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Biosensors for Bacteria for Artwork

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Abstract: *Bacteria have a part in deteriorating, and preserving artwork and paintings. Bacterial processes may produce colored pigments work arts. Bacterial culturing was widely used to explore their pigments by scientists and artists. Bacteria may cause both deteriorative and resolving. A number of bacteria have been utilized in restoring artworks and also in their preservation and stopping their deterioration. The detection of bacteria can be a challenge to the scientific community. The development of biosensing devices to detect bacteria is also very important. In the present review paper we summarize the effect of bacteria in workarts and provide a number of biosensing devices that were developed up to date to detect bacteria.*

Key words: *Bacteria, artwork, art preservation, biosensors*

1. INTRODUCTION

1.1 Influence of Bacteria in Work Arts

An artwork such as a painting can be a good example of a place that microorganisms such as bacteria and fungi can colonize. Bacteria have a part in deteriorating, and preserving artwork and paintings. Bacterial processes may produce colored pigments work arts. Bacterial culturing was widely used to explore their pigments by scientists and artists. The same pigments are also utilized in the processes of coloring various foods, textiles, and paints. Microbes may also play a significant role to deterioration and preservation of art work. The metabolic steps of microbes can affect the ancient cave art and may cause deteriorative harm to these works of arts. Bacteria may cause both deteriorative and resolving. A number of bacteria have been utilized in restoring artworks and and also in their preservation and stopping their deterioration.

Bacteria compose molecules of pigments in their cells particularly in the cell membrane part. The bacterial colors are soluble or insoluble in water, but oxygen in the form of O2 is needed in order to only their aerobic bacterial molecules to be colored. Anyhow, coloring depends on a number of factors such as light, pH and temperature. Pigmentation in bacteria take place in combination with morphology, cellular activities and pathogenesis. In a very alike manner to chlorophyll photosynthesis, there are some bacteria that produce similar functions. Pigments in bacteria are able to absorb UV irradiation to protecting cells.

Another use of bacteria is their utilization as antibiotics to targeting phytopathogenic fungi, yeasts and human pathogens. Bacterial pigments can shield their cell by using an antibacterial resistance (i.e., they form a barrier in the cell that stops antibiotics to reacting with the wall of the cell membrane). Bacterial pigments may be used as sensors for water, soil, and air pollution. Bacterial pigments may show a number of range of colors which includes the whole range of the rainbow. The use of the produce of the pigments bacteria may be utilized as colorants for foods, textiles, and paints.

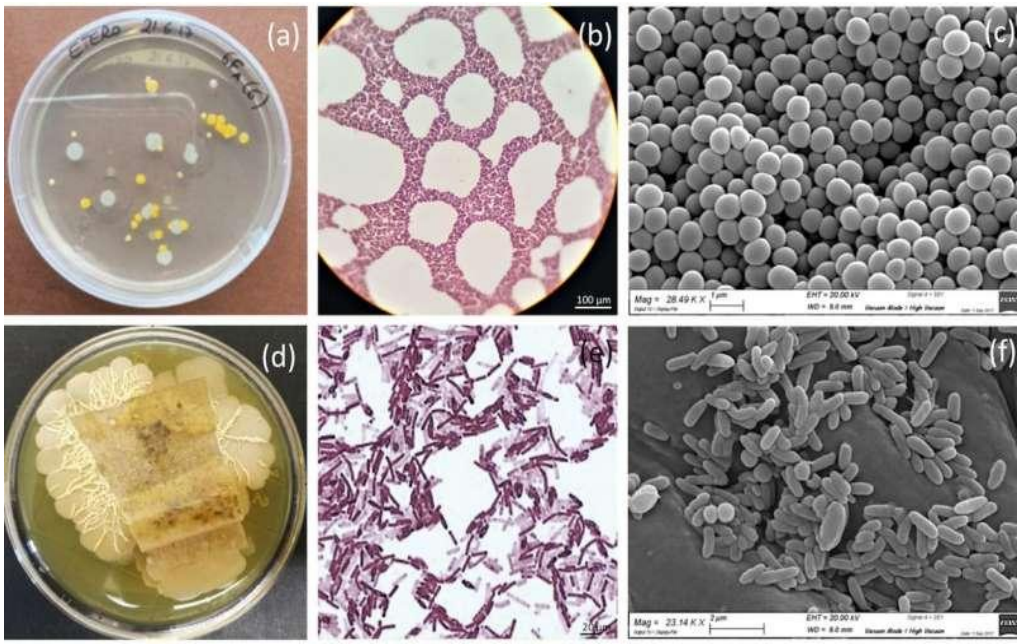
Below we provide some typical bacterial colors:

