

Identification of Bacterial Isolates Originating from the Human Hand

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The human body provides habitat for a diversity of bacterial species that are part of the normal human microbiota. Identification of various members of the normal microbiota to the species level requires a combination of biological staining procedures, biochemical tests, and molecular techniques. In this experiment, ten bacterial isolates originating from the hands of nine students and one faculty member at USC Salkehatchie were identified. Classification to a general taxonomic group was accomplished with standard staining and biochemical tests. Sequences for the 16S ribosomal RNA section of DNA for each isolate were analyzed with BLAST to generate a list of potential species identifications. Species associated with confidence levels greater than 98% were considered positive identifications. The samples were then analyzed using Matrix Assisted Laser Desorption Ionization-Time of Flight Mass Spectrometry (MALDI-TOF MS). Five isolates were identified as *Bacillus megaterium* (2 isolates), *Bacillus thuringiensis*, *Paenibacillus alvei*, and *Micrococcus luteus*. Four isolates were identified as *Bacillus* and *Brevibacterium* species. One isolate had conflicting identifications based on molecular and MALDI-TOF MS and is only listed as a *Bacillus* species. In addition to contributing to the study of the human normal microbiota, the diagnostic properties and identities of each isolate will be incorporated into a laboratory resource used by microbiology students at USC Salkehatchie.

Introduction

The human body provides for a diversity of bacterial species collectively referred to as the normal microbiota. As commensals, the normal microbiota occur and derive nourishment from the host body without causing any harm (Gould, 2012). Resident bacteria can withstand repeated disinfection and can lodge themselves deeper within the epidermis, underneath the fingernails or within hair follicle ducts. They are difficult to dislodge but are not considered pathogenic. Transient bacteria situate themselves superficially as a result of direct contact from the environment through touching or from other people. Transient skin microbiota tend to include pathogenic bacteria (Gould, 2012). Resident microbes are essential normal microorganisms preventing the entrance of transient microbes and colonizing pathogens (Jeppu et al., 2017). Resident microbes can be of concern for the immunocompromised or hospitalized individuals as the resident microbiota can become pathogenic (Sureshkumar et al., 2018).

Identification of various members of the normal microbiota to the species level requires a combination of biochemical staining procedures, biochemical tests and molecular techniques.

Bacterial staining procedures like the Gram stain continue to be one of the most useful differential staining techniques used as it distinguishes between bacteria that are Gram positive (Gram +) or Gram negative (Gram -). Gram reaction designation and morphology provide a starting point from which further diagnostic testing can be determined (McClelland, 2001). A variety of biochemical tests along with special media are used to further differentiate Gram + and Gram - cultures.

Molecular techniques and methods utilized in microbiology for bacterial identification are quite diverse. Some of these techniques include real-time polymerase chain reaction, partial or whole genome sequencing, molecular typing, microarrays, broad range PCR, and multiplexing (Sibley et al., 2012). Polymerase chain reaction (PCR) technology has become a very affordable and attractive means for diagnostic microbiology, especially in the identification of infectious diseases.

The idea for this project stemmed from a class experiment performed by students enrolled in the lab section of Microbiology (BIOL 250L) at the University of South Carolina Salkehatchie (USC Salkehatchie). Each semester, during the first lab, the students start a bacterial culture from their hands and perform a consistent routine of staining procedures and biochemical tests on the culture to work toward a possible identification. The regimen of stains and tests over a 10-year period produced a group of bacterial isolates from the human hand with recognizable cell morphology and cultural characteristics. The primary objective of this project was to identify ten of these isolate types.

Methods

Ten bacterial isolates originating from the hands of nine students and one faculty member at USC Salkehatchie were purified and inspected with standard cultural methods and staining techniques. Samples were isolated from bacteria grown from student fingers. Students rubbed their fingers directly on plated tryptic soy agar (TSA) before handwashing and after handwashing. Bacteria were isolated and purified from these samples and grown on TSA for 24 hours and the following morphological features were determined for each isolate: cell shape (e.g., cocci [spherical], bacilli [rod]), cell arrangement (e.g., strepto [chain], diplo [paired], staphylo [grape-like clusters], tetrad [cuboidal packet]), relative colony size (small, moderate, or large), colony color (e.g., pigmentation and color or lack of pigmentation [cream]), colony form (e.g., irregular, circular, rhizoid), colony margin (e.g., undulate, entire, lobate), and colony elevation (e.g., flat, convex, raised, umbonate).

