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**Bacterial culture through selective and non-selective conditions: the evolution of culture
media in clinical microbiology**

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agents.

1 **Abstract**

2 Microbiology has been largely developed thanks to the discovery and optimization of culture
3 media. The first liquid artificial culture medium was created by Louis Pasteur in 1860.

4 Previously, bacterial growth on daily materials such as some foods had been observed. These
5 observations thus highlighted the importance of the bacteria's natural environment and their
6 nutritional needs in the development of culture media for their isolation. A culture medium is
7 essentially composed of basic elements (water, nutrients), to which must be added different
8 growth factors that will be specific to each bacterium and necessary for their growth.

9 The evolution of bacterial culture through the media used for their culture began with the
10 development of the first solid culture medium by Koch, allowing not only the production of
11 bacterial colonies, but also the possibility of purifying a bacterial clone. The main gelling
12 agent used in solid culture media is agar. However, some limits have been observed in the use
13 of agar because of some extremely oxygen sensitive bacteria that do not grow on agar media
14 and other alternatives were proposed and tested. Then, the discovery of antimicrobial agents
15 and their specific target induced the emergence of selective media. These inhibiting agents
16 thus make it possible to eliminate undesirable bacteria from microbiota and select the bacteria
17 desired. Thanks to a better knowledge of the bacterial environment, it will be possible to
18 develop new culture media and new culture conditions, better adapted to certain fastidious
19 bacteria, difficult to isolate.

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25 **Introduction**

26 The discovery of culture media allowed the development of microbiology in the 19th century
27 (1). Bacterial culture was the first method developed to study the human microbiota (2) using
28 an artificial medium that allows growth and isolation of bacteria. The first to have cultured a
29 bacterium in a reproducible way was Louis Pasteur in 1860 thanks to the development of the
30 first so-called artificial culture medium (3). Recently, after the emergence of molecular
31 techniques in the 1970s, such as PCR, sequencing and more particularly metagenomics,
32 microbiologists have favoured these innovative techniques to the detriment of culture.
33 Nevertheless, metagenomics presents certain disadvantages and in particular a depth bias, due
34 to the lack of sensitivity of the primers used, since it does not detect bacteria present at
35 concentrations below 10^5 bacteria per gram of stool (2). Moreover, these techniques only
36 detect DNA: it is impossible with these techniques to differentiate DNA belonging to living
37 bacteria, from that of the transient bacteria of the microbiota studied or from that of dead
38 bacteria.

39 A few years ago, culturomics, a new culture technique, which uses a very large number of
40 culture media and culture conditions to extend the repertoire of bacteria, was developed in our
41 laboratory (2). This technique demonstrates the complementarity between metagenomics and
42 culturomics. Therefore, the metagenomic identification of bacterial species existing in a given
43 microbiota can be exploited by culturomics through the optimization of new specific culture
44 media for the isolation of these species. This complementarity allows culturomics to become a
45 targeted technique.

46 Nowadays, new culture media mimic the natural environment of bacteria by adding different
47 elements in culture medium to cultivate bacteria previously uncultivated.

48 We propose here a bibliographical review of culture media and techniques evolution through
49 the development of microbiology over time.

50 **I. Empirical approach of microbiology**

51 **I.1. Observational microbiology**

52 Microbiology is defined by the organisms it studies, but also by the tools used to study them.

53 The first observation of a bacterium was made around 1673 by the German microscopist

54 Anton van Leeuwenhoek thanks to the microscopes he had developed. Those enlarged from

55 50 to 300 times what he observed (1, 3).

56 In the course of his research, he highlighted small structures that he called "animalcules" (4),

57 because he thought he was observing small animals (5). Throughout his observations, he

58 described and designed yeast cells, filiform fungi, microscopic algae and protozoa (4).

59 Leeuwenhoek was also the first to observe a parasite, *Giardia lamblia* (3). However,

60 microscopy alone cannot address all the questions about the microorganisms studied. For

61 about 200 years, microbiology has been stagnating until the development of microbial

62 isolation techniques in pure cultures. This is another important milestone in the history of

63 microbiology.

64 **I.2. Cultural microbiology**

65 **I.2.1. The birth of culture broth**

66 In the 13th century AD, 400 years before Leeuwenhoek, a blood-like substance appeared on

67 the communion bread and, into beliefs, this red substance was assimilated to the blood of the

68 Christ. Bartholomeo Bizio, an Italian pharmacist, solved this mystery in 1817 thanks to

69 advances in microbiology and showed that it was not blood, but a microorganism that he

70 named *Serratia marcescens*. This bacterium appeared as red colonies on bread when stored in

71 a warm and humid atmosphere (6). It is one of the first natural cultures of a bacterium. The

72 origins of culture media date back to the 19th century. Many bacteriologists have tried, with

73 varying degrees of success, to grow bacteria on the food or material on which the
74 microorganism was developed in the first place.

