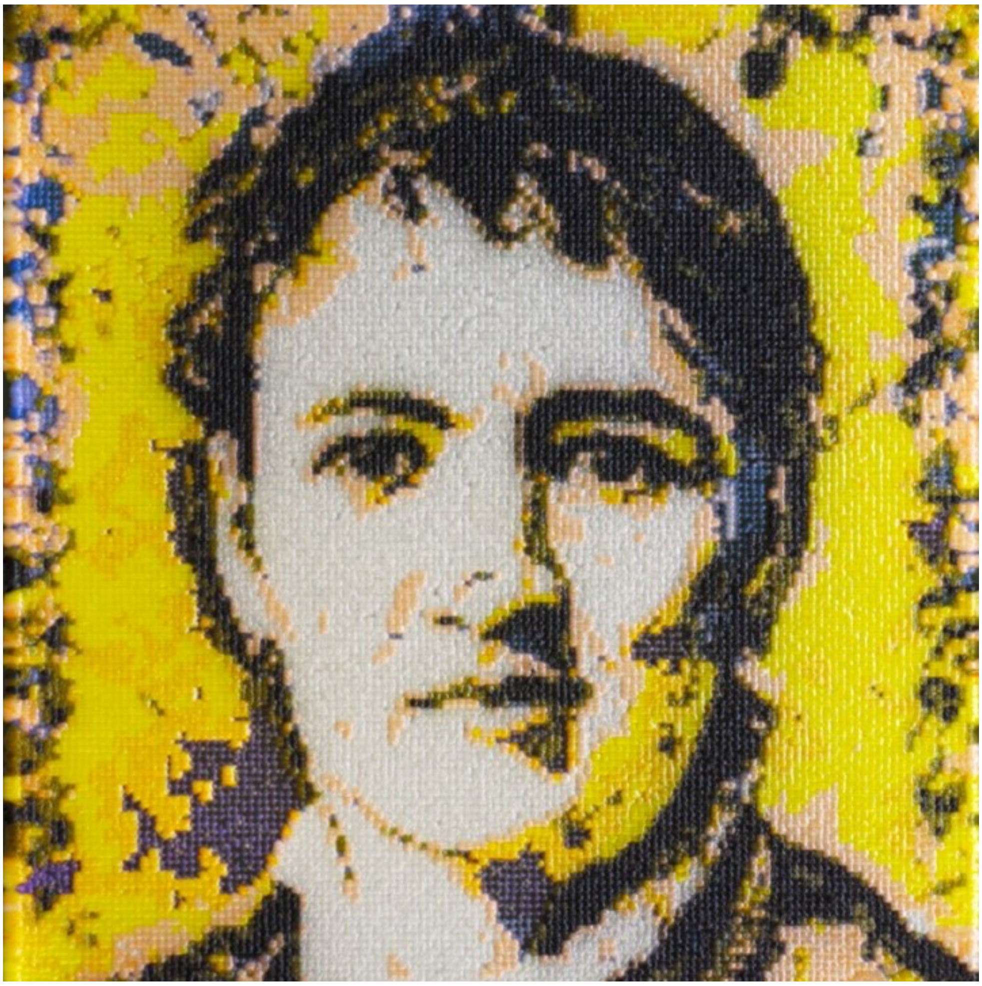
MicroDiscoverer Hero: Fanny Angelina Hesse (1850-1934, USA/Germany)

(Corrado Nai)



Fanny Angelina Hesse portrait (from Wikipedia) reproduced in "Yeast Art", a form of "agar art". One "pixel" is one colony of yeast growing on agar (Credit: Aleksandra Wudzinska & Jef Boeke, NYU Langone Health).

