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ORIGINAL PAPER

Petri dish versus Winogradsky column: a longue durée perspective on purity and diversity in microbiology, 1880s–1980s

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Abstract Microbial diversity has become a leitmotiv of contemporary microbiology, as epitomized in the concept of the microbiome, with significant consequences for the classification of microbes. In this paper, I contrast microbiology's current diversity ideal with its influential predecessor in the twentieth century, that of purity, as epitomized in Robert Koch's bacteriological culture methods. Purity and diversity, the two polar opposites with regard to making sense of the microbial world, have been operationalized in microbiological practice by tools such as the "clean" Petri dish versus the "dirty" Winogradsky column, the latter a container that mimics, in the laboratory, the natural environment that teems with diverse microbial life. By tracing the impact of the practices and concepts of purity and diversity on microbial classification through a history of techniques, tools, and manuals, I show the shifts in these concepts over the last century. Juxtaposing the dominant purity ideal with the more restricted, but continuously articulated, diversity ideal in microbial ecology not only provides a fresh perspective on microbial classification that goes beyond its intellectual history, but also contextualizes the present focus on diversity. By covering the period of a century, this paper outlines a revised longue durée historiography that takes its inspiration from artifacts, such as Petri dish and the Winogradsky column, and thereby simple, but influential technologies that often remain invisible. This enables the problem of historical continuity in modern science to be addressed and the accelerationist narratives of its development to be countered.

Keywords Microbiology · Pure culture · Purity · Classification · Microbial ecology · Petri dish · Winogradsky column

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1 Introduction: mud flasks, microbiomes, and diversity in twenty-first century microbiology

Microbes are becoming popular. While they existed primarily as "invisible enemies" throughout most of the twentieth century, natural history museums have recently started to put them on show in another way: Micropia, Amsterdam's "microbe museum," has devoted an entire building to the invisible life of microbes, while the American Museum of Natural History (AMNH) in New York recently presented a show on "The Human Microbiome"; that is, the communities of microorganisms that populate the human gut, skin, etc.[1] Both of these exhibitions focused on microbial diversity as revealed by genomic analyses of microbial environments. Both shows also featured an exhibit of the Winogradsky column, named for the Russian French microbiologist Sergei N. Winogradsky [Vinogradskii], which gives a perspective of the microbial world as abundant, mixed, and interconnected (see Fig. 1).

A Winogradsky column basically consists of a long jar—the AMNH'S "Make your own microbial medley" instruction recommends a plastic bottle—filled with mud from a pond, to which nutrients (shredded newspaper, egg yolk) and water are added.[2] When placed next to a window for several weeks, different levels in the column will take on different colors, ranging from pitch black to green or purple, for the different communities of microbes (photosynthetic, aerobic/anaerobic, sulfur-metabolizing, etc.) that accumulate under the ecological conditions of the respective zones. The Winogradsky column is a device that enhances growth of certain microbial types in zones of the column, thereby spatially separating them, and rendering them visible to the bare eye by their metabolic activities. The AMNH's accompanying text, however, also uses the column as an analogue to understand the human gut, which is populated by various, specialized bacterial communities.[3] The microbial communities within the column exemplify interacting miniature ecosystems that exchange substances and energy between the different zones they occupy.

Abundance, mixture, variability, and interdependency, in short: diversity—these are the tropes of how the microbial world is pictured by scientists and medical practitioners of our times, and the museums' curators have not been shy about putting a literal piece of dirt and teeming decay on show. What a far cry from the microbes rendered visible by Robert Koch's bacteriology—pure isolates forming characteristic cultures on sterilized surfaces of growth media contained within the

[1] "The secret world inside you," exhibition at the AMNH, Nov. 7th, 2015–Aug. 16th, 2016; on Micropia, see the post on the microbiology blog "Small things considered" at http://schaechter.asmblog.org/schaechter/2016/01/take-your-kids-to-the-bugs-on-a-sunday-afternoon-micropia-the-worlds-only-microbe-zoo-in-amsterdam.html. Last access Aug 28th, 2017.

[2] http://tumblr.amnh.org/post/142650634919/make-your-own-microbial-medley-a-famous.

[3] "The microbiome of your gut"; "Make a home for microbes. How to make a Winogradsky column," texts on the AMNH's website at http://www.amnh.org/exhibitions/the-secret-world-inside-you/the-microbiome-of-your-gut; http://www.amnh.org/explore/ology/microbiology/make-a-home-for-microbes. Last access Mar. 29th, 2017). On microbial communities as models for ecosystems and life in general in recent science, Paxson and Helmreich (2014).

Fig. 1 A Winogradsky column. A laboratory glass cylinder was filled with mud, a carbohydrate source and pond water and incubated at a window sill. The different colors of the column (green, orange-red, black) are indicative of metabolically differing microbes. The microbial ecosystem has been stable for 20 years in a Berlin microbiological laboratory, with the sun as the sole energy source, and only evaporated water refilled. Photo by author

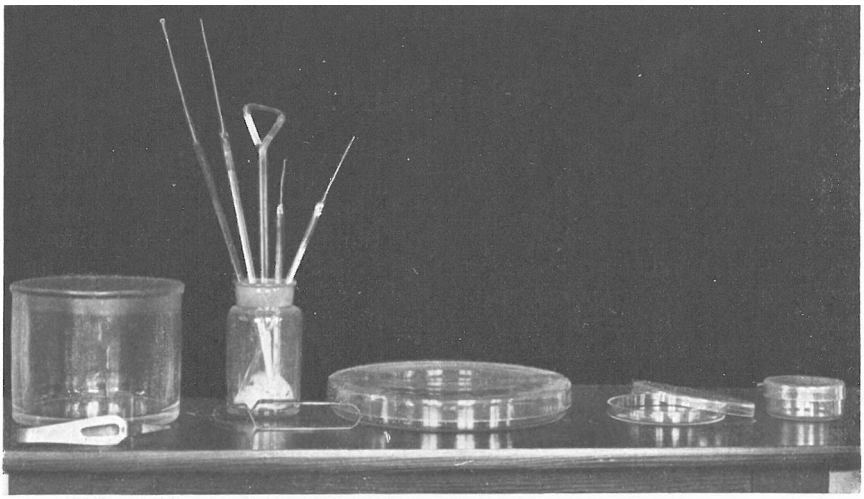


Fig. 1. Von links nach rechts: Glasdose nach Hammerl zur Anaërobenzüchtung. Cornetsche Pinzetten von oben und von der Seite. Platinnadeln und Platinösen; dreieckig zugebogener Glasstab zum oberflächlichen Ausstreichen auf Nährboden. Doppelschale nach v. Drigalski-Conradi. Petrischale (mit abgenommenem Deckel). Kleine Glasdoppelschale.

Fig. 2 The tools of microbial purity at the beginning of the twentieth century. The photo from a bacteriology manual shows inoculating loops, a "Drigalski spatula" (triangular glass rod, standing in a jar) as well as different types of glass dishes for culturing. The classical Petri dish is in the center, with the lid lifted. Reproduced from Kisskalt and Hartmann (1907), p. 2

glass walls of Petri dishes (see Fig. 2). These were the agents of disease that could be isolated as "species" from their environments in a pure state, and that needed to be contained and eradicated for health reasons.

The Petri dish and the Winogradsky column have both been important tools used by researchers to transfer microbes from samples of water, wounds, soil, skin, etc., into the lab and to obtain "cultures," i.e., in vitro growth of various germs that could then be characterized. By juxtaposing the history of the Petri dish and the Winogradsky column, this paper attempts to conceive of the history of practices to move microbes from nature into the laboratory. Since the debut of modern microbiology in the late nineteenth century, these practices have allowed microbiologists to operationalize concepts of purity. Whereas the conceptual counterpart of purity in the vocabulary of many actors has been impurity, here I counterpose it to diversity instead. This is because I will not only focus on microbial systematics—that is, the subfield that has attempted to distinguish and classify microbes in analogy to botanical and zoological taxonomy—, but also on microbial ecology.

Since in most cases, microbes found in situ were too few and/or too mixed for analyses of their characteristics to be conducted, culturing has, until recently, remained a major precondition of taxonomic and other analyses. Furthermore, most serological or biochemical tests, which are important methods to differentiate microbes (e.g., in diagnostics since the 1930s), have been performed on cultured

material that shows some degree of purity. One main problem in microbial taxonomy was, therefore, the degree to which pure cultures—after all, they relied on artificial environments—still resembled the species as found in the "wild." The Petri dish and the Winogradsky column stand in for different approaches to solve this problem whose prominence shifted during the last century. My take on these developments starts off in Germany during the formation of bacteriology in the 1870s and 1880s, and switches over to the United States at the beginning of the twentieth century. Although this is certainly a limited perspective that would need to be complemented, for example, with discussions of microbial systematics and ecology in the context of the "Delft school," framing the story in this way starts with one of the birthplaces of classical bacteriology—in my case, Koch's school is especially important—and follows its global expansion, with the United States arguably the most important place wherein its methods and strategies were taken up. Whereas the Petri dish became a widespread and omnipresent tool in this context, the Winogradsky column, and the ecologically inspired approach behind it, has remained much less prominent until recently.