75 The evolution of microbiology has been made possible by challenging the theory of
76 spontaneous generation, according to which living organisms could develop from dead or
77 decaying matter and thus spontaneously appear in cultures. So it was inconceivable to obtain
78 pure cultures. In 1861, Louis Pasteur, a French microbiologist (3), solved this problem
79 through an experiment. He placed nutrient solutions in flasks, heated their necks with flame
80 and stretched them in various ways, keeping the end open to the air. Pasteur boiled the
81 solutions for a few minutes and cooled them. No growth appeared even if the contents of the
82 flasks had been exposed to air. Pasteur noticed that there was no growth because dust and
83 germs had been trapped on the walls of the curved tubes. If the tubes were broken, growth
84 began immediately. Pasteur thus demonstrated the spontaneous generation nonsense and also
85 found ways to keep sterile solutions (3).

86 The first to have cultured bacteria reproducibly in a liquid culture medium was Louis Pasteur.
87 In 1860, he developed a culture medium containing "yeast soup", ashes, sugar and ammonium
88 salts (7, 8). His objective was to create a fermentation medium to demonstrate that each
89 fermentation (alcoholic, acetic, lactic ...) was associated with the development of a particular
90 microorganism (9). The presence of these different elements in the medium allowed him to
91 observe that some of these components could promote or inhibit the growth of certain bacteria
92 and that they could also allow the emergence of certain bacteria compared to others (8).

93 Indeed, in the course of the study of fermentation of beer, he was confronted with a problem:
94 when the beer was healthy, the microscope only showed brewer's yeast, but when the beer
95 was acid, Pasteur noticed that the tanks contained "tiny rod-shaped objects" producing lactic
96 acid. This fermentation medium therefore made it possible to highlight the multiplication of
97 this bacterium (1, 3).

98 In 1881, Robert Koch demonstrated optimal growth of bacteria when they were incubated in a
99 broth composed of fresh beef serum or meat extract. However, the use of a liquid culture
100 medium did not produce pure bacterial cultures. Koch therefore sought a way to solidify the
101 media (8) (figure 1).

102 **I.2.2. Emergence of pure cultures in solid media**

103 First, he tested coagulated egg albumin, starch paste or an aseptically cut slice of potato.
104 However, not all these techniques allowed him to isolate colonies. Koch then added gelatin to
105 his broth and poured it all over a flat glass plate. However, gelatin had disadvantages since it
106 liquefied at temperatures above 25°C and could be consumed by gelatinase, an enzyme
107 produced by certain bacteria. Thanks to the wife of one of his assistants, Fannie Hesse, who
108 used agar to solidify his jams, he replaced gelatin with agar, which allowed him to obtain firm
109 agars and isolate bacteria. In 1887, Julius Richard Petri replaced the glass plate with a circular
110 culture box, the Petri box has just been created, and is still used today (8). This allowed him to
111 obtain and observe isolated colonies and to limit contamination.

112 **II. How to design an enriched culture medium?**

113 An enriched culture medium is a medium to which have been added elements essential for the
114 growth of bacteria.

115 **The nutrients**

116 In order to grow, bacteria need a minimum of nutrients: water, a carbon source, a nitrogen
117 source and some mineral salts (10).

118 **Water**

119 Water plays a fundamental role in solubilizing nutrients, transporting them and ensuring
120 hydrolysis reactions. Some bacteria need free water for their growth. If evaporation occurs

121 during the incubation of the agar, there may be a loss of this water, resulting in a decrease in
122 colony size and inhibition of bacterial growth (10, 11).

123 **Carbon sources**

124 Carbon is the most abundant constituent element in bacteria. It is essential for bacteria to
125 produce carbon molecules, such as fats, carbohydrates, proteins and nucleic acids. Bacteria
126 can use inorganic carbon sources, such as carbon dioxide, or organic sources such as sugars
127 and alcohols (10, 12).

128 **Nitrogen sources**

129 As for nitrogen sources, they are numerous and can be found in a large number of compounds
130 used in the composition of a culture medium. It is found in the organic form, corresponding to
131 protein hydrolysates, particularly in case of hydrolysate, proteose-peptone or tryptone (12),
132 but also in an inorganic form, nitrates (13). Nitrogen allows bacteria to synthesize their
133 proteins.

134 Finally, among the common mineral salts, phosphate, sulphate, magnesium or calcium (11)
135 are regularly found.

136 **Energy sources**

137 There are two types of bacteria, phototrophic bacteria, such as *Thiocapsa roseopersicina* (14)
138 which uses light as an energy source by transforming it into an electrochemical gradient of
139 protons (14) and chemotrophic bacteria which uses the energy of oxidation of mineral or
140 organic compounds as energy sources (15, 9). Among these bacteria, we can find *Listeria*
141 *monocytogenes* (16).

142 **Growth factors**

143 The use of a minimal medium does not allow the growth of certain bacteria that need specific
144 elements to grow. It is sometimes necessary to add growth factors to culture media in order to
145 boost the multiplication of bacteria. Growth factors are elements that bacteria are unable to
146 synthesize from the nutrients available in the environment (10, 17). Growth factors are
147 required in small quantities in the culture medium and their need is justified by the absence or
148 blocking of a metabolic pathway in the bacterium.

