Genetic Stability of Microorganisms During Preservation and Propagation

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Abstract

Post-preservation propagation of microorganisms is known to create genetic variability that might adversely affect the quality of downstream applications like quantitative analytical assays. In this study, we investigated the genetic stability of microorganisms under different passage conditions following long-term stabilization via lyophilization. Here, wild-type *Escherichia coli* (ATCC® 8739™) was used as a model microorganism, and next-generation sequencing was used to evaluate genetic variability. From our analysis, we observed that lyophilization did not significantly impact genetic stability while an increased number of passages did. These findings underscore the need to restrict the use of model microorganisms to low-passage numbers.

Introduction

Most of the quality control methods used in microbiology depend on the genetic integrity of the microorganisms. In this study, we used a model bacterial strain (*E. coli*, ATCC® 8739™) to investigate stability following lyophilization and post-lyophilization culture. Our preliminary results indicate that while preservation processing using lyophilization does not have a significant impact on genetic makeup, post-lyophilization propagation for more than three passages does.

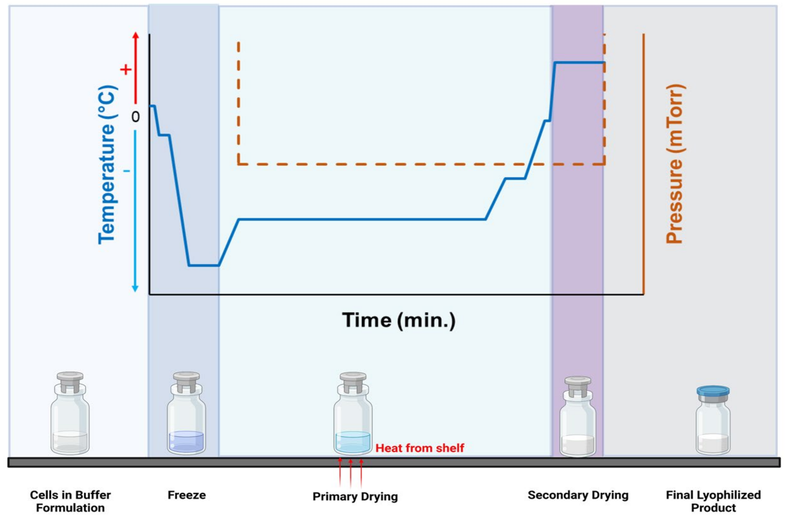


Figure 1: Overview of microbial lyophilization. The process allows ice to change from a solid to a vapor without going through a liquid phase. This process comprises three major steps: freezing the sample, primary drying (ice sublimation), and secondary drying (unfrozen water desorption).

Materials and Methods

Bacterial culture and lyophilization: *E. coli* were grown and cultured in conformance to the established standard techniques at ATCC®. Lyophilization and preservation was performed using two buffers developed internally at ATCC®: Buffer #1 (B#1) and Buffer #2 (B#2).

Bacterial viability assay: Bacterial viability was determined via a plate-based evaluation method.

Genome sequencing analysis: Genomic sequencing and analysis of *E. coli* was performed using the NextSeq™ platform (Illumina) (Figure 2). The *E. coli* genome sequence before and after lyophilization and after post-lyophilization propagation was compared to the reference genome sequence found on the ATCC® Genome Portal to identify the total number of variants, major variants, SNP counts, and indel mutations.

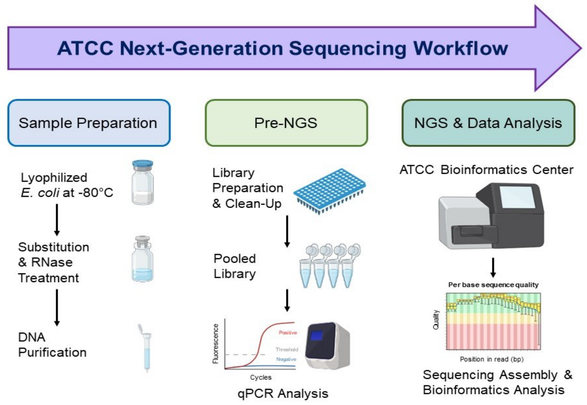


Figure 2: Flow chart depicting the steps in our bioinformatics pipeline, from sample preparation to genome sequencing.

Results

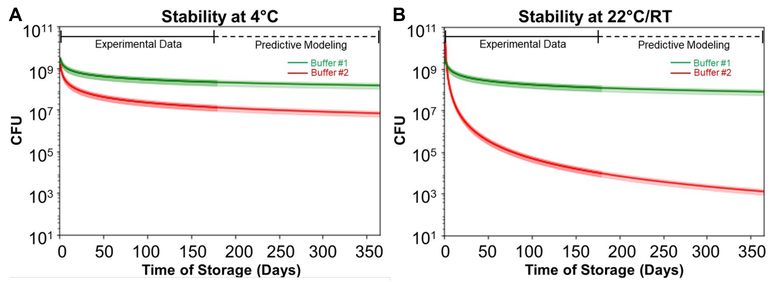


Figure 3: Viability of *E. coli* in two different formulations. (A-B) Stability of *E.coli* in B#1 and B#2 at 4°C and 22°C for one year. Experimental data was collected for 6 months, and predictive modeling done for a year. CFU: Colony formation unit.