The historiographic horizon of my analysis is provided by developments in microbial genetics, which started in the 1980s, and became widespread after 2000. Techniques to study microbial diversity in situ, e.g., by sequencing the DNA of uncultivated and potentially uncultivable microbes (metagenomics), have unquestionably contributed to the conceptual shift from laboratory purity to intrinsic diversity. Such studies have suggested the existence of a far higher number of microbial taxa than came to be known by previous studies relying on culturing. Furthermore, the relevance of horizontal gene transfer in specific ecological niches or mixed microbial communities has had a significant impact on microbial systematics in the recent past (O'Malley 2014). Jan Sapp, therefore, understands the ascent of molecular phylogeny as a "profound revolution in biology" that brought microbes from the periphery to the center of biology, with microbial diversity leading to a novel conception of the evolutionary process in general (Sapp 2009, p. 314, see also O'Malley and Dupré 2007).

In light of diversity, as well as the community- and exchange-based ontology of microbes, some scientists have cast doubt on the applicability of traditional species concepts in microbiology, and prefer to speak about ecologically-defined taxa (ecotypes), or dynamic communities of microbes exchanging genes. That is, with the displacement of culturing by sequencing, the idea that stable taxa of microbes in analogy to those of plants and animals even exist has lost credence. A more traditional concept of microbial species (as defined by a number of strains in pure culture that share biochemical, physiological, or morphological traits) has given way to that of "genomospecies" (i.e., isolates showing a certain degree of DNA homology).[4] Many of today's scientists picture the microbial world as a dynamic

[4] In an introductory essay to Bergey's Manual, the most important taxonomic reference work of microbiology, a microbial species has traditionally been defined as a distinct group of strains with distinguishing features and bearing close resemblance, which are "made up of the descendants of a single isolation in pure culture, and usually made up of a succession of cultures ultimately derived from an initial single colony" (Garrity et al. 2007, pp. 27–28).

and variable continuum that shows at least some family resemblance to conceptions of microbes from before the era of stable species in pure culture (Doolittle 2014).

By contrasting the development of pure culture dominance (viz., the Petri dish) in the twentieth century with the ups and downs of a very different strand of working with microbes (viz., the Winogradsky column), I seek to understand the long-term history of the practices and concepts of purity and diversity that informed the ontology of microbes *before* the genomics shift occurred. My story of how microbes have been conceived of over a century of research, from the 1880s to the 1980s, leads, in a very broad historical narrative, from a "messy and polluted" bacteriology before Koch, through the "pure culture era" and its critics, up to a present in which, as the Winogradsky columns on display reveal, the diversity, mixture, and dynamic change of microbes have gained unprecedented prominence in microbiology.[5]

This paper thus faces a historiographical challenge, as I propose to analyze the development of an aspect of microbiology over the extended period of almost a century. This perspective will reveal continuities in the techniques and concepts of purity and diversity over a timespan that we normally associate with dramatic changes in the life sciences. To understand phenomena of continuity or persistence, the analysis of which remains a desideratum of the history of modern science, I suggest adopting a modified version of Fernand Braudel's concept of the *longue durée*. Historians of science frequently use the *longue durée* in a casual manner when taking into account developments over timespans that cross received historiographical divisions or transcend the narratives of academic disciplines. Yet, in spite of renewed interest in such histories, motivated partly by shortcomings in the prevalent microhistorical approach, exactly how this should be done in a historiographical landscape that has changed significantly since the days of the *Annales* school, remains an open question (Holmes 2003, see also de Chadarevian 2009).

Braudel's most well-known examples of histories constrained over long periods by geographical or climatic conditions, for example, speaks only little to the history of science as a cultural activity, and least of all to that of the twentieth century, in which rapid change appears as a hallmark (Braudel 1966; Grote 2015). Braudel, however, did mention science briefly, when he spoke of mental frameworks (cadres mentaux) as "prisons of the longue durée," such as the "Aristotelian universe" (Braudel 1958, pp. 731–732, my translation). By contrast, the *longue durée* I am taking into view here centers neither on nature nor on ideas, but rather on artifacts and concepts in a relatively recent and yet extended past, which makes the issue of temporality appear in a different light.

Whereas the question of what timespan qualifies as "long" may appear unproblematic in Braudel's examples, it becomes more intricate the further we move toward the present, and the more we focus not on big pictures but on partial histories such as those of practices and concepts. In my case, the period of observation from c. 1880 to 1980 qualifies as extended because I analyze continuity against the background of a generally accelerating development in the life sciences.[6]

[5] On the "messy and polluted bacteriology" (Gradmann 2000), see next section. The term "pure culture era" was used by Cowan and Hill (1978); see Sect. 4.

[6] On social and technological acceleration, see Rosa (2005) and my conclusion.

Whereas a plethora of new microbiological methods were introduced throughout this century, the formative techniques to culture microbes remained in place rather unchanged for a surprisingly long time. In short, the absolute number of years does not tell us much about what period qualifies as "long", but a historiographical background characterized by accelerated change does. Thus, my focus on continuity of technique, on "old" tools with persisting influence in new environments, and on slowly occurring changes in how to think about microbes, may also be read as an antidote for the prevailing innovation-centric narrative of scientific development (Edgerton 2008).

I will proceed in my analysis of this long history of microbiology in the following manner: In Sect. 2, I will be looking into the development of the technique and concept of pure culture in bacteriology's formative period, which saw the development of the famous dish by Richard Julius Petri. Its impact throughout the twentieth century will be dealt with in Sect. 4. I will counterpose this way of dealing with microbes with Winogradsky's approach in Sect. 3. The impact of this way of thinking and working on microbial ecology in the post-war period, leading to the use of the Winogradsky column as a miniature ecosystem since the 1970s, is the subject of Sect. 5. The conclusion will return to the general problem of continuity, longue durée historiography, and acceleration in science.

2 The establishment of pure culture and the Petri dish, c. 1880–1920

2.1 Pure culture and the species problem

The history of the practices and discourse of purity related to microbes starts well before the rise of classical bacteriology. When German mycologist Oscar Brefeld (1839–1925) advocated a "pure culture" of microscopic fungi such as the mold *Penicillium* in the 1860s, he referred to the observation of a single cell throughout different stages of its complex life cycle (Gradmann 2001; Schlegel 2004). Brefeld's proposal should be understood as part of a larger controversy about the nature of microbial organisms, and the adequate way to study them. One main point of debate at the time was the degree to which microorganisms (comprising modern prokaryotes and unicellular fungi) displayed species akin to those of higher organisms. Whereas Louis Pasteur had, shortly before, argued against the spontaneous generation of microbes, and highlighted the specificity of fermenting agents such as yeasts, what one could call a transformationist perspective on microbes was still very much à jour: The Swiss-German biologist Carl von Nägeli held that the morphology of what he called "Spaltpilze" (fission fungi, i.e., modern bacteria) was variable, with different types able to transform into each other (Mazumdar 2002).[7]

The position that constant microbial species did not exist at the expense of a spectrum of various discernible forms was later labeled as "pleomorphism"; however, caution is warranted regarding the exact meaning of this term at various

[7] For brevity's sake, scientists' dates of birth and death are only provided where this is deemed relevant.

points in history. When comparing Nägeli's and others' ideas from the second half of the nineteenth century, which were theoretically grounded in a dynamic, evolutionary understanding of nature, with theories of bacterial variability from the 1920s and 1930s, it is clear that this latter pleomorphic theory was much more restricted in scope then earlier ones.[8] For Nägeli, as much as for the infamous bacteriologist Ernst Hallier (1831–1904), the extreme pleomorphist perspective implied on a practical level that the continuum of morphologically, physiologically, and pathologically different microbes found in a natural sample could not and should not be separated. Thus, it is not surprising, and is perhaps even consequential, that Hallier studied microbes under varying conditions, using complex natural media. The mixtures of microbes found therein were described morphologically.

This background provides a clearer idea of where Oscar Brefeld was heading when he proposed to follow the developmental cycle of one fungus cell over time in pure culture, or what he meant with his oft-quoted dictum that without pure cultures all one would get was "nonsense and *Penicillium glaucum*" (the latter fungus was not only Hallier's object of study, but also known as the most frequent contaminant in lab work; Schlegel 2004, p. 42). With his technique, Brefeld was directly opposing the research program of Nägeli, Hallier, and others, or the "messy and polluted" biology of microbes in the 1860s and 1870s, in which harmless germs could be transformed into virulent pathogens (Gradmann 2000, 158, 165).

