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Yong-qing Li

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7. PERFORMING ORGANIZATION NAMES AND ADDRESSES
East Carolina University
2200 South Charles Blvd
Suite 2906
Greenville, NC 27858 -4353

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14. ABSTRACT
We have investigated the aspects of the release of Ca-dipicolinic acid (DPA), protein denaturation and cellular heterogeneity of single Bacillus spores during wet-heat treatment that are commonly used in spore killing and inactivation. Achievements include: (1) determined kinetic change in spore state and Ca-DPA levels in single spores of Bacillus and Clostridium species during heat activation; (2) measured the rates of Ca-DPA release and protein denaturation of individual spores when incubated in water at elevated temperatures; (3) determined the

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Report Title

Inactivation of spores of Bacillus species by wet heat: studies on single spores using laser tweezers Taman spectroscopy (Final Report)

ABSTRACT

We have investigated the aspects of the release of Ca-dipicolinic acid (DPA), protein denaturation and cellular heterogeneity of single Bacillus spores during wet-heat treatment that are commonly used in spore killing and inactivation. Achievements include: (1) determined kinetic change in spore state and Ca-DPA levels in single spores of Bacillus and Clostridium species during heat activation; (2) measured the rates of Ca-DPA release and protein denaturation of individual spores when incubated in water at elevated temperatures; (3) determined the effects of wet heat-treatment on the germination of individual spores and identified the damages of key proteins; (4) developed useful methodologies to analyze multiple individual spores during wet-heat treatment and germination.

Enter List of papers submitted or published that acknowledge ARO support from the start of the project to the date of this printing. List the papers, including journal references, in the following categories:

(a) Papers published in peer-reviewed journals (N/A for none)

Received		Paper
01/11/2012	23.00	Lingbo Kong, Pengfei Zhang, Peter Setlow, Yong-qing Li. Multifocus confocal Raman microspectroscopy for rapid single-particle analysis, Journal of Biomedical Optics, (12 2011): 0. doi:
05/01/2011	8.00	L. Kong, P. Zhang, G. Wang, J. Yu, P. Setlow, Y.-q. Li. Characterization of bacterial spore germination using phase contrast and fluorescence microscopy, Raman spectroscopy and optical tweezers, Nature Protocols, (04 2011): . doi:
05/11/2010	3.00	S. Ghosh, P. Zhang, Y.-q. Li, P. Setlow. Superdormant spores of Bacillus species have elevated wet heat resistance and temperature requirements for heat activation, Journal of Bacteriology, (01 2009): . doi:
05/11/2010	6.00	P. Zhang, P. Setlow, Y.-q. Li. Characterization of single heat-activated Bacillus spores using laser tweezers Raman spectroscopy, Optics Express, (09 2009): . doi:
05/11/2010	5.00	W.H. Coleman, P. Zhang, Y.-q. Li, P. Setlow. Mechanism of killing of spores of Bacillus cereus and Bacillus megaterium by wet heat, Letters in Applied Microbiology, (02 2010): . doi:
05/11/2010	4.00	P. Zhang, L. Kong, P. Setlow, Y.Q. Li. Characterization of wet heat inactivation of single spores of Bacillus species by dual-trap Raman spectroscopy and elastic light scattering, Applied and Environmental Microbiology, (03 2010): . doi:
05/23/2011	11.00	P.F. Zhang, L.B. Kong, P. Setlow and Y.Q. Li. Multiple-trap laser tweezers Raman spectroscopy for simultaneous monitoring of the biological dynamics of multiple individual cells, Optics Letters, (10 2010): . doi:
05/23/2011	13.00	Lingbo Kong, Pengfei Zhang, Jing Yu, Peter Setlow, and Yong-qing Li. Monitoring the Kinetics of Uptake of a Nucleic Acid Dye during the Germination of Single Spores of Bacillus Species, Analytical Chemistry, (10 2010): . doi:
05/23/2011	12.00	Lingbo Kong, Pengfei Zhang, Peter Setlow, and Yong-qing Li. Characterization of Bacterial Spore Germination Using Integrated Phase Contrast Microscopy,Raman Spectroscopy, and Optical Tweezers, Analytical Chemistry, (05 2010): . doi:
05/23/2011	9.00	Guiwen Wang, Pengfei Zhang, Peter Setlow, and Yong-qing Li. Kinetics of Germination of Wet-Heat-Treated Individual Spores of Bacillus Species, Monitored by Raman Spectroscopy and Differential Interference Contrast Microscopy, Applied Environmental Microbiology, (05 2011): . doi:
05/23/2011	10.00	Pengfei Zhang, Lingbo Kong, Guiwen Wang, Peter Setlow, and Yong-qing Li. Combination of Raman tweezers and quantitative differential interference contrast microscopy for measurement of dynamics and heterogeneity during the germination of individual bacterial spores, Journal of Biomedical Optics, (10 2010): . doi:
05/26/2011	14.00	Lingbo Kong, Pengfei Zhang, Jing Yu, Peter Setlow, and Yong-qing Li. Rapid confocal Raman imaging using a synchro multifoci-scan scheme for dynamic monitoring of single living cells, Applied Physics Letters, (01 2011): . doi:
07/14/2011	15.00	L. Kong, P. Zhang, J. Yu, P. Setlow, Y.Q. Li. Rapid confocal Raman imaging using a synchro multifoci-scan scheme for dynamic monitoring of single living cells, Applied Physics Letters, (01 2011): . doi:
08/11/2012	24.00	L.B. Kong, P. Setlow, Y.Q. Li. Analysis of the Raman spectra of Ca2+-dipicolinic acid alone and in the bacterial spore core in both aqueous and dehydrated environments, Analyst, (05 2012): 3683. doi:

