares the shift in descriptions of prokaryotic species from the Western to the Eastern hemisphere (from 65:35 in 1999 to 37:63 in 2007) within the past 10 years.

Figure 3. Distribution of species descriptions per country according to origin of first author.
a., 1999; b. 2007. Data from IJSB/IJSEM. Most productive countries in bold.

a.



b.



It will be seen later (section 1.3) that of the 183 Collections, listed in the World Federation of Culture Collection (WFCC) database, only 12 international public collections are actually used as main repositories. Among these, 6 are located in West Europe. Their superior reputation to maintain resources by an appropriate management system is the main rational for being used as target collections

The following paragraphs summarizes the extent of holdings and strength of those four public collections are provided which focus of prokaryotic strains

2.2 Holdings of collections of prokaryotes

This paragraph will not comment on the presence of duplicated strains hold in collections. As covered in the EU-funded EBRCN project (QLRT 2001-02806) duplication in collections is considered functional and the decision of whether or not to maintain copies of strains depends upon a wide range of scientific and user-related interest. The following compilations of information gathered from the Microbial Culture Collection of the Institute Pasteur (CIP, Paris), the LMG collection of the Belgian Coordinated Culture (University of Gent, LMG), the Colección Espanola de Cultivos Tipo (CECT, Valencia), INRA-UMR STLO, Rennes, France, and the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig).

The DSMZ covers a broad range of described genera (74%) and type strains (81.6%). With an average of 2.56 strains per species the DSMZ concentrates on type strains rather than in-depth holdings of diversity at the strain level. This is more pronounced in the collection of the Institute Pasteur (CRBIP) and University Ghent (LMG) which hold less types strains but per strain a significantly higher number of strains (see below for details). The CECT collection began only recently to explore biodiversity, hence the number of type strains and genera covered are much smaller. The INRA collection is an example of a very focussed research collection, concentrating on a few genera of significance in food microbiology. The numbers in Table 1 do not allow to conclude on the efficiency of curators as the handling of prokaryotic organisms require different skills and curators have often tasks (administration, research, customer service, teaching) in addition to their curatorship which in other collections are managed by special staff.

The collection holdings of these five public collections have been ordered according to a hierarchic structure of prokaryotic genera. The hierarchy was adopted from several sources, such as the Bergey's Manual, Vols 1-3 (Archaea, deep branching phyla, Proteobacteria, Firmicutes), Bergey's Taxonomic Outline for the phylum Bacteroidetes and the recent outline of the phylum Actinobacteria. Each collection listed the number of type strains and total number of strains of each genus covered against the total number of species according to Euzéby's List of Prokaryotic names with Standing in Nomenclature (<http://www.bacterio.cict.fr/>) (Table 2). This Excel compilation allows rapid access to the numbers of species and strains per genus (though not to the species identity) as covered by each of the four collections, thus giving an easy overview of coverage, and of individual strength and focus on particular groups of organisms. It is obvious that collection show a similar range of diversity in certain taxa, while they differ widely in taxonomic groups. This phenomenon has been described before in the EBRCN project where 58% of species were duplicated among the CABRI partner collections, especially in the economically import genera *Pseudomonas*, *Streptomyces*, *Lactobacillus* and *Streptococcus*. This was and can be still today be explained by the individual mandate and research emphasis (Final report).

Table 1. Some numbers of strains, as extracted from the information of Annex 1, and curator equivalents (scientists and technical staff [TA]). Data June 2009

Number of	DSMZ	CRBIP	LMG	CECT	INRA CIRM-BP	INRA CIRM-BIA
Type strains	7.696	4.083	3.036	829	164	113
Strains	19.678	21.318	16.748	2.658	2.239	3.082
Average number of strains/species	2.56	5.22	5.52	3.2	13.7	27.3
Genera	1.331	792	540	240	60	13
Curators	17.25	6.0	6.05	1.5	2.0	1.0
Aver. number of strains covered by curators	1.140	3.553	2.768	1.772	1.120	3.082
Aver. number of genera covered by curators	77.2	132	89.3	160	30	13

It should be noted that the 9.435 type strains listed in column 8 of Annex 1 do not reflect the actual number of type strains. This is due to the fact that the name some species appear in two or more genera (synonyms), depending on the number of reclassifications (names once validly published or notified will remain valid irrespective its present classification). The number of synonyms is presently 1.555, hence the number of type strains is 8.882. For the same reason the number of type strains listed in the following columns referring to the holdings in the four collections are slightly too high, in case they were not corrected according to the most recent taxonomy. The number of genera is 1.811, corrected for synonyms 1.712. The deviations from the actual numbers will remain uncorrected as the visualization of strength and gaps of the individual collections will be slightly affected only.