The cornerstone of the instrumental-conceptual assemblage of pure culturing was the assumption that stable species also existed for these small organisms, and that they could be cultivated by tools such as Brefeld's transparent surfaces of gelatin. These premises informed work in the laboratory of the Breslau plant physiologist Ferdinand J. Cohn (1828–1898). This foremost Linnaean taxonomist of the microbial world outlined the microbes' morphological genera, such as *Micrococcus*, *Bacterium*, *Bacillus*, or *Vibrio*, with reference to differences in microbial cell shape (Mazumdar 2002). His lab adapted mycological strategies to the study of the even smaller bacteria, by using surfaces such as cooked, sterilized potatoes to grow and examine them (Löffler 1983 [1887]). Beyond cell morphology, a characteristic trait used to follow non-pathogenic microbes over time and under different conditions was their color, as in the case of the strikingly red *Monas prodigiosa* (the "miracle monad," nowadays *Serratia marcescens*).[9]

[8] Some hints at the ambiguities inherent in this concept, which has always functioned as a stratagem to sort out bacteriologists, probably as much as bacteria, are provided by, for example, the concise summary on the topic in Lafar's *Handbuch der technischen Mykologie* (1907), which sees Nägeli as much as Theodor Billroth, Edwin Klebs or Hallier as historical proponents of pleomorphism, but also concedes the confusion stemming from the fact that every researcher in the field would understand the concept differently from others. Lafar, standing firmly within the bacteriological framework of Pasteur and Koch (see below), suggests calling an organism pleomorphic if it possibly displays different self-contained cycles of development ("*verschiedene in sich abgeschlossene Entwicklungskreise*," ibid., p. 47; unless indicated otherwise, all translations are mine). This comes closer to what "pleomorphism" may have referred to in discussions about bacterial variation in the 1920s and 1930s (Méthot 2015).

[9] Serratia's crimson-red growth on, for example, food had inspired tales of blood miracles over centuries, and therefore had great relevance to early nineteenth century protozoologists going back to Christian Gottfried Ehrenberg (1795–1876; Breed and Breed 1924).

The general principle of using a solid surface to immobilize and thereby separate germs present in a liquid sample was adopted by Robert Koch around 1880. Koch had demonstrated anthrax infection shortly before, which allowed him to enter the realm of medical research (Gradmann 2005). He then introduced media of defined chemical composition for culturing bacteria, which were made by adding gelatin or agar–agar as jellying agents to liquid nutrient bases, such as heat-sterilized broth. An early bacteriological manual, *Die Methoden der Bakterienforschung*, described Koch's innovation as a synthesis of prior innovations in culturing, such as those by Brefeld, Pasteur, and Cohn's lab, and this seems entirely plausible (Hueppe 1889, p. 101; Collard and Collard 1976). Nevertheless, the name and fame for pure cultures went entirely with Koch—his publication has been designated a "recipe book for the whole world" (*ein Rezeptbuch für die ganze Welt*) and the beginning of bacteriology's golden age (Schlegel 2004, p. 44). Liquid media had previously led to continuous mixture of the microbes grown therein, which rendered it impossible to determine which cell had come forth from which other. By contrast, the solid medium of culture plates allowed to obtain "colonies," i.e., masses of fixed bacteria forming little dots. Such colonies were understood as pure lines stemming—at least in theory—from a single cell that had divided billions of times.

This simple innovation allowed Koch to establish an easily workable new type of pure culture that differed from Brefeld's single cells—namely, the mass growth of clonal lines under controlled conditions. In conjunction with the concept of stable bacterial species and animal experimentation (animal bodies also served Koch as a "culture apparatus" to multiply and purify pathogenic bacteria), the technique was used as a powerful argument in favour of monomorphism; that is, Cohn's Linnaean assumption that stable bacterial species existed (Mazumdar 2002). As it became possible to separate and study colonies of microbes on plates, the extreme pleomorphists of earlier microbiology could be said to simply have lacked proper methods to isolate the species, the existence of which they denied.

This argument, however, was based on a few background assumptions: The pure culture technique implied, first, that microbes could be generally grown apart from their original environments (i.e., that one would catch what was present in a sample), and second, that growing a parasitic microbe on a plate, for example, would not significantly change its characteristics (Hueppe 1889, p. 108). For Koch, this corresponded to his experiences from medical bacteriology. The colonies obtained in pure culture were characteristic of the respective germs in situ and he reported not having observed any "morphological or physiological change" (*morphologische oder physiologische Wandlung*; Koch 1881, p. 144). In the same passage, Koch argued that bacteriology would now follow the principles of botany and zoology according to which organisms stably differing in one or more character were understood as separate species until their interdependence had been demonstrated.

Koch's was a radical version of monomorphism that did not allow for much variation within species, for example. Taxonomically speaking, he was thus an extreme "splitter" as opposed to extreme "lumpers" such as Nägeli or Hallier. Pauline Mazumdar has caught this distinction in her opposition between adherents of specificity, who advocate a division of organic beings into clearly delineated

categories, versus those of unitarianism, who think of the latter in terms of continuity (Mazumdar 2002).

Critics such as Winogradsky, as we will see in Sect. 3, objected that the arguments for extreme monomorphism and pure culture were, at least to a degree, circular. The plates' standardized environment, they maintained, might artificially produce the respective stability of an isolate, and inferences to the natural situation were problematic—note that what was characteristic of Brefeld's pure cultures, namely to follow the development of a single or a few cells over time, was hardly possible with Koch's plates. In practice extreme monomorphism, pure cultures and the Koch school vindicated each other (Mazumdar 2002, p. 101). Culture plates allowed to literally produce ever more new bacterial species, as represented by each germ that could be bred purely and stably on a plate, and that (in medical cases) could be related to a disease. (Schlegel 2004; Gossel 1989; on Koch's postulates, Gradmann 2014). Moreover, bacterial purity in the late nineteenth century was not restricted to medical bacteriology alone; in the work of Emil Hansen at Copenhagen's Carlsberg laboratory, pure yeast cultures for beer production were obtained by picking one cell and having it reproduce vegetatively under controlled conditions.[10] This was a breakthrough for the brewing industry: Pure yeast rapidly replaced the mixed natural leaven used before. This type of pure culture literally mass-produced identical organisms in series, as known from the industrial production of chemical substances, and thus inserts microbiology into a broader history of industrialization (Bonneuil 2016).

It is important to keep in mind that pure cultures as pure lines in the sense of Koch or Hansen were not a matter of degree, but absolute: One had to start with *one* cell, which would then divide to form boundless masses of identical organisms.[11] This absolute purity did not allow for variation within the line, as any later change of a culture's characters could be understood as revealing its initial impurity (i.e. more than one cell had been present at the start) or its later contamination. Phenomena such as cellular development obviously found no place in this conception either.

2.2 The tools and skills of purity

Purity as a matter of adequate technology, that is, of innovative tools and their careful and sterile usage, quickly became part of the heroic narrative of progress in German bacteriology. Technological advancement was pitched by its proponents against the "dirty" earlier days, even if it seems clear from what has been stated above that the rise of monomorphism and pure culture worked only in conjunction. The construction of an impure past, which seems to have started in the 1880s and 1890s in ongoing bacteriological controversies, is of interest since it reflects a

[10] Hansen's approach represented a fusion of Pasteur, Brefeld, and Koch: Whereas it started with one selected cell (rather than obtaining isolation indirectly as Koch did for bacteria, by streaking them out), he also adapted Koch's solid media. The concept of microbial purity as shaped in Europe also had an impact on Japanese fermentation technologies, where an interesting interplay of the purity ideal with traditional approaches using mixed microbial biota existed. See Ceccatti (2001), Lee (2015), and Teich (1983).

[11] On pure lines in research on heredity, Müller-Wille (2007).

characteristic of purity discourse that has been concisely put forth by anthropologist Mary Douglas: Any delineation of a realm of purity (be it with regard to food, bodily hygiene, or laboratory work) co-creates an "other," that is, an impure and dangerous realm of contamination or mixture (Douglas 1985 [1966]). As the early historiography of bacteriology shows, this realm was represented in the sense of both literally impure cultures and a rejected past (Löffler 1983 [1887]).[12]

The solid culture plate was only one among the tools to establish pure laboratory cultures. Another was the plates' containers, such as most famously, the Petri dish. Introduced by Julius Richard Petri (1856–1921), a member of Koch's group, in 1887, the twin glass dishes that fit loosely into one another quickly became a standard container, helping to protect colonies from contamination, to inspect them under the microscope, or to store them (Grote forthcoming). Just as other tools of pure culture, the Petri dish rapidly turned into a staple of bacteriological laboratories. A 1915 catalogue of the Berlin instrument maker *F. & M. Lautenschläger*, a purveyor to laboratories and medical facilities, advertised different versions of the dish among a whole range of other products needed for pure culturing. Among these were the inoculating loop, a piece of circular platinum wire attached to a handle that allowed for the sterile transfer of microbes from one vessel to another, or the Kolle-flask, which also became used in cell biology (Fig. 2; Lautenschläger 1915). These tools seem to have been devised in a relatively short period during bacteriology's formative period, and they became identified with the names of their respective inventors.