Claim to fame: Use of agar to grow microbes

Background: description of the problem faced

Microbes in the environment are gross mixtures of different species and it is impossible to assign responsibility for a particular activity to one species, that is: to establish a causal relationship. Microbiologists always try to establish causal relationships so that they can focus just on the microbe that expresses the activity of interest and learn about its mechanism. To solve the problem of mixtures of microbial species, microbiologists developed the technique of 'dilution to extinction'. This means that a mixture of microbes (say 10 million) is successively diluted (say 10-fold dilutions, by adding 1 ml of sample to 9 ml of water or medium) until the concentration is so low that single cells can be isolated. Growth of these single cells separate from one another provides single species for study, a 'pure culture'.

There are basically 2 methods to achieve dilution to extinction. One is that already described: successive dilution in liquid such that the final tubes of solution contains only a few cells which, when plated on a solid medium grow as well separated single colonies. The other is to 'streak' the culture in a line on a solid surface; then to use a sterile instrument to streak across the first streak; then to repeat this once more. Each streak carries fewer and fewer cells, such that the final streak deposits well separated cells which then grow into single colonies (https://www.youtube.com/watch?v=_1KP9zOtjXk).

Agar is used in every modern laboratory to solidify nutrient-rich media and grow microbes as pure cultures (single colonies). The "culture plating technique" developed in Robert Koch's laboratory involves streaking or plating cells of microbes on agar plates in Petri dishes (developed by Julius Petri in 1887). But before Fanny Angelina Hesse introduced agar to microbiology, researchers used media such as meat broth, coagulated egg whites, blood serum, potato slices, or gelatine. These media have one or more disadvantages: they are not transparent (hence not suitable for microscopic analysis), they are consumed by microbes (hence not stable), or they become liquid at temperature used to grow many bacteria (hence hamper isolation of single colonies). Without single colonies, microbiologists have a very hard time in linking what they observe with a particular microbe.

The Approach taken

In his work in the early 1880s, Robert Koch started using gelatine following examples from colleagues studying fungi. Gelatine is transparent and semi-solid, and offered advantages over potato slices or meat broth. However, Koch was studying disease-causing bacteria which unlike fungi grow at human-body temperature, which liquefies gelatine. And even without that, many microbes can degrade gelatine into a liquid glob. Robert Koch needed an alternative substance. Fanny Angelina Hesse provided this substance: agar.

The Breakthrough

Agar (a name derived from "*agar-agar*," meaning "jelly" in the Malay language) is a complex sugar present in red algae (seaweed) from the genus *Gelidium*. In Asian and South-East Asian countries, agar is used in cuisine as gelling agent for savoury and sweet dishes. The ingredient is of non-animal origin and low concentrations of it are enough to give dishes a firm bite. Because of her Dutch heritage, Fanny Angelina Hesse, whose husband Walther had worked in the lab of Robert Koch, knew about the use of agar in Java, at the time a colony of The Netherlands.

Agar is transparent, has higher melting (i.e. 96°C) and solidifying temperatures (i.e. 40-45°C) than gelatine, and can be sterilised and handled easily in the lab. Its complex sugar chemistry makes agar non-digestible by most microbes (and by us!). Since its introduction in the life sciences in 1881, agar has become the staple ingredient in every microbiology laboratory. Alternative gelling agents exist (xylan, phytagel, gellan gum, and others) but so far none has proved superior to agar.

Its Application

Walk into any microbiology laboratory and you will find agar. Countless protocols and techniques involve agar, including: creation and maintenance of culture collections (the storage of different microbes for research purposes), replica plating (the transfer of colonies from one agar plate to another), antibiotic testing (the evaluation of antibiotics' efficacy), biochemical and enzymatic essays (the analysis of biological molecules and enzymes in cells), cloning (the transfer of genes between cells), diffusion and motility essays (the observation of movements of chemicals and cells), bacteriophage assays (the quantification and handling of viruses which infect bacteria), microbial identification and discovery (the description of species or strains based on macroscopic morphology), co-cultivation studies (the concomitant incubation of different microbes to analyse how they interact with each other), and more.

An illustration of fungi and bacteria on solid media by Fanny Angelina Hesse for a publication from Walther Hesse (1884) on microbes in the air.

