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Seeing Red: The Story of Prodigiosin

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I. Bread, Blood, and Bacteria

Bread, both leavened and unleavened, plays a crucial nutritional, religious, and emotional role in human lives. In the Old Testament, bread is said to "strengtheneth man's heart" (Psalms 104:15[1]), and in the Lord's Prayer the request is "Give us this day our daily bread" (Matthew 6:11). In Judaism, unleavened bread is the centerpiece of the Passover meal. In Christianity, the Eucharist or sacrament of the Lord's Supper is celebrated through the consecration and consumption of bread and

[1]Biblical quotations are from the Authorized King James Version.

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wine: "And as they did eat, Jesus took bread, and blessed, and brake it, and gave to them, and said, Take, eat: this is my body" (Mark 14:22).

Bread is also, especially when not dried out, an excellent culture medium for the growth of many microorganisms, so much so that many present-day commercial breads contain calcium propionate "added to retard spoilage." In the pre-antibiotic era, microbial contamination of bread was used to good effect: the healing of wounds was facilitated by application of preparations made from moldy bread. A specific and early example is documented in an English herbal of 1760. Such preparations may well have contained penicillin, patulin, or other antibiotic materials formed by the fungi (Wainwright, 1990).

However, in most cases when microbes use bread as a substrate for their growth, the result is spoilage. Contaminated breads can be detected by repellent flavors and distinctive coloration. Most spoilage of bread is caused by fungi: *Aspergillus niger* forms black colonies, many members of the genus *Penicillium* are blue or green, while certain yeasts and bread molds such as *Neurospora crassa* form pink to red pigments. Bacteria are less commonly associated with deterioration of bread; however, under warm and humid conditions some strains of *Serratia marcescens* form distinctive red colonies on this substrate. The red color derives from the presence of the pigment prodigiosin and/or related materials (see later). As the bacterial colonies reach maturity, they dissolve into a fluid and viscous state with a mucilaginous appearance and an uncanny resemblance to blood. Indeed, from early times, there are many records of the appearance of "blood" on bread, beans, and other starchy foods such as polenta and potatoes.

Like bread, blood is a substance with profound cultural implications beyond its physiological role. Human and animal sacrifice were practiced in many societies with the intent to propitiate the wrath of an all-powerful deity. The victim's blood was often associated with a mystical power. For the Aztecs, the sun god (Huitzilopochti) drove back the moon and stars each day. To carry out this tremendous task, he had to be nourished with human blood. In some cultures, prisoners of war were sacrificed and their blood consumed by the executioners, while in other cultures the drinking of blood was taboo (e.g., the ritual slaughter of animals by exsanguination as practiced by the Jews).

The Old Testament is filled with blood imagery and stories of ritual sacrifice. During the momentous Passover devastation of all the first-born (both men and beasts) in the land of Egypt, the Israelites were protected by the blood of an unblemished, 1-year old, male lamb spread on the side and upper door posts of their houses (Exodus 12). The paschal (Passover) lamb was a term later applied symbolically to Christ.

To this day, many Christians proclaim that for believers redemption from sins is only possible by way of "the blood of the Lamb". At the Last Supper, Christ used wine to symbolize his blood: "This cup is the new testament in my blood, which is shed for you" (Luke 22:20). The Roman Catholic faith has embraced the belief in transubstantiation, whereby the bread and wine of the Eucharist actually turn into the body and blood of Christ. This formal doctrine was specifically defined at the Fourth Lateran Council (1215) and reaffirmed at the Council of Trent (1551) (Cross and Livingstone, 1974).

As noted above, mature colonies of pigmented *Serratia* are eerily bloodlike in appearance. More than a few microbiologists have hypothesized that the growth of these bacteria could be interpreted, in certain religious or symbolic contexts, as the miraculous appearance of blood. This paper discusses the possible role of *Serratia marcescens* in forming bloodlike material on starchy foods, and reviews many of the unusual properties of this fascinating bacterium and the red pigment(s) that it and other microorganisms form. In reviewing the historical record, we have made extensive use of previous publications (Harrison, 1924; Reid, 1936; Gaughran, 1969; Yu; 1979; Cullen, 1994).[2]

II. Early Instances of "Blood" on Bread

It is impossible to know who first observed foodstuffs apparently carrying drops of blood. Red-spotted bread was probably observed in many parts of the world; however, only in European countries is there an extensive written record and only there did it come to play a role in religious controversy. The first known recorded report dates from Alexander the Great's siege of Tyre in 322 BCE. The disgruntled Macedonian troops were tired of the siege, when a soldier noticed a trickle of blood inside a piece of broken bread. A soothsayer named Aristander interpreted the event as a good omen, opining that, had the droplets of blood been on the outside, the Macedonians would have been endangered. Since the flow was from the inside, it was an omen that Tyre would fall. Almost certainly, Aristander was well compensated for his ability to both calm the troops and prophesy the future.