08/11/2012	25.00	G.W. Wang, D. Paredes-Sabja, M.R. Sarker, C. Green, P. Setlow, Y.Q. Li. Effects of wet heat-treatment on the germination of individual spores of Clostridium perfringens, J Appl Microiol, (08 2012): 0. doi:
08/19/2009	2.00	L. Peng, D. Chen, P. Setlow, Y. Li. Elastic and Inelastic Light Scattering from Single Bacterial Spores in an Optical Trap Allows the Monitoring of Spore Germination Dynamics, Analytical Chemistry, (2009): . doi:
08/29/2010	7.00	P.F. Zhang, L.B. Kong, P. Setlow, and Y.Q Li. Characterization of wet-heat inactivation of single spores of Bacillus species by dual-trap Raman spectroscopy and elastic light scattering, Applied Environmental Microbiology, (03 2010): . doi:
08/29/2011	16.00	Pengfei Zhang, Lingbo Kong, Guiwen Wang, Peter Setlow, Yong-qing Li1. Monitoring the wet-heat inactivation dynamics of single spores of Bacillus species by using Raman tweezers, differential interference contrast microscopy, and nucleic acid dye fluorescence microscopy, Applied Environmental Microbiology, (07 2011): 4754. doi:
11/13/2011	20.00	J.-L. Sanchez-Salas, B. Setlow, P. Zhang, Y.-q. Li, P. Setlow. Maturation of Released Spores Is Necessary for Acquisition of Full Spore Heat Resistance during Bacillus subtilis Sporulation, Applied and Environmental Microbiology, (08 2011): 0. doi: 10.1128/AEM.05031-11
11/13/2011	21.00	J. Liu, J. R. Faeder, P. Setlow, X. Yi. Synergism between Different Germinant Receptors in the Germination of Bacillus subtilis Spores, Journal of Bacteriology, (07 2011): 0. doi: 10.1128/JB.05343-11
11/13/2011	22.00	Keren K. Griffiths, Jingqiao Zhang, Ann E. Cowan, Ji Yu, Peter Setlow. Germination proteins in the inner membrane of dormant Bacillus subtilis spores colocalize in a discrete cluster, Molecular Microbiology, (08 2011): 0. doi: 10.1111/j.1365-2958.2011.07753.x
TOTAL:	**21**	

Number of Papers published in peer-reviewed journals:

(b) Papers published in non-peer-reviewed journals (N/A for none)

Received	Paper

TOTAL:

Number of Papers published in non peer-reviewed journals:

(c) Presentations

Number of Presentations: 0.00

Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

Received	Paper

TOTAL:

Number of Non Peer-Reviewed Conference Proceeding publications (other than abstracts):

