**History of Gelling Agents in Microbiology & Colonial Growth Characteristics**

Basu, S., Bose, C., Ojha, N., Das, N., Das, J., Pal, M., & Khurana, S. (2015). Evolution of bacterial and fungal growth media. *Bioinformation*, *11*(4), 182.

Microorganisms are almost omnipresent, very diverse and indispensable to human survival. Preparation of suitable culture media is one of the prerequisites to study them. **Different microorganisms thrive at different environments and have variety of growth requirements; like nutrients, pH, osmotic conditions and temperature.** **Due to the lack of sufficient variability of media composition, replication of the exact environmental conditions in the laboratory, nearly 99% of all microorganisms are still unculterable, for example Trephonema pallidum, Candidatus liberibacter, Tropheryma whippelii, Bartonella henselae [1].** Given the current limitations of microbial growth in lab, formulation of newer media should be a much needed thrust area of modern biology.

**Microbial culture media can be of different type, depending on the nutritional growth requirements of the microorganisms.** Microorganisms require about 10 macroelements namely (**C, O, H, N, S, P, K, Ca, Mg and Fe**). The first six components are used in the synthesis of **Carbohydrates, Lipids, Proteins and Nucleic acids** and the remaining four exist in the cell as cations and play a variety of roles. In addition to macroelements, all microorganisms require several microelements like (**Mn, Zn, Co, Mo, Ni and Cu**). These are generally part of enzymes and cofactors. Microorganisms also require growth factors, which are organic compounds.

Bacterial media can be formed into **simple, synthetic or complex media**, where **they vary in nutritional make-up**. **Simple media** facilitates the growth of non-fastidious bacteria and the exact chemical compositions of simple media are known. **Synthetic media** is composed of minimal ingredients needed for the growth of the microorganisms, for example Davis and Mingioli Medium. However in **complex media**, the exact chemical composition is not known, for example in Tryptic Soy Broth. Bacterial media can be of different consistency, solid nutrient agar medium, Stuart’s semi solid media and nutrient broth liquid media. (p. 182)

In the earlier days, **isolation of bacteria for preparation of pure culture was very difficult in liquid media**. Often any attempt to prepare pure culture using liquid media would result in contamination because of the slow and tedious steps. Thus came the need of a solid media, which was achieved by adding a gelling agent to the liquid broth. The first gelling agent used was gelatin by Robert Koch in 1881 to prepare solid medium. **But as it melted at a temperature around 35˙C and was digested by the bacteria, he faced problems with its use**. These problems led to the discovery of an alternative gelling agent agar. Angelina Fanny Eilshemus, wife of Walther Hesse, an associate of Koch, first proposed the use of agar as a substitute to prepare solid culture medium. **Gradually various advantages of agar made it very popular amongst the scientists, as it is stable at wide range of temperature (solidified at 32˙C to 42˙C, melted at 85˙C) and thus is suitable for the growth of mesophilic organisms. Additionally agar does not have any toxic effect on bacteria, has good diffusion characteristics and is not digested by most bacteria. Agar is also found to have good clarity and is metabolically inert.** But on the flip side, agar is not suitable for culturing thermophilic microbes and inhibits PCR in a concentration dependent manner [6]. Besides, **high cost of agar has made research work very expensive and due to its high usage in laboratories, the natural resources of agar (i.e. Gelidium sp., Gracillaria sp. and Pterocladia sp.) are being over-exploited and have made scientific community to look for some other alternative sources of gelling agents.** (p. 183)

Hesse, W., & Gröschel, D. H. M. (1992). Walther and Angelina Hesse-early contributors to bacteriology. *ASM news*, *58*(8), 425-428.

Walther’s wife, called Lina in the family, was his major supporter in many different projects. Aside from her duties in the house and in the education of three sons, she became well acquainted with Walther’s scientific work and assisted him like a present-day medical technologist. The profession was not then known. However, this period ushered in many changes for women in Germany, and they were just starting to step out of the domestic environment into professional life.

Working with her husband, Lina soon played a major part in the magnificent development of medical illustration. She was not the only talented artist of the family. Her grandfather was the-Swiss painter Leopold Robert, and her younger brother, Louis Eilshemius, achieved some fame as a New York painter. She used her talent to prepare drawings of microscopic preparations for her husband’s publications. The last publication of Walther Hesse from 1908 described a quantitative method for the culture of intestinal bacteria with special attention to stools from typhoid fever patients. **[Hesse] drew pictures of the magnified colonies on agar plates during different growth phases and colored them with watercolors in a highly accurate way, indicating her thorough understanding of bacteriology and microscopy.**(The author is proud to mother’s original drawings)

In the summertime, both Schwarzenberg and Dresden had **temperatures that caused liquefaction of gelatin**, the solid culture medium used to coat glass tubes used for the studies. Furthermore, **gelatin-liquefying bacteria often destroyed the cultures**.