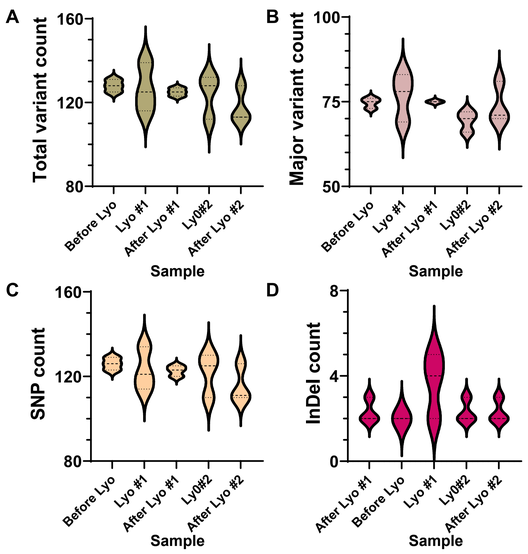
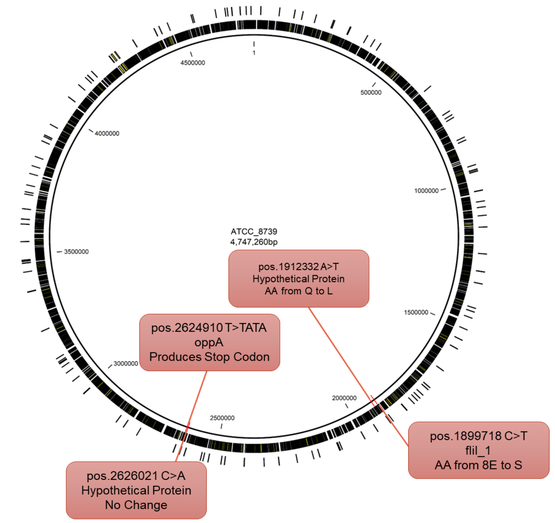
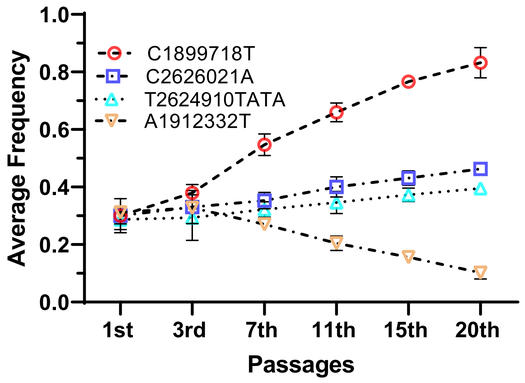


Figure 4: Representative categories variation among the samples. Samples used in this analysis were before lyophilization (Before Lyo), lyophilized in buffer #1 or #2 (Lyo#1, Lyo#2), and one passage after lyophilization (After Lyo#1, After Lyo#2). (A) Total variant counts, (B) major variant counts, (C) SNP (single nucleotide polymorphism) counts, and (D) indel counts were evaluated. Welch's t-test indicated no significant difference regardless of samples.

A. Genomic variation in *E. coli* after twenty passages



B. Variants present in samples at each passage



C. Variant identity and possible changes in the genome

Variant	Prokka Annotation	Impact of change
T2624910TATA	Periplasmic oligopeptide-binding protein (2624091-2625765)	Produces a stop codon
A1912332T	Hypothetical protein (1912039-1913758) – upstream of FLiA, FliZ, and FliY flagellum related-genes	Conversion of amino acid from Q to L
C1899718T	Flagellum-specific ATP synthase (1898367-1899741)	Conversion of amino acid 8E to S
C2626021A	Hypothetical protein (2626003-2626300)	NA

Figure 5: Genome analysis of *E. coli* after passaging in nonselective medium. (A) Visual representation of significant changes in the *E. coli* genome. (B) The allele frequencies of 4 variants changed more than 10% over serial passaging. (C) Identity of the variants and possible changes in the genome.

Conclusions

Our results indicate that the genomic stability of *E. coli* was not significantly impacted when lyophilized using the lyophilization buffer formulations. However, the stability of the lyophilized product significantly improved following storage at 4°C and 22°C if B#1 is used. While lyophilization didn't cause any appreciable genomic instability, propagation of *E. coli* in nonselective medium for more than three passages did result in some genomic variation. Further investigation is needed to determine the impact of these genetic mutations on stability and the global protein expression profile.

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