

**SIGNIFICANCE OF MICROBES IN THE ESTABLISHMENT OF CERTITUDE IN
FORENSIC SCIENCE.**

Dr Amit Chauhan¹, Cyriack CH. Sangma², Sneha Gupta³, Amrita Shill⁴, Dr S K Shukla⁵.

**Amity Institute of forensic Sciences, Amity University Sec-125, Noida, Uttar Pradesh, India-
201313.**

Corresponding Author: Amit* Chauhan

Email address: amit_chauhan777@yahoo.in

ABSTRACT

Microbes or microorganisms are the microscopic organisms that can exist as unicellular, multicellular or even in a cluster form. These microbes can be found everywhere on the earth and could be identified with the help of microscopes. Microbes are also be found on or inside the human body. These microbes vary from individual to individual depending upon gender, age, occupation, environment, etc. Before the birth of an individual, more or less, we are sterile or maybe no microbes are present. But along with age, different species of microbes colonize on the human body. These microbes differ from age to age depending upon the environment we are exposed to the world. It is considered as individualistic as fingerprints. In the recent years, microbial forensic has been used for forensic investigation. In recent studies, microbes are found to be very much useful in criminal investigation. Microbes present on the individual's pubic hair can also be used as trace evidence in sexual assault cases. Microbes can estimate time since death (also known as microbial clock), it could become one of the most powerful tools for crime scene investigation, as after death in late changes of body is due to microbial activity that can help us in estimation of time since death.

Keywords: Microbes, DNA Extraction, putrefaction, diatoms, forensic investigation, etc.

INTRODUCTION

Microbes are the microscopic organism which can be unicellular or multicellular or can even be present in cluster of cells. These microbes are also known as microorganism. These are one of the first microorganisms originated on the earth and the universal ancestor to other unicellular and multicellular organisms. Dr Manuel Delgado-Baquerizo and his colleagues elaborate about the soil microbial feasibility in determination of rates of soil respiration and specially denitrification function¹. In addition of it, Wieder explained about direct effect of the fungal and bacterial abundance that help to control the respiration of microbial community in structural equation modelling analysis².

The Microbial forensics is an arising with imminent branch emphasizing in the microbial forensics to undertake research or forensic studies on microbes. With this advancement in the biological scenario that includes microbiological assay, microbial forensics is inclining out and accepted to be an integral part of the forensic studies³. Now a days, microbial forensic is explored in legal medicine also in order to determine crime spectacle and time since death. Its implementation is highly increasing in m molecular characterization of bacteria from crime scene exhibits. Microbial forensic is also used in determination of probable role of bacteria in degradation of biological fluid that affect the resultant even obtained by use of PCR based sensitive techniques i.e. DNA fingerprinting. It is also used to arbitrate the link between the personal belongings like a cell phone or laptop with the help of bacterial microflora present on the fingertip⁴.

MICROBIAL STRUCTURE

In some aspects of forensic science, microbes are inclining out very beneficial such as soil bespeak, methodical, etc. In case of soil clues, these are the most diverse and abundantly found community which plays a very important role in many ecological and ecosystem functions. These microbes play a very crucial role in decomposition, nutrient cycling, climate regulation, etc. Microbes required suitable pH, temperature, dissolved gases, osmotic pressure and water availability for growth. Such factors help in resolving the crime, determination time since death, purification/ decomposition of dead bodies or skelton remnants etc.

A single microbe can multiply millions of times in appropriate conditions. Microbial growth reflects the biological processes of microorganisms of evidences i.e. DNA extraction, quality and quantity and diatoms⁵.

MICROBIAL SIGNIFICANCE IN CRIME

In the process of identification of a crime scene, microbes help to determine as it depends on the surface where evidences were left or came in the contact of surface. According to a latest study, microbes help to solve a case of burglary. In this research, microbial signatures were used as a trace evidences that were collected from the articles of the house before and after the burglary. As a result of this research, researchers found distinct types of microbial assembly which exactly match to the burglar's house article at 75 % of accuracy. It was the first implementation of microbes used as a forensic tool in an investigation and that can be further used with an improvement. In another aspect of forensic identification, the microbes of pubic hairs were used as a microbial signature to identify the involvement of perpetrator in sexual assault cases. Forensic science always searches for individuality by which an accused can prove guilt or in other words a culprit or assaulter can get his/her deserved punishment⁷.