- Purple: Spirillum rubrum
- Indigo: Janthinobacterium lividum
- Violet: Chromobacterium violacein

- Blue: Streptomyces coelicolor
- Green: Chlorobium tepidum
- Yellow: Xanthomonas campestris
- Orange: Sarcina aurentiaca
- Red: Serratia marcescen
- Brown: Rhiobium etli
- Golden: Staphylococcus aureus
- Black: Prevotela melaninogenica
- Silver: Actinomyces sp.
- White: Staphylococcus epidermidis
- Cream: Proteus vulgaris
- Pink: Micrococcus roseus
- Maroon: Rugamonas rubra
- Fluorescent yellow: Pseudomonas fluorescens

The preparation and characterization of pure forms of pigments that are used for coloration is made by solvent extraction. Genes may be utilized as additives to bacteria to give specific colors. For example, E. coli which grows with an easiness provides an example as an additive to give pigments after is modified. Various colored pigments afterwards are been produced after the addition to E. coli.

A group of researchers from Italy (lead scientist was E. Caselli) have discovered various strains of microorganisms that were colonized in an old painting and they discovered that these bacteria and fungi could both destroyed and preserved the artworks. These scientists also reported that the painting microenvironment could result in detecting a forged painting. Used both microscopical and microbial culture methods and have identified the following bacteria: Aspergillus, Penicillium, Cladosporium, and Alternaria genera. They have also discovered spores of Bacillus microorganisms.



Microorganisms that were found on the painting: These samples were brought from the right hand (a, b, c) and the left (d, e, f) of the canvas. a) represents a colony of S. aureus spp. on an agar plate; b) the same sample that is seen by optical microscopy after Gram staining (100X) and c) by Scanning Electron Microscopy. d) colonies of Bacillus on agar; e) a sample of the microorganism as it seen by optical microscopy following Gram staining (100X) and f) Scanning Electron Microscopy (from ref. 1).

2. Deterioration of Artworks and Examples

Bacteria can deteriorate and destroy ancient rock and cave colors. The climate inside the caves may not be controlled as compared to the environment of the museums and as a result these paintings in those caves were ruined. Cave artworks were examined for the presence of bacteria and these results have exhibited the presence of a number of bacteria that were not well knownand were harming these old paintings,

While investigations in the past have shown a usual bacteria which exists in the walls of the caves in quantities about 5% of the total bacteria, while a bacteria in the class of acidobacteria feeds on FeO, the main pigment of red coloring elements in those places. Following these discoveries, the authorities in Spain closed these caves in order to stop further damaging of the artworks. Preserving routes now on days are focused on the identification of which metabolic processes occur between the microbes that exist in the cave artworks and what can be made to stop the processes.

In contrary to the paintings in caves, those in museums were found not to be affected by bacteria, mainly due to the fact that the temperature and humidity are in these places are highly regulated and therefore they are preserved. In addition, the metals that exist in the paintings are toxic to a number of bacteria. However, some of these microorganisms exhibit high heavy metal resistance and as a result they cause damage over time.

The 'Bradshaw art' in West Australia was exhibited to contain microorganisms which keep the pigments very strong as compared to the rest old stone artworks which they were having issues with degradation that was caused by the growing of bacteria. Experiments exhibited that the paintings had no more paint, but instead showed that they were destroyed. Various bacteria kept the surface of the paintings intact for very long time. The nutrients at the surface of the paintings provided the water and have initiated the interactions between the organisms (i.e., black fungi and red bacteria) in which each species benefits. DNA sequencing will show in the future the age of the paintings. Another similar example is that the bacterial infestation was shown beneficial in the maintaintance of the appearance of the Bradshaw art. On the contrary, rock art maybe seriously distroyed by "Desert Varnish," a pigment that exists when small size particles stick fast to the stone. The tiny parts of the rock contain Mn or Fe, and microorganisms that live on the stone are oxidized and therefore ruin the work of arts on the rock surface.

