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Microbial Fingerprinting - An Emerging Tool in Forensics: A Review

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Abstract:- The human microbiome has been used to create microbial profiles, which is an intriguing field of forensic science research that can be used to identify a person. According to recent research, each person has a highly customized microbial fingerprint that can be used in forensics to recognize or connect a person with any criminal activity. Since this technique is still in its early stages, using a source tracker to locate the sink (microbial population) can be useful. On the basis of phospholipid, DNA, or RNA, microbial fingerprinting methods are being used to isolate and classify an individual community of microbes.

Keywords:- *Human microbiome; microbial profiling; source tracker; phospholipid; contamination.*

- ABBREVIATIONS
  - DNA - Deoxyribonucleic acid
  - RNA - Ribonucleic acid
  - OTUs - Operational Taxonomic Units
  - CO_2 - Carbon dioxide
  - MOD – Manner of Death
  - COD – Cause of Death
  - AKP - Anna Karenina Principle
  - BV - Bacterial vaginosis
  - PCR - Polymerase Chain Reaction
  - STR - Short Tandem Repeat
  - SNP - Single Nucleotide Polymorphism
  - REP - Repetitive Sequence
  - RAD - Relative Aitchison Difference
  - C – Celsius
  - S. – Staphylococcus
  - Sp. – Species
  - PLFA - Phospholipid fatty acid
  - SIP - Stable Isotope Testing
  - DGGE – Denaturing Gradient Gel Electrophoresis
  - T-RFLP - Terminal Fragment Length Polymorphism
  - TPPR - Transmission, survival, prevalence, and recovery
  - nDNA – Nucleotide DNA
  - REAC – Restriction Endonuclease analysis of chromosome
  - RAPD – Random Amplified Polymorphic DNA
  - REAP – Restriction Endonuclease analysis of plasmid DNA

I. INTRODUCTION

We as a human, are home of trillion and billion types of microorganisms including bacteria, fungi, protists, and viruses. The composition of these microbes on or within our bodies varies. As a result, microbes provide us with a special fingerprint that varies from person to person. When it comes to distinguishing microbes, it's often essential to distinguish them among themselves or between classes, which is known as "microbial fingerprinting." These microbes can be distinguished by their specific characteristics such as form, size, nature, color, cell wall, reproduction, or cellular or biomolecular components such as DNA, RNA, and phospholipids. Microbes are being used in massive ways for the betterment of the world and culture in the twenty-first century. Once the microbe and its mechanism, as well as the wonders it can perform, have been examined using microbial fingerprinting, it can be used in a variety of ways, including examining microbes that can influence an individual's health or trigger diseases. Drug manufacturing, such as antibiotics and vaccines; Producing ethanol and enzymes for use in the cosmetics, fruit, and agricultural industries; Cleaning up oil spills and radioactive waste, as well as studying the human microbiome. In our bodies, there are ten times more bacterial cells than human cells (Scientific American, 2007), implying that we only have 43 percent human cells (James Gallagher) and 57 percent bacterial cells (Sarkis Mazmanian). Thus, the human microbiome serves a variety of purposes, including answering questions about a person's wellbeing or resolving microbe detection using different microbial fingerprinting techniques.

II. ROLE OF HUMAN MICROBIOTA

Joshua Lederberg coined the word "human microbiota" in 2001. Many researchers have been perplexed by the definition of the human microbiome, which is described as the microbial taxa linked to humans and “microbiome,” which is defined as the catalog of microbes and their genes, and these words are often interchangeable. Understanding the consistency of a personality's microbiome, the concept of the OTUs (Operational Taxonomic Units) that frame the microbiota, and whether someone has one microbiome or several are all used to determine the definition of the human microbiome. Since each person carries 100 trillion symbiotic microbial cells, the human microbiome is made up of the genes carried by these cells.