ROLE OF MICROBES IN FORENSIC SOIL EVIDENCE

Microbes can be connected to the crime scene as they are depending upon the soil surfaces or any surfaces. The analysis of the soil that is found from the crime scene is usually performed based on their physical, chemical and mineral content of the soil. The soil evidence that is found in the crime scenes can be of great significance and they are accepted to undergo forensic investigation and they also brought justice in the criminal justice system. Various research studies found out that the bacteria which is in the soil are used to generate the DNA profiles. However, customarily many agencies have undergone analysis in order to know the chemical constitution but the outcome came out to be unorganized due to their comparable database in the lab. These happen due to their problem in culturing microbes as they are extremely difficult but the advancement in modern scientific technology lessens and reduces the problems.

The microbial community in the soil are the most diverse and abundantly found community which plays a very important role in many ecological and ecosystem functions. They play a very crucial role in decomposition, nutrient cycling, climate regulation, etc. The vast and their diverse metabolic community signifies their varied activities in contribution to the cycling of various nutrients (e.g., C, N, P) and organic matter production. The microbes which are present in the soil mobilizes the nutrients from the insoluble minerals in order to support the plants.

The microbes play a very crucial role in the cycling of nitrogen which exclusively differentiates between nitrogen fixation, nitrification, and denitrification. The soils absorb the toxic substances (detoxify) and recycles the waste products which is thrown out by the agrochemicals, effluents, and oil spills by the factories and industries thus reducing the harmful effect on the human beings. The process responsible for the recycling waste products and detoxification are mineralization and immobilization however the availability of the soil nutrients may limit the detoxification.

The microbes regulate the soil services and enable humans and the entire biodiversity to live stability and in reliance on each other. Some of the regulations which bring the environment resilient are buffering the water flows, nutrient cycling, recycling of toxic and harmful waste products, contaminants filter, habitat for biodiversity, biological control of pest, weed, and pathogens, carbon storage and regulation of greenhouse gas emission. Recent studies are mainly focused on the microbes of soil contents by using 16-S ribosomal RNA gene sequencing, a single marker using Terminal Restriction Fragment Length Polymorphism analysis (T-RFLP) or Amplicon Length Heterogeneity Polymerase Chain Reaction (ALH-PCR)⁸. TRFLP techniques is also used which is focused on the specific fragments for the identification of individual genotypes with the enhancement of restriction enzymes which cuts a specific site⁸.

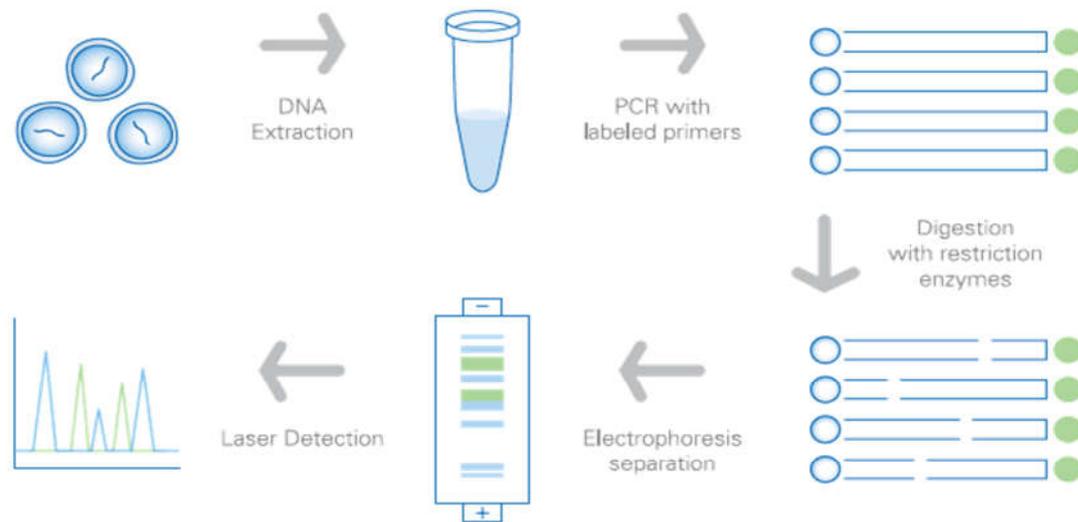


Figure 1; flow chart of Terminal Restriction Fragment Length Polymorphism analysis.

For this purpose, very small amount of soil samples is required for analysis, but the reliability of this method could be affected along with the change in the community of soil microbes even at a short distance also. Therefore for this analysis, it is suggested to target the restricted community such as nitrogen-fixing rhizobia⁹.