Christian Gottfried Ehrenberg (1795–1876) collected almost 100 European reports of the occurrence of blood, starting in the eleventh century; an English summary was provided by Gaughran (1969). Significantly,

[2]Unless otherwise apparent, the simple word "blood" will carry the meaning of "blood" or "bloodlike materials" to avoid much repetition. The genus abbreviation *S.* will refer to *Serratia*, and *Streptomyces* will not be abbreviated.

blood was frequently observed on, or flowing from, the bread or wafers used as the Host in the Eucharistic liturgy. The prototype of many examples was recorded from Alsen, Denmark, in 1169. A village priest saw blood on a Host. Upon reporting this event to his superiors, the Chief Priest predicted the imminent shedding of Christian blood. A few days later, a pagan army overthrew churches, drove people into slavery, and killed those who resisted.

Within several decades after the Alsen event, a strange myth grew up around reports of blood on Communion hosts. In 1247, near Berlin, a woman removed a consecrated wafer from her mouth and sold it to Jews, who "stabbed it," resulting in the appearance of blood. The wafer was returned to the church, bringing it much fame, and the Jews were apparently unharmed. However, in other similar stories, Jews were persecuted and killed. Thus, in 1296 near Frankfurt, a purportedly stolen wafer was sold to Jews, stabbed, and yielded blood. A mob subsequently marched with banners, attacking Jews in Nuremberg, Rothenburg, Würzburg, and elsewhere, with a reported death toll of 10,000.

Similar stereotypic reports of bloody Communion wafers led to repeated tormentations and executions of Jews for at least 200 years. Ominously, the geography of the persecutions overlapped that of the atrocities of the Holocaust several centuries later, with most of the incidents reported from cities in Germany and Poland. The number of those who perished will never be known. Scientists who believe that the explanation for bleeding hosts is the growth of *Serratia* are fond of quoting Scheurlen (1896), an early German observer: "dieser Saprophyt mehr menschen umgebracht hat als mancher pathogene Bacillus," or, in Isenberg's translation (1995), "This saprophyte has killed more humans than some pathogenic bacilli."

In retrospect, whether or not the stories of red spots on Communion wafers were real or fabricated, the interpretation of the events and the reprisals against the Jews seem to make little sense. The Middle Ages was, of course, a time of many superstitions and profound religious belief. One wonders, however, why the Jews were not hailed as heroes for showing that the Host would bleed, thus providing a vivid demonstration of the truth of transubstantiation. It was nonsense to think that Jews wished to drink any sort of blood, since such an action was specifically forbidden to them. Deeper irrationalities were involved, with the incidents serving as a pretext to express a violent antisemitic prejudice. Eventually, the legend of the "blood libel" developed, which held that Jews needed the blood of children to make bread for Passover or for sorcery-related medicinal purposes; more recently, this libel was part of the many grotesqueries incorporated into Nazi propaganda (Trachtenberg, 1943).

On the other hand, in a Christian context, the presence of blood on sacramental bread was interpreted in support of the doctrine of transubstantiation. In fact, one such incident became perhaps the most celebrated miracle of the thirteenth century (Cullen, 1994). According to Church history, a German priest with doubts about the doctrine of transubstantiation once celebrated mass at the Church of Santa Cristina in Bolsena, Italy in 1263. When blood dripped from the Host onto the altar linen and his vestments, his doubts were resolved and he sought absolution for his lack of faith. This event became celebrated as "The Miracle of Bolsena" and was later depicted in a Vatican fresco by Raphael. To commemorate the miracle, Pope Urban IV issued a bull that instituted the Feast of Corpus Christi and later decreed the construction of a new cathedral in Orvieto in which the host and vestment linens are preserved to this day. It has been suggested that the relics provide a tantalizing experimental opportunity. If enough DNA could be isolated from them, the polymerase chain reaction could be used to test the hypothesis that *Serratia marcescens* was involved in this medieval miracle.