Molecular systematic techniques were used to identify the isolates to genus, and where possible, to species. The use of 16S rRNA genes are routinely used to identify strains of bacteria (Barghouthi, 2011). Cultures grown on TSA for 72 hours were used to extract DNA by a modified Chelex method (Turan et al., 2015) using InstaGene™ Matrix (BioRad #732-6030). To extract DNA from each isolate, a tiny amount of each colony was placed into 200 µL of InstaGene. To do this, a sterile P200 pipette tip was used to pick up a small amount of colony on the tip, then the tip was swished into the InstaGene mixture and vortexed. The tubes were placed in 100°C dry bath for 10 mins, then placed on ice for 2 mins. The samples were then vortexed and spun at 13,000 rpm for 10 mins. The DNA within the supernatant was then transferred to sterile tubes.

The species were identified by PCR amplification and sequencing approximately a 1300 bp region of the 16S rRNA gene. The primers selected were B-103F (5'-AGAGTTTGATCCTGGCTCAG-3') and BMBC-R (5'-ACGGGCGGTGTGTRC-3'). Each 50 µL of PCR reaction mix contained 10 µL DNA sample, 10 µL reaction buffer (5X GoTaq® Reaction Buffer; Promega, USA), 1 µL dNTPs (10 mM), 1 µL of each primer at 10 µM concentration, 26.75 µL sterile dH₂O, and 0.25 µL GoTaq® (Promega, USA). The reaction started at 94°C for 2 min, and continued for 35 cycles at 94°C for 30 sec, 55°C for 15 sec, and 72°C for 1 min. The final extension was at 72°C for 5 min. PCR products were then cleaned using EXOSAP-IT, a kit containing enzymes that degrade residual primers. The purified PCR products were sequenced at the University of Tennessee Knoxville Genomics Core Laboratory, using B103-F primer.

Sequences were analyzed and trimmed using Geneious software (Drummond et al., 2010). Sequence results were submitted to GenBank BLAST searches and the most probable taxonomic match was recorded. If the confidence level of the BLAST match was greater than 98%,

isolates were considered identified to the species listed in the BLAST match (modified from Barghouthi, 2011). If the confidence level of the BLAST match was between 95-98%, the isolate was identified to genus or possible species identities. When base substitutions occurred in more than 5% of the region, isolates were identified to the closest genus only. Accession numbers for the sequences are in Tables 1-3.

The samples were then analyzed using matrix assisted laser desorption ionization-time of flight mass spectrometry (MALD-TOF MS) (Singhal et al., 2015). MALDI-TOF MS directly analyzes ionized biological molecules. It is rapid, requiring less than an hour to perform, low cost and efficient (Hou et al., 2019). Microbial ribosomal proteins result in a mass spectrum with a characteristic mass and intensity distribution pattern, likened to a “molecular fingerprint”. This fingerprint is species-specific for many bacteria and can be used for identification of a test specimen that can be compared with the patterns recorded in the reference library for this system. MALDI-TOF MS technology served to complement the initial molecular work and provide further confidence for species identification (MALDI Biotyper CA System).

Results

Eight of the 10 isolates were identified to species or genus level and had a 99% match during BLAST, with the other two isolates (5 and 10) having less than 98.7% matches. Seven isolates were Gram + streptobacilli, two isolates were Gram + bacilli, and one was a Gram + tetrad. Using molecular data and confirmation by MALDI-TOF-MS, five isolates were identified to species level and include various species of *Bacillus* as well as *Paenibacillus alvei* and *Micrococcus luteus* (Table 1).

Four isolates were identified to genus level to potential species within *Bacillus* using BLAST (Table 2). Although high confidence levels were observed for more than one species, tentative species identifications are provided in Table 2. In addition, MALDI-TOF MS analysis did not provide a species identification for these four isolates (Table 2).

One species had conflicting results for identification using molecular data and MALDI-TOF-MS analysis (Table 3). Tentative identifications are listed for both processes in Table 3 and indicate the isolate is a *Bacillus* species.

Discussion

Research indicates that molecular identifications are not always 100% accurate due to variability in bacterial genomes (Barghouthi, 2011; Větrovský and Baldrian, 2013) and that MALDI-TOF MS also is not 100% accurate as not all bacterial species are in the databases that are used by the software of this analytic process (Rudrik et al., 2017). The information sheet for the specific MALDI-TOF MS analyzer used

indicates the unit can identify 333 species groups which covers 424 clinically relevant bacteria and yeast species that are relevant to hospital infections, specifically 98% of typical bacterial species (MALDI Biotyper CA System).