PORTRAYAL OF MICROBESTO HUMAN

Pathogen is a microbial organism the can cause the disease. It enters through the respiratory tract, urogenital tract, gastrointestinal tract, or through cut on skin surface. These microbes affect our immune system which results in weak immune response. Such microbes are easily transferred from one surface to another surface by touch, as in the case of viral infection or viral fever. It is caused due to infectious microbes that is also known as viruses i.e. COVID-19. These viruses attack multiple conglomerate cells and in the response of it, human body's temperature increases by resulting the discoloration in form of viral fever. Contamination of blood can be occurred by sharing used injection or during intercourse (HIV). During acute HIV infection, virus rapidly multiplies and spread in our body. These virus attacks and destroys our normal cells and make them infectious cells. AIDS is the final stage, as the immune system won't able to fight back to these microbes and these results in the death of the person.

EXTRACTION OF BIOLOGICAL FLUIDS FROM SOIL

During the investigation of spectacle, a forensic expert deal with the traces evidences that are left behind by the perpetrator unknowingly or transferred to other materials such as clothing, knives, and guns etc. However, most often the biological evidences get contaminated in the soil surfaces in the cases of outdoor crime scene which may be only evidences that would lead to solving the crimes. There are certain commercial DNA extraction kits which is an appropriate method of extracting the quality DNA from soil contaminated blood such as PrepFiler Forensic DNA Extraction kit, Promega DNA IQ Kit and Blood Miniprep kit. These kits are considered to be an effective way of extracting the contaminated blood from soil¹⁰.

ROLE OF MICROBES IN SEXUAL ASSAULT CASES

In forensic investigation, the identification of biological aqueous help to determine the identity of perpetrator and confirm the course of events during the crime. Hence, numerous preliminary tests are performed to identify such biological aqueous, but sometimes, these can't help to precise the result of investigation. In a sexual assault cases, the tests don't produce plentiful reliability among the vaginal fluid and saliva that is considered extensive in the corroborating of allegations. The vaginal aqueous consist of a variety of bacterial content which are also dominated by a few species belonging to the genus *Lactobacillus*. In 1990, Rendon Lopez suggested that females have a unique bacterial flora. Later on in 2009, Nakanishi and his colleagues suggested that Saliva can also be detected through a microbial content known as *Streptococcus salivarius* will be a reliable means of identifying stains as saliva. *Streptococcus salivarius* is a lactic acid bacterium that is confronted mainly in the tongue of the human beings. This particular bacterium was the first commensal bacterium that has found to be appeared in the first-born child's oral cavity and it colonizes to the respiratory tract. This presence of adhesions systems to the oral epithelial cells such as pili, fibrils, saliva-binding proteins and host-cells binding proteins along with its high competition rate helps this particular bacterium species to hold on to the mouth of the human being lifetime¹¹. In sexual cases, bacteria's present in human pubic hairs act as a microbial signature of the perpetrator that is individualistic by nature. The best thing in microbes present in the human body is it is highly individualistic¹³.

ROLE OF MICROBES IN FORENSIC ENTOMOLOGY

Microbes are the living organisms that are acquaint in the environment including the internal organs of human body. These microbes/ unicellular organisms that are present in the human body, are basically bacteria i.e. viruses, fungi and other micro-organisms. A few Bacteria's named as Bacillus subtilis helps to protect the human skin. The microbes present in the nasal helps in determination of the reaction pattern of the mucosal and systematic immune system. Microbes help in digestion, absorption, and assists with waste excretion, Moreover, human reproduction is getting possible because of the presence of healthy microbes present in the reproductive tract.

The essential and foremost application of microbes in forensic science is to determine the time since death also referred to as minimum post-mortem interval. It is conducted by measuring the extent of putrefaction caused by the microbes and determining the growth of microbes in the dead body. According to Fredetter, the decline of viability occurring during a variable period preceding death rendering the individual susceptible to invasion by endogenous micro-organisms. The endogenous bacteria continue multiplying and migrate throughout the body only after death. In drowning cases, the presence of diatoms in human body proceed that death was caused by drowning. Sometimes, such cases raised a question whether the body is drowned before death or after death. In this process, if the diatoms are observed in the dead body's lungs and bone marrow, it means the victim was alive after drowning and the death can be caused by drowning because the diatoms present in the water is transported to the lungs by reparation process. On another phase of it, if the diatoms are not found in lungs and bone marrow than it is post mortem drowning means death is occurred due to other reasons i.e. after the murder, the body was disposed there. Microbes are also helpful in determining whether dead body was moved from one place to another or not? As per the earlier discussion, microbial community differs from distinct places. Presence of two or more microbes can't grow at same place in the same body which provide a clue about the movement of dead body after death¹².