149 **Purine and pyrimidine bases**

150 There are different categories of growth factors among which we find purine and pyrimidine
151 bases. They are necessary for synthesis of nucleic acids. Indeed, some lactic acid bacteria
152 need adenine, guanine, thymine or uracil for growth (17). This is the case in particular for the
153 bacterium *Leuconostoc mesenteroides*, for which guanine is essential for its growth (18).

154 **Amino acids**

155 Amino acids are also growth factors and are used for protein synthesis. Dunn et al (19)
156 showed in 1946 that only 2 amino acids were essential for *Leuconostoc mesenteroides*,
157 glutamic acid and valine, while for *Lactobacillus brevis*, no less than 15 amino acids were
158 necessary for its growth. This high requirement for amino acids can be explained by the fact
159 that the base medium used at the time was not rich in common nutrients. Indeed, nowadays,
160 *Lactobacillus brevis* grows on a COS agar (Columbia Blood Agar) (Biomérieux, France), not
161 supplemented with amino acids (20), but composed of casein hydrolysate and peptone
162 proteose, themselves sources of amino acids (11).

163 **Vitamins**

164 Vitamins are also part of growth factors. They are coenzymes or precursors of coenzymes. A
165 vitamin is an organic substance, necessary in small quantities for the metabolism of a living
166 organism, which cannot be synthesized in sufficient quantity by that organism.

167 *Faecalibacterium prausnitzii*, which is a tedious bacterium, requires a large number of
168 vitamins to grow, such as biotin, folic acid, riboflavin or vitamin B12 (21). This bacterium
169 also requires the presence of other growth factors such as volatile fatty acids (acetic acid,
170 propionic acid or valeric acid) (21).

171 **Blood use and its derivatives**

172 Blood and its derivatives will also boost the growth of certain bacteria. In culture media, it is
173 common to use sheep's blood or horse's blood. The role of blood is to act as a protective agent
174 against toxic oxygen radicals (22), but also as a nutritional supplement. For instance, cooked
175 sheep's blood provides the factor X (heme) necessary for the growth of many pathogenic
176 species, including *Haemophilus influenzae* (23). Most often, 5% blood is added to the culture
177 media (22). Serum, such as fetal calf or lamb serum, can also be used as a growth factor by
178 providing a large number of elements such as lipids, vitamins, triglycerides, minerals and
179 others (24). This blood has been cleared of cells, platelets and clotting factors. Fetal calf
180 serum, which is most commonly used in microbiology (25, 26), must be inactivated by heat
181 before use (56°C for 30 min) in order to inactivate all components of the complement system
182 present in this serum (27, 28). It must be stored at -20°C and heated to room temperature
183 rather than in a water bath to avoid deterioration of the proteins it contains.

184 **Rumen fluid**

185 Finally, it is also possible to use the rumen fluid (29, 2) to promote the growth of certain
186 bacterial species by mimicking their natural environment. The rumen fluid used corresponds
187 to the rumen of the sheep. To prepare it, it is first necessary to recover the juice from the
188 fermented plants in the stomach by filtering it through a fine cloth. This juice is then
189 centrifuged (10,000 rpm for 90 min) and the supernatant collected. Then, it undergoes 3
190 successive filtrations at 0.8µm, 0.45µm and 0.22µm (2, 30). 5% rumen juice is usually added

191 to the culture media. The rumen fluid can promote the growth of certain *Treponema*, such as
192 *Treponema hyodysenteriae* and *Treponema innocens* (31).

193 **Antioxidants**

194 Some anaerobic bacteria are fastidious to grow and new culture strategies have been
195 developed to isolate them. Indeed, anaerobic bacteria are most abundant in the human
196 intestinal microbiota, accounting for up to 99.9% of total bacteria (32) and are extremely
197 sensitive to oxygen, which is toxic to them. Antioxidants have therefore been added to
198 culture media to allow the culture of strict anaerobic bacteria under aerobic conditions (33,
199 34). A number of antioxidants have been tested and have shown satisfactory results. This is
200 the case for ascorbic acid and glutathione (34) or uric acid (35). The addition of these
201 antioxidants to the culture medium and its incubation under aerobic conditions allowed the
202 growth of 135 strict anaerobic bacteria, 12 microaerophilic bacteria and 22 strict aerobic
203 bacteria (35).

204

205 **III. How can we have a selective culture medium?**

206 A selective culture medium is used to isolate a particular bacterial species or genus. After the
207 addition of a number of inhibitors to the culture medium, the objective of this type of media is
208 to eliminate unwanted microbial flora. The selective medium is composed of a basic medium
209 to which antibiotics, chemicals, dyes, antiseptics, sodium salts or phages can be added (36).