The species of *Bacillus*, *Paenibacillus*, and *Micrococcus* identified in this experiment are commonly found on skin surfaces, including hands (Edmond-Wilsons, 2015; Grady et al., 2016). These resident skin microbiota occur as commensals on the human body and have a role in maintaining general immunity for the host; however, in the immunocompromised these species may function as opportunistic pathogens. For example, *Bacillus megaterium* was the culprit behind a gangrenous foot infection that was initially diagnosed as being caused by *Bacillus anthracis* (Loong et al., 2017). *Bacillus subtilis* has been implicated in cases of food spoilage and food-borne diseases as *Bacillus* species are capable of producing resistant endospores (Mikkola et al., 2000; Apetroaie-Constantin et al., 2009). *Paenibacillus alvei* was not known to cause human disease until recently when a patient with chronic kidney disease was diagnosed as having a urinary tract infection caused by *P. alvei* (Sanghamitra et al., 2013).

Research has also supported the benefits of the normal microbiota. *Bacillus subtilis*, the same microbe that may be a food spoiler may also produce antibiotics (Stein, 2005). Other species of *Bacillus* may also produce beneficial antibiotics and may be found on skin, such as *Bacillus amyloliquefaciens* which can produce metabolites that have the capability of inhibiting the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) and may show potential in the prevention of MRSA skin colonization (Nasrin et al., 2015). As bacteria naturally occur in the environment and soil, bacterial species have beneficial properties that aid in the control of plant diseases and control of fungal growth (Grady et al., 2016; Rabbee et al., 2019). One such example is *Bacillus velezensis*, which is valuable for promoting plant growth and suppressing the growth of microbial pathogens and nematodes (Rabbee et al., 2019). Microbial skin microbiota are vital components of human biology with recent research indicating that they play a much greater role than previously thought, having great implications in human health and disease (Stein, 2005; Kali, 2015; Nasrin et al., 2015).

Conclusions

This study used a combination of traditional and emerging molecular techniques that identified 10 bacterial cultures isolated from the human hand. Several of the species identified are known to be commensal on the human body. In addition to contributing to the study of the human normal microbiota, the diagnostic properties of each isolate will be incorporated into a laboratory resource used by microbiology students at USC Salkehatchie. Additional research exploring the microbiota of the human body would add to the knowledge base of the relationship between normal microbiota and human health.

Table 1. Specimen identification, cell morphology (shape and arrangement), and colony morphology (size, pigmentation, form, margin, and elevation). Identifications based on BLAST data and confirmed with MALDI-TOF MS.

Isolate (Accession#)	Identification	Cell Morphology	Colony Morphology
2 (MN128550)	<i>Paenibacillus alvei</i>	streptobacilli	small, cream, irregular, undulate, convex
3 (MN128551)	<i>Bacillus megaterium</i>	streptobacilli	moderate, pale yellow, irregular, undulate, umbonate
6 (MN128554)	<i>Bacillus thuringiensis</i>	streptobacilli	moderate, cream, irregular, lobate, umbonate
9 (MN128557)	<i>Bacillus megaterium</i>	streptobacilli	moderate, pale yellow, circular, entire, convex
10 (MN128558)	<i>Micrococcus luteus</i>	cocci, tetrad	small, yellow, circular, entire, raised

Table 2. Isolates with possible genus/species identifications (listed in order of highest possibility), cell morphology (shape and arrangement), and colony morphology (size, pigmentation, form, margin, and elevation). Identifications are based on BLAST data but not confirmed with MALDI-TOF MS.

Isolate (Accession#)	Possible Identification	Cell Morphology	Colony Morphology
1 (MN128549)	<i>Bacillus velezensis</i>	bacilli	large, cream, rhizoid, lobate, raised
4 (MN128552)	<i>Bacillus</i> sp. or <i>Brevibacterium frigiditolerans</i>	bacilli	moderate, cream, circular, undulate, convex
5 (MN128553)	<i>Bacillus</i> sp. Either <i>B. aquimaris</i> or <i>B. vietnamensis</i>	streptobacilli	small, red, circular, entire, convex
8 (MN128556)	<i>Bacillus</i> sp. Either <i>B. licheniformis</i> or <i>B. flexus</i>	streptobacilli	moderate, cream, irregular, undulate, raised

Table 3. Specimen identification, cell morphology (shape and arrangement), and colony morphology (size, pigmentation, form, margin, and elevation). Identification based on BLAST data conflict with MALDI-TOF MS.

Isolate (Accession#)	Identification	Cell Morphology	Colony Morphology
7 (MN128555)	<i>Bacillus</i> sp. (MALDI) – <i>Bacillus subtilis</i> (DNA) – <i>B. velezensis</i> or other <i>Bacillus</i> sp.	streptobacilli	moderate, cream, irregular, lobate, raised

Notes and References

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