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Rapid advancements in molecular sequencing and analytical approaches are causing a paradigm shift in how we research such microbes (Li et al., 2012; Segata et al., 2012). For example, it is no longer necessary to culture microbes in order to detect them, and the rapidly developing science of metagenomics now allows characterisation of the thousands of microorganisms that make up an ecosystem's microbial population, or "microbiome" (Human Microbiome Project Consortium 2012a, b; Yatsunenko et al., 2012). Similarly, it is now possible to more accurately study the communication chains of specific bacterial strains and plasmids (MacConaill & Meyersen., 2008; Nakamura et al., 2008; Pallen & Loman., 2011). Microbes' ubiquity and diversity suggest that they may be a source of forensic evidence. In reality, the word "microbial forensics" is now used to describe the study of how microorganisms can help with forensic investigations (Breeze et al., 2005). The appearance of a particular microbial population, for example, may be used to characterize an individual microbe, substance, or position. Furthermore, certain microorganisms are pathogenic, and although most infectious diseases are easily diagnosed, they can play a role in some controversial situations where someone dies for no apparent reason. Microbes have often been distributed carelessly or maliciously, necessitating a connection between the accused and the petitioner and a specific strain. Furthermore, the diversity of microbial metabolism ensures that their actions change most organic materials and certain inorganic compounds, thereby affecting the recovery of other types of forensic evidence. Lastly, a body may be a host of contagious microorganisms and must be treated with caution to minimize the possibility of infection (Malik & Singh., 2010).

A. Decomposition Process

Somatic death and cellular death are the two types of death. Somatic death results in the loss of a person's sentient identity, but reflex nervous function also continues. Cellular death occurs as the body's cells stop functioning, lose metabolic activity, and are unable to survive by aerobic respiration. The body remains flaccid and blood begins to flow until heart function is lost. The “classic triad” of livor, rigor, and algor mortis are well-documented modifications that occur in the body. That is, blood drains to the lower parts of the body, stiffening and cooling the body until it reaches a comfortable temperature. The rate at which these changes occur is largely determined by environmental factors, especially temperature, as well as microbial load and diversity (R.C. Janaway et al.). When physical and immunological barriers begin to break down, microorganisms such as bacteria and fungi that live on the skin and in the gut spread across the body. As a result of the increased ambient temperature, the microorganisms found on and inside a body can multiply quicker, causing the pH of the blood to become acidic, as well as the fluids and tissues to become anaerobic. Since high humidity promotes the growth of microorganisms, decay occurs more quickly in the tropical tropics than in cold temperate climates. Since bacillus has a doubling time of just 8 minutes under ideal conditions (Spicer, 2000), the Gram-positive bacillus has the potential to dominate the microbial population. Bacterial putrefaction causes the loss of body tissues (and hence possible evidence) as well as the production of gases such as CO_2, hydrogen sulphide, and methane, which cause the bloat stage of decomposition.

B. Post-Mortem Toxicology

The study of the presence, dissemination, and quantification of a xenobiotic after death is known as postmortem toxicology. This data were used to account for a xenobiotic's physiologic consequences at the time of death, based on its quantification and distribution within the body at autopsy. Several factors may cause differences in xenobiotic concentrations between the time of death and the subsequent autopsy, as well as the storage period between the time of sampling and the time of examination. Toxicologists and forensic pathologists are often called upon to analyze postmortem xenobiotic concentrations to determine if the identified values are meaningful and, if so, if these compounds were unintentionally or intentionally involved in the cause of death (Rama B. Rao & Mark A. Flomenbaum). The identification and quantity of medications and toxins found in postmortem specimens was expected to aid in determining the conditions underlying people's deaths. Due to the decomposition of human remains, microbial activity can influence this quantitation by (i) reducing the volume and quality of biological specimens available for analysis; and (ii) modifying medication, toxin, and metabolite concentrations. Although autopsy specimens are also preserved at low temperatures and with preservatives to prevent decay, the circumstances before the corpse is delivered to the mortuary are uncontrollable. As a consequence, microbial behavior can make it difficult to interpret analytical findings, particularly if the examined substances' soundness in a putrefactive environment is unknown (Castel et al.,2017). Microbes dissolve some medications and produce metabolites that are confused for markers of pre-mortem drug intake during the decay phase. Nitrobenzodiazepines, such as Clonazepam and Nitrazepam, are easily converted to amino compounds by bacteria and are impossible to detect in the blood, particularly after the victim has died of an overdose (Robertson & Drummer, 1995; Drummer, 2007). Despite the fact that morphine-3-glucuronide is currently transformed to free morphine by bacteria, morphine can be found in buried bodies up to eight years after death (Skopp, 2004). Methamphetamine use is increasingly growing on a global scale, increasing its forensic importance. Several gastrointestinal microorganisms, such as those from the genera Enterobacterium, Enterococcus, Lactobacillus, Clostridium, and Bacterioides, are responsible for N-demethylating methamphetamines and transforming them to amphetamines (Castle et al. 2017; Rommel et al. 2016).

