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Microbial culturomics: The next generation culture for identification of the human gastrointestinal microbiota

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Abstract

The maintenance of human health and the development of disease are both significantly influenced by the gut microbiota. The development of omics technologies improves the understanding of the gut microbial ecosystem. Metagenomics has emphasized the diversity of the gut microbiome; however, it does not provide reliable insight on the dark matter of microorganisms or the minor populations. As a result of the rebirth of cultural techniques in microbiology; the field of "culturomics" is created to cultivate the unidentified bacteria that reside inside the human gut. In the 21st century's discipline of clinical microbiology; microbial culturomics becomes a promising strategy that may be used to cultivate hundreds of novel microorganisms linked to human; thus, opening new insights on the host-microbial relationships. Novel taxa and species will be detected by optimizing the culture conditions; followed by quick identification using mass spectrometry or molecular next generation sequencing. Culturomics of the human gut microbiota can be used as a bactriotherapy for the inflammatory bowel diseases and the respiratory illnesses like COVID-19, and as an immunomodulatory agent for cancer therapy. Furthermore, culturomics is a big store for discovering new antibacterial agents and resistance genes. The aim of this review was to highlight the background; methodologies, and future use of culturomics to study the human gut microbiota.

Keywords: Microbiota, Culturomics, MALDI-TOF, Sequencing, Gastrointestinal tract

1. Introduction

In several articles; the word “microbiota” is sometimes confused with the word “microbiome”. Microbiota refers to all the living microorganisms located in a certain environment such as the human gut; while microbiome, is the genomic information of these microbial communities (Martellacci *et al*., 2019). A deep understanding of the bacterial diversities and the roles of microbiomes in the environment; can

provide a comprehensive and directed insights needed for manipulation of these microbial communities. In the agro-environment, this can be used to inoculate the soil with certain microorganisms to improve the plants growth; where this approach is called synthetic microbial communities (Lian *et al*., 2023).

Development of metagenomics helps in understanding the vast microorganisms in a complex environment by direct application of the next generation sequencing, and is also able to replace the traditional microbial cultures. However; about 80 % of the microbial sequences detected by metagenomic studies have not been cultured before. Moreover; metagenomics cannot detect a minor bacterial population that is less than 100.000 cells/ g. For example, the human gut microbiota; representing approximately 10^{12} bacterial cells/ g of stool, contains a major pathogen (*i.e*., *Salmonella typhi*), which can't be detected by such traditional methods (Eckburg *et al*., 2005).

All microorganisms can be cultivated as long as the culture tools are optimized. Hence, the introduction of culturomic studies, including the use of diverse culture conditions to simulate the natural bacterial environment; followed by their identification using matrix assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry, has expanded the bacterial reservoir (Dubourg *et al*., 2014). Approximately, 1700 bacterial species have been detected until the 1980; however, after the application of culturomics in the laboratory; more than 12000 species have been recorded. In addition; taxonogenomics have evolved to detect the novel microbial species by using genome sequencing in addition to the classical criteria (Lagier *et al*., 2015a).

The objective of this review was to outline the background; current methodologies, and future applications of culturomic studies, which are currently used to expand the human gastrointestinal microbiota. In addition, this study highlighted the possible effects of culturomics on the human health and disease.

2. History of "Omics" in microbiology

Culture was the early method used for studying the gut microbiota during the 1970s. The identified species are related mainly to the *Enterobacteriaceae* and *Veillonellaceae* families; with the predominance of the anaerobic bacteria (Rajilic-Stojanovic *et al*., 2007). However, the conventional culture methods are time and work intensive and unable to support the growth of certain microorganisms on the artificial media (Nowrotek *et al*., 2019).

After the development of metagenomics in the 1998; it superseded the traditional culturing techniques used in studying the genomic materials of the multiple microorganisms, which are explored from the natural specimens. The major obstacle facing metagenomics is the recovery of indefinite number of unknown sequences that are not related to known microorganisms (Lagier *et al*., 2012b). These observations have directed the environmental microbiology scientists toward the design of a next generation culture techniques; to fulfill the identification of the human gut microbiota. A previous study conducted by Bollmann *et al*., (2007) developed a culture method that simulates the natural environment of the microorganisms by the help of the diffusion chamber technique. In 2009; the MALDI-TOF mass spectrometry was introduced for microbial identification in the clinical microbiology laboratories (Seng *et al*., 2009).