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Received	Paper	
08/29/2011	17.00	Pengfei Zhang, Lingbo Kong, Peter Setlow , Yong-qing Li1. Monitoring of germination dynamics of multiple individual bacterial spores by multiple-trap Raman tweezers and differential interference contrast microscopy, CLEO 2011, Conference on Lasers and Electro-Optics, Baltimore, MA, USA. 2011/04/30 12:00:00, . : ,
08/29/2011	18.00	Lingbo Kong, Pengfei Zhang, Peter Setlow, Yong-qing Li1. Combining Phase Contrast Microscopy and Laser Tweezers Raman Spectroscopy to Characterize Germination of Single Bacterial Spores, OSA/CLEO 2011, Conference on Lasers and Electro-Optics, Baltimore, MA, USA. 2011/04/30 12:00:00, . : ,
08/29/2011	19.00	Lingbo Kong, Pengfei Zhang, Guiwen Wang, Peter Setlow, Yong-qing Li1. Using Integrated Multiple Microscopies to Monitor the Kinetics of SYTO 16 Dye Uptake during the Germination of Single Bacterial Spores, OSA/CLEO 2011, Conference on Lasers and Electro-Optics, Baltimore, MA, USA. 2011/04/30 12:00:00, . : ,
TOTAL:	**3**	

Number of Peer-Reviewed Conference Proceeding publications (other than abstracts):

(d) Manuscripts

Received	Paper

TOTAL:

Number of Manuscripts:

Books

Received Paper

TOTAL:

Patents Submitted

Patents Awarded

Awards

Graduate Students

NAME	PERCENT_SUPPORTED	Discipline
Zhan Chen	0.20	
FTE Equivalent:	**0.20**	
Total Number:	**1**	

Names of Post Doctorates

NAME	PERCENT_SUPPORTED
Pengfei Zhang	1.00
Lin Ling	0.10
FTE Equivalent:	**1.10**
Total Number:	**2**

Names of Faculty Supported

NAME	PERCENT_SUPPORTED	National Academy Member
Yong-qing Li	0.17	
FTE Equivalent:	**0.17**	
Total Number:	**1**	

Names of Under Graduate students supported

NAME	PERCENT_SUPPORTED
FTE Equivalent:	
Total Number:	

Student Metrics

This section only applies to graduating undergraduates supported by this agreement in this reporting period

The number of undergraduates funded by this agreement who graduated during this period:	0.00
The number of undergraduates funded by this agreement who graduated during this period with a degree in science, mathematics, engineering, or technology fields:.....	0.00
The number of undergraduates funded by your agreement who graduated during this period and will continue to pursue a graduate or Ph.D. degree in science, mathematics, engineering, or technology fields:.....	0.00
Number of graduating undergraduates who achieved a 3.5 GPA to 4.0 (4.0 max scale):	0.00
Number of graduating undergraduates funded by a DoD funded Center of Excellence grant for Education, Research and Engineering:	0.00
The number of undergraduates funded by your agreement who graduated during this period and intend to work for the Department of Defense	0.00
The number of undergraduates funded by your agreement who graduated during this period and will receive scholarships or fellowships for further studies in science, mathematics, engineering or technology fields:	0.00

Names of Personnel receiving masters degrees

NAME

Total Number:

Names of personnel receiving PHDs

NAME

Total Number:

Names of other research staff

NAME PERCENT_SUPPORTED

FTE Equivalent:

Total Number:

Sub Contractors (DD882)

Inventions (DD882)

Scientific Progress

The goal of this project is to study aspects of the release of Ca-dipicolinic acid (DPA), protein denaturation and cellular heterogeneity during treatment of single Bacillus spores with wet heat. Specific objectives include: (1) determination of the kinetic change in spore state and Ca-DPA levels of single spores of various Bacillus species during heat activation; (2) measurement of dynamic properties of individual Bacillus spores during spore killing by wet heat; (3) determination of the kinetics of germination in response to different nutrient or non-nutrient germinants of individual spores that were treated by wet heat and retained their Ca-DPA. We have successfully accomplished the goals of this project during the past year. Specific achievements include the followings:

1. Development new methodologies for single-cell analysis and imaging.We have developed a number of novel single-cell techniques to observe heat activation, wet heat-killing dynamics and germination dynamics of single bacterial spores.

(1a) Raman scattering spectroscopy and elastic light scattering intensity (ESLI) technology was developed to simultaneously measure levels of Ca-dipicolinic acid and changes in spore morphology and refractive index during germination of individual B. subtilis spores with and without the two redundant cortex-lytic enzymes (CLEs) [Anal. Chem. 81, 4035 (2009)].

(1b) We developed a methodology that combines external phase contrast microscopy, Raman spectroscopy, and optical tweezers to monitor a variety of changes during the germination of single Bacillus cereus spores with a temporal resolution of ~2 s. Phase contrast microscopy assesses changes in refractility of individual spores during germination, while Raman spectroscopy gives information on changes in sporespecific molecules [Anal. Chem. 82, 3840 (2010)].