C. As a cause of death

As a replicated picture of antemortem health status, the postmortem microbiome has significant forensic implications for postmortem interval assessment. It also has the ability to assist in manner of death (MOD) and cause for death (COD) determination. To dig deeper into this connection, we looked at beta-dispersion, or microbiome heterogeneity, in the sense of the “Anna Karenina Principle” (AKP). The basic premise of AKP is that stressors have unexpected effects on microbiomes, increasing population

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beta-dispersion. Cases with known M/CODs were expected to have different population beta-dispersion that mirrored antemortem factors, with disturbed and/or natural deaths having higher beta-dispersion than other deaths (e.g., injuries, drug-related deaths) (Kaszubinski et al., 2020). Since calculating M/COD is error-prone, microbial population measurements could theoretically help health workers and other certifiers of death (referred to as "medical examiners"). MOD embraces only five major categories: normal, accident, suicide, murder, and indeterminate. While COD encompasses a wide range of causes relating to the injury/disease by which a person died, MOD only encompasses five major categories: natural, accident, suicide, homicide, and indeterminate (Randy et al., 2002). Considering the relevant data, medical experts qualify their MOD determination with incremental degrees of certainty (Randy et al., 2002). Given the possibility of a discrepancy between the MOD determination and the real MOD, the postmortem microbiome may be another piece of proof to support the M/COD determination. One microbial species recovered from body fluids at autopsy indicates infection occurred during life, while mixed species profiles indicate post-mortem invasion. One of the most prevalent diseases caused by dysbiosis of the microbiota is infection. Importantly, communicable disease and its treatment have a significant effect on the human microbiota, which decides the communicable disease's final outcome inside the human body (Wang et al.,2017). Bacterial vaginosis (BV), for example, is linked to a number of negative health effects, including preterm birth and, as a result, the development of sexually transmitted infections. BV is considered to be a vaginal microbiota ecological condition. Using culture-independent polymerase chain reaction (PCR), denaturing gradient gel electrophoresis (DGGE), and barcoded 454 pyrosequencing methods, (Ling et al.) observed a profound shift in the absolute and relative abundances of bacterial species present within the vagina in a very comparison of populations related to healthy and diseased conditions. Three phyla and eight genera were clearly a difference in the absolute and relative abundances of bacterial species present within the These genera are also used as targets for molecular methods to diagnose clinical BV. Staphylococcal septicaemia and meningitis are another case.

D. Natural Transmission

Natural transmission is the spread of pathogens through a variety of routes, including direct contact (individual-to-individual), indirect contact (contact with a contaminated surface), insect vectors such as biting flies, mosquitoes, and ticks, and aerosol spread (contaminated droplets spread by coughing or sneezing that are either inhaled or available in direct contact) (Equine Disease Communication Center). Normal spread of microbial infections is a major concern for public health officials. When pathogens linked to bioterrorism are discovered, however, police intervention may be required. Anthrax, for example, has killed a number of opioid addicts in the United Kingdom and elsewhere in Europe (Christie, 2010; Knox et al., 2011). Those that inject the opioid rather than smoke it tend to be at the highest risk (Palmateer et al., 2012). The majority of heroin used in the United Kingdom and Europe comes from Afghanistan, where Bacillus anthracis is endemic. A number of people were killed by anthrax inhalation, which is exceedingly rare in normal human infections (but would be a characteristic of the disease if it is used as a biological weapon). Naturally acquired pathogens, especially those with a limited geographic range, may be used as a forensic indicator of geographic origin. Wild animals and plants, for example, often carry parasites and diseases that are missing in captive-bred humans due to treatment or the lack of an adequate vector. This may be helpful in cases of suspected wildlife crime where the origin of a species is a point of contention. Indeed, the illegal trade has been identified as a potential vector of new diseases (Rosen & Smith, 2010).

E. Nosocomial Transmission

Nosocomial transmission (also known as "Healthcare Associated Transmission" or "Patient Acquired Infections") occurs when an illness is spread within a medical facility such as a hospital or as a result of a procedure such as an injection or the placement of a urinary catheter. Nosocomial infection may be endogenous, meaning it arises from an agent already existing in the bloodstream of the patient, or exogenous, meaning it is spread from another source within the facility. Nosocomial infections do not occur within the time frame of admission to the hospital, but community-acquired contamination of patients or workers is a significant cause of nosocomial infection (Celia Aitken & Donald J. Jeffries,2001). Inevitably, hospitals and emergency centers are crammed with sick patients, and medical operations often include puncturing the body and/or installing instruments. As a result, the chances of disease transmission are high, and although it's difficult to completely prevent them, there are generally consistent rules and precautions in place. When a patient contracts an infection as a result of these not being followed, the consequences for the patient may be serious, if not lethal. In such a condition, a petition for medical malpractice may be made, and there may be reasons for court action in certain situations. It's crucial to show when a patient got their infection before being examined, and whether an infection can be traced back to a certain member of medical staff or medical equipment. For example, in the United States, many cases of bacterial meningitis caused by the oral bacterium Streptococcus salivarius have been attributed to an anaesthetist who gave spinal injections without wearing a facemask (Shewmaker et al., 2010).