Culturomics was first recorded by Lagier *et al*., (2012a) to investigate the human gastrointestinal microbiota. This study has tested about 212 different culturing conditions and successfully isolated 32,000 bacterial colonies from the human feces, which belong to 340 diverse bacterial species. In the next year; Pfleiderer *et al*., (2013) study has tested and approved about seventy best culture conditions to expand the human gut repertoire. MALDI-TOF mass spectrometry has been approved as an integral step in the culturomic studies for rapid and precise colony identification (Seng *et al*., 2010). Later, Kambouris *et al*., (2018) study declared culturomics as a novel kid in

the family of omics, which has described its inter-relationships. Culturomics has been routinely applied by the clinical and environmental microbiology laboratories. In the last decade; the metagenomics assembled genome's (MAGs) have been reported as a single microbial genome that is re-assembled from the metagenome sample. Thousands of MAGs have been recorded by the researchers for identification of the novel and non-cultivable microorganisms in a variety of environments, including humans (Sood *et al*., 2021).

3. Microbial culturomics: new generation culture-dependent methods

3.1. Culturomics versus metagenomics

Culturomics is an advanced culturing technique that consists of different laboratory stages, as revealed by Lagier *et al*., (2018). The first stage involves splitting of the sample obtained from the environment or tissues such as the human gut; followed by culturing on agar medium with the establishment of divert cultural conditions. These conditions improve culturing of the target fastidious bacteria and inhibit the growth of the undesired mass populations. The next stage involves selection of the pure bacterial isolates followed by rapid detection of the bacterial species by MALDI–TOF mass spectrometry. If bacterial detection by MALDI-TOF fails; 16S ribosomal RNA (rRNA) or RNA polymerase B subunit (*rpo B*) sequencing can be used for bacterial genomic analysis. If the isolates have a 16S rRNA sequence identity of less than 98.65 % when compared with the phylogenetically closely related species; it is categorized as a novel species by taxonogenomics (Martellacci *et al*., 2019; Wan *et al*., 2023). A previous study reported by Abdallah *et al*., (2017) that microbial culturomics has developed to fill the metagenomics gaps. It determines the composition of the living microorganisms in a certain ecosystem, including archaea and bacteria. Moreover; microbial culturomics has evolved as an efficient tool to isolate the large bacterial numbers and the new species; mainly from the human body. The bacterial colonies can be identified by MALDI-TOF spectrometry with the assistance of smart incubation and automated colony picking machines; to constitute the new generation of culturomic approaches. Table (1) summarizes the possible characters; methods, strengths, drawbacks, and future of the culturomics and metagenomics (Greub, 2012).

3.2. MALDI-TOF mass spectrometry and culturomics

3.2.1. Principles of the MALDI-TOF mass spectrometry

MALDI-TOF mass spectrometry becomes the standard method used for microbial diagnosis in the clinical microbiology laboratory. The development of culturomic research to investigate the complex ecosystems has been made possible by MALDI mass spectrometry, which is recognized as a rapid identification technique (Seng *et al*., 2013). Three machines; mainly Vitek MS (bioMérieux, France); Andromas database (Andromas, France), and Bruker Biotyper (Bruker Daltonics, Germany), are used for mass spectrometry identification of the microorganisms. The mechanism of MALDI-TOF spectrometry involves an UV soft ionization theory. The bacterial colonies are mixed with a matrix on metal plates; laser is directed towards the mixture, and then ionization takes place through proton transfer to the bacteria. The time of flight of the ionized bacterial colonies into the tube to reach a final detector is measured as the lighter molecules travel faster. The generated protein mass spectrum represents the number of ions hitting the detector over time. It is compared through a defined automated protein database that permits for bacterial identification (Clark *et al*., 2013).

