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Hot Topic

Culturomics provides critical prokaryote strains for anti-Listeria and anti-cancer probiotics

The identification of a consortium of 11 human bacterial strains that stimulate anti-infectious and anti-cancer immunity by colonizing the cecal and colic mucus and activating CD103$^+$ dendritic cells of the gut mucosa provides a significant opportunity for probiotic-based interventions [1]. This activation is a highly specific and non-inflammatory process, with a highly effective mucosal and systemic IFNγ^+ CD8$^+$ T cells immune response. This immune response is critical against intracellular bacteria, such as *Listeria monocytogenes*, and cancer, particularly in the context of treatment by immune checkpoint inhibitors. Colonization of axenic mice by this consortium was associated with a persistent response in the long-term (up to 6 months). For *L. monocytogenes* infection, colonization by this consortium enabled an evolution towards cure, which was reversed by anti-CD8 monoclonal antibodies. The anti-tumor effect was observed even in the absence of anti-PD1 treatment in favor of a direct anti-cancer effect, even if the synergistic effect of anti-PD1 associated with the microbial consortium was maximal and prevented appearance of the syngeneic tumor after inoculation. This anti-cancer effect was also totally reversible when anti-CD8 antibodies were administered. This illustrates the potential of culturomics in investigating the role of microbiota in immunity and particularly in providing strains for study and treatment [2].

The consortium of 11 human bacterial strains was composed of 4 strains considered as effectors, and 7 strains acting as potentiators. Among these 11 strains, 2 (18%) corresponded to strains isolated recently by culturomics [2]. *Alistipes senegalensis,* isolated in 2012, is one of the 7 potentiator species, and we named *Ihuella massiliensis* ("*Ruminococcaceae* bacterium cv2"), one of the bacterial effectors, isolated in 2015. These bacterial strains are available in our international collection of strains (Collection de Souche de l'Unité des Rickettsies = CSUR) under CSUR P156 and CSURP1486, respectively, and are available to any worldwide researcher or medical doctor for study and treatment. Nevertheless, caution must be maintained in these experimental models because the test of anti-*Listeria* activity of 3 of these strains (*Alistipes senegalensis, Ihuella massiliensis* and *Phascolactobacterium faecium*) against 6 different strains of *L. monocytogenes* was not demonstrated in agar plates (Fig. 1), which indicates an indirect host-immunity-mediated effect cannot be ruled out.

Surprisingly, this interferon response of the digestive mucosa had also been described for the probiotic effect of *Lactobacillus casei* BL23 and *L. paracasei* CNCM I-3689 [3]. However, according to Occam's razor principle, which postulates that a simpler explanation is more likely to be true, this probiotic effect against **orally administered** *L. monocytogenes,* **as in the experiment by Tanoue et al.,** is more likely related to the role of bacteriocins, which are a characteristic feature of lactic acid bacteria [3]. This is worth future investigations because although *Listeria* is no longer a public health problem in Europe and the USA, an epidemic of more than 800 cases and 80 deaths is ongoing in South Africa [4].

Whatever the physiopathological mechanism, culturomics **provides new cultured strains** and is becoming a key partner in the discovery of anti-cancer probiotics **and in other preventive or therapeutic fields**. These strains are **associated with an immune checkpoint inhibitor promoting effect** and belong to *Bifidobacterium* species *(B. longum, B. breve, B. adolescentis), Enterococcus hirae, Bacteroides fragilis, Bacteroides thetaiotaomicron, Barnesiella intestinihominis* or *Akkermansia muciniphila* [5-8].

The availability through culturomics of three other strains that are very similar to *Ihuella massiliensis (Ruminococcaceae* bacterium cv2) in our collection expands the opportunities to reproduce the effect or identify a strain-dependent effect [9,10]. Two new strains very close to *Ruminococcacea* bacterium cv2 have been described by another Russian team, probably by work inspired by culturomics, and have led to the description of a new species but their anti-cancer and anti-*Listeria* effect is worth investigating [11]. These six strains provide an unparalleled opportunity to test and develop new anti-cancer probiotics. This is proof of concept that culturomics offers the probiotics of the future.