The culture plate, Petri dish, and spatula became tied to a specific set of routines on how to obtain pure cultures; that is, to distribute germ samples evenly and to avoid contamination. The "*Gußplattenmethode*" (pour plate method) is a pivotal element of these routines: First, a microbial sample is diluted in liquid agar, it is mixed, and finally poured from a test tube into a sterilized Petri dish. Each viable cell forms an immobilized colony on the solid agar surface. Since Koch first mentioned this method in his 1881 article on pure culture, staining, and microscopy, it has been described and depicted countless times in the instructional literature (Koch 1881). An early example can be found in Emil Aberhalden's *Handbuch der biochemischen Arbeitsmethoden*, a multi-authored method handbook. The respective section meticulously details each of the steps, including small but crucial details such as the proximity of the test tube to the flame or its tilting angle. A series of photographs focusing on the hands of an investigator displays the proper handling of the spatula, test tubes, and Petri dishes (Fig. 3; Fuhrmann 1910).

Instructions for obtaining pure cultures even went down to the level of how the two hands were to hold the required items (test tube, plug, etc.), as a quote from an American manual of the 1920s illustrates:

> The agar is added to the plates by holding the tube or flask in one hand, removing the plug with the other hand, passing the lip through the flame, then lifting the cover of the dish with the hand that does not hold the tube or flask,

[12] See, for example, Löffler (1983 [1887]), or William Bulloch's *History of Bacteriology* (1938); Mazumdar (2002, pp. 98 f.) provides more references.

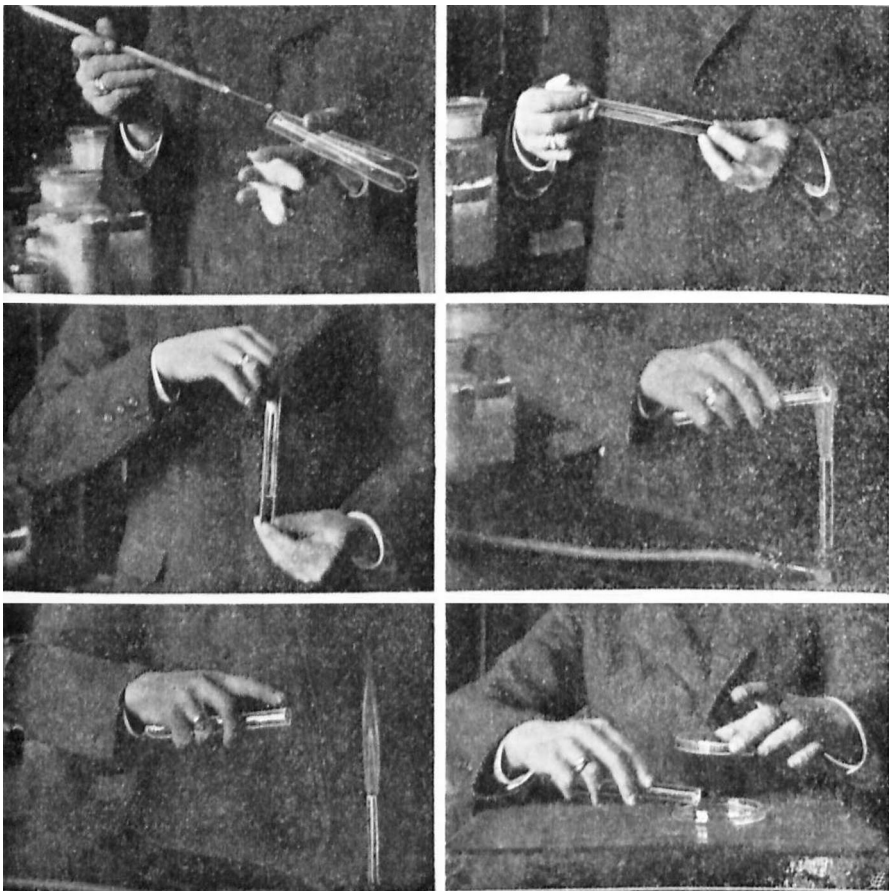


Fig. 3 The technique of purity. A series of instructional photographs displaying Koch's plate method. An investigator first dissolves microbial material in liquid agar within a test tube (upper left), followed by mixing and sterilization routines; finally, one hand pours the test tube's content into a Petri dish while the other lifts the lid. To avoid contamination by airborne germs, the entire process is carried out in proximity to a Bunsen burner flame. This process has been described and depicted numerous times in the instructional literature from the early twentieth century until today. From: Fuhrmann (1910), p. 1232

> pouring the agar into the dish. Then quickly stir, moving the dish to and fro on the table (without tipping, so as not to spill). (Conn 1927, p. 35).

The instructional literature has continuously referred to such pure culture routines, while the dexterity of using one's fingers and tools has been taught to generations of biology and medical students around the globe over decades. In fact, it is still among the skills to be mastered by every undergraduate in order to avoid producing, to return to Brefeld, "only non-sense and *Penicillium glaucum*" in the laboratory.[13] Descriptions vary in detail obviously, and the tools and laboratory environments in

[13] See, for example, Kostka (1924, p. 26); Hauduroy (1947, p. 200); Schröder (1975, p. 20, pp. 59–60); Madigan and Martinko (2006, pp. 119–120). For use of these methods in classification, see Sect. 3.

which pure culturing has taken place have changed over the twentieth century (e.g., from glass to disposable plastic dishes,). Yet, if one compares the skills of pure culturing from the early decades of the twentieth century to their practice a century later, the impression of continuity is striking. What is more, these routines have acquired a ritual-like character: Every student must master them in order to cross the threshold towards becoming a researcher, even if their actual relevance varies considerably. An early review on pure culturing, repeating its impact like a mantra, is revelatory on this point: "A sense for scrupulous cleanliness, a strengthening of perseverance and patience" are mentioned among the "moral successes" that will come about when practicing pure culture techniques.[14]

Thus, purity represents to microbiology far more than a single technology or method. Similar to the value of purity for food production, as discussed by Mary Douglas, purity has developed into a precondition and cultural value of modern microbiology, as the science that claims to have systematized and ordered the microcosm. The continuity of tools and skills involved in pure culturing microbes qualifies as a *longue durée* effect of the culture of microbiology: Whereas much has changed in microbiology since the 1880s, these tools, and the basic principles guiding their use, have remained operative.[15] One may find it relatively unsurprising that cheap and simple routine technologies such as these remained influential over a long period of time, yet, this view—taking simple technologies for granted—means overlooking the fact that there were alternatives, and that they entailed a specific perspective on the microbial world, which looks utterly limited from today's vantage point. As artifacts combined with practices, these technologies represent an "infrastructure" of classical bacteriology that has remained omnipresent and influential throughout the twentieth century, in spite of all other innovations.

3 The Winogradsky column and enrichment culture

If purity creates a true order of things, as Mary Douglas has argued, drawing the boundary of the pure co-creates a realm of matter out of place. In microbiology, this applied not only to the construction of a dirty past of pleomorphism, but it must also have applied, at least in the eyes of the advocates of absolute purity, to the microbiology of soil, water, or food, fields that were always related to mainstream medical bacteriology, if only rather distantly.[16] Following Douglas' dialectic thinking further, I will argue that what may have been perceived, from the medical perspective, as matter out of place not only endangered prevalent conceptions about microbes, but also bore the potential for the re-ordering of these conceptions, and was thus productive from another perspective (Douglas 1985 [1966], 124ff.). Sergei

[14] "Nicht zu verachten sind auch die moralischen Erfolge, die sich unwillkürlich bei der Beschäftigung mit der Reinkultur einstellen, wie der Sinn für peinliche Sauberkeit, die Stärkung der Ausdauer und der Geduld u. dgl. m." (Richter 1907, p. 115).

[15] Moreover, these tools have allowed classical bacteriology to spread around the world, when the equipment and instructions on proper usage, spread by, for example, Koch's famous courses, were transferred within Europe and to North America (Gossel 1989; Kreuder-Sonnen 2012).

[16] On these fields, see Spath (1999) and O'Malley (2014).

N. Winogradsky's (1856–1953) approach, and the device that bears his name today, allows a discussion of central aspects of non-medical microbiology, and he is of special interest here, since he was a vocal critic of pure mass culturing. Yet, Winogradsky also argued against pleomorphisms of various sorts, his technical skills have been highly praised, and following the isolation of a number of environmental microbes, he is considered one of the pioneers of microbiology (Ackert 2012). So, how do we square this figure with the rise of purity, the demise of mixed culturing, and the ups and downs of pleomorphisms since the late nineteenth century that we have heard about?

Around the time when Petri published on his dish, Winogradsky was working with botanist Anton de Bary at Straßburg on sulfur-metabolizing freshwater bacteria named *Beggiatoa*. As historian of science Lloyd Ackert argues, these organisms, which form visible filaments in, for example, wastewater or sulfur-containing springs, were of interest for two reasons: First, because some contemporaries had suspected that they represented a case of pleomorphism, which de Bary and Winogradsky sought to disprove, and second, because *Beggiatoa* cells contained sulfur granules—an uncommon trait among a group of organisms that lacked clearly discernible characters apart from their cell morphology. This set Winogradsky on track to study the peculiar behavior and metabolism of these organisms (Ackert 2012).