3.2.2. Role of the MALDI-TOF in the culturomic studies

For time utilization; MALDI-TOF that annually identifies a mean of 112 distinct microbial species has been compared with the traditional phenotypic methods, which have identified 44 species annually. Furthermore; MALDI-TOF is used to characterize the

Table 1. A comparison between culturomics and metagenomics (Greub, 2012)

	Culturomics	Metagenomics
Characters	Technique that describes the microbe composition by high throughput culture.	Technique that describes microbe genetic composition by high throughput sequencing.
Methods	Use of different selective and/or enriched culturing conditions followed by MALDI-TOF[a] mass spectrometry identification.	Sequencing of 16S rRNA[b] or *rpo B*[c] genes (+/- amplification step).
Strengths	1. Detects all living microbes (*i.e.*, eubacteria and archaea). 2. Detects minor populations.	1. Detects non-cultivable microbes. 2. Integrative workflow.
Drawbacks	1. Skip non-cultivable microbes. 2. Extensive workload.	1. Only detects eubacteria. 2. Viability bias. 3. Skip minor populations (depth bias). 4. Sequences may not assign to known microorganisms. 5. Does not provide strains for further studies. 6. Does not provide information on enzyme activities.
Future	1. Antibiotic resistance research. 2. Automated identification step/ full laboratory automation. 3. Other creative culture conditions.	1. Increased sequencing coverage. 2. Coupling pyro-sequencing with direct metagenomics.

Where; [a]MALDI-TOF: matrix assisted laser desorption ionization-time of flight; [b]16 rRNA; 16S ribosomal RNA; [c]*rpo B*: RNA polymerase B subunit

rare and fastidious bacteria and archaea (Seng *et al*., 2013). In the clinical practice; mass spectrometry has demonstrated excellent microbial identifications, including *Staphylococcus* species; β-hemolytic Streptococci, Enterococci, *Neisseria meningitides*, *N. gonorrhoeae*, *Enterobacteriaceae*, and non-fermenting Gram-negative rods. However, inconsistent findings have been observed for diagnosis of the non-Enterococci group D Streptococci (Lagier *et al*., 2015b). For the anaerobic bacteria; La Scola *et al*., (2011) have identified 61 % of 544 isolates by using MALDI-TOF technology. Several additional uncommon bacteria have been appropriately identified to the species level by MALDI-TOF, including *Brucella*; *Leptospira*, *Pasteurella*, and *Legionella* (Lagier *et al*., 2015b).

3.2.3. Advantages of the MALDI-TOF

According to Seng *et al*., (2010), MALDI-TOF mass spectrometry is considered as a cost and time efficient approach. With the modest level of training; MALDI-TOF spectrometry is based on intact protein and can identify several bacterial strains in a matter of minutes (*i.e*., in about 6-8 min. with an AutoFlex program and 2 min. with the MicroFlex LT program). A single person can easily check 1000 distinct bacterial colonies weekly; as required in the culturomics studies (Lagier *et al*., 2012a). Furthermore; mass spectrometry allows secure bacterial identification and antimicrobial sensitivity testing's with the exclusion of possible culture contamination (Seng *et al*., 2010). In practice, the cost of identifying a single bacterial strain by MALDI-TOF mass spectrometry is extremely low ($1.5 to $2.0); as opposed to the traditional phenotypic methods ($4.5 to $20) and for 16S rRNA sequencing ($100) (Torres-Sangiao *et al*., 2021).

3.2.4. Limitations of the MALDI-TOF

The limited databases are the main challenges for bacterial identification using MALDI-TOF (Verroken *et al*., 2010). Lack of spectra reduces the accuracy of identification of the rare species. Fortunately; this restriction can be bypassed by the growing databases such as those of the *Nocardia* species. The study reported by Werno *et al*., (2012) added that the clinical microbiology occasionally observes differences in the identification of some bacterial species by MALDI-TOF spectrometry. For instance; the differentiation between *Streptococcus pneumoniae* and *Streptococcus mitis* by spectrometry remains unsuccessful. Furthermore, *Salmonella* spp. can't be accurately identified down to the subspecies and serovars levels (Kuhns *et al*., 2012).