2.3 Identify the expertise to handle new additions to their holdings. Obtain lists of taxonomic and methodological expertise of curators of partner collections

The compilation of accession permits to search for collections strength according to diversity covered. The number of genera alone is not valuable indicator for diversity as certain families embrace a high number of genera with similar growth requirements. In this case a small number of staff can handle a high number of species and genera. The number of genera per phylum (Table 2) is more indicative of diversity as phyla, especially the deeply branching ones and those of Archaea, generally cover a broader range of more nutritionally demanding taxa e.g., anaerobic, fastidious and extremophilic members. Most of these taxa often require special technical equipment and taxonomic expertise. This is exemplified by the average number of species and genera serviced by curators in the individual collections (Table 1) which differ by a factor of 2 to 3. Curators of the CRBIP and LMG handle a high number of strains, while those especially of DSMZ have to deal with lower numbers of more fastidious strains. The two research collections focus on certain food-related aspects of microbiology and naturally their holding are much more focused in terms of phylogenetic and physiological diversity. This explains while a small staff number can handle rather large strain numbers.

All four public collections share their focus on 4 phyla, i.e., Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes (Table 2, in red letters). These phyla show the highest annual

increase in numbers of genus and species descriptions and these phyla embrace most of the biotechnologically, medically and agriculturally important taxa. The finer resolution of selected genera of interest to any of the four collections is shown in Table 3. Some species-rich genera, maintained to a greater extent in all collections, are the “classical” genera, described over 100 years ago, e.g., *Lactobacillus*, *Staphylococcus*, *Enterococcus*, *Streptomyces*, *Pseudomonas* and *Vibrio*. All collections complement each other in depth and breadth of holdings which can be explained by the history of the collection: The CIP collection within CRBIP, Institute Pasteur, was founded early 1900, to curate the legacy of L. Pasteur, E. Roux and their successors, placing emphasis on medically related bacteria. The LMG collection, founded in the 1950s by the elevation of the University Collection of J. de Ley, centre around agriculturally and biotechnologically related strains. The phylogenetic diversity of the DSMZ, established in the late 1960s, can be attributed to the union of several different university collections each with different emphasis (O. Kandler, Firmicutes; H. Schlegel, Proteobacteria; H. Kutzner, Actinobacteria; N. Pfennig, phototrophes; F. Widdel, Archaea). The CECT collection was founded 1960 by J.R. Villanueva and continued by F. Uruburu, originally placing emphasis on yeast and fungi but the collection was later broadened to include prokaryotic strains as well.

From the compilation of genera in Table 3 the holding emphasis of each of these four collections is obvious. The CRBIP collection concentrates on aerobic medically and biotechnologically relevant genera (e.g., *Bacillus*, *Listeria*, *Actinomyces*, *Corynebacterium* and relatives, *Bordetella* and *Neisseria*) while the LMG collection focuses on agricultural and biotechnologically important taxa (e.g. *Enterococcus*, N₂-fixing taxa [*Rhizobium*, *Bradyrhizobium*], *Xanthomonas*). Except for *Streptomyces* and *Clostridium*, the DSMZ does not concentrate on in-depth collection of biodiversity but on covering a broad range of biodiversity. In almost all of the genera listed in Table 3 the CRBIP and LMG collections offer a higher number of strains in their catalogues than the DSMZ. As pointed out above, the recent founding date of the CECT collection (when) is the reason for the lower number of holdings, though the collection is strong on *Aeromonas*, *Vibrio*, N₂-fixing strains, *Lactobacillus*, *Streptomyces* as well as *Oenococcus* (see Annex 1). The concentration of the CIP and the LMG collections on many strains of certain pathogen or a given nutritional type explains that the average number of strains handled by curators/TA is about 2.5 to 3.4 times higher than those handled in the DSMZ. The two research collections are defined by specialization.