Yet, let us first see how Winogradsky attempted to get *Beggiatoa* into the laboratory. In 1888, he described the isolation and culturing of this organism in a very palpable manner:

> Considering the acquisition of the necessary material, these bacteria are, as mentioned above, much more frequent than assumed previously. Some of them are present in any swamp or pond, but so scarcely that one does not succeed in finding them through direct microscopic observation of water and mud. […] Yet, I have established the presence of a number of colourless as well as red species in the pond of the botanical garden, […], by having these organisms appear spontaneously in vessels of water, mud etc. sampled from these locations. Usually, I proceeded as follows: A few cut-up, freshly sampled pieces of a rhizome of Butomus [flowering rush, an aquatic plant, M.G.], with mud attached, were put into a deep jar of 3-5 l of volume, a few grams of gypsum were added. After 5-7 days of standing at room temperature, the formation of H_2S starts, which first blackens the mud at the vessel's bottom; then, the liquid starts to opalesce gradually, progressing from the lower to the upper strata, due to the excretion of sulfur; finally, a strong odour of H_2S is perceivable; and a film of sulfur forms on the surface. After 3-6 weeks, one can easily find some forms of sulfur bacteria by microscopy examination; furthermore, under given circumstances, they can reproduce immensely.[17]

[17] "Was zunächst die Gewinnung des nöthigen Materials betrifft, so sind diese Bacterien, wie oben schon erwähnt, viel verbreiteter als man bisher glaubte. Einige von ihnen sind in jedem Sumpfe oder Teiche vorhanden, aber so spärlich, dass es nicht gelingt, dieselben bei directer mikroskopischer Untersuchung des Wassers und Schlammes aufzufinden. […] Dennoch habe ich das Vorhandensein von mehreren farblosen und rothen Arten im Teiche des Botanischen Gartens, […] constatiert, indem ich diese Organismen in Gefässen mit aus den betreffenden Orten entnommenem Wasser, Schlamm etc. spontan erscheinen liess. Ich verfuhr gewöhnlich auf die Weise, dass ich einige zerschnittene Stücke eines frisch

This protocol not only foreshadowed the device that later became known as the Winogradsky column, but the procedure was also diametrically opposed to pure culturing: Instead of a clearly defined medium, Winogradsky started with complex and contaminated natural substances; second, instead of a few days of culturing, his experiment took weeks, revealing the appearance of different microbial forms within the culture; and third, instead of using an artificial surface medium, he mimicked the natural situation in a large jar (apparently without the sterilization process that was a *sine qua non* of medical bacteriology). Therefore, the glass cylinder filled with natural substances and mixed microbes can be seen as a counterpart to the culture plate and Petri dish—it emblematizes an approach that has found different guises in how microbiologists dealing with mixes of organisms that differed in abundance and activity have cultured their objects of study. Not least, it highlights a very different role of the environment for this endeavor.

Winogradsky deliberately used complex mixes of substances, which he conceived as closer to the natural situation than the use of synthetic growth media. Yet, he still intended the conditions to be controlled as far as he thought possible without undue interference, e.g., by further, iterative isolations of microbes from the primary cultures, by varying certain parameters in them (adding a substance in excess), or by mechanically isolating cells for microscopic study or culturing on slides. In a way, the method of "elective cultures" (Winogradsky 1949) that he shaped in his studies of environmental microbes attempted to steer a middle ground between in situ mixture and in vitro purity by mimicking the environment for culturing, and achieving the spatial segregation and accumulation of one suitably adapted microbial type at the expense of others. These methods implied an ideal of *relative* purity, as Winogradsky's aim was to isolate certain microbes to study their physiology and life cycles in order to classify them, or to respond to claims of pleomorphism. Methods based on a similar technique and reasoning were at the time also developed by the Delft microbiologist Martinus W. Beijerinck (1851–1931), and became, using various devices or methods, a mainstay of microbial ecology.[18]

The 1888 *Beggiatoa* work illustrates the specificity of the way in which Winogradsky understood and operationalized purity. Sometimes, he remarked, when growing microbes from a sparsely populated source, one arrived at only a few forms being present in a culture, or by chance at what he admitted to be a "pure culture" (*Reinkultur*; Winogradsky 1888, p. 14). Thereby, he referred to the

Footnote 17 continued
herausgenommenen Butomus-Rhizoms mit dem an demselben anhaftenden Schlamm in ein tiefes, 3–5 Liter Wasser fassendes Gefäss legte und ein paar Gramm Gyps zusetzte. Nach 5-7 tägigem Stehen bei Zimmertemperatur beginnt die H_2S-Entwickelung, wodurch zunächst der am Boden des Gefässes angesammelte Schlamm geschwärzt wird; dann fängt die Flüssigkeit allmählich an, von den unteren zu den oberen Schichten fortschreitend, infolge der Ausscheidung von Schwefel zu opalesciren, und endlich wird ein starker Geruch nach H_2S bemerkbar; auf der Oberfläche bildet sich ein Häutchen welches aus Schwefel besteht. Nach 3-6 Wochen kann man schon bei mikroskopischer Untersuchung ohne Mühe einige Formen von Schwefelbacterien finden; weiterhin können sie sich unter Umständen ganz gewaltig vermehren." (Winogradsky 1888, pp. 11–12).

[18] Ackert (2012) and O'Malley (2014); see also the collection of enrichment methods from environmental microbiology and brewing compiled under the curious title *Ökologie: Anhäufungen nach Beijerinck* ("Ecology: Accumulations following Beijerinck") by Stockhausen (1907).

absence, or more precisely the undetectability, of other microbial forms with a similar physiological activity to those being studied. Here, purity did not designate prior asepsis, or a culture's genealogy stemming from one cell, which could never be safely established in a liquid medium, but, rather, the lack of a disturbing microbe. For Winogradsky, Beijerinck and others working with natural substances and using principles of physiological and environmental selection to obtain microbes from the mixed biota of soil, water, etc., relative purity was clearly desirable, but it represented just one goal to be weighed against others, such as closely mimicking the natural environment, or trying to characterize microbes that would not grow on a culture plate.

Winogradsky's long professional life brought him to an agricultural department of the *Institut Pasteur* in the 1920s, after he had lost his property in Soviet Russia as a result of the October Revolution. There, soil microbiology became his main subject. Like his American colleague, soil and dairy bacteriologist Harold Joel Conn (1888–1975), who adapted Koch's methods for agricultural bacteriology at New York State's Agricultural Experiment Station, he contributed to the development of methods that were to circumvent the impasse between not being able to tackle soil microbes by simple plate technique and pure cultures of some sort still being desirable, if not necessary, to make sense of the diverse and highly variable soil "microflora" (see, e.g., Winogradsky 1949 [1924], p. 441). For this purpose, Conn and Winogradsky developed so-called "direct methods" to study microbes within their natural environment, such as attached to soil grains on microscopic slides, with carefully established control samples, added substances, or the development of in situ staining methods (see Ackert (2012) for more detail and a critique of the alleged "directness" of these methods).

In addition to providing alternative approaches to the problem of how to purify microbes, late in his life, Winogradsky openly criticized the dominance of the pure culture method from medical bacteriology, when he called microbes cultured in laboratory environments *plants de culture* ("cultivated plants"), which one may oppose to the *paysages microbiens* ("microbial landscapes") he suspected to inhabit natural environments (quoted from Ackert 2012, p. 113). In correspondence with the Dutch–American microbial ecologist C.B. van Niel, who had pressed him on the issue in 1932, he denounced pure cultures as "the holy dogma of the microbiological religion" and suggested a thought experiment to illustrate his position:

> Imagine a botanist who isolated some alpine plants from their natural environment to cultivate them in a greenhouse for, say, thirty years. Comparing them to their wild ancestors and remarking that the differential characters of the species, so clear at the outset, have become blurred, would he go as far as to conclude that these characters have never existed? […] Thus, I think a microbiologist ought to refrain from an analogous conclusion, taking into account that 24 h in an incubator count for the microbes possibly as much as a year for a phanerogam.[19]

[19] "Sans entrer dans des details, ce n'est que le principe de la culture – dogme sacré de la religion microbiologique – qui m'intéresse à ce moment." Undated annotated letter from Winogradsky to van Niel, presumably early 1932; "Imaginez un botaniste qui isolerait quelques plantes alpines de leur habitat naturel pour les cultiver dans une serre durant, disons, une trentaine d'années; en les comparant ensuite à

For Winogradsky, pure culturing on plates was one method among others (such as microscopic cultures), which also had a bias and therefore needed to be adapted or complemented to take into view the "'wild' forms" (*formes 'sauvages'*) of microbes. Compared to the low numbers of microbes in environmental samples of water or soil and the lack of nutrients therein, he considered pure mass cultures in the Koch tradition too abundant, a form of "hypertrophy" that was to produce abnormal forms due to the richness and standardization of the growth media. They thus represented an "artifice" of the bacteriologist (Winogradsky 1949 [1937], p. 144 f.). To overstate matters, one could say that, for Winogradsky, environmental microbes suffered from 'diseases of culture' on medical bacteriology's plates, and that what he called the "old botanical method" (i.e., observation of the life cycles of a few cells, close to Brefeld's approach) was more appropriate for the study of their normal forms (ibid., p. 147).