The paradigm has changed (Figure). The first probiotic strain isolated and widely marketed (*L. casei* strain shirota) was described in the 1930s by Minoru SHIROTA using a targeted human stool culture approach. The approach was selective on a bacterium resistant to gastric juice that reached the digestive tract alive. We propose a new model for the discovery of new anti-Listeria and anti-cancer probiotics (Fig. 2):

1. ***The unsupervised extension of the repertoire***: Culturomics aims at the unsupervised extension of the human microbial repertoire. 500+ new human species have been isolated in our center because of this high throughput approach based on ultra-fast identification by MALDI-TOF and spectral clustering. New species are rapidly identified, and genomes are sequenced and deposited in worldwide databases available for the community. No attention is paid to the clinical relevance of any

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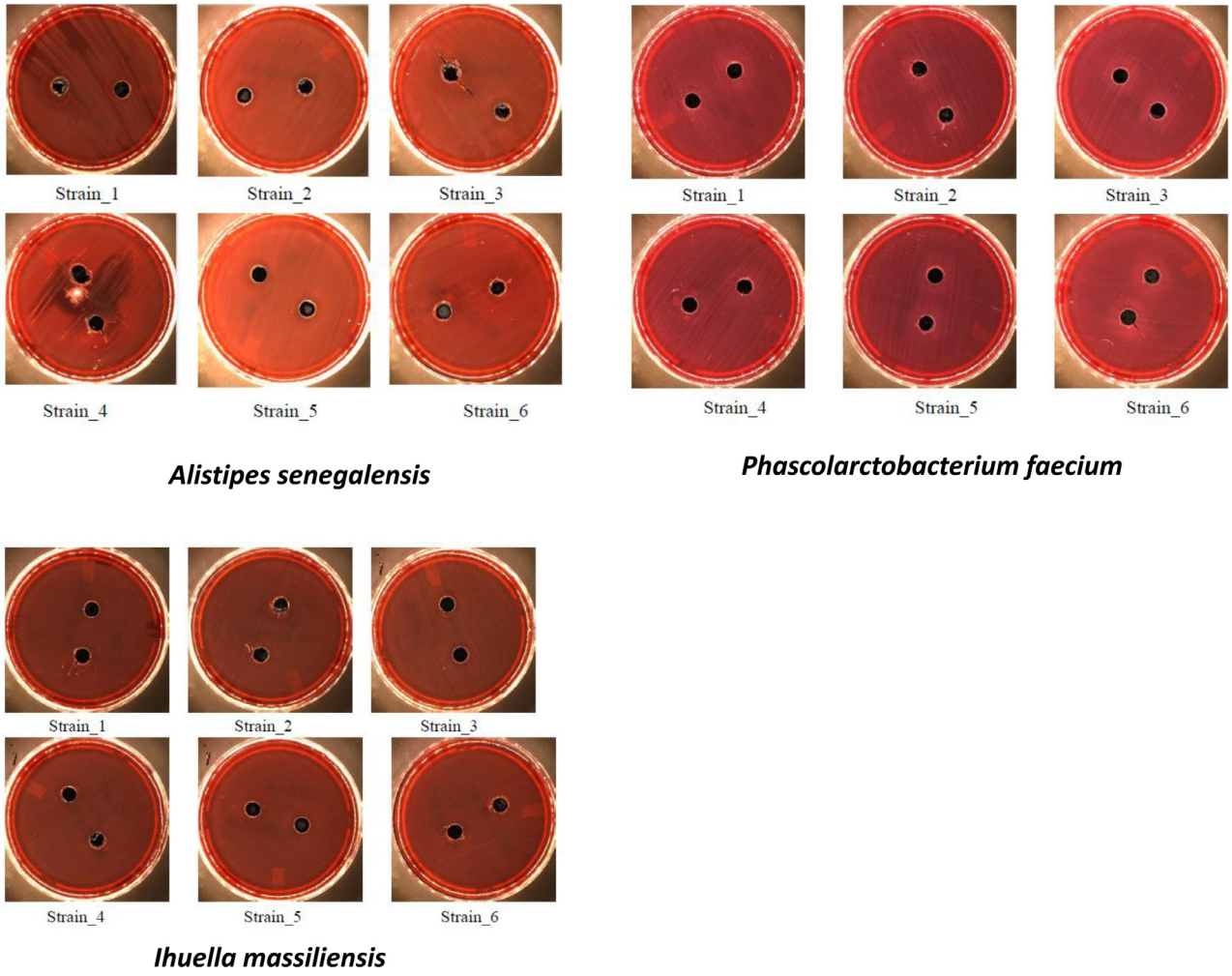

Fig. 1. Summary diagram of the extension of the repertoire to the potential modification of the microbiota by probiotics.