Table 2. Holdings of genera per phylum in the four collections as extracted from the information of Annex 1. Taxa and numbers in red indicate collection strength

Phyla	Genera described	DSMZ	CIP	LMG	CECT	INRA CIRM-BP	INRA CIRM-BIA
Crenarchaeota	26	24	-	-	-	-	-
Euryarchaeota	65	58	-	-	12	-	-
Aquificae	12	12	1	-	-	-	-
Thermotogae	6	5	2	-	-	-	-
Thermodesulfobacteria	4	4	1	1	-	-	-
Deinococcus/Thermus	6	6	2	3	1	-	-
Chrysiogenetes	1	1	-	-	-	-	-
Chlorobia	3	3	3	-	-	-	-
Chloroflexi	13	9	-	-	-	-	-
Thermomicrobia	1	1	-	-	-	-	-

Phyla	Genera described	DSMZ	CIP	LMG	CECT	INRA CIRM-BP	INRA CIRM-BIA
Nitrospirae	3	2	1	-	-	-	-
Deferribacteres	6	4	2	-	-	-	-
Synergistetes	5	2	1	-	-	-	-
Planctomycetes	9	6	-	-	-	-	-
Fusobacteria	9	5	4	1	-	-	-
Chlamydiae	5	1	-	-	-	-	-
Spirochaetes	14	7	4	-	-	-	-
Fibrobacteres	1	-	-	-	-	-	-
Acidobacteria	6	5	-	-	-	-	-
Verrucomicrobia	13	5	3	-	-	-	-
Dyctioglomi	1	1	-	-	-	-	-
Gemmatimonadetes	1	1	-	-	-	-	-
Lentisphaera	1	-	-	-	-	-	-
Bacteroidetes	181	105	107	76	13	5	-
Firmicutes	306	264	146	76	42	1	7
Actinobacteria	238	226	147	79	46	2	3
Proteobacteria	746	572	367	277	126	50	-

**Table 3: Number of strains in selected genera, showing collection emphasis within the four collections.
Taxa and numbers in blue show collection strength**

Phylum	Genus	DSMZ	CRBIP	LMG	CECT	INRA CIRM-BP	INRA CIRM-BIA
Bacteroidetes							
	<i>Flavobacterium</i>	90	115	86	6	-	-
	<i>Chryseobacterium</i>	31	112	77	3	4	-
Firmicutes							
	<i>Bacillus</i>	848	6.592	625	104	-	-
	<i>Listeria</i>	14	819	57	41	-	-
	<i>Lactobacillus</i>	427	685	700	147	-	823
	<i>Staphylococcus</i>	205	296	188	56	8	29
	<i>Enterococcus</i>	78	131	824	35	8	131
	<i>Streptococcus</i>	210	469	549	48	3	268
	<i>Clostridium</i>	380	197	54	42	-	-
	<i>Lactococcus</i>	26	24	149	41	7	293
	<i>Leuconostoc</i>	50	64	98	34	10	240
Actinobacteria							
	<i>Actinomyces</i>	58	155	52	3	-	-
	<i>Corynebacterium</i> <i>+Mycobacterium</i>	628	962	153	78	-	-
	<i>Bifidobacterium</i>	83	35	88	13	-	585
	<i>Streptomyces</i>	1739	99	288	142	-	-
Proteobacteria							
	<i>Aeromonas</i>	59	77	353	200	-	-
	"enterobacteria" ¹	1.354	2.035	1.451	348	1328	-
	<i>Bordetella</i>	15	294	141	7	-	-
	<i>Bradyrhizobium</i>	8	3	160	64	-	-
	<i>Rhizobium</i>	84	55	402	153	-	-
	<i>Burkholderia</i>	67	120	378	8	-	-
	<i>Brucella</i>	-	78	-	-	182	-
	<i>Campylobacter</i>	37	155	548	-	-	-
	<i>Gluconobacter</i>	47	13	183	3	-	-
	<i>Haemophilus</i>	27	295	6	-	-	-
	<i>Neisseria</i>	25	326	11	22	-	-
	<i>Pseudomonas</i>	588	1.223	1.461	113	390	-
	<i>Vibrio</i>	106	216	411	151	-	-
	<i>Xanthomonas</i>	46	119	1.640	9	-	-

¹, The strains of 45 phylogenetically highly related genera are summarized

Gap analysis at the higher taxon level

Comparison of data compiled in Annex 1 and Tables 1-3 show that the majority of phyla are covered at least by some strains. At the genus level most phyla are covered above 80%. Only the monogeneric phyla Fibrobacteres and Lentisphaera are not covered at all and some of the “rare” phyla are represented by a few type strains only. At the genus level level a broad coverage (>85%) is presented in that at least one of the four collections provide material. Obvious gaps detected are within the order Mollicutes (phylum Firmicutes) and within Cyanobacteria. Mollicutes embrace primarily parasites of various animals and plants, living within the host's cells. Their maintenance often require host tissues which is out of range for most resource centers. Collections of cyanobacteria exist in all partner countries. While in Germany and Spain the cyanobacterial collection (SAG, Göttingen and UAM Culture Collection, Universidad Autónoma de Madrid, Spain, respectively) are separate identities, in France and Belgium the algal collections are at least placed under the same umbrella than the collections of heterotrophes.