3.3. Role of the 16S rRNA molecular tools in culturomics

The molecular tools continue to be the benchmark for bacterial identification; especially when the spectra are deficient in the MALDI-TOF database. Sequencing enables validation of the MALDI-TOF spectra and provides accurate addition to the protein databases. Nonetheless; sequencing takes at least 24 h, and its cost is 100 times as much as the MALDI-TOF identification (Seng *et al*., 2013). In parallel; an efficient method for selecting the DNA sequences used for bacterial identification has been made possible by expanding the number of sequences that are available in the different databases, such as the GenBank and Genomes OnLine Database (GOLD) (Fenollar *et al*., 2004). A bacterial identification system that is based on the universal 16S rRNA genome amplification and sequencing has been proposed. A significant potential for describing the new bacterial species has been provided by the 16S rRNA. However; several bacterial taxa, including *Rickettsia*; *Brucella*, *Corynebacterium*, and *Bacillus* species are not discriminatory for this gene (Lagier *et al*., 2015b).

4. Environmental microbiology

4.1. Approaches of using high throughput media for culturomics

4.1.1. Dilution culture approach

The term "great plate count anomaly" was first used by Staley and Konopka, (1985). This theory showed that not all bacterial species present in the environment can be cultivated using the traditional culture conditions. This explains why the number of bacterial cells is greater upon using direct microscopy for investigating an environmental sample than after culturing on the routine agar medium. Scientists specialized in environmental microbiology have exerted efforts in finding a solution to the great plate count anomaly (Epstein, 2013). They used the empirical dilution culture approach to identify the minority populations, which is later applied to explore the human gut microbiota. The dilution culture approach involves serial dilutions of the bacterial population until no further growth is observed (Lagier *et al*., 2018). According to Harwani, (2013), the great plate count anomaly can be explained in a number of ways. For instance, they may not be viable; be viable but uncultivable, be dormant, be rare, grow slowly, and/ or have strict growth needs, such as the necessity for particular physical conditions, including pH; oxygen levels, and/ or incubation temperature.

4.1.2. Diffusion chamber approach

Kaeberlein *et al*., (2002) established a technique for in situ culture of the environmental samples that exist in the same environment; thus, overcoming the limitations of the agar plate-based methods. They employed a diffusion chamber, which has two porous membranes that confine the bacterial cells and prevents the entry of air or soil contaminants, while enabling the environmental nutrients to permeate. This allows to mimicking the natural environments in the laboratory and enhances the number of cultivated colonies about 300 fold, compared to the agar plate method. In their experiment; Gavrish *et al*., (2008) used a trap made up of agar and a diluted environmental specimen that is sandwiched between two semi-permeable membranes (0.02 to 0.03 µm pore size); glue dried, and then set on a wet soil. Most of the bacteria detected by the diffusion chamber belong to Actinomycetes.

4.1.3. Isolation chip (ichip) approach

The isolation chip (ichip) is a high throughput bacterial cultivation device that was created by Nichols *et al*., (2010). It consists of several hundred microscopic diffusion chambers; where each is seeded with a solitary environmental bacterial cell. Microbial diversity obtained by ichip is far greater than that of the traditional cultivation methods; especially for the *Deltaproteobacteria*. The bacterial species obtained using the ichip have also demonstrated taxonomic novelty (Nichols *et al*., 2010). The minitrap device is an example of ichip, which is used for *in vivo* cultivation of the oral microbiota (Sizova *et al*., 2012).

4.1.4. Co-culture chamber approach

The co-culture chamber enabled cultivation of the microorganisms inhabiting various environments without coming into direct contact. Through membranes; this technique permits the indirect inter-bacterial exchange of the growth-promoting agents and the signaling molecules. In this model; mutualism between the bacterial species has allowed the discovery of nutritional and signaling molecules deficits, which are necessary for bacterial growth, and may subsequently be provided to the monoculture of each bacterial strain (Wan *et al*., 2023).

4.2. Applications of the environmental microbiology

4.2.1. Rhizosphere microbiome and SynComs

In the agricultural environment; the rhizosphere microbial community shows diverse interactions with the crops. This can be used to inoculate the soil with certain rhizosphere microorganisms to improve growth of the plants; where this approach is called synthetic microbial communities (SynComs) (Lian *et al*., 2023).

4.2.2. Halophilic microorganisms and the extreme environments

The halophilic microorganisms include the bacteria and archaea. The former are composed of low; moderate, and the extremely halophilic bacteria, which

are restricted to the photosynthetic purple bacteria. Meanwhile, the halophilic bacteria haven't got great attention in the past; except for their adaptation to the salty diets. Nowadays; these microorganisms become important to discover more about living in the extreme conditions, and to study the enzymatic pathways under the unique harsh characteristics. The human gut is found to contain halo archaea. Intestinal biopsies from the patients who have consumed a salty solution prior to a colonoscopy revealed the DNA sequences of the halophilic archaea. In the laboratory; cultivation of such microorganisms from the human gut will be the next challenge (Oxley *et al*., 2010).