3. Bacterial Preservation of Art

Besides the fact that bacteria are harming the works of art, they can are preserve them. Some more harmful bacteria for the artworks can be imbedded to grow by the cellular activities of some bacteria. These protecting bacteria use the available space and they release some chemicals to impede the presence of these harmful bacteria. Some bacteria maybe utilized for the restoration of a wide range of artworks. A group of bacteria which belong to pseudomonas taught to consume the salt efflorescence that was build in the pigment. A technique utilized in Italy, in which the bacteria is spead with a woolcotton; a team in Valencia has further proceeded this method and prepared a gel which when is spread on the artwork, it prevents a gel forms that is expanded all over the surface that also prevents the moisture to spread on the painting. After application of the gel., it is taken out and the surface of the painting is dried and cleaned; through this route, along of moving out the moisture, the bacteria dies. The utilization of microorganisms in the retaining artworks and paintings in a good shape is a rapid growing technique as the least hazardous methods to restore workarts.

4. Examples of Biosensors for Bacteria for Artwork

A group of bacteria and fungi enter the cells and use any resources that surround them in order to survive. A review report appeared in the literature recently describing the progress achieved in the manufacture of novel biosensors to detect microorganisms such as bacteria and fungi [2]. The challenges that exist to develop future devices for this purpose are also described herein. In the report, bacteria, fungi and other pathogens were reviewed. The design and constructions of novel biosensors which were very selective and sensitive has broaden the field for effective biodefense.

Pathogen monitoring is vital and as such a wide range of methods have been described in the literature which were reviewed in a recent published paper [3]. The methods that described included enzyme-linked immunosorbent methods (ELISA) and direct tissue blot immunoassays (DTBIA). DNA-based

techniques such as polymerase chain reaction (PCR), real time PCR (RT-PCR) and dot blot hybridization. However these techniques are time-consuming, request expensive and highly sophisticated instrumentation and are not suitable for real time detection and monitoring. It is therefore mandatory to develop novel nanosensors to monitor these microorganisms and point-of-care systems. This paper reviews all the recent achievements in the development and manufacture of nanosensors for the detection of bacteria that were based on antibodies and DNA receptors. The utilization of various materials that were manufactured by using nanotechnology such as channels with the smallest passage dimension and metallic ultrafine particles with a size between 1 and 100 nm in diameter for the construction of novel and sensitive devices for the detection of pathogens (i.e. bacteria and viruses) at the point-of-care were provided. Plastics and papers were utilized that offer inexpensive devices for rapid on-site detection. A short revision of commercial kits were also described in this review.

Quantum dots (QDs) consisted of CdTe along with Concanavalin A (Con A) were synthesized and used as a novel selective and sensitive nanosensors to detect Lipopolysaccharide (LPS) [4]. The device consisted of CdTe QDs–Con A was utilized as fluorescence small piece of material attached and provided information on how to capture *Serratia* bacteria through the identification between CdTe QDs–Con A and LPS of Serratia member of the genus Serratia, The structure of the window framework. Via a high resolution transmission electron picture appeared and shown to have a crystalline structure of ca. 4–5 nm in size for the CdTe QDs. These outcomes exhibited that the fluorescence of the composite was linear to the LPS concentration (decreasing) between 10 to 90 fetograms/mLhaving a r^2 about 0.97. LPS surrounds the *Serratia* microorganisms and it attaches the composite which leads to quenching the fluorescence intensity of PL. The results have shown that there is a linearity of the relative PL fluorescence and the logarithm of the population of the microorganisms between 1×10 to 1×10^6 CFU/mL at pH 7 having an r^2 of about 0.95.

A fast technique giving grounds to qualification of black-pigmented gram-negative anaerobic rods was reported in the literature [5]. The investigations included monitoring of filter papers of generation of indole, and sialidase, alpha-glucosidase, beta-glucosidase, alpha-fucosidase, and trypsinlike enzyme activities, 100% of Porphyromonas gingivalis, Prevotella intermedia, and Bacteroides levii and 89% of Prevotella corporis isolates were identified at the species level. The technique was not selective towards porphyromonas asaccharolytica and Porphyromonas endodontalis but selective to other species. The method was not also selective between Prevotella denticola, Prevotella loescheii, and Prevotella melaninogenica. 4-Methylumbelliferone derivatives was the basis of these investigations which were performed with easiness and rapidly (ca. 15 min), and can be applied to anaerobic rods that are formed with large difficulty.

