Investigation of the Microbial and Molecular Correlates of Morgellons Disease

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Abstract

Morgellons disease is a complex dermopathy that is controversial in the medical community. Unfortunately, there is not enough evidence to the etiology or transmission of this disease. Due to the lack of information, the debate surrounding Morgellons is considerable. There are currently no accepted markers for diagnosis, which leads patients to a common differential diagnosis of delusional parasitosis or delusional infestation.[1,3] However, with further investigation, potential etiologies can be explored.

In this study:

- Lesions from patients are collected and de-identified so they are anonymous to the researchers.
- Lesions are studied for unusual microbial organisms; specifically, *Bartonella henselae, Helicobacter pylori, Borrelia burgdorferi,* and *Treponema denticola*.
- *Borrelia burgdorferi* have been detected in dermatological specimens, providing a base line for spirochetal cause.[1]
- We hypothesize microbial organisms could be the infectious cause of Morgellons disease.
- Identifying these related strains will help to determine if an infectious etiology of the dermopathy is present.

Introduction

Morgellons is a multisystem infective disease that is characterized as a mysterious condition which is misunderstood in the medical community. This condition can be debilitating and disabling, as non-healing lesions with unique colored fiber-like filaments emerge from open wounds.[1,3] Crawling sensations on and under the skin, with intense itching, severe fatigue, difficulty concentrating, and short-term memory loss are also associated within the sign and symptoms of Morgellons disease. Morgellons disease does not discriminate as it is found within all socio-economic and age groups. The distinct feature is the presence of near microscopic and microscopic subcutaneous fibers. These fibers can range in a multitude of colors and analysis given mixed results ranging from unknown to keratin.[3] Through further DNA analysis[1,3], hopefully, the answers to this unsolved mystery will be revealed and bring a better understanding to the medical community.

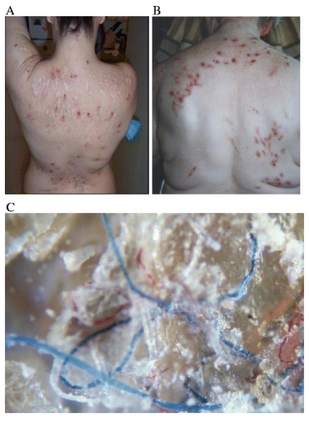


Fig.1. Clinical features of Morgellons disease. A. MD patient back showing lesions covering entire surface, including areas out of patient's reach. **B.** Back of patient with scratching-induced lesions showing distribution limited to patient's reach. **C.** Multicolored fibers embedded in skin callus from MD Patient 2 (100x). Reproduced from Ref.1.

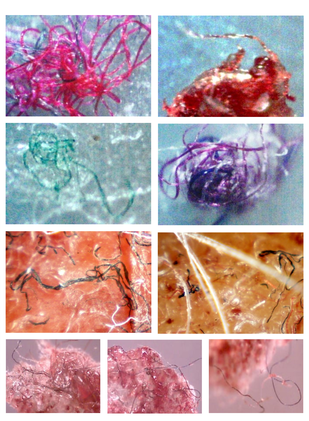


Fig.2. Morgellons Disease Fibers. This figure depicts the multitude of colors found in Morgellons disease epithelial tissue cultures. Microscopic fibers can range in a variety of colors. This photo above shows colors from purple, red, blue, and pink fibers. These images were reproduced from the Charles E. Holman website. Ref. 9.

Methods

Microbial Techniques

❑ MacConkey Agar

MacConkey agar is used to isolate and differentiate members of the Enterobacteriaceae.

- Ability to ferment lactose.
- Selective and differential culture media.
- Selectively isolate Gram-negative and enteric bacilli bacteria.
- Samples are plated using a lawn.
- Colonies are isolated.
- Molecular analysis is used to conduct further research.

Fig. 3. MacConkey Agar. Preparing MacConkey agar for our bacteria of interest. Photograph taken by Samantha Rice

Molecular Techniques

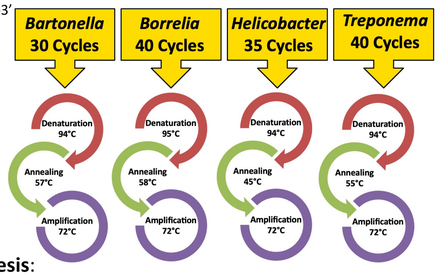
❑ Phenol Chloroform DNA Extraction:

