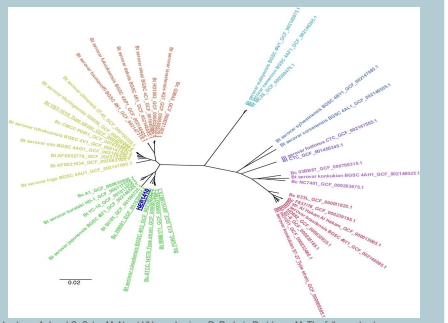
Developing a Method for Rapid and Accurate Identification of *Bacillus* Species in Clinical Isolates Using Polymerase Chain Reaction

Jessica L. Anderson and Casey M. Finnerty, Ph.D.
Biology Department; Winona State University; Winona, MN 55987

Abstract

Bacillus species have caused a variety of diseases in humans throughout history. These include foodborne illness, wound infections, and anthrax poisoning. With over 266 identified species within the *Bacillus* genus, isolates previously described as independent species (e.g. *B. anthracis*, *B. cereus*, *B. thuringiensis*) have been discovered to be so genetically and phenotypically similar that they are often very difficult to discriminate between. Therefore, there have been instances of misidentification reported in scientific literature. Therefore, the majority of clinical tests done may not be precise enough to distinguish between some species of *Bacillus*, as they are so genetically similar. A new software, called MALDI-TOF, has been very successful in species discrimination. However, this technology is relatively new and not widely available for all clinical settings. The misidentification of clinical *Bacillus* isolates from infected patients could have medical significance and negatively affect treatment. By finding improved approaches for correctly identifying and distinguishing between *Bacillus* species, the scientific community can increase its understanding of the pathogenic potential of certain *Bacillus* species and positively impact patient diagnosis and treatment.



Lechuga A, Lood C, Salas M, Noort VV van, Lavigne R, Redrejo-Rodríguez M. The fully resolved genome of Bacillus thuringiensis HER1410 reveals a cry-containing chromosome, two megaplasmids & an integrative plasmidial prophage. Published online May 8, 2020:2020.05.05.080028. doi:10.1101/2020.05.05.080028

This study was intended to develop a technique for better distinguishing between *Bacillus* species. Recent research has suggested that the pycA gene may be the key to separating Bacillus species. A series of specially designed primers will be used to target the pycA gene in *Bacillus thuringiensis* and *Bacillus cereus* strains grown from isolates obtained from the NRRL repository. With the use of these primers, PCR testing will be performed on the various Bacillus strains. This will allow for identification of the isolate strains based on the PCR products and comparison to the initial repository identification. If successful, this approach could prove to be a more rapid and precise way to distinguish between *Bacillus* species in clinical settings, possibly improving the speed of diagnosis and treatment. Data collection and analysis are currently being executed.

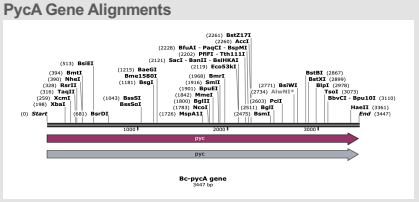
Introduction

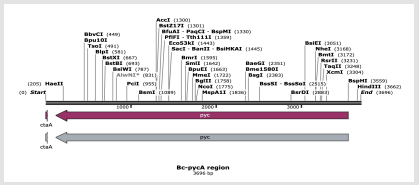
Designing the primers was a crucial part of the experimental design process. The gene of focus was the pycA gene. After reviewing the literature, there seemed to be the most variability between *Bacillus* species in the pycA gene region of the genomes. The pycA gene encodes for pyruvate carboxylase protein.

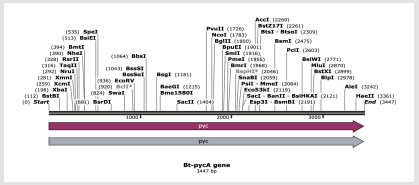
10 primers were carefully chosen using multiple sequence alignment software. The pycA gene sequences of *Bacillus cereus* and *Bacillus thuringiensis* were aligned, and primers were chosen to cover the most variable areas.

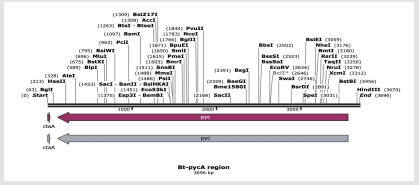
Each primer will be used to preform a PCR analysis followed by gel electrophoresis in order to compare the results from the *Bacillus cereus* and *Bacillus thuringiensis* strains from the NRRL repository.

PycA Gene Alignments



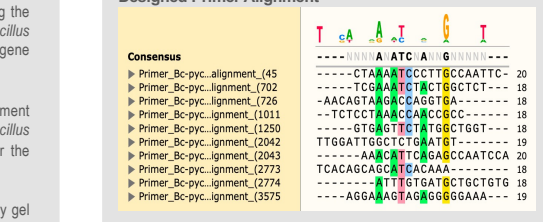






Materials & Methods

Designed Primer Alignment



Procedure

1. **Grow** each of the 20 strains on nutrient agar plates for 12h
2. Transfer loopful of 1 cell colony to 0.1mL H2O in conical tube
3. Boil each tube for 10min
4. Centrifuge (10sec @ 10,000rpm)
5. Combine PCR mixture in tube
   - 15 µL supernatant
   - 0.5-2.5 U taq polymerase
   - 0.1-0.5 µM primer
   - 2.5 mM dNTPS
6. **Amplification**
   - Single denaturation step (2min @ 95°C)
   - 30-cycle program
     - Denaturation (1min @ 95°C)
     - Annealing (1min @ 48°C)
     - Extension (1min @ 72°C)
     - Extension (5min @ 72°C)
7. **Electrophorese** 15µL of each PCR mixture on 3% agarose in 0.5x tris-borate buffer @250V for 30-35min
8. Stain with ethidium bromide

NRRL Isolates

NRRL Bacillus Isolate Strains			
	NRRL ID	Species	Source
1	B-3711	Bacillus cereus	Type Strain
2	B-14724	Bacillus cereus	Food Poisoning Incident
3	B-1425	Bacillus cereus	Food Poisoning Incident
4	B-1426	Bacillus cereus	Food Poisoning Incident
5	B-1427	Bacillus cereus	Food Poisoning Outbreak
6	NRS-1232	Bacillus cereus	Lesion on NRS-243 Injected Mouse
7	NRS-1261	Bacillus cereus	Infection Post-Mastectomy
8	NRS-1595	Bacillus cereus	Bovine Mastitis
9	NRS-202	Bacillus cereus	Blood Culture
10	NRS-599	Bacillus cereus	Spinal Fluid
11	NRS-720	Bacillus cereus	Blood Culture
12	NRS-785	Bacillus cereus	Puncture Wound
13	HD-735	Bacillus thuringiensis	Type Strain
14	HD-1	Bacillus thuringiensis	Diseased Pink Bollworm
15	HD-119	Bacillus thuringiensis	Insect
16	HD-121	Bacillus thuringiensis	Insect
17	HD-15	Bacillus thuringiensis	Silkworm
18	HD-17	Bacillus thuringiensis	Moth
19	HD-203	Bacillus thuringiensis	Diseased Moth
20	HD-27	Bacillus thuringiensis	Insect

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