Table. 4 Coverage of bacterial diversity at the genus level by the six EMbaRC collections

Taxon	Number of described genera	Number of genera covered	% coverage
Archaea	91	86	95
Bacteria			
Aquificae	12	12	100
Thermotogae	5	4	80
Thermodesulfobacteriae	4	4	100
Deinococcus/Thermus	5	5	100
Chrysiogenetes	1	1	100
Chlorobia	3	3	100
Chloroflexi	13	10	77
Thermomicrobia	1	1	100
Nitrospirae ¹	3	2	67
Deferribacteres	6	5	83
Synergistetes	5	3	60
Planctomycetes	9	7	78
Chlamydiae	5	1	20
Spirochaetes	14	7	50
Fibrobacteres	1	0	0
Acidobacteres	6	5	83
Bacteroidetes	181	148	82
Fusobacteria	9	7	78
Verrucomicrobia	13	8	62

Dictyoglomi	1	1	100
Gemmatomonadetes	1	1	100
Lentisphaerae	1	0	0
Firmicutes	312	289	93
Actinobacteria	238	228	96
Proteobacteria			
<i>Alphaproteobacteria</i>	235	188	80
<i>Betaproteobacteria</i>	143	131	92
<i>Gammaproteobacteria</i>	265	232	88
<i>Deltaproteobacteria</i>	84	76	90
<i>Epsilonproteobacteria</i>	15	12	80

¹, Names in red indicate phyla which are yet not well represented

At the species level gaps are obvious in the so called "rare" species where the more specialized collections show their strength, especially among the extremophiles. These species are usually less frequently requested than species of medical and biotechnological interest. Other gaps are also witnessed among some of the genera of obligate endosymbionts and pathogens, as well as among the obligate chemolithotrophs. Type strains of recently described genera have not yet spread among collections. Redundancy among type strains is welcome as it facilitates ordering of resources within national borders without high mailing and custom charges.

2.4 Holdings of collections of yeasts and fungi

Annex 2, displaying the holdings of four collections (CBS, Utrecht, The Netherlands; IMI, Egham, UK; CIRM, Levures, France; CIRM, Marseille, France and DSMZ, Braunschweig, Germany) according to the recent hierarchic structure of Yeast and Fungi. CBS also has holdings of some Protozoa species which are not indicated. The content of Annex 2 must be considered tentative as in some cases only type material but not total number of species or strains are indicated. This will be corrected over the next month. But even in this incomplete way it is obvious that the CBS collection has the widest offer (strains: 67.719, species 48.296, types 6123) both in term of breadth and depth of material. This collection is the leading facility for mycological material world-wide. Some overlap, especially among *Saccharomycetaceae* and *Mucoraceae* exist with the IMI collection but hardly with the other ones which concentrate on a few genera only with which they mainly serve the national market.

Conclusions

The results of the compilation of Tables 1-3, and Annex Tables 1 and 2 allow the following conclusions:

1. The European collections included in this study cover a very broad range of prokaryotic biodiversity at all taxonomic levels and individual collections offer in-depth diversity at the species level. This concentration of diversity in terms of strain, species and genus numbers is unparalleled in the world.
2. The range of gaps at the generic level is rather small. Besides Cyanobacteria and Mollicutes, of all genera described, 12% are not covered by any of the five collections. These are those either recently described, containing obligate endosymbionts, or fastidious pathogens.
3. Collection emphasis are the range of pathogens and reference material (human, animal, plants), relevant strains to biotechnology (food, agriculture, pharmacy) and strains covering diversity in terms of habitat, metabolism and ecology (academia, industry)
4. The size of individual holdings and the expertise of curators are determined by the history of the collections. This history also explains the strength in methods use in-house for authentication and characterization but also offering the skills to the public by providing identification service and offering training courses.
5. Collections differ in the breadth and depth of phylogenetic and metabolic diversity, resulting in different average numbers of holdings per curator. It appears that any expansion of diversity, both in terms of phylogenetic diversity and in depth coverage of genus and species, will require increase of expertise and number of curators and technical staff.
6. All collections face an increasing demand for new accessions as the number of novel species is steadily increasing.
7. In terms of mycological holdings the CBS holds a dominating position world-wide. The other European collections maintain holding in specific areas, either for research or to satisfy the requests of national users

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Annexes

Annex 1: Holdings of prokaryotic collections according to hierarchy

Annex 2: Holding of mycological collections according to hierarchy