Abstract

The number of isolated new microorganisms has dramatically increased after the readaption of culture using the culturomics approach. Each of these microorganisms is deposited in an international strain collection institute, with its name being attributed and published by the scientist who isolated it. The attributed name is of Latin or Latinized origin and chosen on the basis of the geographical location of the sample collection, the institute or geographical region where the project was being performed, the name of a concerned scientist, and characteristics of the sample or the microorganism. Our institution has played an important role in the isolation of new microorganisms, with the first effort reporting 468 new bacterial species (3% of the bacterial species isolated at least once worldwide) and 327 species isolated for the first time from human beings, which in turn resulted in an increase of 30% of the total number of microorganisms isolated. Additionally, more than 100 giant viruses, including seven new species, have been isolated at our institute. In the present work, after recalling the rules of nomenclature, we detail the naming of the new microorganisms chosen at our laboratory. The most common species name was massiliensis, attributed 161 times. We consider it imperative for the cultivators, who have frequently made considerable efforts in the field of microbial culture, to be the ones who name the newly isolated microorganisms, taking into consideration the Latinized nomenclature standards.

Keywords: Archaea, Culturomics, Giant virus, Human microbiota, New bacterial species, Taxonogenomics

Introduction

Out of the 10 million predicted bacterial species [1], only about 150000 have been cultured. When comparing the number of isolated bacterial species to the number of known archaeal species, viruses and eukaryotes, the number cultured seems small [1,2]. However, the number of cultured species has increased dramatically after the reintroduction of culture by Lagier et al. in 2012 [3,4], and approximately 2776 species are currently predicted to be isolated from human samples [2,5]. Our laboratory contributed significantly in the past 30 years in enlarging the repertoire of microorganisms by isolating 468 new bacterial species, mainly from human origins (Supplementary Table S1).

We succeeded in isolating 327 bacterial species from human samples that had previously been reported to be isolated from environmental sources [6]. This led to a 30% increase in the bacterial repertoire associated with humans [6]. In addition, we also increased the number of archaeal species isolated from human beings by adding two new species, one of which was the first halophilic archaeal species isolated from humans. Finally, our laboratory contributed significantly to the culture of giant viruses by the isolation of 100 different isolates [6].

Naming these new microorganisms is a challenge because the species’ name is proposed by the first publication to report its isolation [7]. The name of the new microorganism is chosen by the author reporting its first culture [7]. In this
work, we review the main nomenclature rules for naming microorganisms and discuss the contribution of our laboratory to naming.

Bacteria

Process for identification of a new prokaryote

To confirm the novelty of a bacterial species, 16S rRNA gene sequencing is performed along with a BLAST analysis keyed to the National Center for Biotechnology Information nucleotide database for phylogenetic analyses. A threshold of 98.7% of 16S rRNA gene sequence similarity with the phylogenetically closest species withstanding in nomenclature was suggested by Stackebrandt and Ebers [8] to classify a new bacterial species. The 16S rRNA gene sequence of each isolated new bacterial species is submitted to the GenBank database, and its strain type is deposited in a strain collection institute.

However, DNA-DNA hybridization, a technique previously considered to be the reference standard to classify a new bacterial species, should be considered as outdated, as it is not reproducible among laboratories and is not cost-effective [9]. With the advent of both matrix-assisted desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) and genome sequencing, we recently proposed describing new isolated species by using the taxonogenomic approach [9]. The determination of digital DNA-DNA hybridization as well as the average of genomic identity of orthologous gene sequences are also included [9].

Unfortunately, this process is time-consuming—sometimes several years. Accordingly, we proposed a new format (new species announcement) that reports the 16S GenBank accession number, phylogenetic tree, strain deposit accession numbers and main phenotypic characteristics of the new isolated species [10]. Finally, according to the rules of the International Committee on Systematics of Prokaryotes, the name of the new bacterial species must be officially recognized [6].

Naming rules

The current nomenclature for bacterial species requires a capital letter for the genus name and an epithet beginning by a lowercase letter for the species name [7]. Genera and epithet should be Latin or Latinized; the specific epithet is an adjective that must agree with the gender of the generic name [7]. The name can be derived from a person’s name (frequently a microbiologist), a geographical location, a phenotypic characteristic (growth condition, colour, biochemical characteristics) or any other origin. Before being classified as a species (frequently for bacteria not yet cultivated but with genomic criteria), a species may provisionally be classified ‘Candidatus’.

For instance, the genus of Escherichia coli, one of the most commonly found bacteria, is named after Theodor Escherich, who isolated it, and its species epithet name refers to the colon, the source of its isolation [11]. Another example is *Staphylococcus aureus*. Its genus designation was named after its phenotypic characteristics (the grapelike coccus), and its species was named after the golden colour of its colonies [11]. The four most abundant genera are *Bacillus*, *Paenibacillus*, *Clostridium* and *Corynebacterium* (Fig. 1).