F. Identification of an individual

According to the Federal Bureau of Investigation, trace proof is material that is transported from a suspect to an off-the-books location that is minuscule or where conventional fingerprinting is difficult or impossible to do, but residual details such as hair, fiber, dirt, or other intangible items is retrieved from the crime scene as trace evidence. Modern DNA fingerprinting methods include analyzing Short Tandem Repeat (STR) and SNP gene markers with PCR amplification to reduce contamination and degradation (Roeder K.,1994), but contaminants contribute to the vast majority of detected DNA in trace fingerprint samples. Microbial fingerprinting, on the other hand, has shown fewer blemished findings for trace proof than DNA fingerprinting. When skin flora comes into direct contact with every surface, multiple microbes are transferred

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instantly. Microbial populations on objects handled by hands, in fact, are found to contain approximately 60% to 70% of human skin-associated microbes (Knights et al., 2011).

The human microbiome shows that not only are there significant variations in microbial composition across body areas, but also that there tend to be consistent differences across people (Costello et al., 2009). This has contributed to speculation that people could have distinct microbiomes, which may be used to identify them (Blaser 2010). The microbiota evolves over time as the quantitative concentrations of bacteria change, but the community's makeup continues to remain relatively constant (Caporaso et al., 2011; David et al., 2014). The nature of the microbiome in the ecosystem may be used to determine geographical origin or to link humans, animals, and objects to one another or to a specific site (Walker AR, Datta S. ,2019; Kodama WA et al.,2019). As a result, microorganisms can be used as evidence in a variety of forensic situations, including sex crime cases where no other type of evidence is available (Williams DW & Gibson G. 2019).

The variations in the microbiome's structure and composition may contain valuable information that could be used for forensic purposes. Diet, profession, travel, and prescription usage may all have an effect on the microbiome's composition and function. This suggests that studying the microbial population in and on our bodies could uncover information about a person's lifestyle (Gonzalez et al., 2016; Kuntz and Gilbert, 2017), which could be new trace evidence. Given the significant forensic potential of microbial analysis, standardized operating procedures for the collection, analysis, and interpretation of microbial evidence, as well as solid and complete databases for full implementation within the forensic context, are required, allowing the use of microorganisms as auxiliary evidence in criminal cases to be clarified (Javan GT et al.,2016 ; Uchiyama T et al., 2012).

a) Source Tracker

Microbial source-tracking refers to computational techniques for identifying origins of microbes (contaminants) in a sink microbial community (Knights et al.,2011 & Hagedorn et al.,2011). Construction of microbial profiles using nucleotides, k-mers, or Operational Taxonomic Unit (OTU) counts is used as source monitoring methods. The microbial source detection system is useful in forensic science because it uses the suspect's microbiota as a possible contaminant source and the trace evidence as a drain. Microsatellites are organisms that do not seem to be associated between sources or between the placement of particular markers to identify sources, and hence marker gene sets and REP-PCR strain-specific studies depend on this. However, this approach may not be suitable for two reasons: one, there's a risk the truth source will not be selected correctly, and two, the time gap between sample collections may be important. The location where the samples are collected may have an effect on the human microbiome's temporal stability. Although the microbiota in the human intestine, nose, and throat environments has been shown to be temporally consistent (Martinez et al.,2013 & Brooke et al.,2009), the skin microbiome has been shown to be inconsistent over time and to be perturbed by common activities such as hand washing or contact with others (Tims at el.,2010).

Relative Aitchison Difference(RAD) is a novel approach that employs the Aitchison distance to identify critical suspects/sources, then combines it with current source monitoring algorithms to approximate the proportions of microbial samples originating from those suspects/sources. When evaluating the general Aitchison discrepancy, RAD is calculated separately for each source within a bin, though all observations that are within the same bin because the current source are omitted (Carter KM et al., 2020).

a. Bayesian SourceTracker

In 2011, a Bayesian approach called SourceTracker was proposed for community-based source monitoring, which uses a mixture model of OTU profiles to approximate contamination proportions (Knights D.et al.,2011). Gibbs sampling allows for a probabilistic calculation of mixture proportions while concurrently accumulating dissimilar OTUs into unidentified source components. SourceTracker has been widely used in exploratory studies involving microbial fingerprinting through surface touch, with varying degrees of accuracy, in recent years (Knights D.et al., 2011; Lax S et al., 2014; Lax S et al., 2015). Though Bayesian SourceTracker can change sensitivity through parameter tuning, it isn't designed to verify source selection and significantly increases computation times. When dealing with massive data sets, parameter tuning and Gibbs sampling are both computationally costly.