III. Red Bacteria and the History of Bacteriology

A. Pre-Pasteurian Research

A giant leap forward in the understanding of microbiology in general and the formation of red-pigmented materials on foodstuffs in particular began with yet another event in Italy, this time in Legnaro (province of Padua) in 1819. The affected foodstuff was a bloody polenta (corn mush, corn porridge) found in the squalid home of a superstitious farmer named Antonio Pittarello. Eventually, more than 100 families in the region reported bloodlike materials on polenta or rice soup. A cooked chicken was described as "dripping with blood." Maleficent spirits were blamed for the event, and families who found bloodlike spots on their food were accused of evil activities. The event caused so much publicity that an official investigation was established under the direction of Dr. Vincenzo Sette, the medical officer at Pione di Sacco. Sette concluded that a fungus was responsible, while a botanist-priest, Pietro Melo, "claimed that the phenomenon was due to a spontaneous fermentation of the polenta which caused the corn meal to be transformed into a colored mucilage" (Breed and Breed, 1924). Sette eventually published his report in 1824, calling the organism *Zaogalactina* *imetropha* (from the Greek, "living slime situated on food"). On one occasion, he reddened polenta in a priest's house, thereby, according to the story, disposing of the theory that the phenomenon could only occur in the house of a sinner (it is a tribute to local piety that no one suggested the possibility that the priest was less than perfect!).

Meanwhile, a pharmacist, Bartolomeo Bizio (then a student and later professor at the University of Padua), independently examined the red polenta, giving preliminary and detailed accounts of his work in 1819 and 1823 (Merlino, 1924). Bizio also classified the organism as a fungus and coined the further name *Serratia marcescens*. He used *Serratia* to honor a physicist, Serafino Serrati, who had run a steamboat on the Arno in 1787. Bizio believed that Serrati had a prior claim over "a foreigner" (presumably James Rumsey) as inventor of the steamboat and wished to honor his countryman. The second part of the binomial, *marcescens*, came from the appearance of the mature colonies that dissolved into "a fluid and viscous matter which has a mucilaginous appearance." *Marcescens* is the present participle of the Latin verb meaning "to decay or wither." Bizio performed experiments in which he used paper soaked with the red substance, or bits of red polenta, to transmit "seeds" of his fungus. As did Sette, Bizio made an honest mistake in identifying the causative bacterium as a fungus, but we are indebted to them for laying sure foundations for further investigations. Both Sette and Bizio were the first to provide evidence suggesting that the bloody material on food was due to a living organism, similar to the alga that caused pink snow on mountains, and transmissible from substrate to substrate by inoculation.

Many years later, the spoilage of corn by fungal growth was investigated in connection with pellagra. With whole corn, "sometimes the embryo is colored reddish by *Micrococcus prodigiosus*"—one of the many binomials applied to *S. marcescens* (Black and Alsberg, 1910). These authors, however, did not refer to the spoilage of polenta. They are known for their discovery of penicillic acid in *Penicillium puberulum* and the rediscovery of mycophenolic acid in *Penicillium brevicompactum* (Alsberg and Black, 1913).

Another chapter of the *Serratia* story picks up in 1848, when blood-like spots were found on a boiled potato in a Berlin home. Ehrenberg investigated the phenomenon and became fascinated. A distinguished physician and protozoologist (he described more than 300 new species), Ehrenberg regarded the organism as a "tiny, oval animalcule," and renamed it *Monas prodigiosa* in 1849. Although aware of Sette's prior nomenclature, his historical efforts apparently did not lead him to Bizio. He came to believe that most historical accounts of bloody food could be attributed to the growth of *M. prodigiosa*. His colleague

Scheurlen supported this belief, speaking of the "deceptive red color" of the microorganism and the fact that the hosts were a "particularly agreeable . . . medium" (Scheurlen, 1896: Gaughran, 1969). Cullen (1994) has analyzed retrospectively weather conditions that correlate humidity and warm air temperatures with historical reports of bloody foods.

Another historical aspect may be noted. The laboratory culture of microorganisms on solid or semisolid media (e.g., cooked potato slices, gelatine, agar) developed towards the end of the nineteenth century; Koch's pioneering work with gelatine was reported in 1881. Although Sette and Bizio did not use pure cultures, their work with polenta is probably the first documented use of a solid medium for culturing microorganisms (Bulloch, 1938). A splendid color plate of *S. marcescens* growing on a potato is found in an early text (Crookshank, 1890). The organism is named as *Bacterium prodigiosum* with three other binomials but not including *S. marcescens*; it is also described picturesquely as "Blood Rain."