(1c) We deveoped a methodology that combines fluorescence microscopy, phase contrast microscopy, and laser tweezers Raman spectroscopy to monitor the kinetics of uptake of the nucleic acid dye SYTO 16 during germination of individual Bacillus cereus and Bacillus subtilis spores. The level of dye bound to nucleic acids of individual spores was measured by fluorescence emission, while changes in spore refractility and the level of CaDPA were monitored by phase contrast microscopy and Raman spectroscopy, respectively [Anal. Chem. 82, 8717 (2010)].

(1d) We developed a protocol that combines phase-contrast and fluorescence microscopy, Raman spectroscopy and optical tweezers to characterize the germination of single bacterial spores. The method can also be adapted to use multi-trap Raman spectroscopy or phase-contrast microscopy of spores adhered on a cover slip to simultaneously obtain germination parameters for multiple individual spores [Nature Protocols, 6, 625 (2011)].

(1e) We developed a multiple-trap laser tweezers Raman spectroscopy (LTRS) array for simultaneous monitoring of the biological dynamics of multiple individual cells in physiological environments. This LTRS-array technique was also combined with phase contrast and fluorescence microscopy, allowing measurement of Raman spectra, refractility, and fluorescence images of individual cells with a temporal resolution of ~5 s. We used this technique to monitor multiple Bacillus cereus spores germinating in a nutrient medium for up to 90 min and observed the kinetics of dipicolinic acid release and uptake of nucleic acid-binding stain molecules during spore germination [Opt. Lett. 35, 3321 (2010)].

(1f) We have developed a multifocus confocal Raman microspectroscopy system that allows simultaneous analyses of ~80 individual biological or airborne micro-particles based on a precise image-guided technique. Multiple individual particles adhered in random positions on a coverslip were illuminated by a multifocus excitation pattern formed by rapidly steering a single laser beam with a pair of galvo-mirrors, and their Raman scatterings were synchronously projected with another galvo-mirror to different rows of a CCD chip for parallel spectroscopic analyses. We show that this technique can be used to rapidly identify single airborne particles or bacteria collected on a slide and to monitor germination dynamics of multiple bacterial spores in real-time. [J. Biomed. Opt. 16, 120503 (2011)].

(1g) Rapid confocal Raman imaging using a synchro multifoci-scan scheme for dynamic monitoring of single living cells was developed. A rapid confocal Raman microscopy system for label-free molecular imaging of single living cells was developed, which ~40 times faster than in conventional method. We demonstrated that this system can be used to monitor the germination dynamics of single bacterial spores with about 1.0 min resolution and 2.5 mW power at each focal point. The system could be applied to study wet-heat inactivation process of single spores. [Appl. Phys. Lett. 98, 213703 (2011)].

(1h) Raman tweezers and quantitative differential interference contrast microscopy are combined for measurement of dynamics and heterogeneity during the germination of individual bacterial spores. The DIC bias phase is set properly such that the brightness of DIC images of individual spores is proportional to the dipicolinic acid _DPA_ level of the spores, and an algorithm is developed to retrieve the phase image of an individual spore from its DIC image. We find that during germination, the rapid drop in both the intensity of the original DIC image and the intensity of the reconstructed phase image precisely corresponds to the release of all DPA from that spore. The summed pixel intensity of the DIC image of individual spores adhered on a microscope coverslip is not sensitive to the drift of the slide in both horizontal and vertical directions. This quantitative DIC technique is used to track the germination of hundreds or thousands of individual spores simultaneously. [J. Biomed. Opt. 15, 056010 (2010)].

2. Measurement on individual Bacillus spores during heat activation

(2a) We applied a novel polarized and non-polarized Raman spectroscopy technique combined with laser tweezers and principle component analysis (PCA) to characterize the heat activation of single spores of Bacillus cereus and Bacillus subtilis. The Raman spectra of single spores before, during, and after heat activation were measured. We measured the Raman spectra of single spores without treatment, during heat activation at 65 °C (B. cereus) or 70 °C (B. subtilis), and following heat activation and cooling to 25 °C. Large changes in the Raman bands of Ca-DPA and protein for both B. cereus and B. subtilis spores during heat activation were observed for the first time, indicative of changes in spore core state and partial protein denaturation

at the heat activation temperatures. These changes become smaller once the heated spores are cooled, indicating heat activation being reversible. The kinetic activation of single B. subtilis spores by heat was studied with Laser Tweezers Raman Spectroscopy. The kinetic measurement showed that Ca-DPA structure modification reached maximum at ~55 ºC, which may be the glass transition temperature of spore protoplast and this modification was reversible when spores were cooled down. [Opt. Express, 17, 16480 (2009)].