210 **Antibiotics**

211 Antibiotics are the most commonly used selective agents. Their spectrum of action being well
212 known, it is easier to anticipate their action on bacteria. There are a large number of
213 antibiotics that can be used in culture media, some of which are called antibacterial because
214 they target bacteria and others antifungal because they eliminate fungi and yeasts (37). Some

215 molecules target Gram positives, such as penicillin G, bacitracin or vancomycin, while others
216 target Gram negatives such as colistin or polymixin B. Amphotericin B, cycloheximide or
217 nystatin have an action against fungi and yeasts (Table 1) (39, 10). Several antibiotics can be
218 combined to obtain a more selective medium (37).

219 **Antiseptics**

220 Antiseptics are more rarely used in culture media. However, cetrimide (43, 44) or acriflavin
221 (10) can be found in some culture media. Chlorhexidine can be used to select *Mycobacterium*
222 *tuberculosis* (45, 46) (Table 2). Ethanol can also select bacterial species, including sporulated
223 bacteria (51, 52) such as *Clostridiodes difficile* (51).

224 Cetrimide Agar Base is a culture medium used to selectively isolate and identify
225 *Pseudomonas aeruginosa*. Cetrimide is a quaternary ammonium that inhibits a large number
226 of bacteria, including those of the genus *Pseudomonas*, other than *P. aeruginosa* (10).

227 **Sodium salts**

228 Sodium salts are known for their inhibitory properties. The best known is sodium chloride,
229 used to select halophilic bacteria that resist very high amounts of salts (53). In addition,
230 sodium deoxycholate has a strong solvent action on bacteria (54) (Table 3).

231 The Marine Agar 2216E culture medium is used to enumerate marine heterotrophic bacteria.
232 It is composed of a high concentration of salt, which eliminates a large number of bacteria and
233 preserves marine bacteria of interest (55).

234 **Chemical substances**

235 Chemical substances can be added to culture media to inhibit certain bacteria. These
236 inhibiting substances include potassium tellurite and bile salts, which inhibit Gram-positive
237 bacteria (47, 10, 39) or lithium chloride (10, 39), which eliminates Gram-negative bacteria
238 (Table 4).

239 Brayton *et al* (48) have created a selective culture medium for *Vibrio vulnificus*, which is a
240 pathogenic halophilic bacterium. This medium, VV agar, consists, among other things, of
241 potassium tellurite as selective agent for inhibiting *Enterobacteriaceae*.

242 **Dyes**

243 Dyes can be used as a color indicator in a culture medium or as a selective agent against
244 certain bacteria. Cristal violet is one of the most commonly used dyes to inhibit bacteria (37,
245 49). Malachite green and methylene blue are also used to inhibit Gram positive and negative
246 bacteria and Gram positive bacteria respectively (10, 50) (Table 5).

247 A selective medium of *Streptococcus pneumoniae* has been developed, containing crystal
248 violet. This dye is used to select *Streptococci* and inhibit *Staphylococci* as well as other Gram-
249 positive bacteria (49).

250 **Phages**

251 Bacteriophages are specific viruses of bacteria that can infect and even destroy bacteria, in the
252 case of lytic phages. In order to isolate *Mycobacterium tuberculosis*, the use of phage lysin
253 decontaminates the sputum of other bacteria present in the pulmonary microbiota (56).

254 Sillankorva *et al* (57) worked on permanent urinary tract infections due to *Escherichia coli*. In
255 order to treat these infections, they tested different phages (T1, T4, and phiX174 like phages)
256 against *E. coli*. After 2 hours of treatment, phage T1 reduced the *E. coli* population by 45%,
257 thus demonstrating the efficacy of this selective agent.

258

259 **IV. Disadvantages of gelling agents use**

260 **IV.1 Liquid culture media versus solid culture media**

261 There are two main types of culture media, liquid and solid culture media.

262 In liquid culture media, also called culture broths, nutrients are dissolved in water. The growth
263 of bacteria in this type of medium can be demonstrated by the appearance of a turbidity in the
264 medium, although this is not always the case. It is difficult to isolate a bacterium specifically
265 in this type of medium. Indeed, the bacteria obtained from a sample inoculated into the culture
266 broth are all mixed with each other. In addition, this type of culture medium does not allow
267 the morphological characteristics of bacterial species to be identified (9).

268 However, liquid culture media facilitate access to nutrients for bacteria. These nutrients are all
269 the more accessible as the culture media are incubated under agitation, allowing a renewal of
270 nutrients for bacteria.

271 Solid culture media are obtained by adding a gelling agent, such as agar, to the culture broth.
272 They make it possible to obtain isolated colonies of different bacterial species, which can be
273 identified. The different morphological characteristics of the bacterium can be described from
274 these cultures (9, 58). However, in solid culture media, access to nutrients for bacteria may be
275 limited. Media with high gel content, such as agar, will form smaller colonies than low gel
276 content media because nutrient flow and toxin removal are reduced (7).

277 In addition, it has been shown that agar, in excessive quantities, can inhibit the growth of
278 certain bacteria, highlighting the need to find other gelling agents (9).

279 **IV.2 Different gelling agents**

280 One of the first gelling agents used in culture media was gelatin. The problem with this
281 gelling agent is that it melts at 37°C, which is the incubation temperature of most bacteria.