B. Pigments and Paintings

At the time of Sette's work, a chemist, Pietro de Col, extracted the red pigment and used it to dye silk. He also created yet one more name, *Mucor sanguineus*, for the organism (Harrison, 1924). Similarly, both Sette and Bizio made ethanol extracts of the pigment and used them to dye silk and wool, sometimes with the aid of mordants. Alert to commercial possibilities, they were thwarted by the unfortunate sensitivity of the dye to light. It has to be remembered that in 1819 the major available red pigments were naturally occurring secondary metabolites derived with difficulty from insects (cochineal, kermes, lac) or from plants (madder); not until 1856 did Perkin produce the first synthetic dye, mauve.

More than a century after Bizio and Sette's work, Alexander Fleming found a curious application for the red-pigmented bacterium. He made microbial "paintings" by outlining a drawing on blotting paper, placing it on a nutrient agar plate, and then inoculating with bacterial culture broths. On incubation a colored "germ painting" developed. Six of these "paintings" were reproduced on the endpapers of his biography by André Maurois. Clearly unfamiliar with the vagaries of bacterial nomenclature, Maurois used the superseded name in describing the possible colors—"the *staphylococcus* is yellow, the *bacillus prodigiosus* (*sic*) red, the *bacillus violaceus* (*sic*) blue" (Maurois, 1959). Even more recently, *Serratia* has also been used as "red ink"; at the 1956 Presidential Banquet of the American Society for Microbiologists in Houston, substitute "place cards" were fashioned from Petri plates containing appropriate media on which the names of the officers had been "written" with cultures of red-pigmented *S. marcescens* (Anonymous, 1999).

C. The Genus *Serratia*

The organism with this long and fascinating history is a member of the Enterobacteriaceae (aero-anaerobic, Gram-negative bacteria) and is motile (Blazevic, 1980; Grimont and Grimont, 1984, 1991). Some species and biotypes of *Serratia* produce reddish pigment(s) and, depending on colony age, the color ranges from dark red to pale pink. Pigment production is dependent on specific growth conditions, including medium composition, presence of certain ions and detergents, and temperature. It requires air, and the pigmentation is better developed when *Serratia* cultures are incubated below 35°C or when a low-phosphate agar without glucose (e.g., peptone-glycerol) is used. There is a strong tendency for clinical isolates to be nonpigmented and difficult to distinguish from other coliform organisms (Hejazi and Falkiner, 1997). Nonpigmented *S. marcescens* biotypes seem restricted to hospitalized patients, whereas pigmented biotypes are ubiquitous. In a 1978 review of the genus *Serratia*, three other species (in addition to *S. marcescens*) were recognized (*Serratia liquefaciens*, *S. plymuthica*, *S. marinorubra*), and a fourth, tentatively discussed as "strain 38" (Grimont and Grimont, 1978), was later named *S. odorifera* (Grimont *et al.*, 1978). In the 1984 edition of Bergey's *Manual of Determinative Bacteriology*, *S. marinorubra* became *S. rubidaea*, and a sixth species, *S. ficaria*, was recognized (Holt and Krieg, 1984). In the second edition of *The Prokaryotes*, 10 species were mentioned and are presently known to belong in the genus *Serratia* (Grimont and Grimont, 1991). In addition to those already listed, the four other species are as follows: *S. entomophila*, *S. fonticola*, *S. grimesii*, and *S. proteomaculans*. Of these 10 species, only three—*S. marcescens*, *S. plymuthica*, *S. rubidaea*—produce prodigiosin (Grimont and Grimont, 1991).

Bizio's patriotism in naming the first member of this genus as *S. marcescens* is admirable, and there is a pleasant euphony in the name; however, the steamboating physicist had nothing to do with the contaminated polenta. Bizio might have made a more relevant choice, for instance, the use of the peasant's name in whose house the "fungus" was found: *Pittarella marcescens* also has a fine ring to it! In fact, naming the red-pigmented bacterium became something of a cottage industry among early bacteriologists, with more than 20 names applied to this organism during the 100 years after Bizio's description. There was a tendency to retain the prodigious characteristic invented by

Ehrenberg, probably in view of the supposed association with miracles—for example, *Bacillus prodigiosus* and *Bacterium prodigiosum*. In 1920, the "final" report of The Committee on Classsification of the Society of American Bacteriologists recognized a possible priority for *Erythrobacillus pyosepticus*, which had been preserved as ATCC 275, and suggested the name *Erythrobacillus prodigiosus* (Grimont and Grimont, 1991).