(2b) Raman spectroscopy has been used to study individual superdormant spores. It was found that superdormant spores of Bacillus species have elevated wet heat resistance and temperature requirements for heat activation and the environment of dipicolinic acid in the core of superdormant spores is different from that in dormant spores [J. Bacteriol., 191, 5584 (2009)].

(2c) We have analyzed Raman spectra of single spores of three Bacillus species in different hydration states in both aqueous and dehydrated environments. As a comparison, we also measured the Raman spectra of CaDPA and DPA in different forms including in aqueous solution, and as amorphous powder and crystalline. We also monitored changes in Raman spectra of an individual spore during dehydration under vacuum. The results indicated that: 1) the state of CaDPA in the core of a spore suspended in water is close to an amorphous solid or a glassy state, but still mixed with water molecules; 2) the ratio of intensities of Raman bands at 1572 and 1017 cm-1 (I1572/I1017) is sensitive to the water content in the CaDPA's environment; 3) variations in I1572/I1017 are small (~4%) in a population of dormant Bacillus spores suspended in water; and 4) the I1572/I1017 ratio increases significantly during dehydration under vacuum. Consequently, measurement of the I1572/I1017 ratio of CaDPA in spores may allow a qualitative estimation of the degree of hydration of the bacterial spore's core. [Analyst, 137, 3683 (2012)].

3. Measurement of dynamic properties of individual Bacillus spores during spore killing by wet heat.

(3a) We have characterized wet-heat inactivation of single spores of Bacillus species by dual-trap Raman spectroscopy and elastic light scattering. The dynamic processes during high-temperature treatment of individual spores of B. cereus, B. megaterium, and B. subtilis in water were investigated. Major conclusions included: (i) After spores of all three species were added to water at 80 to 90°C, the level of Ca dipicolinic acid in individual spores remained relatively constant during a highly variable lag time (Tlag), and then CaDPA was released within 1 to 2 min. (ii) The Tlag values prior to rapid CaDPA release and thus the times for wet-heat killing of individual spores of all three species were very heterogeneous. (iii) The heterogeneity in kinetics of wet-heat killing of individual spores was not due to differences in the microscopic physical environments during heat treatment. (iv) During the wet-heat treatment of spores of all three species, spore protein denaturation largely but not completely accompanied rapid CaDPA release. [Appl. Environ. Microbiol. 76, 1796 (2010)].

(3b) We have monitored the wet-heat inactivation dynamics of single spores of Bacillus species by using Raman tweezers, differential interference contrast microscopy, and nucleic acid dye fluorescence microscopy. Dynamic processes during wet-heat treatment of individual spores of B. cereus, B. megaterium, and B. subtilis at 80 to 90°C were investigated. We found that during spore wet-heat treatment, while the spores' CaDPA was released rapidly at a highly variable time Tlag, the levels of spore nucleic acids remained nearly unchanged, and the Tlag times for individual spores from the same preparation were increased somewhat as spore levels of CaDPA increased. The SYTO 16 fluorescence intensity began to increase during wet-heat treatment at a time before Tlag and reached maximum at a time slightly later than Trelease. However, the fluorescence intensities of wet-heat-inactivated spores were ~15-fold lower than those of nutrient-germinated spores, and this low SYTO 16 fluorescence intensity may be due in part to the low permeability of the dormant spores' inner membranes to SYTO 16 and in part to nucleic acid denaturation during the wet-heat treatment [Appl Environ Microbiol. 77, 4754 (2011)].

(3c) We have investigated the mechanism of wet heat killing of spores of B. cereus and B. megaterium. B. cereus and B. megaterium spores wet heat-killed 82–99% gave two bands on equilibrium density gradient centrifugation. The lighter band was absent from spores that were not heat-treated and increased in intensity upon increased heating times. These spores lacked dipicolinic acid (DPA) were not viable, germinated minimally and had much denatured protein. The spores in the denser band had viabilities as low as 2% of starting spores but retained normal DPA levels and most germinated, albeit slowly. However, these largely dead spores outgrew poorly if at all and synthesized little or no ATP following germination. Wet heat treatment appears to kill spores of B. cereus and B. megaterium by denaturing one or more key proteins [Lett. Appl. Microbiol., 50, 507 (2010)].