5. Culturomics for rapid identification of the human gut microbiota

5.1. Types of used culture media

5.1.1. Basic culture media

The environmental microbiologists' empirical dilution model represents the basis for the use of non-selective culture medium, such as 5 % sheep blood agar; to cultivate the human gut microbiota. Each gram of stool is to be dissolved in 9 ml of phosphate buffer saline (PBS) and then inoculated into a basic culture medium; under various conditions in serial dilutions that range from 1/10 to $1/10^{10}$. Temperatures ranging from 4 °C to 55 °C and various atmospheric conditions (*i.e*., aerobic; aerobic with 5 % CO_2, microaerophilic, and anaerobic) are examined. To identify the slow-growing bacterial species; incubation periods ranging from 24 h to two months have been used. Following their primary inoculation; each agar plate is examined after 24 and 48 h of incubation, and once a week. However; the minimal impact of the basic culture media is attributed to the high bacterial abundances in the human gut. In fact, despite serial dilutions; the plates are frequently overloaded; thus, necessitating the development of additional strategies (Lagier *et al*., 2012a; Petakh *et al*., 2023).

5.1.2. Selective culture media "kill the winner strategy"

The objectives of using a selective culture medium are to select and specify the minor bacterial populations in a strategy known as "kill the winner" (Lagier *et al*., 2012b).

Inhibitory antibiotics and dyes: Antibiotics are the most widely applied selective agents; since it is simple to predict how they can damage the bacteria. Colistin and/ or kanamycin are commonly used to prevent growth of the Gram-negative bacteria; while vancomycin and/ or bacitracin are added to inhibit growth of the Gram-positive bacteria. Various dyes and sodium salts, such as bile extracts; methylene blue, methyl violet, sodium citrate, and sodium thiosulfate, are frequently employed in clinical microbiology to help in identifying the enteric bacteria (Bonnet *et al*., 2020).

Filtering techniques: A previous study conducted by Lagier *et al*., (2012a) reported that for active filtration; syringe filters with pore diameters ranging from 5 to 0.2 µm are employed to get bacteria with different sizes. After filtering the environmental sample for multiple times using filters with increasing pore sizes; the filtrate in each time is seeded in 5 % sheep blood and brain heart infusion agar media at 37°C under aerobic and anaerobic environments, respectively. However, the passive filtration is used to isolate the spirochetes from the human fecal samples. Passive filtration is used to select the motile bacteria; using cell culture plates and 0.4 µm membrane filter. On one side of the membrane is the diluted feces mixed with broth; while on the other side is the sterile broth. Every day; the supernatant is examined under a dark-ground microscope to look for motile spirochetes (Lagier *et al*., 2012c).

Heat shock technique: Heat shock is used to select the human gut bacteria that belong to the spore forming *Clostridium* and *Bacillus* spp., as previously reported by Pfleiderer *et al*., (2013). Two heating protocols, including 65 °C for 20 min. and 80 °C for 20 min. are applied in the first culturomic assays. Later, the heat shock is extended for one hour; after

which the diluted stool sample is inoculated into a blood culture bottle (Lagier *et al*., 2016).

Phage therapy: Bonnet *et al*., (2020) revealed that humans have included the phages for therapeutic purposes as in the culturomic studies. Stool samples that are cultivated using culturomics showed that the gut is overloaded with *Escherichia coli*. Thus; Type 1 and Type 4 lytic phages are applied to inhibit growth of the *E. coli* in the fecal samples. Bacterial growth is reduced by 50 %; allowing the discovery of novel bacterial species (*i.e*., *Enterobacter massiliensis*).

5.1.3. Enriched culture media

Pre-incubation in blood culture bottles: The stool samples are pre-incubated in both aerobic and anaerobic blood culture bottles for 2 to 30 d before culturing onto various agar media. This action encourages growth of the bacteria from the human gut; especially the anaerobic bacteria. Fifty novel gut bacterial species have been isolated using this technique (Lagier *et al*., 2016).