282 Moreover, the presence of an enzyme in certain bacteria, gelatinase, causes the digestion of
283 gelatin and therefore its degradation. Agar was then used in culture media. However, over-
284 consumption of agar has led to a reduction in its source, red algae (59), thus increasing costs.

285 In addition, agarase, present in some bacteria, destroys agar, thus preventing the isolation of
286 these bacteria (60). Moreover, agar can inhibit the growth of some anaerobic bacteria because

287 inhibitory growth compounds can be produced from autoclaving phosphate with agar (61).
288 Finally, agar can cause inhibition of PCR of fungal DNA when extracted directly from the
289 solid culture medium (62). For all these reasons, new gelling agents have been sought. These
290 gelling agents include κ -carrageenan, ι -carrageenan, sodium alginate, HM (high-methoxyl)
291 and LM (low-methoxyl) pectins, gellan gum (60, 63). All these gelling agents have different
292 properties and particular needs to gel (Table 6).

293 **Carrageenan gums**

294 κ -carrageenan and ι -carrageenan are part of the carrageenan gums.

295 The first will form a firm gel with a rapid mass build-up when combined with potassium ions.
296 It allows the growth of some bacteria. This gelling agent resists very alkaline pH values above
297 12.5, thus isolate highly very alkalineophilic bacteria (64). It can be used to replace agar
298 because many bacteria grow on κ -carrageenan-based media (65).

299 ι -carrageenan is rarely used because it gives elastic gels that make bacterial culture difficult
300 (65).

301 **Sodium alginate**

302 This gelling agent is produced from brown seaweed extract and form a flexible gel in the
303 presence of calcium ions. However, this gelling agent does not provide a gel firm enough to
304 grow bacteria (63).

305 **HM and LM pectins**

306 HM pectins require sugar and high acidity to gel. Gel setting is slow and results in the
307 formation of a "spreadable" gel.

308 LM pectins form brittle gels in the presence of Ca^{2+} (62).

309 **Gellan gum**

310 Gellan gum is a polysaccharide produced by a bacterial genus, *Sphingomonas spp.* According
311 to Tamaki *et al* (66), 108 bacteria tested on media with gellan gum as gelling agent showed
312 growth.

313 The use of these different gelling agents could allow the culture of new bacteria, which do not
314 grow on the agar, because of the presence of an agarase for example or because the agar
315 forms a network too dense to allow motility and optimal growth of certain bacteria (9).

316 **IV.3 Colony size**

317 The amount of nutrients available in a culture medium will determine the size of bacterial
318 colonies (58). An overly firm culture medium, due to a high concentration of gelling, causes a
319 decrease in the flow of nutrients and thus a decrease in the access of these nutrients to bacteria
320 (7, 9). On the other hand, in some culture media, the amount of nutrients available is too high
321 and can be toxic for certain bacteria that necessitate a poor culture medium to grow (68).

322 Microcolonies are colonies that are barely visible to the naked eye (between 100 and 300 μm
323 in diameter). To obtain larger colonies, it is sometimes necessary to mimic the bacterium's
324 natural environment by providing it with specific elements. This is the case, for example, for
325 *Phascolarctobacterium faecium* and *Phascolarctobacterium succinatutens*, which form
326 microcolonies. However, when the medium is supplemented with succinate, the colonies have
327 a diameter ranging from 0.8 to 1.2 mm (69, 70).

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333 **Conclusion**

334 After a stagnation in the development of new culture techniques, due to the rapid evolution of
335 new microbiological methods such as metagenomics, bacterial culture is experiencing a new
336 boom. In recent years, culturomics, with the use of new culture media and new culture
337 conditions, has enabled the enrichment of the bacterial repertoire through the isolation of new
338 bacterial species. This shows that, despite the abandonment of culture by a large number of
339 microbiologists, culture media remain a fundamental tool for bacteriologists for the isolation
340 of commensal but also pathogenic bacteria.

341 Studying the natural environment of bacteria that remain uncultivated to date would be
342 interesting because it would provide the essential elements for the bacteria to grow. Indeed,
343 although there are many enriched culture media, each bacterium is unique and requires
344 specific needs. The use of new gelling agents could also allow the isolation of new species for
345 which agar was not suitable for their growth. Although many gelling agents have been tested,
346 few are still used in commercial culture media and therefore in laboratories. Many
347 developments in bacterial culture are therefore still to come, making it possible to enrich the
348 bacterial repertoire and gain a better understanding of certain diseases.

349 In addition, intracellular bacteria such as *Coxiella burnetii* or *Tropheryma whipplei* require a
350 host cell to survive and multiply. Some of these bacteria cause severe diseases and pose a
351 diagnostic problem because of their fastidious growth or lack of growth on conventional
352 media (71). It would therefore be interesting to develop culture media that allow faster and
353 easier detection of these bacteria. In addition, as the microbiota plays an increasingly
354 important role in human health (72, 73), the development of probiotics is on the rise (74). The
355 use of targeted culture media to select certain bacteria with an important medical role
356 therefore remains a priority.