RAD is often used as a preprocessing technique for Gibbs sampling-based Bayesian source monitoring. It is possible to increase the speed and predictive precision of this approach by extracting non-important sources before sampling (Carter KM et al., 2020).

b. FEAST Source Tracker

Another source monitoring tool, FEAST, was proposed in 2019, and it uses an expectation-maximization approach with two parameter sets: mixture proportions and underlying relative abundance (Senhav et al.,2019). This method works in the same way as the Bayesian SourceTracker, but it has a much quicker run time, minimizing run times by a factor of 30 or more. It, like SourceTracker, needs parameter tuning to achieve optimal efficiency and isn't intended to select sources (Carter et al.,2020). To

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improve accuracy, the source pool is often parsed to include only certain sources that lead to the sink. Senhav et al. (Senhav et al., 2019) used RAD to restrict the source pool for the FEAST expectation-maximization algorithm. The underlying formula is similar to the Bayesian model used by Bayesian SourceTracker, in that the effects are optimized by the relative abundance and combination proportion merchandise (Carter KM et al., 2020).

b) Case Study

Grice et al. (Grice et al., 2009) studied the temporal heterogeneity of the skin microbiome in 2009. They selected 20 skin sites that represented different niches and obtained follow-up samples from five healthy people after four to six months. The microbial makeup of the external meatus, inguinal crease, alar crease, and nares remained unchanged over time. The microbiota of the popliteal fossa, volar forearm, and buttock, on the other hand, demonstrated significant variation between the two sampling periods. As a result, the authors concluded that skin microbial communities' longitudinal resilience is site-dependent. They also found that bacterial composition in the nares and backs was consistent for all people, while interdigital network fields, toe webs, axillae, and umbilici displayed a large degree of interpersonal heterogeneity, appearing to be the least similar locations, according to OTUs-based study (Tozzo et al.,2020).

Fierer et al. is the first to show that microbiome research could be used for forensic identification in 2010. They demonstrated how the study of a person's skin microbiome can be used to connect them to an item they touched, demonstrating that through comparing the bacterial communities generated by an individual's skin to an object's surface, it's possible to link touched surfaces to the people who touched them. They conducted two experiments that were interconnected: the keyboard study and the electronic mouse study. It was the aim of the "keyboard research" to compare bacterial communities on the keys of three personal computers to the communities generated by keyboard owners' fingertips. , Fierer and associates swabbed all individual keys and the fingertips of the owner and almost exclusive operator of three notebook machine keyboards (25–30 keys per keyboard). 30 minutes prior to sampling, the keyboards were last reached. To compare the bacterial populations on the three keyboards to those on 15 other private and public computer keyboards, main keys from 15 other private and public computer keyboards were swabbed. After storing all swabs at -80°C for less than a week, DNA was removed. The bacterial population composition was determined using a barcoded pyrosequencing protocol. This revealed that the bacterial communities on the fingertips of a keyboard owner are similar to the communities on the owner's keyboard, and that bacterial communities on a particular individual's fingertips or keyboard are even more similar to one another than fingertips or keyboards from other individuals.

They concluded that variations in keyboard-associated communities are likely caused by direct transmission of fingertip bacteria because inter-individual differences in fingertip and keyboard communities outweigh differences in bacterial communities on the fingers and keyboards of a single individual.

The developers of the "machine mouse" report enlisted the help of nine adults who all served in the same house. Then they swabbed the mobile device's entire exposed body, as well as the palm surface of every owner's dominant side, which is most likely going to switch the button. The owner has not touched any of the mice in over 12 hours. Until DNA extraction, all swabs were held at -80°C. The authors compared bacterial communities found on computer mice to a database that included bacterial communities from the mouse's owner's hand as well as 270 other hands belonging to healthy male and female volunteers aged 18 to 40 who had never touched the mouse. They were able to show that bacteria found on a private object are more similar to the owner's skin bacterial populations than to a general population microbiome, such as that found in the volunteers. This result was obtained using the UniFrac algorithm, which uses the degree of phylogenetic overlap between any pair of communities with estimated points representing samples of identical bacterial communities to calculate phylogenetic distance.