(3d) We found that maturation of released spores is necessary for acquisition of full spore heat resistance during Bacillus subtilis sporulation. The first ~10% of spores released from sporangia (early spores) during Bacillus subtilis sporulation were isolated, and their properties were compared to those of the total spores produced from the same culture. The early spores had significantly lower resistance to wet heat and hypochlorite than the total spores but identical resistance to dry heat and UV radiation. Early and total spores also had the same levels of core water, dipicolinic acid, and Ca and germinated similarly with several nutrient germinants. The wet heat resistance of the early spores could be increased to that of total spores if early spores were incubated in conditioned sporulation medium for ~24 h at 37°C (maturation), and some hypochlorite resistance was also restored. The maturation of early spores took place in pH 8 buffer with Ca2 but was blocked by EDTA; maturation was also seen with early spores of strains lacking the CotE protein or the coat-associated transglutaminase, both of which are needed for normal coat structure. Nonetheless, it appears to be most likely that it is changes in coat structure that are responsible for the increased resistance to wet heat and hypochlorite upon early spore maturation. [Appl Environ Microbiol. 77, 6746 (2011)].

4. Analysis of the germination properties of wet heat-treated Bacillus and Clostridium spores.

(4a) We have measured kinetics of nutrient and nonnutrient germination of individual untreated and wet-heat-treated spores of B. cereus, B. megaterium, and several isogenic B. subtilis strains using Raman and DIC microscopy. We have found that: (i) More than 90% of these spores were nonculturable but retained their dipicolinic acid (CaDPA) when incubated in water at 80 to 95°C for 5 to 30 min; (ii) Wet-heat treatment significantly increased the time, Tlag, at which spores began release of the great majority of their CaDPA during the germination of B. subtilis spores with different nutrient germinants and also increased the variability of Tlag values; (iii) The time period, Trelease, between Tlag and the time, Trelease, was also increased in wet-heat-treated spores. (iv) Wet-heat-treated spores germinating with nutrients had higher values of Irelease, the intensity of a spore's DIC image at Trelease, than did untreated spores and had much longer time periods, Tlys, for the reduction in Irelease intensities to the basal value due to hydrolysis of the spore's peptidoglycan cortex, probably due at least in part to damage to the cortex-lytic enzyme CwlJ. These results indicate that (i) some proteins important in spore germination are damaged by wet-heat treatment, (ii) the cortex-lytic enzyme CwlJ is one germination protein damaged by wet heat, and (iii) the CaDPA release process itself seems likely to be the target of wet-heat damage which has the greatest effect on spore germination. [Appl Environ Microbiol. 77, 3368 (2011)].

(4b) Effects of wet heat-treatment on the germination of individual spores of Clostridium perfringens have been measured. Raman spectroscopy and DIC microscopy were used to monitor the dynamic germination of individual untreated and wet heat-treated spores of C. perfringens with various germinants. When incubated in water at 90-100°C for 10-30 min, > 90% of spores were inactivated but 50-80% retained their CaDPA. The wet heat-treated spores that lost CaDPA exhibited extensive protein denaturation as seen in the 1,640 -1,680 cm–1 (amide I) and 1,230 - 1,340 cm–1 (amide III) regions of Raman spectra, while spores that retained CaDPA showed partial protein denaturation. Wet heat-treated spores that retained CaDPA germinated with KCl or L-asparagine, but wet heat treatment increased values of Tlag, Trelease, and ΔTlys, during which spores initiated release of the majority of their CaDPA after mixing with germinant, released > 90% of their CaDPA, and completed the decrease in their DIC intensity due to cortex hydrolysis, respectively. Untreated C. perfringens spores lacking the essential cortex-lytic enzyme (CLE), SleC, exhibited longer Tlag and ΔTrelease values during KCl germination than wild-type spores, and germinated poorly with CaDPA. Wet heat-treated wild-type spores germinating with CaDPA or dodecylamine exhibited increased Tlag, Trelease, and ΔTlys values, as did wet heat-treated sleC spores germinating with dodecylamine. This study provides information on the germination of individual C. perfringens spores and improves the understanding of effects of wet heat treatment on spores. [J. Appl. Microbiol. 113, 824 (2012)].

Technology Transfer