357

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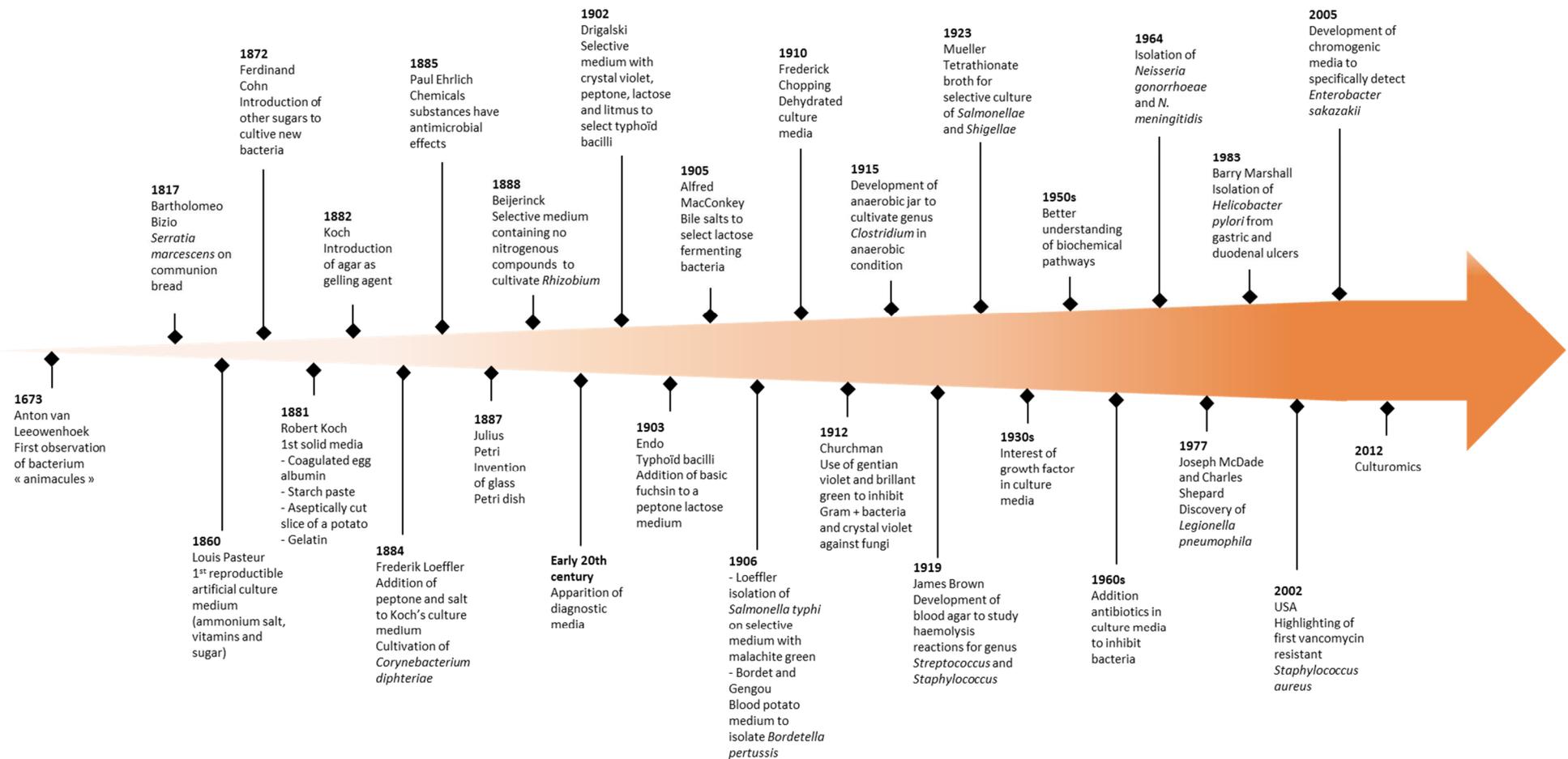


Figure 1: Evolution of culture media: from the first bacterial culture (1673) to culturomics.

Table 1: Antibiotics agents used in bacterial culture: ✓ Action against bacteria or fungi ✗ No action against bacteria or fungi