Addition of stool filtrate: Fresh stool filtrate is employed in the culturomic research as growth factors for culturing the archaea and the anaerobes from the human gut. A stool mixture is generated from various fecal samples donors; lyophilized, and crushed using a hammer and pestle. The powder is re-dissolved in dist. water (2 g/ 100 ml) and centrifuged at low speed for 10 min. Sonication of the supernatant are carried out two times, which are interrupted by a French press treatment. The solution is filtered using a 0.2 µm filter. The medium is prepared by adding agar; 20 % stool filtrate, and two antibiotics; mainly vancomycin and colistin (Lagier *et al*., 2015b).

Addition of rumen extract: Rumen fluid is employed in culturomics studies to isolate the gut spirochetes; archaea, and the Bacteroides. This substance made it possible to cultivate the various bacterial strains that are not recovered using the conventional culture methods (Goodman *et al*., 2011). To make a rumen fluid; sixteen distinct digestive contents from the sheep are employed. Rumen fluid can be stored at -80 °C. It is utilized by adding 5 ml to a blood culture bottle or at 5 % to the culture medium. A total of 30 new bacterial species have been discovered using rumen extract out of those 91 discovered by culturomics (Bonnet *et al*., 2020).

Addition of Media lipids: The diluted stool samples are mixed with 5 ml of 20 % Media lipids (medium chain triglycerides and soya oil), and then added into anaerobic blood culture bottles for 2 to 30 d. After incubation, the broth is anaerobically inoculated into sheep blood agar. Using this nutrient; *Bacillus okuhidensis* has been firstly discovered in the human intestine (Lagier *et al*., 2015b).

Addition of antioxidants: Ascorbic acid and glutathione have been utilized as antioxidants for the Spirochetes and several other anaerobic bacterial species, which are very sensitive to oxygen. *Enterococcus canintestini* has been first identified from the human gut by this technique. The fecal sample is inoculated into a blood culture bottle with rumen extract and 500 g/ ml of ascorbic acid; or the same amount of ascorbic acid is added to sheep blood agar. These antioxidants are added to enable culturing of the obligate anaerobes under aerobic conditions (La Scola *et al*., 2014).

5.2. Consequences of the culturomic studies

5.2.1. Reduction of the culture conditions

The first step in standardizing the culturomics is to reduce the workload and numbers of the used culture conditions. Lagier *et al*., (2012a) study initially investigated about 212 diverse culture conditions and discovered 340 novel microbial species, including 170 species that have never been isolated before from the human gut. Later on; the diverse culture conditions have been reduced to 70 out of 212, which have been applied in the subsequent studies with different stool specimens. In order to standardize the culturomics; the researchers have chosen the most efficient twenty culture conditions, which allowed them to identify 70 % of the bacteria that they have grown in their studies (Pfleiderer *et al*., 2013; Dubourg *et al*., 2013).

5.2.2. Expansion of the human gut repertoire with new taxa and species

The integration of culturomics in various projects; particularly the study of human microbiota, has led to the submission of novel species in MALDI-TOF spectrometry databases; thus, expanding the number of bacteria that can be recognized (Hugon *et al*., 2015). Culturomics is used to cultivate 1055 species from the 690 prokaryotic species that have been previously recorded in the human gut; thus increasing the repertoire of the human gut microbiota (Lagier *et al*., 2016; Khelaifia *et al*., 2016). Furthermore; culturomics led to a shift in the techniques used to describe the unknown bacteria with the integration of different databases, such as MALDI-TOF mass spectra and genome sequencing (Hugon *et al*., 2015).

5.3. Future applications of culturomics

5.3.1. Fecal microbiota transplants and bacteriotherapy

Several alterations in the ecology of the gut microbiota have been linked to diseases. Culturomics may play a vital role in the development of bacteriotherapy to treat these changes (Lagier *et al*., 2018).