**One day the frustrated scientist asked Lina why her jellies and puddings stayed solid at these temperatures. She told him about agar-agar. Agar-agar had been known as a gelling agent in warm climates**. For example, East Indian swallows use agar for making their nests-the bird’s nests of the famous Chinese soup. Lina had learned about this material as a youngster in New York from a Dutch neighbor who had immigrated from Java. The practical application of this kitchen secret was to bring major recognition to the Hesses, more today than during their lifetime. It contributed to Walther’s success with his air studies, and it was an essential contribution to the development of modern bacteriology. **The thermal stability of agar, its resistance to microbial enzymes, and the ability to sterilize the medium and store it for a long time permitted long-term cultures,** especially important in tuberculosis research and diagnosis.

Walther reported this finding to Robert Koch, who immediately included the new medium in his studies of the tubercle bacillus. **Although Koch mentioned agar-agar in a short sentence in his 1882 preliminary note on the tubercle bacillus, he did so without giving credit to the source.** (p. 427)

Hitchens, A. P., & Leikind, M. C. (1939). The introduction of agar-agar into bacteriology. *Journal of bacteriology*, *37*(5), 485.

We can imagine the elation of Dr. Hesse when he set up and studied his tubes lined with his wife's new medium and found that his troubles were at an end. **Now he could prepare a substrate, solid, transparent and sterile, which would retain its consistency at all temperatures at which bacteria could grow and which, furthermore, would not be liquified by any of the organisms he encountered in his studies.** Without delay the discovery was communicated to Robert Koch by letter, probably late in 1881. Koch recognized its value and made it his own. In 1882, in his now classic preliminary note on the tubercle bacillus, **Koch made what is the first printed reference to the use of agar-just one short sentence for a technical improvement so fundamental and epoch making.** No formal paper was ever published. (p. 491)

Mortimer, P. (2001). Koch's colonies and the culinary contribution of Fanny Hesse. *Microbiology Today*, *28*, 136-137.

**Gelatin was inconvenient for several reasons. Various bacteria were found to liquefy it and on hot days it melted spontaneously**. **For the same reason gelatin could not be incubated at the temperature that most human pathogens needed to grow in a convenient time span**

Fanny Hesse has been portrayed as ‘just a housewife’, but the historical record contradicts such a dismissive comment. She was born Fanny Eilshemius in New Jersey in 1850 to a first generation immigrant family who had prospered sufficiently to be able to send her, when she reached her early twenties, on a tour of Europe. In Germany she met and married Dr Hesse and, like others who were the wives of the pioneers of bacteriology, she acted as his laboratory assistant and technical artist. What has immortalized her, though, was not her exercise of these skills but her modest proposal that agar should be used in the growth medium for isolating bacteria. **She had first learnt about agar from friends of her mother who had lived in the East Indies, where the seaweed extract originates. There it was, and is, widely used as a cooking ingredient.**

Her contribution was not acknowledged. Soon, however, **it was realized that it was more convenient to grow bacteria not in but on the surface of agar and to have the medium pre-poured into a shallow circular ‘Petri’ dish. A loopful of bacterial suspension could then be swept back and forth across the surface of the set agar until the number of cells transferred from the loop was so few that they grew as single colonies. It is an isolation technique that has never been changed; there has simply been no need.  (p. 136)**

Fanny Hesse, does deserve everlasting credit for bequeathing to bacteriology a simple and enabling technique for isolating and growing bacterial species. **Visualization of bacterial colonies remains essential for clinical diagnosis and they are sources of pure microbial DNA and proteins…Other living cells can be suspended in it, immunological reagents will diffuse through it and large molecules can be separated electrophoretically in it. (p.137)**

**Questions**

**Answer the following questions.**

1) Why is nutrient agar necessary in the isolation of bacteria?

2) Why is it that bacteriologists do no rely on gelatin agar when cultivating bacteria on solid mediums?

[***Click here to visit the "View Online" version of the OpenStax Microbiology textbook.***](https://openstax.org/books/microbiology/pages/1-introduction)***Search for the name “Fanny Angeline Hesse,” or any variation of the name you may have read in the discussion post text. Search the name "Robert Koch."***

3) Is there any mention of Fanny Hesse in the book?

4) Can you find Robert Koch’s name in the book?

5) Why do you think curricular materials continue to exclude such important individuals despite calls to acknowledge the diverse makeup of individuals in the scientific enterprise?

6) Why do you think Robert Kock failed to acknowledge Hesse in the use of agar as a solidifying agent?

7) What is the cultural origin of agar-agar? Where is it found and what is it used for? Why is there value in culture as it relates to scientific discovery and innovation?

8)What do we call microorganisms that are “finicky” and difficult to grow?

9) Describe three different types of media (simple, complex, synthetic).

10) Describe the different media used in this lab (nutrient agar plates, nutrient agar slants, nutrient agar broths, differential media)

11) Why is it useful to grow bacteria on a solid medium rather than relying solely on liquid mediums?

12) Is agar only used in bacteriology? What else is it used for?

13) Why is it important to find alternative resources by today’s standards given agar-agar is derived from a natural resource?