Agar is so important that during World War II Britain, faced with import shortages from Japan (the major producer at the time), issued a national emergency and started foraging seaweed along its coast to find for alternative sources of agar. Without agar, production of antibiotics would have stopped! Agar is also used in industry, medicine... and art! “Agar art” is a form of “bioart”” created with living cells: colourful (pigmented) microbes are the colour, and agar is the canvas (https://www.smithsonianmag.com/science-nature/how-microbiologists-craft-stunning-art-using-pathogens-180977261/)!

Its Significance and how it changed microbiology and humanity

It is not an exaggeration to say that there is hardly any innovation in microbiology which has been so pervasive and so durable as the use of agar. Agar plates are a staple in every microbiology laboratory, and invariably used at the beginning (to select single colonies) and end of most experiment (to isolate and preserve desired strains). Many microbiology testings are done on agar plates (see: *Its Application*). Countless lifesaving and life-changing experiments have been done on agar plates, like for example diagnosis of the pathogens causing infections and determination of their antibiotic sensitivity/resistance to inform the clinician about treatment options, antibiotic discovery, vaccine testing and production, and the discovery of CRISPR/Cas9. Agarose is a derived from agar and is used in numerous molecular biology protocols, like DNA and protein electrophoresis.

A scientific study commemorated Fanny Angelina Hesse in an uncommon way by naming a genus of bacteria *Fannyhessea*. It's hard to tell if this honour would have pleased Fanny Angelina Hesse: one of the bacteria carrying her name, *Fannyhessea vaginae*, is a cause of vaginal infections.

Fanny Angelina Hesse – the person

Fanny Angelina was born Eilshemius in 1850 in New York from a Dutch merchant father and a Swiss mother emigrated to the US the year before. She was the oldest of five siblings and grew up in a spacious property in New Jersey (near New York). At 15 she was sent to a finishing school in Neuchâtel (Switzerland) to learn home economics and decor, as common for daughters of wealthy families at the time. In New York and then later in Switzerland she met the German physician Walther Hesse, whom she married in 1874 in Geneva. The couple lived most of their life in Saxony (Germany), and spent the Winter of 1880-81 in Berlin where Walther worked as a scientific assistant in Robert Koch's lab. There Walther learned new bacteriological methods and struggled (like other colleagues) with gelatine to grow pure cultures of bacteria. In the Summer of 1881 in Schwarzenberg (near Dresden) the couple figured out that agar is more suitable than gelatine, and communicated this per letter to Koch in Berlin. Robert Koch mentioned agar for the first time in the scientific literature in his famous lecture on tuberculosis bacteria without crediting the Hesse but, notably, the importance of agar eluded him. For many years, microbiologists investigated the benefits of agar versus gelatine for growing microbes. As often occurs in science, progresses are gradual.

Walther (1846-1911) and Fanny Angelina Hesse (1850-1934) in 1897 in Saxony (Germany), unpublished (Credit: Estate of family of Dr. Wolfgang Hesse).

While her historical and societal role has been that of a “Deutsche Hausfrau” (a German housewife), and while recent accounts in popular articles and blogs often refer to her as an unpaid assistant in Robert Koch’s lab or as a family cook, Fanny Angelina Hesse was much more than that. At home, she had the role of raising three kids. Throughout the career of her husband Walther, she supported him in his scientific work, and took over two major roles in preserving and documenting scientific results. Over decades she archived Walther’s publications and other historical documents which are still preserved to this day. Among these are astonishing scientific illustrations drawn by her own hand showing growth of microbes on solid media. Her legacy and contributions qualify her as a scientist in her own right. From information available about her life, we know that Fanny Angelina was a humble person and she never spoke about her revolutionary innovation in the life sciences, even though already during her lifetime her name appeared in textbook as the person introducing agar to microbiology. Fanny Angelina Hesse died in 1934 in Dresden (Germany).