The authors came to the conclusion that each individual leaves a unique bacterial "fingerprint" on surfaces they contact. Bacterial DNA can be retrieved from these surfaces and traced to the individual who touched it, passing bacteria from their fingertip. Since skin-associated bacterial communities can survive on specimens for up to two weeks after they've been treated under normal indoor conditions, the object's microbiome is often examined for forensic purposes. This type of research can help you recognize an object, particularly if you can't get clear fingerprints on it, like from fabrics or smudged surfaces (Tozzo et al.,2020).

Wilkins et al. (Wilkins D et al., 2017) conducted research in 2017 with the aim of linking the skin microbiome to areas of domestic residence. The taxonomic composition of the surface samples revealed that the bulk of the microbiota on household surfaces came from the occupants' blood. Moraxellaceae was the most common family in all of the tests, dominated by the skin-colonizing genus Acinetobacter. The Staphylococcaceae, Micrococcaceae, Corynebacteriaceae, and Streptococcaceae, all related to human skin, were among the ten most common families on surfaces. Sphingomonadaceae, Methylobacteriaceae,

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Pseudomonadaceae, Rhodobacteraceae, and Xanthomonadaceae, for example, have abundant populations possibly derived from natural factors such as soil and vegetation. The authors have discovered that the bulk of the taxonomic units studied remained on the skin or surfaces for a limited amount of time before becoming indistinguishable. Because of these factors, the authors suggest that, while microbiota traces can have forensic significance, they are not static and, as a result, decay in such a way that their valuable characteristics for recognizing individuals are lost (Garcia et al., 2020).

Park et al. (Park et al., 2017) investigated the diversity of microbial species found in the palms of 15 people and assessed their ability for human identity. The authors show how the genus Staphylococcus was found in all of the participants, while Micrococcus and Enhydrobacter were found in the rest of them (87 percent and 80 percent of the cases, respectively). Staphylococcus epidermidis (14 subjects) has the highest proportion of Staphylococcus, which is known as one of the most common skin bacteria. S. capitis subsp. capitis (11 subjects), S. warneri (9 subjects), S. hominis subsp. hominis, and S. hominis subsp. novobiosepticus were the organisms that came in second and third, respectively (8 subjects). Micrococcus sp., especially Micrococcus yunnanensis, were also prevalent (11 subjects). Oceanobacillus caeni (1 subject), Paracoccus sanguines (1 subject), Enterobacter aerogenes (1 subject), and Cornenebacterium striatum (1 subject) were the organisms that exhibited personal variations (1 subjects). The authors conclude that certain minor organisms were endemic to certain individuals and thus had the ability for private recognition based on these findings. They also emphasize that the majority of species, especially Staphylococcus species that showed spread, can be used as molecular biological markers at the subspecies stage, according to the participants. This is why the authors believe the cutaneous microbiota of the palm of the hand has a strong propensity for private recognition (Garcia et al., 2020).

c) Microbial Fingerprinting Methods

Microbial forensics has emerged as an interdisciplinary area of microbiology devoted to the creation, evaluation, acceptance, and application of techniques to distinguish and fully depict microbial specimens comprising a natural specialist or its segments. The primary goal of a criminological microbiologist is to distinguish between possible pathogens and identify their DNA marks in order to establish the probable source's base. Another logical train, microbial fingerprinting, has been developed under microbial legal sciences in recent years with the specific aim of strengthening the law implementation reaction, especially in a bioterrorism situation. Microbial fingerprinting strategies are a class of methods for distinguishing microorganisms or groups of microorganisms based on novel characteristics of a bio particle's general portion or region (e.g., phospholipids, DNA, or RNA). Some microbial fingerprinting techniques are used to distinguish subsets of microorganisms, while others are used to provide a general profile of the microbial community (Moumita Sinha & I. Arjun Rao, 2017).