Massed out to 0.1g deidentified epithelial tissue samples, added 100-200µl of Molecular Grade DI Water and place in a 1.5µl microcentrifuge tube. Spin at 12,000 x g for 3 minutes. Suspend pellet with 250µl of resuspension media. Vortex to mix. Set tubes in a dry bath at 37°C for approximately 30 minutes or until the liquid becomes viscous. After incubation period, add 250µl TNE solution to each tube and vortex to mix. Incubate in a dry bath at 65ºC for 1 hour. Add 500µl Phenol: Chloroform: Isoamyl Solution to each tube. Vortex mixture and spin at 10,000 x g for 3 minutes. Remove supernatant to clean microcentrifuge tube and add equivalent amount of chloroform: isoamyl. Vortex and spin again at 10,000 x g for 3 minutes. Remove supernatant to blood culture tube. Add 2 volumes of 95% ethanol and tilt. Remove DNA clot from supernatant with loop and place in a clean microcentrifuge tube containing 100-200µl of TE.

❑ Polymerase Chain Reaction:

PCR was used to amplify our gene of interest.

- ***Borrelia* 16S ribosomal RNA small subunit or CTP synthase gene** F: 5'-CCTGGCTTAGAACTAACG-3'; R: 5'-CCTACAAAGCTTATTCCTCA-3'
- ***Treponema* specific 16S ribosomal RNA** F: 5'-AARCATGCAAGTCGARCGGCAAG-3'; R: 5'-TCCATTGCGGAATATTCTTA-3'
- ***Bartonella* 16S rRNA** F: 5'-CCTCCTTCAGTTAGGCTGG-3'; R: 5'-GAGATGGCTTTTGGAGATTA-3'
- ***Helicobacter pylori* Urease gene** F: 5'-GCCAATGGTAAATTAGTT-3'; R: 5'-CTCCTTAATTGTTTTTAC-3'



❑ Gel Electrophoresis:

Ten microliters of each PCR product was run on a 1% agarose gel at 75 Volts for 2 hours. The gel was stained using Ethidium Bromide (EtBr) solution for 5 minutes and viewed with a Bio-Doc IT UV transilluminator.

❑ Gel Purification:

Gel purification was conducted with a kit provided by Invitrogen. After, purification 12µl of purified DNA are stored in a clean 0.5µl tube.

❑ Nanodrop:

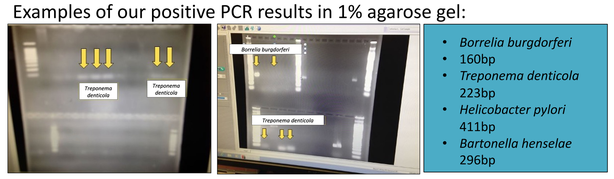
1µl of DNA sample was used to measure the nucleic acid concentration.

❑ DNA Sequencing:

DNA is sent for sequencing with subsequent bioinformatics analysis.

Results

Examples of our positive PCR results in 1% agarose gel:



- *Borrelia burgdorferi* 160bp
- *Treponema denticola* 223bp
- *Helicobacter pylori* 411bp
- *Bartonella henselae* 296bp

Fig. 3. Images of 1% Agarose Gel Electrophoresis. This figure depicts images of 1% Agarose Gel Electrophoresis with lanes 5, 6, 7, 11, and 12 with positive bands of *Treponema denticola* on the left and positive bands *of Borrelia burgdorferi* in lane 1,4 on the right top portion of the image and positive bands of Treponema denticola in lanes 2,4, and 5. All images were taken by Samantha Rice

Helicobacter pylori strain 26695-1MET, complete genome

GCTCTCCGGGAACTTTTGCGCATGTAACACGCTCTTTAGCTCTGTGTAAAGCAATTTTTTTG
CTTTCGTTGTCTGCTTGCCTATCAACCAACGCGTTAAATCCAAAGATTCTTCTGTTACCGCC
AATGTCAATCAATTCTACGGATTTTTCTTCGCCAGGCTCAAACCTTACCGCTGTCCCGCTCG
CAATGTCTAAGCGTTTACCGAAAGTTTTTTCTCTGTCAAAGTCTAAGCATCTATTCACTTCAA
AGAAATGGAAGTGTGAGCCGATTTGAACCGGTCTGTCGCCAACATTTTTAACTTTCACGCTA
ACGGCTTTTTTGCCTTCGTTGATAGTGATGTCTTCATTTTTTAAGAACAACTCACCAGGAACT
AATTTACCATTGGCA