Clostridium difficile infections: Several studies investigating the changes in gut microbiota that are associated with *C. difficile* infections (CDIs) primarily revealed a decline in the microbial diversity (Chang *et al*., 2008). The fecal microbiota transplants (FMT) can restore this diversity. The process of FMT involves injecting a liquid suspension of the intestinal bacteria into the gastrointestinal tract (GIT) of the patient, which can prevent the recurrence of CDI (Masucci *et al*., 2017). Furthermore; administering a non-toxic *C. difficile* spores to CDI patients seems to be a potential treatment strategy. Culturomic studies will find novel spore-forming bacteria that may be effective in treating CDI (Gerding *et al*., 2015). FMT have more recent roles in management of the other inflammatory bowel diseases (Weingarden and Vaughn, 2017).

Neonatal necrotizing enterocolitis and *Clostridium butyricum*: Neonatal necrotizing enterocolitis (NEC), which affects up to 10 % of the neonates with extremely low birth weight, has been linked to the gut *C. butyricum* isolates. Using culturomics; an over-expression of this bacterium and toxin secretion has been detected in the NEC patients. Bacteriotherapy used to restore the commensal gut microbiota may be a promising therapy for this severe illness (Cassir *et al*., 2015).

Severe acute malnutrition: Nutrition is a major determinant of the gut microbiota diversity. It has been observed that an immature gut microbiota is related to severe acute malnutrition. A cohort of people affected by kwashiorkor disease from the Niger and Senegal has been subjected to a culturomic study. Compared with the controls; culturomics observed 45 missing microorganisms in the affected individuals. However; twelve of these species are thought to be suitable for future bacteriotherapy (Alou *et al*., 2017).

***Akkermansia muciniphila* and obesity**: In 2004; Derrien *et al*., (2004) study has got a benefit from the environmental microbiology and is enabled to grow the mucin-degrading bacterium in the human gut "*Akkermansia muciniphila*". The diluted stool samples are inoculated under an anaerobic medium with mucin. This bacterium has made up to 1 % of the total bacteria in the human gut. It has been observed that the obese people have lower numbers of *A. muciniphila*, compared with the controls. According to an experiment that has been conducted on mice; the use of *A. muciniphila* may be protective against obesity and its related metabolic problems (Lagier *et al*., 2015b).

5.3.2. Oncomicrobiotics

Oncomicrobiotics are gut microbiota that augment the efficacy of immunomodulatory treatments of the tumors. In an experimental setting; it has been discovered that the absence of microbiota reduced the synthesis of the tumor necrosis factor. This in turn reduced the effect of the anti-cytotoxic T lymphocyte-

associated protein 4 (CTLA4), which is used as an antibody tumor immunotherapy. It has been demonstrated that the absence of certain bacterial species (*i.e*., *Bacteroides fragilis* and *Burkholderia cepacia*) reduces the efficacy of tumor immunotherapy; however, oral intake of these isolates has recovered the antitumor activity of the anti-CTLA4 antibodies (Vétizou *et al*., 2015). Furthermore, it has been shown that antibiotics intake before immunotherapy significantly reduces the activity of the programmed cell death protein 1 (PD-1) based immunotherapy against the advanced epithelial tumors. Culturomics can help in the development of novel anticancer therapies, which will depend on the immune modulation of the microbiota (Routy *et al*., 2018). A recent study conducted in Egypt by Mahmoud *et al*., (2023) highlighted the reduction in prevalence of some *Lactobacillus* species among patients with breast cancer, compared to the healthy control.

5.3.3. Novel antibacterial therapy

O'Shea *et al*., (2011) highlighted that several categories of antibiotics that are frequently used to treat the bacterial illnesses in humans are mostly derived from bacteria. By using culturomics to increase the gut bacterial diversity; new antibiotics will be developed. Bacteriocin lantibiotics can be generated by members of the human gut microbiota. This may also be supported by the development of lugdunin antibiotic at 2016; a promising peptide generated by a nasal strain of *Staphylococcus lugdunensis*. Lugdunin inhibits *Staphylococcus aureus* skin colonization in the human and rodents by direct killing and by triggering the innate immunity in the skin. People who carry *Staphylococcus lugdunensis* are six times less likely to get *Staphylococcus aureus* in their nasal passages. Moreover; this peptide is active against several Gram positive and drug resistant bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA); Vancomycin-resistant *Enterococci* (VRE), *Listeria*, and *Pneumococci* (Zipperer *et al*., 2016; Bitschar *et al*., 2019).

