Electro-Mechanical Stresses on *Bacillus Atrophaeus* Bacterial Spore Germination Induced by Pulsed Electric Fields

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Abstract

In this work, the electro-mechanical stresses induced by pulsed electric fields (PEF) on bacterial spore germination was modeled and empirically tested using *Bacillus atrophaeus* spores. Specifically, a new model, termed as Qiu-Wu’s electro-mechanical (QW’s EM) spore model, was derived to analyze the effect of electro-mechanical stresses on spores subjected under electric fields. A non-linear inverse relationship was found between electric fields and thickness change of spore coat. SEM, pH, hydrophobicity and electrochemical measurements were implemented for verification of the model. PEF-treated spores germinated with a faster rate and a higher degree of homogeneity. The longer the treatment time, the better the homogeneity. The speed of dipicolinic acid (DPA) release was around 20% faster in PEF-treated samples, while the peak intensity of terbium-DPA from PEF-treated samples was up to 80% lower. Theoretical analysis and empirical results were consistent to show that PEF introduces electro-mechanical stresses to expedite spore germination. The significance and impact of this study is obvious: bacterial spore is implicated in food spoilage and foodborne diseases primarily *via* the process of germination, and PEF technology has been introduced to inactivate microorganisms in food. Understanding the mechanism of germination under PEF can provide deep understanding for inactivation of foodborne pathogens and better food preservation methods.

Full Text

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Tables

Table 1 Germination parameters monitored by phase-contrast microscopy

Germination Parameters	Treatment Condition						
	Non-PEF treatment	7.5 kV cm ⁻¹ , 1 pulse in DI water	7.5 kV cm ⁻¹ , 50 pulses in DI water	7.5 kV cm ⁻¹ , 1 pulse in saline	7.5 kV cm ⁻¹ , 50 pulses in saline	15 kV cm ⁻¹ , 1 pulse in saline	15 kV cm ⁻¹ , 50 pulses in saline
Mean of T_{lag} (min)	12.4	8.3	11.2	5.2	1.3	6.8	2.3
Distribution of T_{lag} (min)	13.8	7.6	11.1	5.2	1.6	2.5	2.2
$\Delta T_{release}$ (min)	2.5±0.9	2.3±0.6	2.3±0.7	2.1±0.5	2.0±0.4	2.3±0.6	2.4±0.8

Figures

Figure 1

Spore simulation during a time-course of 50 ms. Surface stress and morphology after PEF treatment at 1 pulse (a) and 50 pulses (b).

Figure 2

SEM images of PEF-treated spores at 15 kV cm⁻¹. The scale bar of the figures and the panels is 5 μm and 1 μm respectively. (a) Control, (b) 50 pulses in DI water, (c) 1 pulse in saline and (d) 50 pulses in saline.

Figure 3

pH measurement of PEF-treated spores with treatment conditions of 15 kV cm⁻¹ in DI water and saline at three different concentrations: (●) 2×10^7 , (Δ) 2×10^5 and ($\square$) 2×10^3 . (A) Control in DI water, (B) Control in saline, (C) 1 pulse in DI water, (D) 50 pulses in DI water, (E) 1 pulse in saline, (F) 50 pulses in saline.

Figure 4

Linear sweep voltammetry measurement of PEF-treated spores in DI water (a) and in saline (b). (dashed line) Non-PEF treatment, (dotted line) 15 kV cm⁻¹, 1 pulse and (solid line) 15 kV cm⁻¹, 50 pulses.

Figure 5

Germination kinetics monitored by phase-contrast microscopy. Germination ratio of PEF-treated spores and control during 60 min. (●) Non-PEF treatment, (△) 7.5 kV cm⁻¹, 1 pulse in saline, (□) 7.5 kV cm⁻¹, 50 pulses in saline, (●) 15 kV cm⁻¹, 1 pulse in saline and (▲) 15 kV cm⁻¹, 50 pulses in saline.

Figure 6

Germination kinetics monitored by Tb-DPA luminescence assay. DPA release kinetics during spore germination. (●) 7.5 kV cm⁻¹, 1 pulse in DI water, (△) 7.5 kV cm⁻¹, 50 pulses in DI water, (□) 7.5 kV cm⁻¹, 1 pulse in DI water and (▲) 7.5 kV cm⁻¹, 50 pulses in DI water.

1 pulse in saline, (●) 7.5 kV cm⁻¹, 50 pulses in saline, (▲) 15 kV cm⁻¹, 1 pulse in saline and (■) 15 kV cm⁻¹, 50 pulses in saline.

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