Against this background, it may be surprising to learn that Winogradsky remained a partisan of monomorphism, from his early days with de Bary to the end of his life. In the 1888 work on sulfur bacteria, his reaction to alleged pleomorphism and the shortcomings of Cohn's morphological species concept comprised the introduction of new culturing methods, microscopic observation, and physiological inquiry, so that he closed his argument with the bold assertion that "the longstanding controversy on bacterial species has to be considered as finally closed."[20] In a similar vein to Koch's adherents, he argued that much of the observed variability in cultures was due to improper technique, which, in contrast to Koch, he did not want to standardize but continuously attempted to adapt to specific circumstances. Even when debates on bacterial variability, life cycles, and pleomorphism resurfaced in the interwar period, he remained critical of the "doctrine of pleomorphism" (thus the title of an essay from 1937). He held that the claims of novel pleomorphists were grounded on faulty technique and overinterpretation of findings, all the while conceding that *regular* life cycles and various forms of bacteria were to be found, with "rigid uniformity being unthinkable in living organisms" (Winogradsky 1949 [1937], p. 139).

In line with what has been said about variation in medical bacteriology, Winogradsky's case shows that the polar opposites of monomorphism versus pleomorphism do not capture the full picture (Mendelsohn 2002; Méthot 2016). Here, the point is that Winogradsky decoupled what went together for Koch;

Footnote 19 continued
leurs ancêtres sauvages et en ayant constaté que les caractères différentiels des espèces, si nets à l'origine, sont devenus brouillés, irait-il jusqu'à affirmer que ces caractères n'ont jamais existé? […] Et bien, je crois que le microbiologiste devrait aussi se garder d'une conclusion analogue en songeant que les 24 heures d'étuve comptent pour les microbes possiblement autant que toute une année pour une phanérogame." Annotated letter from Winogradsky to van Niel, 9.7.1932; handwritten corrections of the typescript have been included. All letters from: Fonds Winogradsky WIN.2, Archives de l' Institut Pasteur, Paris. Van Niel tried to establish pure cultures of environmental microbes at the time, using enrichment methods as a first step. He was interested in ecology and classification as well as in obtaining organisms to study certain physiological phenomena such as phototaxis or salt tolerance in the lab. On van Niel and the Delft school, see Spath (1999, Ch. 3.)

[20] "Die langwierige Controverse über die Bacterienspecies muss damit als endgiltig abgeschlossen betrachtet werden." Winogradsky (1888, p. 115).

namely, pure mass culture and monomorphism. Taking environments, population density, physiological types and interactions as well as development into account called for a different operationalization and value of purity, and a species concept that had more in common with botany than with the characterization of pathological agents. Therefore, the methods of enrichment culture that he and others pioneered, and the device that was to receive his name, represent a tension within twentieth-century microbiology—between the indisputable power of the pure culture approach and the insight that this approach brought about other constraints. Thus, pure mass cultures had a double edge, especially when studying environments where microbes are found in varied and mixed forms, or in specific ecological niches. Interestingly, in more recent times, these premises have increasingly been shared by medical microbiologists, and it seems to have been in this context that Winogradsky's column became a model for human microbiomes (Anderson 2004; Méthot 2015; see Sect. 5).

4 Microbial classification in the "pure culture era," 1920s–1980s

Notwithstanding the development of environmental microbiology, the tools and skills of pure culturing continued to have a strong impact on the understanding of microbial diversity. The classification of microbes developed in a way that was in many respects different from the way in which the classification of plants and animals developed. Cohn and others transferred the conventions of Linnaean nomenclature (binomials) and taxonomy (the hierarchy of ranks and the prevalence of morphological criteria) to organisms that differed quite strongly from macrobes both in their external appearance and in their reproductive behaviour. A natural historical tradition of collecting, describing, and classifying of microbes existed, but it was not comparable in its dimensions to that for plants during the unfolding of experimental bacteriology. Moreover, this natural history of bacteria was overshadowed by the considerable impact of diagnostics on bacterial systematics: Medical bacteriologists were not very versed in the principles of classification, such as the rules of nomenclature or priority, and sometimes, diagnosis and taxonomic analyses appeared conflated (on botanical systematics, see Daston 2004; Müller-Wille forthcoming). Names and data on the many "species" that were isolated by pure culturing were therefore not only published in diverse medical or botanical journals, but also in a plethora of monographs (e.g., Lehmann and Neumann 1907; see Sapp 2009). All this provoked the impression of taxonomic confusion, as the following retrospective statement illustrates: "The discovery of the principles of pure-culture study resulted in such a sudden burst of investigation that it was a lost month in which a new organism was not described, catalogued, and laid away, very frequently in the wrong grave".[21]

[21] Perkins (1928, p. 124). Richter (1907) makes a strong argument for the impact of pure culturing on the classification of various microbes, listing dozens of methods, many of which are variations of Koch's technique.

In this situation, the Society of American Bacteriologists (SAB) commenced efforts to standardize classificatory procedures.[22] From 1905, so-called "descriptive charts" were published and sold among bacteriologists, which specified different traits of the organisms to be assessed and described in a uniform way. This would help to compare findings, facilitate diagnosis and, not least, education (Kupferberg 2001). Nearly all of these tests relied on pure cultures, since microbes, as found in the environment, were potentially mixed and often scarce. Characters such as "colony shape" were by definition tied to microbes on standardized agar surfaces (Fig. 4).

In 1923, the first edition of *Bergey's Manual of Determinative Bacteriology* was published, a multi-authored, diagnostic and taxonomic manual that resulted from one of the SAB's committees (ibid.). *Bergey's* described more than 800 bacterial species using bullet points that covered many of the characters stipulated in the previous descriptive charts, and by providing references to their first descriptions. Despite criticism pertaining to its eclectic supraspecific taxonomy, *Bergey's* became a frequently re-edited and widely used reference work.[23]

In addition to the charts and the taxonomic manual, the SAB's committee for "Bacteriological Technic", to which agricultural microbiologist Harold J. Conn was central, also edited a "how to"-manual (Committee on Bacteriological Technic 1923; on Conn, see Sect. 3). Through re-editions over almost four decades (starting with a loose leaflet continuation service and, from 1946 on, as a bound volume), the "*Manual of Methods for Pure Culture Study of Bacteria"* kept bacteriologists up to date about received and improved methods for bacterial systematics, which pertained to obtaining and assessing pure cultures, but also to testing their traits and storing them in the proper way.[24] In addition to recipes for culture media or staining reagents, the manual included separate sections on serology or biochemistry.

Pure culture was considered "a necessary prelude to bacterial taxonomy"; however, the methods it described also served many other fields of microbiology (Committee on Bacteriological Technic 1952, I52–3). The link between pure culturing and classification was reinforced in the post-war period when a deposition of pure type cultures in collections, similar to type specimens in botany or zoology, was stipulated as a requirement to validly publish new bacterial species (Buchanan et al. 1948).

[22] I will analyze the role of pure culture in classification for the American case, mostly for the reason of the availability of sources. Whereas different national and/or personal approaches to the subject existed in the first half of the century (see below), the situation became globally more homogenous after 1945, and the American institutions and manuals, such as *Bergey's Manual of Determinative Bacteriology*, were internationally authoritative.

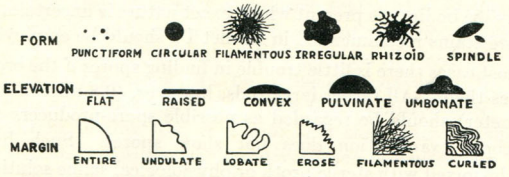
[23] Bergey (1923); further issues date from 1925, 1930, 1934, 1948, 1957, 1974, and 1984; for Bergey's history, see Murray and Holt (2001).

[24] Committee on Bacteriological Technic (ed.) (1946). Similar to the descriptive charts and *Bergey's*, the *Manual of Pure Culture Study* developed into a successful, long-term publication. Reports read by Conn at the SAB's Annual Meetings on behalf of the Committee document the growth and revisions of the manual, as well as the numbers of copies being sold and the revenue generated for the society (e.g., Anon. 1931, p. 2). In 1946, the first bound volume was printed, to be followed by the re-named *Manual of Microbiological Methods* as a 7th edition in 1957 (Committee on Bacteriological Technic (ed.) 1957).

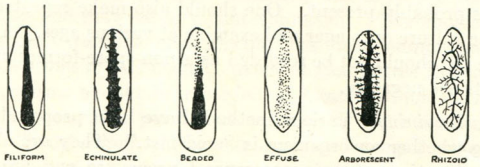
ROUTINE TESTS FOR THE DESCRIPTIVE CHART V51-7

CULTURAL CHARACTERISTICS OF BACTERIA

COLONIES

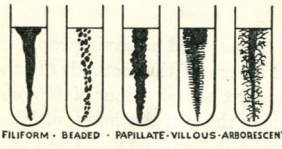


AGAR STROKE - FORM OF GROWTH

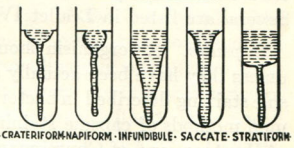


GELATIN STAB

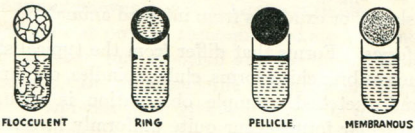
LINE OF PUNCTURE



LIQUEFACTION



NUTRIENT BROTH - SURFACE GROWTH



(Copies of this chart on sale by Biotech Publications, Geneva, N. Y.)