In order to highlight the naming preferences of new isolates by their cultivators, Table 1 details the 31 officially recognized *Rickettsia* spp. Thirty-four (42%) were named after a geographic location, 12 (39%) in honour of a zoologist or a microbiologist, four (13%) after a vector name and two (6%) after a clinical characteristic of the disease caused by the microorganism.

Our contribution

Over the last 30 years our laboratory has isolated 468 new bacterial species (more than 3% of the bacterial species isolated at least once), mainly from humans and few from animals and environmental samples (Supplementary Table S1).

Most of the species isolated by our team were named in reference to the place where they were first isolated. For instance, 161 bacterial species were named *massiliensis* and 22 *massiliense* for Marseille (Southern France), 38 *timonensis* and seven *timonense* for the La Timone hospital in Marseille and eight *ihumii* or *ihuae* for our institution (IHU, Institut Hospitalo Universitaire Méditerranée Infection). When comparing species epithet names, *massiliensis* was the most abundant (Fig. 2). When combining together genus and species names, *massiliensis* remains the most represented (Fig. 3).

Additionally, some species were named *bouchedorhornensis* or *bouchedorhornonense* (seven species) after the department of Bouches-du-Rhône. *Pacaensis* or *pacaense* were also attributed to four species after the name of our region, Provence Alpes Côtes d’Azur. In addition, certain species were named after the geographical region where samples were collected. For example, 15 species were named *senegalensis* or *senegalense* for Senegal, Western Africa; five *jeddahensis* or *jeddahense* for Jeddah, a city in Saudi Arabia; and three *saudii* for Saudi Arabia. Geographic locations were also used for naming new genera (e.g. *Massilia timonae*, *Timonella senegalensis*, *Ihubacter massiliensis*, *Jeddahella massiliensis*) [12,13]. Also, we named *Dielma fastidiosa* and *Nidopella massiliensis* after two rural villages (Dielmo and N’Diop), where our research institution has been involved for the past 10 years (Senegal and Western Africa) [14]. Finally, diverse combinations of the places where the samples were collected and the places where the strains were isolated were used. For example, for *Senegalemassilia* spp., three species were named *massiliosenegalensis*, and others were...
Eleven new bacterial species were named in honour of some famous microbiologists or technicians particularly involved in culture at our laboratory (e.g. Lactobacillus raoulti, Corynebacterium lascolaeense, Affisia birgae) [6]. In addition, 18 new genera were named after famous microbiologists (e.g. Raoultibacter spp., Drancourtella spp., Medianikovella massiliensis), other scientists (e.g. Millonella massiliensis, Khelaiabacter massiliensis) or students working on culturomics (e.g. Huganella massiliensis, Ndangobacter massiliensis). Beyond our institute, we honoured other scientists with Legionella rowbothamii, named in honour of Timothy Rowbotham, who isolated the majority of known Legionella-like amoebal pathogen strains, and Garbachella massiliensis, in honour of the famous microbiologist Sherwood Gorbach at the Tufts University School of Medicine, Boston, MA, USA (Supplementary Table S1).

The characteristics of the individuals from whom the samples were collected have been used to name some new species. For example, three bacteria were isolated from children with marasmus (Bacillus marasmi, Blautia marasmi, Paenibacillus marasmiensis), and one was isolated from a child with kwashiorkor (B. kwashiorkori). Other clinical characteristics, such as obesity, were used for three new species (obesi or obesiensis). In addition, a new genus was named Enorma massiliensis because it was isolated from the stool sample of an obese woman. Finally, we named Kallypiga massiliensis after the Greek epithet kallipygos, referring to a statue of Aphrodite having beautifully proportioned buttocks [15]. Other names pertained to the type of sample tested, such as Merdibacter massiliensis or Clostridium merdae isolated from stool samples, Actinomyces urinae from urine samples and Colinsella vaginalis from vaginal samples (Supplementary Table S1).

Finally, phenotypic characteristics such as the type of atmosphere required for growth (Senealemassilia anaerobia) or the form of the bacteria observed during Gram staining or its shape (Soleaferrea massiliensis is horseshoe shaped) were taken into account for naming. Sometimes a combination of a phenotypic characteristic and the type of sample (Duodenibacillus massiliensis) or the geographic place (Libanicoccus massiliensis, Gabonibacter massiliensis) was used (Supplementary Table S1).

Occurrence of new bacterial species among different microbiota
Among the large panel of new species described by culturomics [16], 80 have been isolated in at least one other type of sample.
Occurrence of different species epithets of new species isolated by culturomics (Supplementary Material S1). Word cloud generated by Wordle (http://www.wordle.net/). Name size of each species is relative to its occurrence in list reported in Supplementary Material S1.

