Culturomics: a new approach to study the human microbiome

G. Greub
Institute of Microbiology, University Hospital Centre and University of Lausanne, Lausanne, Switzerland
E-mail: gilbert.greub@chuv.ch

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Humans are heavily colonized by approximately $10^{14}$ bacteria, and the composition of our microbiota has been shown to be associated with various diseases, such as obesity, diabetes, atopic dermatitis, and bacterial vaginosis [1]. The medical importance of our microbiota, which has even been considered as a ‘human organ’[2], has thus led to a growing number of descriptive studies, mainly comparing the microbiomes of healthy and ill individuals. As the large majority of microbial communities are located in the gut, an especially high number of studies have tried to define the composition of its core microbiota, as well as to identify specific alterations that might be associated with various pathologies, such as ulcerative colitis, colorectal cancer, and necrotizing enterocolitis [3–5].

During the last 10 years, most of these studies have been performed with metagenomic approaches, which allow relatively fast assessment of the microbial composition by high-throughput sequencing (Table 1). Despite completely missing the microbial species present in the studied ecosystems at a low concentration ($<10^4–10^5$ mL), direct metagenomics has progressively replaced molecular techniques based on PCR amplification steps, such as sequencing libraries of cloned ribosomal amplicons or pyrosequencing of 16S rRNA amplicons, as these PCR-based approaches were limited to eubacteria, and did not provide any information on the metabolic capacities of the studied microbiota. Microarrays have also been used to define the microbial composition of the intestinal human microbiota; however, this approach also has major limitations, including a low depth of analysis, and the fact that it only identifies the known bacterial species, for which corresponding sequences are present on the chip [6].

Thus, ‘culturomics’, the new approach depicted in the article by Lagier et al. [7], represents a completely new approach to the study of complex microbial ecosystems, such as the human intestinal tract, that: (i) has the potential to detect minority populations; (ii) is not restricted to eubacteria; and (iii) provides strains that allow extensive characterization of new species and allows the study of interactions between different bacterial strains present in a given microbiota (Table 1). Another additional advantage of using culture instead of molecular approaches is the additional information on the viability of detected microorganisms.

Providing strains for downstream studies is not trivial. Indeed, as metagenomics only provides sequencing data, metagenomic-based research investigating the impact of the microbiome on a given disease (e.g. obesity) had to be split into two parts: first, bacterial species that are probably involved in weight gain were identified by metagenomics; then, animal experiments were performed with a strain of the same species, but generally recovered from completely different ecosystems, and thus possibly not containing the bacterial genes that are important in triggering obesity. In contrast, with culturomics, it is possible to directly test the strain originating from the patient microbiota presenting the disease of interest.

Culturomics may be defined—by analogy with metagenomics—as an approach allowing an extensive assessment of the microbial composition by high-throughput culture (Table 1). Thus, Lagier et al. have identified as many as 32,500 different colonies recovered from three human stools [7]. Such very high-throughput identification was only possible because of the availability of matrix-assisted laser desorption ionization time-of-flight mass spectrometry, which not only represents a revolution in clinical diagnostic laboratories [8,9], but also represents a revolution in microbial ecology, especially when it is coupled to smart incubators and automated colony-picking systems to constitute the next generation of culturomic approaches. Indeed, culturomics will further improve, thanks to automation, miniaturization, and improved technology.

It is noteworthy that Lagier et al. not only propose a new concept, i.e. ‘culturomics’, but also, more importantly, they provide in this milestone article a proof-of-concept. They applied 212 different culture conditions, and successfully cultured 340 different bacterial species, as well as five fungi and the largest virus ever found in a human sample [7]. Moreover, 32 new species have been discovered.
is a true breakthrough, as these 32 new species represent approximately one-third of all new validated species recovered by culture from the human intestinal tract during the last decade.

Lagier et al. also performed a metagenomic analysis of the same three stools by pyrosequencing of 16S rRNA amplicons, and a total of 638 phylotypes were identified, including 282 known bacterial species [7]. Interestingly, only 51 species identified by 16S rRNA sequencing were among the 340 cultured species. Thus, given such a major discrepancy, culturomics appears to be an ideal complementary approach to metagenomics, as it has the ability to increase by one-third the identified microbial repertoire. This important discrepancy is partially attributable to the extraordinary amplification power of culture, but is also related to the restricted biodiversity observed when starting from 16S rRNA amplicons.

It is important to stress that Raoult’s team not only provided a new approach and a proof-of-concept, but also used highly innovative culture conditions. Thus, to circumvent the very high rate of growth of fast-growing Enterobacteriaceae, and because faeces may contain a huge microbial biodiversity, they used various highly selective approaches, such as: (i) the removal of Escherichia coli by specific lytic phages; (ii) various antibiotic cocktails; (iii) heat destruction of non-sporulated bacteria; (iv) different selective media; and (v) amoebal co-culture.