Inhibitors			Microorganisms			Example of culture media	References
			Gram +	Gram -	Fungi, yeast		
Antibiotics	Penicillins	Penicillin G	✓	✗	✗	*A7 Agar modified	ECN Pilly 2018 (38) Power et al, 2009 (11)
		Cephalosporins	Cefalotin (1st generation)	✓	✓ <i>Enterobacteria</i>	✗	*Cefalotin buffered dextrin broth
	Cefamandole (2nd generation)		✓	✓ <i>Enterobacteria</i>	✗	*BCYE supplemented with Cefamandole	Bartram et al, 2007 (42)
	Cefixime (3rd generation)		✓ Cocci	✓ Bacillus	✗	*MacConkey Sorbitol agar (CT-SMAC)	Delarras, 2014 (39)
	Ceftazidime (3d generation)			✓	✗	*Palcam medium	Delarras, 2014 (39)
	Carboxypenicillins	Ticarillin	✓	✓ Aerobic bacillus	✗	*PC agar	Power et al, 2009 (11)
	Diaminopyrimidines	Trimethoprim	✓	✓	✗	*Bolton broth * Preston Agar and Broth	Delarras, 2014 (39)
	Nitrofurane	Nitrofurantoin	✓ Especially those of the urinary tract	✓ Especially those of the urinary tract	✗	*Differentiation test	Delarras, 2014 (39)
	Phenicolos	Chloramphenicol	✓	✓	✗	*Yeast Extract Glucose Chloramphenicol Agar *Chloramphenicol Sabouraud Agar	Delarras, 2014 (39)
	Rifamycines	Rifampicin	✓	✓ Other than Enterobacteria	✗	*Preston Agar and Broth	Delarras, 2014 (39)
Tetracyclines	Oxytetracycline	✓	✓	✗	*OGYE Agar Base	Power et al, 2009 (11)	
Macrolides	Erythromycin	✓	✓	✗	Gallery API Campy Biomérieux	Delarras, 2014 (39)	

Inhibitors		Microorganisms			Example of culture media	References	
		Gram +	Gram -	Fungi, yeast			
Antibiotics	Polypeptides	Polymyxin B	✗	✓ Aerobic	✗	*PALCAM Medium Base *SPS Agar *TSN Agar	Power et al, 2009 (11)
		Colistin	✗	✓ Aerobic	✗	*Oxford Agar *Campyloel Agar *Thayer-Martin Selective Agar	Power et al, 2009 (11)
		Bacitracin	✓		✗	*Chocolate II Agar with Bacitracin *Haemophilus Isolation Agar with Bacitracin	Power et al, 2009 (11)
	Quinolones	Nalidixic acid	✗	✓ Enterobacteria	✗	*Todd Hewitt Broth with Gentamicin and Nalidixic Acid *UVM Modified Listeria Enrichment Broth *Columbia CNA Agar	Power et al, 2009 (11)
	Aminosides	Neomycin	✓ Aerobic	✓ Aerobic	✗	*Lecithin Lactose Agar *Neomycin Blood Agar *TSN Agar	Power et al, 2009 (11)
		Gentamicin	✓ Aerobic	✓ Aerobic	✗	*Todd Hewitt Broth with Gentamicin and Nalidixic Acid	Power et al, 2009 (11)
	Glycopeptides	Vancomycin	✓	✗	✗	*Vancomycin Screen Agar *Anaerobe Laked Sheep Blood Agar with Kanamycin and Vancomycin	Power et al, 2009 (11)
		Fosfomycin (Natural antibiotic)	✓ Aerobic	✓ <i>Enterobacteria</i>	✗	*Oxford Agar	Power et al, 2009 (11)
		Novobiocin	✓	✓ Cocci	✗	*CIN Agar *Yersinia Antimicrobial Supplement CN *EC Medium, Modified	Power et al, 2009 (11)
	Antibiotics Antifungal	Amphotericin B	✗	✗	✓	*Campylobacter Antimicrobial Supplement Blaser *Butzler Agar *Campyloel Agar	Power et al, 2009 (11) Delarras, 2014 (39)
		Cycloheximide	✗	✗	✓	*Brain Heart CC Agar *Dermatophyte Test Medium Base *m E Agar	Power et al, 2009 (11)
		Nystatin	✗	✗	✓	*Thayer-Martin Selective Agar	Power et al, 2009 (11)

Table 2: Antiseptics used in bacterial culture: ✓ Action against bacteria or fungi ✗ No action against bacteria or fungi

Inhibitors		Microorganisms			Example of culture media	References
		Gram +	Gram -	Fungi, yeast		
Antiseptics	Chlorhexidine	✓	✓	✗	*Selection of <i>Mycobacterium tuberculosis</i>	Asmar et al, 2015 (44) Asmar et al, 2016 (45)
	Cetrimide	✓	✓	✗	*Cetrimide Agar Base *CN Agar	Power et al, 2009 (11) Delarras, 2014 (39)
	Acriflavin	✓	✗	✗	*Fraser Broth Base *Listeria Enrichment Broth *PALCAM Medium Base	Power et al, 2009 (11)

Table 3: Sodium salts: ✓ Action against bacteria or fungi ✗ No action against bacteria or fungi

Inhibitors		Microorganisms			Example of culture media	References
		Gram +	Gram -	Fungi, yeast		
Sodium Salts	Sodium azide	✗	✓	✗	*Azide Blood Agar Base *Azide Dextrose Broth *m E Agar *EVA Broth	Power et al, 2009 (11)
	Sodium chloride	✓ Except halophilic and halotolerant bacteria	✓ Except halophilic and halotolerant bacteria	✗	*Fraser Broth Base *Mannitol Salt Agar *Marine Agar 2216	Power et al, 2009 (11)
	Sodium deoxycholate	✓	✓	✗	*m TEC Agar *TT Broth Base, Hajna *XLD Agar	Power et al, 2009 (11)
	Sodium citrate	✓	✗	✗	*APT Agar *DCLS Agar *Desoxycholate Citrate Agar	Power et al, 2009 (11)
	Sodium selenite	✓	✓ Other than <i>Salmonella</i>	✗	*Selenite Cystine Broth *Selenite Broth	Power et al, 2009 (11)
	Sodium tetrathionate	✗	✓ Other than <i>Salmonella</i> and <i>Proteus</i>	✗	*MKTn Broth	Delarras, 2014 (39)