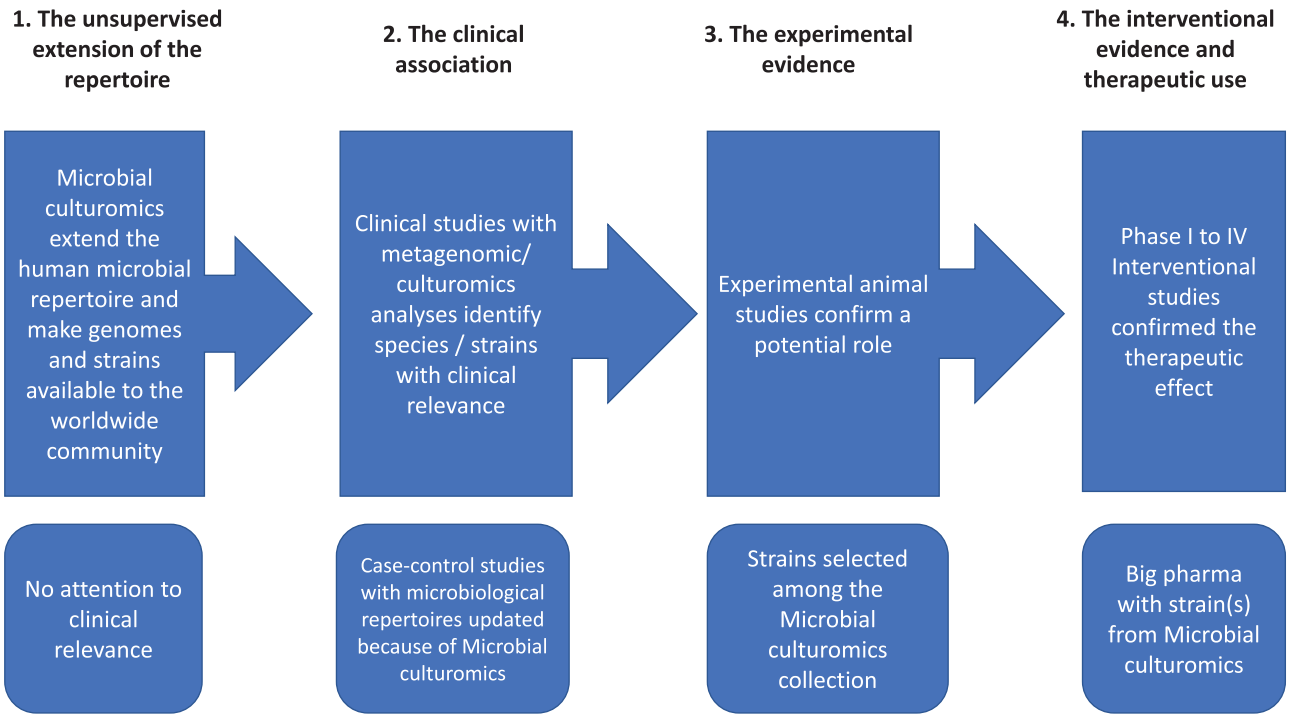

Fig. 2. In vitro activity of *Alistipes senegalensis, Phascolarctobacterium faecium*, and *Ihuella massiliensis* against 6 *Listeria monocytogenes* strains.

strain or species. The goal is to discover all the human microbes, whatever their effect.

2. ***Clinical association***: Clinical studies on different diseases using untargeted strategies (culturomics or metagenomics) identify species / strains with clinical relevance. This is best done with case-control studies with microbiological repertoires updated because of culturomics. For example, the Silva_132 (latest release) database includes increasing numbers of culturomic species. In the study by Tanoue et al. [1], six human donors were included, discriminating those with high or low IFNγ^+ CD8$^+$ T cell response in transplanted axenic mice.
3. ***Experimental evidence***: Experimental animal studies confirm a potential role of species / strain identified in clinical association studies. These experimental studies use strains isolated in the previous step or available in the culturomic collection.
4. ***The interventional evidence and therapeutic use***: Phase I to IV interventional studies confirmed the therapeutic effect. Big pharma can use strain(s) available in the culturomic collection.

This work once again highlights the need to obtain microorganisms in pure culture as a starting point for studies investigating the relationships between human microbiota and human diseases. Culturomics has been striving for nearly 10 years to explore the gut microbiota in depth. Indeed, culturomics has made it possible to isolate more than 1200 bacterial species from the human gut, including 501 new bacterial species [12]. This study should prompt more teams to develop culturomics.

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Declarations

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Declaration of Competing Interests

None.

Ethical Approval

Not required.

Jean-Christophe LAGIER*, Matthieu MILLION*, Amadou H. TOGO, Saber KHELAIFIA, Didier RAOULT#

Aix Marseille Univ, IRD, Microbes, Evolution, Phylogeny and Infection (MEPHI), Marseille, France

IHU Méditerranée Infection, Marseille, France

#To whom correspondence should be addressed. Prof. Didier RAOULT, IHU Méditerranée Infection, 19-21 boulevard Jean Moulin, 13385 Marseille cedex 5, France, Tel: +33 (0)4 13 73 24 01, Fax: +33 (0)4 13 73 24 02.

* contributed equally

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