The main cause of the infections and diseases appears to be Staphylococcus aureus. The toxic parts of Staphylococcus aureus are around the vessels of the humans and cause illnesses that threaten life. The most common techniques for their identification are bacterial culturing techniques that provide results after many days. DNA-based techniques are costly and must be performed by skilled scientists. To overcome these boundaries, a device was manufactured made from paper [6]. The mechanism of signal generation was investigated and was found to be due to activation of the enzymes that catalyze proteolysis, i.e., the breakdown of proteins that were between the magnet beads and the Au interface on the filter into smaller polypeptides or single amino acids. A magnet was positioned at the sensor surface so that an acceleration of the liberation of the proteins far from the device to be achieved when the blank dropped. The pigment alteration that resulted from parting apart the magnetic beads was monitored with an eye and analysed with the help of Image analysis software so to receive quantitative results. The detection limits were on the order in the order of CFU/mL. A wide range of Listeria monocytogenesis 19115 and E. coli O157:H7, methicillin-resistant S. aureus (MRSA), Candida albicans) and Pseudomonas aeruginosa 15692 pathogens were tested as interferents. The results have shown that the method was highly selective except for MRSA. This method was rapid and used to monitor the pathogen in contaminated samples.

A paper was reported in the literature using gold nanoparticles (AuNPs) and a spectrophotometric method to determine Staphylococcus aureus [7]. The device was designed on Protein A in genome and a PCR technique made in optimized experimental conditions. The nanosensors were incorporated in the device and, then, the s-s gene was placed on the device for the substrates to be hybridize. The evaluation was made with the pigment alteration seen by the human, spectrometry, and TEM. As an

end result, the characteristics of the device were estimated. The experiments exhibited that a 390 bp band of the matrix of agarose, that acquired the presence of Protein A genomes on the bacterial strains . A comparison was made between the PCR and spectrophotometric methods. The limit of detection of these techniques were on the ng μL^{-1} range. The limit of detection (LOD) was equal to 8.73 ng μL^{-1}.

An ultra-sensitive immunosensor was described in the literature to monitor Staphylococcus aureus [8]. The "receptor" was a commercial anti-S. aureus antibody. A monolayer of thiolamine was formed by absorption and organization on a device that was covered by Au. Protein A was immobilized to the thiolated monolayers via the technique of glutaraldehyde. The anti-S. aureus was positioned by affinity to the Protein A and a blocking took place by Bovine Serum Albumin (BSA),. Polarization Modulation Reflection Absorption Infrared Spectroscopy (PM-RAIRS) and Quartz Crystal Microbalance with Dissipation (QCM-D) were used to monitor every stage of these experiments. Initially, the characteristics of immunosensor construction became optimum using rabbit IgG. The polyclonal anti- Staphylococcus aureus was then positioned and the device layer was used to detect the microorganism. The evaluation of the specificity and the device response was made by fluorescent imaging. The sensitivity was enhanced with PM-RAIRS as a transducer and the detection limit was found 105CFU mL^{-1}.

An electrochemical device based on antibody-antigen interactions for the rapid monitoring of the microorganism *Staphylococcus aureus* ATCC25923 recently was reported in the literature [9]. The anti-Staphylococcus aures was placed on a self assembled monolayer of 3-Mercaptopripionic acid. The monolayers were instantly formed when immersing in a solvent of organic nature that contained the self-assembled monolayers and waere attached on the gold electrode surface. Cyclic voltammetric and electrochemical impedance spectrophotometric techniques were used to characterizing on how this immunodevice was constructed and functioned. A buffer consisting of phosphoric composition was used as media and the redox was ferrocyanide/ ferric ions. The electrochemical impedance spectrophotometric technique was utilized to complete to complete the techniques and the appropriate binding. The calibration graph related the electron transfer resistance (R_{CT}) and the logarithm of *Staphylococcus. aureus* in the range of 10 and 10^6 CFU/mL. The sensitivity was 10 CFU/mL, and the relative standard deviation was ca. 8%. The selectivity of the device to monitor this bacteria against *E. coli* and *S. epidermidis* was shown to be excellent.