EXECUTION IN DETERMINATION OF TIME SINCE DEATH

As a preliminary examination of dead body, the changes in the appearance of facial pallor, changes in skin, primary flaccidity of the muscles, changes in the eye, by algor mortis (post mortem cooling), livor mortis, color of hypostasis, rigor mortis, cadaveric spasm etc. help to determine the time since death. While the late changes are determined with the help of microbes and microbial activity in the corps. The condition of dead body like decomposition or autolysis of the stage because decomposition doesn't occur at the same time in all body parts, some tissues start to decompose at the early phase while some occurs later which may extend to several weeks. Putrefaction is the final stage of dissolution which results in the breaking down of complex organic body constituents into simpler inorganic ones. This process includes few steps described below

Autolysis firstly takes place intrinsically in the parenchymatous and glandular organs. Rupture of stomach and esophagus occurs by cause of auto-digestion by the gastric juice in some newborn after death.

Bacterial action in which both the aerobic and anaerobic bacteria place a dominant role in putrefaction. After death bacteria invade by tissues clostridium welchii is chiefly present from the bowel. Bacteria comes from several places including the respiratory tract and open skin wounds. In blood, bacteria grow rapidly at such organs where blood concentration is highly present hence, at such place's putrefaction occurs predominantly. Lecithinase produced by clostridium welchii helps in post-mortem hydrolysis and hydrogenation of body fat. This bacterium can grow at lower temperatures even below at 70°F. The microbial action on dead body will conflict with the tropical areas and the growth of micro-organism will be different according to the temperature of the region. Because the growth of bacteria depends on temperature also. Therefore, it become essential to determine the place of death and the prevailing presence of the dead body.

Site of appearance changes in color of skin, Putrefaction appears in the dead body when the rigor mortis disappears. The skin of the anterior abdominal wall and right iliac fossa gets greenish discoloration and manifestation becomes the place are rich in bacteria and fluid contents. In fair skin patch of discoloration occurs in 12-15 hours in summer and 24-48 hours in winter.

By the time greenish color appears in the chest, neck, face, legs and arms and these patterns gradually deepen get purple than dark blue and at last coalesce together. At this phase, the bacteria only originate from the intestine that obtained nutrients from blood and infested the venous system. As a resultant of this process, marbled appearance occurs after a period of 36-48 hours after death.



Figure 2; Post-mortem stains after 12-15 hours of death..

Development of foul-smelling gases, in this process, the body actualizes to emit a nauseating smell owing to the gradual development of putrefaction gases. Over a period of 2 to 3 days, skin slippage starts to ensue in form of the loosening of the epidermis. while after a span of 3 to 7 days, the putrefying gases conceive utmost pressure over the tissue, teeth, skin, nails getting loosening can be easily pulled out. liquefaction occurs over a time period of 5-10 days and as a resultant of it, body tissues change into semi-solid black masses.

Skeletonization, normally the human body starts to get skeletonized within in 2-4 weeks, but it depends on condition of body, temperature of the region or weather also. In case of wild animal's attack over the body then, time gets reduced.



Fig3; - Skeletonization of dead body after 2-4 weeks of death.

SIGNIFICANCE OF MICROBIAL STUDY IN FORENSIC INVESTIGATION

Microbes of human body are individualistic by nature. various attributes and distinct amount of microbes help to determine the individuality. In forensic field, only individualistic aspects are considered to demonstrate the involvement of an individual for any particular crime. In this scenario, microbial studies help abundantly to provide link among evidences, crime or suspect. Now a days, criminals have become smarter and don't leave any trail of evidences that could help the investigator to nab them. As a resultant of it, perpetrators are using precautions (condom) in sexual assault cases, therefore, no semen traces are recovered from pubic hairs of victim. In such cases, the microbes can help to reveal the identity of accused of the crime¹⁴. As per our earlier discussion, microbes and its types and amount vary from one individual to another, even the growth are also distinct in each and every individual. In cases of burglary, missing of belongings etc. microbes can help to identify it because microbes play a crucial role in the nitrogen cycle and in nitrogen fixation. In forensic field, microbial studies help to determine the time of occurrence, determination of time of an injury, sexual assault, time since death of a dead body are based on the microbial concentration. Diatoms take a crucial part in post-mortem and anti-mortem drowning while microbes help to find out whether the body is moved after death or not. Moreover, the aspects of microbial studies are emerging in various field of forensic science.