5.3.4. Novel human gut resistome

The term "resistome" refers to the antibacterial resistance genes allocated on the pan genome of the gut microorganisms. In this context; culturomics may be the perfect supplementary approach to metagenomics in studying the antibacterial resistance. Culturomics identifies the human and environmental microbiome, which is a diverse repertoire of the antibiotic resistant genes. It introduces enriched media with different culture conditions, which is useful to detect the novel antibiotic resistant microorganisms and the novel resistant mechanisms (Nowrotek *et al*., 2019). The usage of culture-based techniques for identifying drug resistance in the agro-environments has been described by McLain *et al*., (2016); Lian *et al*., (2023). In addition; the individual bacterium expressing antibiotic resistant genes must be cultured to identify the multi-drug resistant strain. Culturomics and metagenomics surveys of the donor's gut resistome can be added as screening tests before the FMT process (Nowrotek *et al*., 2019). A recent study conducted in Egypt by Mahmoud and El-Kazzaz, (2021); used an antibiotic containing culture medium for rapid screening of the Colistin resistant *Enterobacteriaceae* in the fecal samples.

5.3.5. Gut microbiota in patients with COVID-19

Recently, Petakh *et al*., (2023) have used the dilution culture method to evaluate the correlation between dysbiosis in the gut microbiota and the severity of COVID-19 infection; especially in the diabetic patients. The pathogenesis is potentially attributed to an inflammatory dysfunction that is triggered by the gut dysbiosis. They added that patients with COVID-19 have increased Enterococci and decreased Lactobacilli, compared to the controls. El-Sabbagh *et al*., (2022) also demonstrated that certain *Lactobacillus* spp. may have a link with the development of diabetes mellitus. These findings may carry a potential treatment option through using the probiotics or FMT to restore the balance to the gut bacteria.

6. Taxonogenomics

Taxonogenomics is a novel concept that incorporates a poly phasic strategy, including the latest phenotypic and genotypic knowledge; to define a new bacterial species. First phase; bacterial isolates that have a 16S rRNA sequence identity of less than 98.65 % when compared with the phylogenetically closely related species, are categorized as novel species. In addition, the DNA-DNA hybridization; GC content, and the average nucleotide identity (ANI), are genotypic criteria employed in the bacterial taxonomy. Next phase; the phenotypic characters (*i.e*., Gram stain; primary culture, and metabolic activity); MALDI-TOF mass spectrum, and the genomic characters (such as RNA genes; mobile genetic elements, and signal peptides) of the bacterial isolates, are compared with those of the most closely related bacterial species with existing nomenclatures (Tindall *et al*., 2010; Ramasamy *et al*., 2014). Based on the approaches mentioned above; all the novel microbial species that are discovered using microbial culturomics have been described. The creation of potent tools that are able to examine the newly discovered microbial species by culturomics will be made possible by the development of more genome sequencing techniques (Lagier *et al*., 2018).

Conclusion

Microbial culturomics is the next generation culture method that tries to mimic the natural microbial ecosystem. It consists of serial dilutions of the sample with seeding of medium under multiple culture conditions; followed by spectral identification by MALDI-TOF mass spectrometry. Culturomics has developed to complement the metagenomics sequencing techniques. It has a wide detection range that includes all the living eubacteria and archaea bacteria. MALDI-TOF mass spectrometry represents the corner stone in the workflow of the culturomic studies, which permits fast; accurate, and cheap microbial identification. Pyro-sequencing is still authorized for bacterial identification; especially when MALDI-TOF fails. Through several approaches; the environmental microbiology represents a rich and renewable source of media for the clinical microbiology and culturomics. Exploring the huge repertoire of the human gut microbiome represents a great challenge that is achieved by culturomics. High throughput enriched media with the addition of selective materials are mandatory to isolate the minor communities. Culturomics followed by taxonogenomics for the novel microbial species will design the future of the microbiological research. Culturomics of the human gut microbiota can be used as a bactriotherapy for the inflammatory bowel diseases and as an immunomodulatory for cancer therapy. Moreover; culturomics is a big store for discovery of the new antibacterial agents and the antibacterial resistance genes.

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Conflict of interest

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The author (N.M.M.) did Conceptualization; Data curation; Investigation; Supervision; Validation; Roles/Writing-original draft; Writing - review & editing.

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