Microbial fingerprinting techniques may offer a comprehensive analysis of a microbial population. Hereditary strategies allow for the separation of evidence of primary individuals from the microbial community to the family or class level. It denotes unique microorganisms or groups of microorganisms that are recognizable as one-of-a-kind properties of a shared thing or region of a bio atom (for example, phospholipids, DNA, or RNA). It also reveals microbial differences and provides information about the types of metabolic processes taking place on the site, allowing a subset of the microorganisms incorporated into the specimen to be distinguished. It may identify the microbial greenery of the precise topographical region that is exclusive to that area in advance. This is significant because any microorganism has its own signature flora, which can influence a person's surrounding atmosphere during a criminal investigation (Moumita Sinha & I. Arjun Rao, 2017). The following are some of the microbial fingerprinting methods:

a. PLFA Analysis

Phospholipid fatty acid is found mostly on the membranes of all living cells and disappears immediately when the cell dies. As a result, the mass of PLFA is an immediate estimate of the specimen's feasible biomass. Although phospholipids are found in all cell layers, not all life forms or groups of creatures have the same PLFA types to the same degree. Some species produce one-of-a-kind or "label" PLFA. Measuring these PLFA assemblages in this way creates a profile or special finger impression of the appropriate microbial community and provides insight into some important microbial utilitarian gatherings (e.g., iron-and sulfate-decreasing microscopic organisms).

In ecological examples, PLFA analysis is similar to that of other concoction mixes present as blends (e.g., dysfunctional natural mixes): (1) extraction, (2) detachment by gas chromatography with fire ionization discovery, and (3) affirmation of identifiable evidence by spectrographic analysis, if necessary. By calculating the consolidation of the stable isotope mark into biomass, PLFA research can be combined with stable isotope testing (SIP) to show that biodegradation is taking place (Moumita Sinha & I. Arjun Rao, 2017).

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b. DGGE Analysis

DGGE is a nucleic acid corrosive (DNA or RNA)–based technique for creating a microbial group's genetic special mark and potentially distinguishing overwhelming microorganisms. DGGE profiles are commonly used to search for differences or improvements in microbial community quality and structure between samples, over time or space, or in response to treatment.

The four stages of DGGE are typically: (1) DNA or RNA extraction, (2) intensification, (3) detachment and representation, and (4) grouping recognizable evidence. Polymerase chain reaction (PCR) is used in the enhancement process to have an excessive number of duplicates of a variable location within an objective quality. Per relatively minute living being has a different DNA course of action in this variable area. As a result of the PCR step, a combination of the standard bits for each creature bunch appears inside the primary illustration. The third stage of DGGE involves using an electrical current (electrophoresis) and a denaturing procedure to isolate the mixture based on the DNA collected, resulting in a profile, or exceptional check, of the microbial collection. DGGE is a cost-effective option (Moumita Sinha & I. Arjun Rao, 2017).

c. T-RFLP Analysis

T-RFLP (terminal fragment length polymorphism) analysis is a technique for quickly profiling mixed populations of homologous amplicons (i.e., diverse sequences of one gene). It integrates automatic sequencing gel technologies with fragment analysis of a PCR-amplified gene marker. T-RFLP, like DGGE, is a macromolecule (DNA or RNA)–based technique that creates a microbial species fingerprint and can be used to classify unique microbial communities.

T-RFLP is a four-step procedure that includes isolation of DNA or RNA, PCR amplification, enzyme digestion, and fragment recognition. Following the separation of the mixed group DNA or RNA, PCR amplification with a fluorescent-labeled PCR primer is used to create various duplicates of objective consistency, and the PCR products are then processed with confinement proteins that cut the DNA particle at known arrangements. The length of each corresponding terminal confinement fragment is unique to a particular microorganism. The sensitivity of T-RFLP is higher than that of DGGE (i.e., it should detect microorganisms that are present at lower numbers during a sample). T-RFLP is commercially available (Moumita Sinha & I. Arjun Rao, 2017).

d) Challenges

Human DNA transmission, survival, prevalence, and recovery (TPPR) research is also needed in relevance microbial DNA specific to human microbiomes since the human microbiome may be used to link or classify persons for forensic investigation. However, the complexity of what constitutes a microbiome complicates such studies, since microbiomes include ecological relationships that show temporal changes (Zang Y. Et al., 2017; Flores et al., 2014). The transfer and survival of the human microbiota is complicated and influenced by a variety of intrinsic and extrinsic factors such as nutritional supply, season, oral antibiotic use, and host diet. It would be claimed that microbial profiling of people for forensic purposes may just suggest the possible affiliation of particular microbes with an individual's microbiome, rather than providing evidence of the microbe's inherent participation in that microbiome. If the aim of microbial identification is to link an individual to illegal crime and/or warn inquiries, this may be a source of concern (Ana Neckovic et al., 2020). Furthermore, certain considerations can make determining whether or not microbial contamination has occurred in a forensic environment more difficult. Though human nDNA databases can be used to determine whether staff contamination occurred in a forensic setting (e.g., at a crime scene or in a forensic laboratory), a staff microbiome database, and by extension a criminal microbiome database, may not have the same value, depending on if an individual's microbiome has the potential to vary in minor or significant ways (Oh, J et al., 2016).