ETHICAL CONSIDERATION: NA

CONFLICT OF INTEREST: NA

SOURCE OF FUNDING: NA

REFERENCES

1. Delgado-Baquerizo, M., Grinyer, J., Reich, P. B., & Singh, B. K. (2016). Relative importance of soil properties and microbial community for soil functionality: insights from a microbial swap experiment. *Functional Ecology*, 30(11), 1862-1873.
2. Wieder, W. R., Boehnert, J., and Bonan, G. B. (2014). Evaluating soil biogeochemistry parameterizations in Earth system models with observations. *American Geophysical Union, Global Biogeochem. Cycles*, 28, 211–222.
3. Pala A, Arora L, Venkatesh C, Vekaria S, Shah A, Mehta A. Microbial Forensics: An Integral Part of Forensic Study. *International Journal of Oral Health and Medical Research*, 2016; 3(3): 87-89.
4. Shrivastava P., Dash H.R., Kakkar S., Gupta M.K., Jain T. (2018) Unique Individualistic Microflora: The Future of DNA Fingerprinting Technique. In: Dash H., Shrivastava P., Mohapatra B., Das S. (eds) *DNA Fingerprinting: Advancements and Future Endeavors*. Springer, Singapore.
5. Desmond, A. U., Nicholas, O., and Emmanue, O. O. (2018). Microbial Forensics: Forensic Relevance of the Individual Person's Microbial Signature. *International Journal of Life Science and Scientific Research by Society for Scientific Research under a CC BY-NC 4.0 International License*; Volume 04; Issue 05, Page 2037.
6. Madeline Drexler; Institute of Medicine (US). *What You Need to Know About Infectious Disease*. Washington (DC): National Academies Press (US); 2010.
7. Bishop, A. H. (2019). The signatures of microorganisms and human and environmental biomes can now be used to provide evidence in legal cases. *FEMS Microbiology Letters*, Volume 366, Issue 3, February 2019,

8. Shrivastava, P., Jain, T., and Gupta, M. K. (2015). Microbial Forensics in Legal Medicine. SAS Journal of Medicine. Volume-1; Issue-1(May-Jun, 2015); p-33-40.
9. Ahrenholtz, I., Harms, K., De Vries, J. Wackernagel, W. (2000). Increased killing of *Bacillus subtilis* on the hair roots of transgenic T4 lysozyme-producing potatoes. *Appl Environ Microbiol*, 66: 1862–1865.
10. Gibbons R.J., van Houte J. (1980) Bacterial Adherence and the Formation of Dental Plaques. In: Beachey E.H. (eds) Bacterial Adherence. Receptors and Recognition, vol 6. Springer, Dordrecht.
11. Nakanishi, H., Kido, A., Ohmori, T., Takada, A., Hara, M., Adachi, N., and Saito, K. (2008). A novel method for the identification of saliva by detecting oral streptococci using PCR. *Forensic Science International* 183 (2009) 20–23.
12. Roger, P., Delettre, J., Bouix, M. and Be'al, C. (2011). Characterization of *Streptococcus salivarius* growth and maintenance in artificial saliva. *Journal of Applied Microbiology* 111, 631–641.
13. Redondo-Lopez, V., Cook, R. L., Sobel, J. D. (1990). Emerging role of lactobacilli in the control and maintenance of the vaginal bacterial microflora. *Rev Infect Dis.* 1990 Sep-Oct; 12(5):856-72.
14. Committee on Science Needs for Microbial Forensics: Developing an Initial International Roadmap; Board on Life Sciences; Division on Earth and Life Studies; National Research Council. Science Needs for Microbial Forensics: Developing Initial International Research Priorities. Washington (DC): National Academies Press (US); 2014 Jul 25. 1, Introduction: What Is Microbial Forensics and Why Is It Important. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK234883/>.
15. Introduction to Criminal Investigation: Processes, Practices, and Thinking. Retrieved on January, 31st 2020.

16. Jacoby R, Peukert M, Succurro A, Koprivova A and Kopriva S (2017) The Role of Soil Microorganisms in Plant Mineral Nutrition—Current Knowledge and Future Directions. *Front. Plant Sci.* 8:1617.
17. Brugger SD, Frei L, Frey PM, Aebi S, Mühlemann K, Hilty M (2012) 16S *rRNA* Terminal Restriction Fragment Length Polymorphism for the Characterization of the Nasopharyngeal Microbiota. *PLoS ONE* 7(12): e52241.