NCBI Blastn and Blastx results

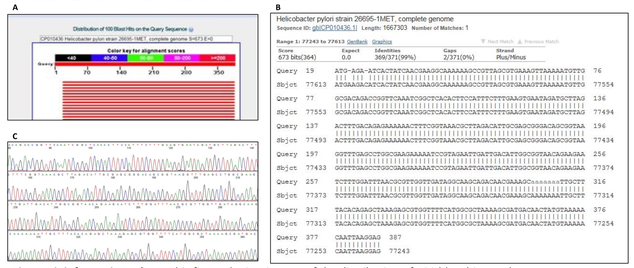


Fig. 4. Bioinformatic Tools. A. This figure depicts images of the distribution of 100 blast hits on the query sequence for the *Helicobacter pylori* strain. **B.** Sequences producing significant alliances for *Helicobacter pylori* strain 26695-1Met, matching in GenBank. **C.** Nucleotide sequence of chromatogram of specimen as viewed on Finch TV. All images were taken by Samantha Rice.

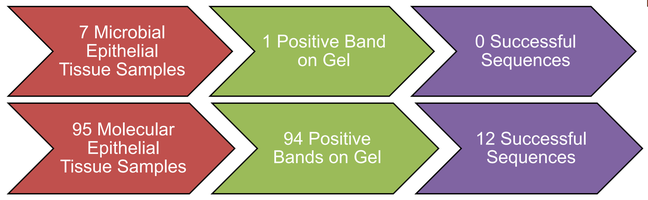


Table 1. Unusual microbial organisms found in Morgellons disease epithelial tissue samples: positive bands and successfully sequenced DNA using bioinformatic tools..

Bacteria of Interest	Positive bands in Agarose Gel	Sequenced DNA Using Bioinformatic Tools
Bartonella henselae	#1, BIF, BM2, 1E, 1F, 1G, 1P, 1U, 1W, 1X, 1Y, 2O, 4A, 4E, 4H	
Borrelia burgdorferi	B1, B2, B6, BIF, BOR1, BIG, #1, 1A, 1B, 1C, 1F, 1G, 1J1, 1K, 1M, 1U, 2B, 1, 4A, 4B, 4B1, CC, 4D, 4D1, 4E	#1, 1F, 2B, 1
Helicobacter pylori	MB2, H1L, H17, H9, #1, 1E, 1G, 1K, 1L, 1P, 1U, 1X, 1Y, 2L, 2M, 2N, 2O	H17, 1E, 1G, 1K, 1L
Treponema denticola	BM2, Y2, TM1, T4, 3S, TH6, 16W, P35, TP4, T1, #1, #2, 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1K, 1L, 1M, 1O, 1P, 1R, 1S, 1U, 1V, 1W, 1X, 1Y, 1Z, 2F, 2K, 2L, 2M, 2P	T4, TN6, 1M

Table 2. Environmental negative controls to show our bacteria of interest is not readily found in the environment.

Environmental Contaminant Controls	Controls Were Negative
Subject 1 – BJ (Epithelial Cells)	Negative
Subject 2 – C (Epithelial Cells)	Negative
Subject 3 – S (Epithelial Cells)	Negative
Subject 4 – W (Epithelial Cells)	Negative
Autoclave – A Barson Building	Negative
Women's Bathroom – B	Negative
Stairwell – ST (Main)	Negative
Water Fountain	Negative

Conclusion

- Unusual microbial organisms have been identified in Morgellons epithelial tissue samples.
- Over 95 molecular epithelial tissue samples were analyzed with each bacteria of interest, with 94 positive bands, and 12 successful sequences.
- DNA analysis of *Bartonella henselae, Helicobacter pylori, Borrelia burgdorferi,* and *Treponema denticola* have successfully been extracted from epithelial tissue samples and sequences identified in GenBank®, the NIH genetic sequence database.
- Environmental contaminant controls were implemented to show these bacterial agents are not readily found in the environment.
- Research suggests there may be an infectious etiology of the dermopathy is present.
- The core facility suggested looking into different gel purification techniques for a higher success of DNA sequencing.
- Purification from PCR products may yield a higher recovery rate than from gel purification.

Future Research

- Future research needs to be conducted to continue exploring etiologies to support our findings and replicate these results.
- Future research includes testing more epithelial tissue samples.
- We want to explore more primers for different bacterial species that could be present.
- Create a standardized kit for environmental controls.
- Create a database of samples brought into the lab.
- Culture epithelial cells to study fiber growth and study the pH levels of *H. pylori*.

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