Table 4: Chemical substances

Inhibitors		Microorganisms			Example of culture media	References
		Gram +	Gram -	Fungi, yeast		
Chemical substances	Bile salts	✓	✗	✗	*Bile Esculin Agar *EC Medium *m FC Agar and Broth	Power et al, 2009 (11)
	Oxgall	✓	✗	✗	*Bile Esculin Agar *Brilliant Green Bile Agar	Power et al, 2009 (11)
	Lithium chloride	✗	✓	✗	*VJ Agar *Baird-Parker Agar Base *Giolitti-Cantoni Broth Base	Power et al, 2009 (11)
	D-cycloserine	✓	✓	✗	*Cycloserine-cetoxitin-fructose agar *Tryptose Sulfite Cycloserine Agar	Power et al, 2009 (11)
	Irgasan	✓	✓ Other than <i>Pseudomonas</i>	✗	*CIN Agar *Pseudomonas Isolation Agar	Power et al, 2009 (11)
	Tergitol 7	✓	✓	✗	*TTC and Tergitol 7 Lactose Agar	Delarras, 2014 (39)
	Potassium tellurite	✓	✗	✗	*Serum Tellurite Agar *Tellurite Glycine Agar	Power et al, 2009 (11)
	Lauryl sulfates	✓	✗	✗	*m Endo Agar LES *Lauryl Tryptose Broth *Lauryl Sulfate Broth	Power et al, 2009 (11)

✓ Action against bacteria or fungi ✗ No action against bacteria or fungi

Table 5: Dyes: ✓ Action against bacteria or fungi ✗ No action against bacteria or fungi

Inhibitors		Microorganisms			Example of culture media	References
		Gram +	Gram -	Fungi, yeast		
Dyes	Methylen blue	✓	✗	✗	*Eosin Methylene Blue Agar *Levine EMB Agar	Power et al, 2009 (11) Delarras, 2014 (39)
	Eosin	✓	✗	✗	*Eosin Methylene Blue Agar *Levine EMB Agar	Power et al, 2009 (11) Delarras, 2014 (39)
	Crystal violet	✓ Cocci	✗	✗	*MacConkey Agar *Mitis Salivarius Agar *Drigalski medium	Power et al, 2009 (11) Delarras, 2014 (39)
	Ethyl violet	✓	✗	✗	*EVA Broth *Litsky Broth	Power et al, 2009 (11) Delarras, 2014 (39)
	Brilliant green	✓	✓	✗	*Brilliant Green Bile Broth *SS Agar	Power et al, 2009 (11) Delarras, 2014 (39)
	Malachite green	✓	✓	✗	*Mycobacteria 7H11 Agar *Wallenstein Medium	Power et al, 2009 (11) Delarras, 2014 (39)

1 Table 6: Gelling agents used in culture media (60, 63, 67)

2

Origin	Type	Gel texture	Necessary Ions	Gelling temperature	Melting temperature
Red seaweed extracts	Agar	Firm, brittle, transparent Acid-resistant (up to pH 3.5)		< 35°C	> 80°C
Red seaweed extracts	Carrageenans (kappa, iota)	· Kappa Elastic or firm (depending on concentration), transparent, glossy Very fast gel setting	K ⁺	< 40°C	> 65°C
		· Iota Elastic, transparent, reformable	Na ⁺ or K ⁺		
Brown seaweed extracts	Sodium alginate	Flexible gel	Ca ²⁺	Whatever the temperature	Thermo-irreversible
Extracts of vegetable by-products	HM Pectin	Gels in an acidic environment (pH<3) and in the presence of sugar. Slow gel setting "Spreadable" gel		< 65°C	Thermo-irreversible
Extracts of vegetable by-products	LM Pectine	Brittle, transparent	Ca ²⁺		Thermo-reversible
Biosynthetics	Gellan gum	Transparent, shiny, firm. Stable up to pH=3		< 90°C	> 90°C
Animale	Gelatin	Elastic gel, transparent		< 20°C	> 40°C
<i>Xanthomonas campestris</i> bacteria	Xanthan gum (+ carob bean gum)	Stable over a wide temperature and pH range Soft elastic gel in combination with carob bean gum and in the presence of salts			270°C thermo-reversible
Plant exudates	Arabic gum	Soft gel (>10% of final volume) Stable in acidic medium Heating must be limited in time as there is a risk of loss of the gum's gelification capacity			
Animal	Egg			Thermo-irreversible	

3