For example, *Ezakiella massiliensis* [17], which was isolated for the first time from a vaginal sample, has been then cultivated from stool, urine and respiratory samples. *Actinomyces ihimii* [18] and *Butyricimonas phoceensis* [19] were both first isolated from stool samples and then later isolated from vaginal and respiratory samples. *Olegusella massiliensis* [20] has been isolated in vaginal, stool and urine samples. *Enterococcus massiliensis* [21] was originally isolated from a stool sample and afterwards from urine and vaginal samples.

### From commensal to potentially pathogenic bacteria and vice versa

The microorganisms’ repertoire should include the totality of species isolated from human body at different sites or environment because any commensal can become pathogenic in certain conditions. For example, *Rickettsia parkeri* was detected for the first time in a clinical case in 2004—in other words, 39 years after its first isolation [22]. Being able to detect new species isolated by culturomics in clinical samples or vice versa emphasizes the fact that any commensal can acquire pathogenicity at a certain stage. For instance, among the new bacterial species first isolated from the human gut microbiota by culturomics, 12 were isolated 57 times from clinical samples at our clinical microbiology laboratory, with *Peptoniphilus grossensis* isolated 18 times from diverse samples (including abscesses) [23]. On the other hand, *Paenibacillus provencensis*, first isolated from the urine sample of a patient with a urinary infection [24], has been isolated from a stool sample for the first time from a vaginal sample.
sample by culturomics. Similarly, *Paenibacillus massiliensis*, initially isolated from a blood culture bottle in a clinical microbiology laboratory [25], has since been isolated in a stool sample analysed by culturomics. Indeed, the boundary between commensal and pathogenic bacteria remains indistinct, so efforts must be sustained by both clinical microbiology laboratories and culturomics studies in order to increase the identification of new microorganisms. This also highlights the need to update and share MALDI-TOF MS databases for the optimal identification of bacterial species during clinical or research studies [26].

**Archaea**

Our laboratory isolated seven methanogenic Archaea and two halophilic Archaea, including three new archaeal species that were named after a geographical location (*Haloferax massiliensis, Methanobrevibacter massiliense* (for Marseille, the city where the strain was first isolated) and *Methanomassilicoccus luminyensis* (for Luminy, the place where the species was isolated in Marseille)) [27–29] (Supplementary Table S1). In addition, we first isolated in humans two other Archaea (*Methanobrevibacter*...
arboriphilicus and Haloferax alexandrinus), and we isolated Methanobrevibacter oralis and Methanobrevibacter millerae for the first time from the human gut [6].

Giant viruses

Our laboratory has developed the culture of giant viruses and has succeeded in isolating more than 100 different isolates [6]. Among these isolates, Mimivirus was named after its ability to ‘mimic’ microbes, which previously led to its misidentification as a bacterium [30], and after ‘Mimi the amoeba,’ a tale about evolution invented by the father of one of our scientists (DR) and told to him when he was a child (Fig. 4). Marseilleivirus was named after the geographical location of its first culture and description, Marseille [31] (Fig. 4). In addition, we cultured Faustovirus, which was isolated from sewage by a sewage worker named Fausto [32], as well as Kaumoebavirus, which was isolated in a sample from Saudi Arabia by a PhD student using amoeba co-culture and which was supported by a grant from King Abdulaziz University [33]. Cedratvirus was named after its shape, similar to a lemon known as a cedrat (Citrus medic) [34] and Pacmanvirus, which, given its broken-looking capsid, resembles Pac-Man, from the arcade game [35] (Fig. 4). Recently a student isolated a virus from a sewage sample, and because the student compared himself to Orpheus, the hero of the Greek legend who travelled to the underworld to bring back his dead wife, Eurydice [36], the virus was named Orpheovirus. Finally, Tupanvirus, an extraordinary giant virus isolated in Brazil, was named in honour of the Indian Amazonian god Tupa (Abrahão et al., personal communication).

Conclusion

It is impossible to predict infectious diseases and consequently, as demonstrated here, to anticipate the evolution of a new microorganism. It is essential to allow the cultivators to choose the name of new isolates because their considerable efforts made their cultivation possible. Contrary to the beliefs of some scientists [37], the use of geographical locations in the naming of bacteria has been known for more than a century. The genera Rickettsia is a perfect example, with 13 bacteria named after geographical locations, including five species isolated more than 50 years ago and three others isolated more than 25 years ago (Table 1). Naming species on the basis of a geographical location does not represent a contradiction with the literature or previous efforts within the field.

Acknowledgements

We thank all the contributors involved in culture and those who work in our laboratory. This work has benefited from the support of the French state, managed by the ‘Agence Nationale de la Recherche,’ including the ‘Programme d’Investissement d’avenir’ under the reference Méditerranée Infection 10-IAHU-03. This work was supported by Région Provence Alpes Côte d’Azur and European funding FEDER PRIMI.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.nmni.2018.08.006.

Conflict of interest

None declared.

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