However, at that time, bacteriological nomenclature was governed by the International Botanical Code, which contained a priority principle requiring the oldest validly published name to be used. *Erythrobacillus prodigiosus* contradicted rules of priority and never gained acceptance outside the United States (Breed and Breed, 1924). It took the American bacteriologist Buchanan to apply the principle of priority and revive *Serratia* as the valid name. The first edition of Bergey's *Manual of Determinative Bacteriology* legitimized Bizio's priority more than a century after he had wished to honor Serrati (Bergey *et al.*, 1923). It has been retained in subsequent editions of the *Manual*. Ironically, there is no proof that what is now called *Serratia* corresponds to Bizio's organism. The genus *Serratia* has the distinction in bacteriology of being outranked in age only by the genera *Vibrio* (1773) and *Polyangium* (1809).

Even so, the acceptance of *Serratia* as a valid name has attracted considerable dissent. Specimens viewed by early microbiologists tended to be mixed cultures. The small size and morphological monotony of most bacteria provided few clues to the diversity of species. There was an unfortunate tendency to call all red-pigmented microorganisms *Serratia* simply because of their color. Red bacteria appearing on salted fish are a case in point. Such halophilic species do not ferment carbohydrates and are probably species of *Halobacterium* (Ayres *et al.*, 1980). Several species of the yeast genus *Rhodotorula* form shiny, pink to red colonies on bread and other starchy foods and may also have been responsible for some of the incidents of bloody bread. In his delightful essay "Heretical Taxonomy for Bacteriologists," Cowan (1970) devoted a section to "The heresy of *Serratia marcescens*" referring to the uncertainty of applying names from the "pre-bacteriological era." Cowan felt that it was better to change the rules of nomenclature than to use pre-Pasteurian descriptions that were "in the modern sense, nothing short of the farcical" (Cowan, 1956). Later, in his posthumously published *A Dictionary of Microbial Taxonomy*, Cowan (1978) stated his opinion authoritatively:

> In my view it is a waste of time to try to find useful bacteriological information from observations made before bacteria were clearly distin-

guished from algae, filamentous fungi and yeasts, and I believe we shall not lose anything by ignoring all work before the pioneer work of Pasteur.

Nevertheless, many scientists, especially microbiologists, will have confidence that *S. marcescens* was responsible for most of the incidents involving blood on foodstuffs. There are other microorganisms forming pink or red colonies such as the yeasts *Rhodotorula*, *Sporobolomyces salmonicolor*, and *Candida pulcherrima*; the latter at least is ruled out since its pigment, pulcherrimin, is an iron complex and is insoluble in the usual organic solvents. Moreover, the characteristic dripping or flowing of *S. marcescens* cultures is not associated with the yeasts or fungi having red pigmentation. On the other hand, since other bacteria produce prodigiosin or prodigiosin-like materials, some of the observed cases of blood-spotted food may have been due to organisms other than *S. marcescens*. The phenomenon was readily reproduced on "host bread" using *S. marcescens* cultures by Ehrenberg in the nineteenth century and more recently on polenta, unconsecrated Communion wafers (both Catholic and Protestant), and not-for-Passover matzos (Karp, 1988; Cullen, 1994); Protestant wafers gave the best results (Bennett, 1994). One of us has pointed out the lack of an appropriate control in these experiments; simple crackers or preservative-free bread without religious significance should have been included! (Bentley, 1997).

It may be noted that there have been reports of red spots on the cream layer of milk (Grimont and Grimont, 1978). Moreover, the range of materials subject to the development of red-spotted areas has been extended by the discovery of a "red spot disease" on culture beds of the kelp *Laminaria japonica* cultivated in the ocean around Hokkaido and used in the production of "makonbu" (Sawabe *et al.*, 1998). The dried kelp, more colloquially known as "konbu," is usually used to flavor broth and soups, being then discarded. An aerobic, polarly flagellated, marine bacterium was identified as the causative agent of the red spot disease and the name *Pseudoalteromonas bacteriolytica* sp. nov. was proposed for it. It produces a prodigiosin-like pigment.

IV. Prodigiosin and Related Compounds

A. Structures

The major pigment of *S. marcescens*, originally named prodigiosine, was isolated in 1902, but a choice between alternate structural possibilities was not possible until its chemical synthesis was achieved in 1960, nearly a century and a half after the entrepreneurial hopes of Bizio and Sette had been dashed. Now termed prodigiosin, it is a very