◀**Fig. 4** Pure culturing as a basis for microbial systematics. Instructional chart displaying and naming different morphologies of how bacterial cultures grow on the surface of agar plates (first three rows), on agar in test tubes (middle rows), or in liquid media (lower row). These characteristics have been used in *Bergey's Manual of Determinative Bacteriology* to distinguish microbes in diagnostics and for classification. The trait "culture morphology" is by definition bound to pure mass cultures and can rarely be observed for microbes outside of the laboratory. "Table displaying characteristics of microbes in pure culture" (Leaflet V 1951) reproduced from: Committee on Bacteriological Technic (ed., 1946), *Manual of methods for pure culture studies of bacteria*, 9th ed., Geneva (N.Y.): Biotech Publications; American Society of Microbiology Archives, University of Maryland, Baltimore County, Baltimore, MD, p. V51–V57. With kind permission of The American Society of Microbiology Archives

The continuity of methods in the various editions of the manual (that nevertheless included new approaches) is striking, and corresponds to the continuity of technique described in Sect. 2. This methodical "conservatism" may be of special importance to systematics as a way to maintain a coherent body of knowledge that allows researchers to repeat and compare analyses and use findings published in older literature. At the level of the scientists, a striking long-term continuity also exists: From early reports on pure culture published in 1918 to the 1957 volume, Conn occupied a central position as editor or organizer of the manual's various formats and editions.[25] Even in 1981, when the American Society of Microbiology (ASM; successor to the SAB) re-published a "how to"-manual, the relevance of basic bacteriological technique was stated upfront:

> Bacteriology became a science only after unique methods were developed, and they are responsible for its continuing influence on and expansion into subsequently developed fields such as virology, immunology, and molecular biology. Koch's introduction of pure culture technique and Pasteur's use of immunological response and chemical analysis remain as influential now as then. (Gerhardt and ASM 1981, p. 1).

In sum, the paper trail of charts, taxonomic and laboratory manuals from the 1920s to 1980s reveals deep continuities in how bacterial systematics was tied to pure culturing for a large part of the twentieth century. Even if a plethora of methods to obtain cultures of, for example, anaerobic, aquatic, or parasitic microbes existed beyond Petri dish and standardized growth media, these latter and the clonal lines which they produced have remained a cornerstone of microbial classification. The "pure culture era" that set in at the beginning of the century thus enabled and stabilized taxonomy. By contrast, what existed before and beyond it—impurity—endangered it.[26]

This perspective was not undisputed, as Winogradsky's approach has already illustrated. However, he was not the only one voicing his discontent: The shortcomings of pure culture were also discussed by medical bacteriologists. Ludwik Fleck, for one, had already found clear words on this issue in his essay "About the concept of species in bacteriology," a review article resulting from a

[25] Conn, who also published a handbook on staining procedures, managed the publication of charts and manuals through a publisher at his home institution (Lillie 1977).

[26] The era's onset depended on which species were considered. The term "pure culture era" seems to have been coined by Samuel T. Cowan (1905–1976), curator of the British National Culture and Type Collection (NCTC) and important bacterial taxonomist in the post-war decades. Cowan and Hill (1978, p. 12).

talk he had given at a meeting of a medical association in the Polish city of Lwów in 1930. Regarding the isolation of certain pathogenic microbes, *Rickettsia*, from their hosts, Fleck noted that the "artificial media" (*künstliche Nährböden*) may have bred various "forms" (*Gestalten*) of the microbe, and that culturing microbes in this way was not, as classical bacteriology had presumed, "an isolation, obtaining a pure breed, but an artificial 'delivery' from the organism."[27] In his main work, *The Genesis and Development of a Scientific Fact*, Fleck added in a similar vein that bacteriology had almost lost its connection to botany, displaying an "unbiological" thought style that revealed itself in pure culture methods as well as in a neglect of morphology and population study (Fleck 1980 [1935], p. 161). Yet, it is notable that Fleck's own laboratory work on variability in *Streptococci*, which found its way into *Genesis*, relied on exactly the methods of classical bacteriology to isolate and characterize microbes he thus criticized—agar plates, inoculations from one plate colony to another, and pure lines. There is a clear ambivalence between the methods and the detection of variability: Only on the basis of an established technique supplying pure genealogical lines and a general monomorphic stance was it possible to clearly differentiate variants, whereas that same technique may also have produced them as artifacts of culturing. It is beyond the scope of this paper to go further into this matter, which may, however, have a bearing on the debates around bacterial variability in classical bacteriology and the interwar period (Mendelsohn 2002; Méthot 2016).

5 From enrichment culture to "totem pole": The Winogradsky column and microbial ecology, 1920s–1980s

Fleck did not specify who, or what, exactly he had in mind when stating that bacteriology had *almost* lost its connection to botany due to its "unbiological" thought style. However, in the microbiology of soil and water as presented in Sect. 3, that connection was clearly visible. Remember that Winogradsky had lamented the decline of the "old botanical method" at the expense of the pure culture approach (Winogradsky 1949 [1937], p. 147). In the following, I will present some elements of the story of how this branch of microbiology fared in the second half of the twentieth century and how it connects to present-day microbial ecology.[28]

Through the work of Martinus Beijerinck, elective or enrichment culturing methods became central tools for the environmental microbiology of the Delft School, which was influential in American post-war microbiology through van Niel (Spath 1999). The connection between this approach and molecular biology was also visible at a 1964 symposium on the topic held by German microbiologist Hans-

[27] Fleck (2011, p. 108): "Jenes Züchten aus einem kranken Organismus ist nicht, wie das die klassische Bakteriologie will, eine Isolierung, die Gewinnung einer reinen Züchtung, sondern ein künstliches "Entbinden" aus dem Organismus, eine Umwandlung des Erregers in einen wilden Proteus." The term *Entbinden* is German in the Polish original; the text was translated into German and republished in Fleck (2011).

[28] For further detail on environmental microbiology and microbial ecology, see Ackert 2013; O'Malley 2014.

Günther Schlegel (1924–2013).[29] The work of Winogradsky and Beijerinck ranged high on the agenda of an historical lecture on the topic, as did Delft microbiologists; moreover, the program contained contributions on sulfur and nitrate bacteria as much as on algae, yeast, or luminous bacteria. Whereas here it was mostly about putting to use ecological methods in a new age and context, around the same time, the American microbial ecologist Thomas D. Brock (*1926; author of the most influential American microbiology textbook in recent decades) returned to the fundamental issue of culturing and purity in ecology as raised by Winogradsky. Making an epistemological argument, Brock brought the techniques of microbiological observation into perspective. In analogy to quantum physics, he argued, the relationship between microscopic and macroscopic events, and the techniques of amplification establishing that relationship, needed to be taken into account to understand what microbiologists did when culturing microbes in the lab: "In physics, a Geiger tube or a photographic plate is such a device [for the amplification of microscopic events]; in microbiology, the agar plate on which colonies […] develop is quite analogous" (Brock 1966, p. 24). As amplification was a matter of degree, whether a pure culture was necessary depended "on how closely we wish to study [a microbe, M.G.]" (ibid., 25). Brock's analogy between an elementary particle and a cell hinged on the relevance of apparatus and scale: Culturing as an inescapable, but necessarily interventionist method led him to a pragmatic differentiation of concepts of purity that mirrored aspects of Winogradsky's earlier position: Brock distinguished between "pure cultures" (in Koch's sense, absolute and genealogical) and "axenic cultures." The latter term, literally meaning "free from foreign elements" in ecology and parasitology, came into use in microbial taxonomy to differentiate the issue of purity as required for the study of an organism from that of genealogical cell lines on culture plates.[30]

Brock's and Schlegel's work have contributed to the shaping of ecological microbiology. Their textbooks make repeated mention of enrichment cultures and devices similar to Winogradsky's. However, an interesting change in the usage and connotation of the device can be observed: From around 1970, these devices were not only used to cultivate microbes by mimicking their environments, but they became models of microbial ecosystems; that is, displays of how coexisting microbial communities in the column would (re-)cycle substances (Brock 1970; Veldkamp 1970; Schlegel 1976). With the sun as an energy source, such closed jars containing microbial niches (aerobic, anaerobic, dark, illuminated, etc.) formed ecosystems that remained stable for years. Less than being a research tool, under the name of "Winogradsky column," these jars now acquired a demonstrative function in high school and undergraduate (micro-)biology education. They were models for the entanglement of biological and geochemical processes in the age of environmental concern (Corner 1992; Schlegel 2004).

The original perspective on the microbial world that the Winogradsky column as a culturing device entailed (diversity, mixedness, interdependence, and relevance of

[29] The symposium's title was "*Anreicherungskultur und Mutantenauslese*" (enrichment culture and mutant selection); see Schlegel (1965).