If microbial profiling of human skin-associated bacteria is to be used for forensic use, forensic scientists must also consider the possibility of microbial contamination events within forensic settings (i.e., crime scene to laboratory) and use this information to design new, or improve existing, contamination-prevention protocols to ensure that they are suitable for microbial profiling (Ana Neckovic et al., 2020). For example, storing evidence in cold, dry environments may allow specific microbes to thrive while others become non-viable, or exposure to low temperatures may cause changes in microbial growth and structure, as the skin-associated bacteria Staphylococci has shown (Onyango, L.A et al., 2012). Furthermore, drying cotton swabs or aerating swab containers may cause foreign microbes to enter the swab surface, and the evidentiary packaging itself may be a source of additional microbial contamination. Standard protocols such as these which trigger microbial population changes over time; as a result, the impacts of the different protocols made accessible by comprehensive testing should be disclosed, and sufficient steps should be taken to properly remedy any negative consequences. However, since a ‘expected' microbial population composition cannot be identified, just as ‘ground fact' cannot be established in a criminal investigation, all microbial

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analysis performed after the collection and review of an evidentiary object should be approached with caution.

The extraction of microbial DNA, which will be used for sequencing and interrogation of a microbial population of interest, is an important part of microbial profiling. However, there is a growing understanding of the effects of DNA extraction on microbial profiling (Bjerre, R.D et al., 2019; Teng, F et al., 2018; Ducarmon, Q.R et al., 2019), especially in terms of sample profile reproducibility when a single kit and protocol is used (Vera-Wolf, P et al., 2018), as well as the varying proportions of extracted microbial DNA from (Teng, F et al., 2018). It may be argued, then, that extracting microbial DNA from an evidentiary sample does not reliably reflect the microbial population of interest, the proportions of which may be used to decide how closely a sample represents a person's microbiota, and hence the person's affiliation with illegal behavior. Furthermore, scientific reproducibility is a persistent problem in microbiome study (Schloss, P.D., 2018), making it difficult to breed microbial communities from similar source samples utilizing the same extraction technique, or to breed the same outcome from one sample using new or modified extraction methodologies. Low-biomass samples have the same amount of microbial DNA as negative extraction (or blank) samples; as a result, any microbial DNA found in the sample will easily be outcompeted by external/contaminating microbial DNA (Eisenhofer, R. Et al., 2019).

Even where negative extraction controls are used and sequenced to theoretically distinguish background contamination emanating from extraction kits, additional microbial contamination can occur during the sequencing process. When non-ligated adapters from one sample bind to free DNA from another sample on the same sequencing run, index-hopping may occur; this would be particularly troublesome for low biomass samples, in which there could be an absence, or low DNA template samples (Hornung, B.V.H. et al., 2019). Index-hopping has been stated to occur in 1–10% of collected sequencing results, but this varies depending on the Illumina sequencer used (Sinha, R. et al., 2017) Finally, this may result in the incorrect assignment of sequencing data from one sample to another, resulting in microbial duplication between samples. Because of index-hopping results, a negative extraction control could represent the same microbial profile as a sample of interest, which could be troublesome (Hornung, B.V.H. et al., 2019). Innocent transfer may also be described as the deposit of uncountable bacterial cells from an individual's microbial cloud into a built environment, which may later be identified as a criminal crime scene, or the indirect transfer of one individual's microbiome to another by their involvement in a shared space or the handling of an object.

III. CONCLUSION

The human microbiome serves a variety of functions, ranging from body decomposition to individual identification. Microbial fingerprinting is a reliable method of identifying an individual, but it is still in its early stages in the field of forensics due to the many problems associated with contamination and its durability. The most popular challenge is that the true source might not be selected correctly, and the second explanation is that the time gap between sample samples may be high, resulting in sample contamination. To address these issues, scientists are developing or improving microbial source trackers and microbial fingerprinting methods. Due to its high sensitivity in detecting less abundant microorganisms in the sample, T-RFLP is the most widely used microbial fingerprinting process. Several advanced techniques, such as REAC, REAP, and RAPD, can also be used for potential research in this area. The skin is the main organ of the physical organ and is colonized by the millions of microorganisms, who have an endogenous host structure (i.e. age, race, etc.) and exogenous environmental computations (i.e., diet, geography, etc.). The forensic usefulness and association ship of individuals with crimes in research and development is still in the infancy of microbial profiling, but the above-mentioned shortcomings must be addressed and investigated prior to consideration of inclusion of the casework.

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