Ameoba co-culture is a cell culture method that uses amoebae as cells in a cell culture system [10]. This approach, initially developed by Rowbotham [11], has the ability to selectively grow amoeba-resistant microorganisms, such as Legionella, mycobacteria, and chlamydiae, from heavily contaminated environments [12–14], and was previously applied successfully to isolate a Legionella strain from a stool sample [15]. Here, amoebal co-culture was successfully used to isolate Senegalivirus [7], a giant virus related to Marseillevirus and Lausannevirus [16,17].

In addition to these selective conditions, Lagier et al. used various atmospheres and incubation temperatures, as well as a large variety of enrichment broths and media. Here again, the present study was highly innovative, as it used rumen fluid and sterile human stools in order to have an enrichment broth that is as similar as possible to that encountered by the microorganisms present in our intestinal microbiota. Similar studies are now needed to define which enrichment broths and culture conditions are the most successful, in order to define optimal culturomic protocols.

In conclusion, Raoult’s team invented culturomics, and proved that this new approach is feasible, providing completely new insights into the microbiota. In the future, culturomics will certainly be one major approach to study the human microbiome, besides metagenomics. Besides descriptive studies, culturomics may also have very specific applications, such as detailed characterization of faecal contents, which will be used for ‘controlled faecal transplantation’. Thus, instead of inoculating raw faeces to treat Clostridium difficile colitis [18], we might inoculate a controlled mixture of bacteria recovered from faeces of relatives. Moreover, culturomics will probably be more widely used in the future, with advances in automation and when the most effective culture conditions have been defined.

TABLE 1. Comparison between metagenomics and culturomics

<table>
<thead>
<tr>
<th>Metagenomics</th>
<th>Culturomics</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Methodology</strong></td>
<td><strong>Methodology</strong></td>
</tr>
<tr>
<td>Pyrosequencing of 16S rRNA amplicons and/or direct metagenomics</td>
<td>Use of various selective and/or enrichment culture conditions coupled to MALDI-TOF MS identification</td>
</tr>
<tr>
<td>Direct metagenomics without amplification step</td>
<td>Masses so-called ‘non-cultivable’ microorganisms</td>
</tr>
<tr>
<td><strong>Limitations</strong></td>
<td><strong>Limitations</strong></td>
</tr>
<tr>
<td>Does not provide a strain for further studies</td>
<td>Misses minority populations</td>
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<tr>
<td>Misses minority population (depth bias)</td>
<td>Major workload</td>
</tr>
<tr>
<td>Only detects eubacteria</td>
<td>Detects minority populations</td>
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<tr>
<td>Does not provide information on enzymatic abilities</td>
<td>Open approach</td>
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<tr>
<td><strong>Advantages</strong></td>
<td><strong>Advantages</strong></td>
</tr>
<tr>
<td>Detects ‘non-cultivable’ microorganisms</td>
<td>Detects only viable bacteria</td>
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<tr>
<td>Increased depth of sequencing because of new technology</td>
<td>Approximately 100 bacterial species/sample</td>
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<tr>
<td>Coupling pyrosequencing with direct metagenomics</td>
<td>Automated detection of microbial growth</td>
</tr>
<tr>
<td><strong>Rate of success</strong></td>
<td><strong>Rate of success</strong></td>
</tr>
<tr>
<td>Approximately 200 bacterial species/sample</td>
<td>Automated identification</td>
</tr>
<tr>
<td><strong>Possible future developments</strong></td>
<td><strong>Possible future developments</strong></td>
</tr>
<tr>
<td>Coupling pyrosequencing with direct metagenomics</td>
<td>Miniaturization</td>
</tr>
<tr>
<td>Coupling pyrosequencing with direct metagenomics</td>
<td>Other innovative culture conditions</td>
</tr>
</tbody>
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MALDI-TOF MS, matrix-assisted laser desorption ionization time-of-flight mass spectrometry.

1Limitation of direct metagenomics (no amplification step).
2Limitation of pyrosequencing of 16S rRNA amplicons.
3Relevance of dead microorganisms lower than that of viable microorganisms (no metabolic activity).
4Mean numbers of bacterial species recovered from one stool sample by Lagier et al. [7] when comparing both approaches.
5Smart incubators, pH indicators in broth, microcalorimetry, etc.
6Automated colony picking coupled to MALDI-TOF MS and/or full laboratory automation.
Transparency Declaration

No conflict of interest to declare.

References