[30] The concept is used in taxonomy also by, for example, Starr et al. (1981, p. 155).

the environment) have been around throughout microbiology's history, but never as the mainstream up to the very recent times of genomics. Possible reasons for this are the institutional dominance of medical microbiology, the fact that the tools of absolute purity were deeply entrenched in microbiological practice, as well as the promise that such cultures could help to better understand the causal processes in diseases (e.g. infection—think of Koch's postulates), as they seemed to exclude disturbing factors. Thus, when two American microbiologists called the (well-known) observation that many more microbes could be counted in environmental samples than one would be able to cultivate on plates the "great count plate anomaly", they may have unintentionally hit the nail on the head: All subtleties aside, pure culturing remained the perceived paradigm of microbiology, and observations that would not fit in with this scheme were seen as anomalies.[31] To make things worse, a historiography of science neglecting fields such as agriculture or geology—certainly of equal importance than medicine—may have reinforced rather than helped to correct this impression.

We must await detailed historical analysis regarding how the tide has turned since the 1980s, that is how the relevance of populations, environments, development and intrinsic diversity has moved into focus and how culturing was partly displaced by other methods of analysis. Certainly, the rise of sequencing and computational tools to detect and characterize microbes in situ was the main technological factor that made pure cultures look like an exception. They now appeared as a laboratory artifact that only captured a minute part of the microbial diversity and that has therefore skewed biologists' view of what microbes are and how they live (Doolittle 2013; O'Malley 2014; Sapp 2009). This shift in perspective seems to have gained in strength since the 1990s, when Schlegel dubbed the Winogradsky column microbiology's "totem pole," thereby attributing a symbolic value to the device, albeit with a connotation that is very different from that which the Petri dish had acquired decades before (Schlegel 2004, p. 67). Whatever technologies, or social and cultural factors, may have contributed to this shift in the recent past, the columns on display in natural history museums today bring environmental thinking to the fore. They also highlight the complexity of microbial communities and their exchanges with human hosts, which pertain to current conceptions of health, hygiene, and even selfhood (such as discussions on the gut microbiome's effect on the mind). However, the most dramatic changes concern classification: The species identified on the basis of pure culture isolates in books such as *Bergey's Manual* appeared, all of a sudden, as just the tip of the iceberg of microbial diversity, and in fact, many distinctions that had been central to microbiology have since been revisited.[32]

[31] Staley and Konopka (1985) trace this observation back to Winogradsky's students in the 1930s.

[32] Such as, for example, the proposal of the Archaea as a third domain of life on the basis of RNA analyses around 1980 (Sapp 2009) or the blurring of species boundaries such as between the enteric bacteria *Escherichia* and *Shigella* (Lan and Reeves 2002).

6 Conclusion: purity and diversity over the *longue durée*

Over the last century, purity and diversity have represented to microbiologists both aims achieved by technologies as well as ideals regarding the right way to study microbes. The polar opposites to either obtain genealogical lines of cells mass-cultured under standardized laboratory conditions (Koch) or to study microbiological diversity in complex environments (Hallier) represent only the ends of a spectrum, with Winogradsky's approach situated in between. The insight that one may well accept clearly delineated microbial species, but not neglect environment, development etc. allows to critically assess Mazumdar's binary of specificity (Cohn and Koch's monomorphism) versus unitarianism (such as Nägeli's and later pleomorphism) for later periods (Mazumdar 2002).

Moreover, this paper has shown how the persisting or changing conceptions of the microbial world in between purity and diversity was related to the use of technique: The "pure culture era" set in with the establishment and broad use of Koch's plates towards the end of the nineteenth century and the demise of the earlier, pleomorphist bacteriology. Even if alternatives such as enrichment culture or Brock's axenic cultures were important in some contexts, absolutely pure culture remained dominant in microbial classification, and presumably in most of microbiology. However, ecological thinking seems to have played a role as well—taking this into account may complexify the overall picture (Anderson 2004). Since the 1980s, the advent and spread of genomic technologies changed the role of culturing, which increasingly came to be seen as a bottleneck, if not an outright obstacle to the study of microbial diversity. Presumably not only for technological reasons, the diversity, mixedness, and heterogeneity of microbial populations became a focus of interest much more generally among microbiologists and an ideal in its own right. The concept of the "microbiome" is indicative of this trend, as much as ecologically-inspired concepts of species (ecotypes; see O'Malley 2014). Novel in situ culturing methods such as microbial "traps" in the soil, looking like revenants of Conn and Winogradsky's direct method, combine the advantages of pure culturing with those of in situ analyses and genomic studies (Epstein 2013).

The new diversity ideal, as epitomized in the Winogradsky columns on display at Micropia and the AMNH, certainly should not be understood as the return of a messy and polluted bacteriology from before the 1880s. The techniques and concepts of purity are still important for classifying microbes; not the least since a deposition of a pure type culture in a collection remains a requirement to validly publish a new bacterial species.[33] Rather than speaking of a complete displacement of practices and concepts, I propose to understand the historical development sketched out in this paper as a transition between approaches to study microbes

[33] "Whenever possible, the type of a species or subspecies is a designated strain. A type strain is made up of living cultures of an organism which are descended from a strain designated as the nomenclatural type. The strain should have been maintained in pure culture and should agree closely in its characters with those in the original description (see Chapter 4C). The type strain may be designated in various ways (see Rule 18b, c, and d). For a species which has not so far been maintained in laboratory culture or for which a type strain does not exist, a description, preserved specimen, or illustration (see also Rule 18f) may serve as the type" (Lapage et al. 1992, Rule 18a).

conceived of as either dominant and paradigmatic or marginal and partial. The outcome of a prior historical phase (such as the pure culturing techniques) was not lost, but changed status at a later time, which has, in turn, led to a more complex situation in the present.

To conclude, I shall briefly return to the question of what may be gained for an understanding of science by taking a *longue durée* perspective on practices and concepts. My take differs historiographically from Braudel's because I focus on artifacts, in terms of how their use has shaped the scientists' perspective on microbes. The long history of the two devices highlighted above and their continuous impact may be taken as reminders of what David Edgerton has called the "shock of the old" in the history of technology; that is, the persistent impact of simple technologies from a long bygone age (Edgerton 2008). Thereby, these examples share similarities with histories of other simple but influential techniques in science: The aquarium, for example, has served both as a gateway and an instrument to biologists since the nineteenth century, as have the various glass containers, first and foremost the test tube, that populate every laboratory (Reiß 2012; Espahangizi 2015; Jackson 2015). Comparative analyses of what may be called techno-scientific infrastructures (omnipresent, often invisible, but nevertheless formative) may help to distinguish more general patterns of continuity and change in research. This could reveal different speeds of development within science, or the coexistence of phenomena of different ages beyond innovation alone, for example, the presence of the Petri dish and Koch's plate in the age of genomics, or the return of the Winogradsky column within ecology.

At this point, there is a connection to a current topic that has repercussions beyond the history of the life sciences—that is, acceleration. Acceleration has been singled out as a hallmark of modernity, pertaining to a speeding up of social processes, rhythms of individual life, and technological change (e.g. Rosa 2013). Science and technology obviously play an important role: Technological innovation appears as an important factor of acceleration—from trains to telephones to airplanes and the internet. And so it seems for science itself: For biology, one may think of the last century as a seemingly ever-accelerating chain of technological innovations—from the ultracentrifuge around 1930, to the electron microscope after World War II, to the impact of molecular genetics, to cloning, sequencing, and computational studies in more recent decades.

Yet, it may be illuminating to turn the tables on this argument and to ask what the histories of simple technological infrastructures, or of continued practices, may tell us on this issue. Such histories of continuity in spite of an environment characterized by change indicate a temporal heterogeneity of developments, in which the "old" also has its place and its relevance. Therefore, they may help to counter the monolithic accelerationist narratives of scientific development that focus solely on development and change, which generally prevail in the historiography of modern sciences. And as science and technology frequently figure as unquestioned pillars in theories of social acceleration, this may even be of wider importance.

Following the transitions from contamination to purity to diversity in microbiology over more than a century may also enable us to conceive of how these concepts have been interwoven diachronically within broader discourses. As much

as the practices of a serial mass production of identical organisms by Koch's plate technique, e.g. in medical research or in the brewing industry, can be considered as part of the cultural, social and economic developments of the late nineteenth century (concepts of purity, industrialization), the present focus of diversity, which has unfolded since the 1980s in conjunction with novel concepts of cells, organisms, and evolution, is certainly part and parcel of broader developments as well: One may think of ecological thinking, for example, of networks and their ontologies, or perhaps the deskilling of labor and the rise of post-industrial modes of production (see e.g. Grote 2016; Paxson and Helmreich 2014). While this must be spelled out elsewhere, when a glass cylinder filled with mud and billions of different microbes becomes part of a hygiene exhibit, or when contemporary craft brewers celebrate the diversity of yeasts, which, a 100 years earlier, were considered a dangerous contamination, it is clear that the era of pure culture is over. Or, to be fair, over in some quarters.

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