The detection methods for Staphylococcus Aureus include a number of techniques that culture the bacteria; however, these methods require many days. There are also other techniques that are based on DNA, but hese are too costly and the personnel has to be trained. A report recently has been published in the literature that describes a device based on a paper and on the activity of the proreases of this bacteria on a peptide which was placed between magnet microbeads and a gold electrode and the whole unit was positioned on a paper [10]. This report overcomes the above described limitations. A magnetic field was applied at the back rear on the device to speed up the moieties freedom for out from the interface of the magnet-paper. When these moieties have dissociated there was an alteration of the color which could be easily detected by even a human eye.. These alterations in the color were investigated by an image analysis technique in order to obtain quantification of the bacteria. The detection limit of this technique was in the range of a few decades of CFU/mL in all samples. Interference studies were made and included *Listeria monocytogenesis* 19115, *E. coli O157:H7*, methicillin-resistant *Staphyloccocus aureus*, *Candida albicans* and *Pseudomonas aeruginosa* 15692 microorganisms. No interferences were noticed in all cases except in the case of methicillin-resistant *Staphyloccocus aureus*, *Candida*. This technique had an analysis time of a few minutes and it can be used as a rapid method to detect this bacteria.

A review paper appeared in the literature that describes devices that are used to detect methicillin resistant *Staphylococcus aureus* (MRSA) based on microfluidics [11]. These techniques are characterized by a good sensitivity and specificity. It is certain that in the near future, MEMS and nanotechnology based detection methods will take the place of current methods in clinical diagnosis. Other advantages of these techniques are low cst, small sized, and can be disposable.

A report appeared in the literature that describes a device for the sensitive detection of *Pseudomonas aeruginosa* [12]. Polydopamine-polyethyleneimine (PDA-PEI) copolymer dots were constructed through self-polymerized and were cross-linked with are prepared via the self-polymerization of

dopamine and cross-linking with polyethyleneimine. The polydopamine-polyethyleneimine polymer dots were stabilized at high pGH and ionic strength values. Therefore a fluorescent device was fabricated to rapidly and sensitively detect and quantify *Pseudomonas aeruginosa* (*P. aeruginosa*). A dual polydopamine-polyethyleneimine polymer dots device was manufactured that was more sensitive than a single one. This optical device shows a sensitive linearity in the analyte at concentrations of 10^1–10^7 cfu mL^{-1}. The LD was 1 cfu mL^{-1}. The time for analysis is ca. 1.5 h.

Whole-cell devices that could detect the responses of strains to a stimulus were reported recently in the literature. These devices could detect and monitor the produce of analytes with an activity against microbes. These biosensors were based on chemiluminescence and Bacillus subtilis and could be able to detect chemicals that were active towards cell walls [13]. Colonies of strains were produced and were positioned on the reporter molecule. This has permitted the quantification of substances with antimicrobial activity.

The use of devices for the detection of *Bacillus subtilis* for their antibacterial uses were studied and reported in the literature. These devices could not sense tRNA synthetase inhibitors mupirocin, indolmycin, and borrelidin, some inhibitors of peptidoglycan synthesis, and membrane-damaging agents. These devices although could detect the modes of action of RNA polymerase inhibitors and DNA intercalators; they also have given evidence of the action of iprofloxacin, anhydrotetracycline, corralopyronin, 8-hydroxyquinoline, and juglone [14].

A fast and accurate device for the detection of Alternaria panax Whetz was recently reported in the literature [15] and was used on a single-tube nested PCR-lateral flow biosensor assay (STNPCR-LFBA) [15]. This STNPCR-LFBA device had a sensitivity larger by 100-fold than the common used PCR technique. The specificity of the biosensor towards Alternaria panax Whetz was very high and there were no cross-reactions for other similar samples. The LD was down to 0.01 pg of analyte. This single-tube nested PCR-lateral flow biosensor assay was utilized in real samples with high accuracy and rapid resonse times.

5. CONCLUSION

While the role bacteria play in the degradation of artwork is well documented, its use to create art, as well as to restore and preserve it, is still being researched. Further research may allow us to produce natural paints that serve as an alternative to synthetics and are more environmentally sustainable. As we increase our understandings of the ways bacteria interact with existing artwork, we will be better able to apply this knowledge to the preservation and restoration of art in both museums and in more unstable environments such as caves. Until then, scientists will continue to explore the complex ways in which bacteria interacts with art.

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