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Press Release

New Publication: Mechanism of *Bacillus subtilis* spore inactivation by and resistance to supercritical CO_2 plus peracetic acid. Setlow *et al., J Appl Microbiol* 120:5769.

NovaSterilis and Professor Peter Setlow at the University of Connecticut collaborated on an NIH-funded study of the mechanism of spore inactivation in the NovaSterilis sterilization process. The results published in the Journal of Applied Microbiology describe the mechanism of spore inactivation both in a liquid suspension and on a dry surface, and reaffirm the uniquely strong synergistic sporicidal activity observed between $scCO_2$ and the NovaKill™ additive.

With the addition of 1 mL NovaKill™ additive in the 20 L Nova2200 vessel, spores in suspension were inactivated to SAL6* within 30 minutes of supercritical treatment. Peracetic acid was measured at 35 to 55ppm in these suspensions. For comparison, a 50ppm peracetic acid solution does not show any sporicidal activity within 5 hours under normal temperature and pressure, and it takes over 4 hours for a 300 ppm solution to achieve SAL6 spore inactivation. For spores dried on a glass slide, SAL6 was achieved within less than 30 minutes when adding 16 mL NovaKill™ additive in the 20L Nova2200 vessel. This highlights how efficient the NovaSterilis process is for the sterilization of different sample types.

Damage to the spore coat induced by $scCO_2$ alone or in combination with the NovaKill™ additive could provide an explanation for the strong synergy in spore kill. Experiments described in this article however show clear evidence of damage to the spores inner membrane rather than the coat. Many of the observations made in this study are reminiscent of what we know of the sporicidal action of peracetic acid in a solution, and the mechanism involved in the remarkable synergy produced by the supercritical treatment remains in part unexplained.

Spores inactivated with the NovaSterilis process do not fully lose the ability to germinate. While they do lose the ability to germinate upon AGFK induction via the germination receptors GerB and GerK, the GerA receptor can be partially induced with L-Valine and calcium-DPA release remains inducible with dodecylamine. Importantly, those inactivated spores with inducible germination exhibit a severe outgrowth defect, in which they are not able to produce a viable cell. Spore coat mutants are more sensitive to the NovaSterilis process, confirming the role of the coat in making spores a challenge organism to any sterilization process. No significant increase in DNA mutation was seen in inactivated spores, and *recA* spores, severely affected in their ability to repair DNA,

were not more sensitive to sterilization, indicating that DNA damage does not play a key role in the NovaSterilis sterilization process. However, mutant spores lacking the DNA-protective SASP proteins were more sensitive to inactivation in dry conditions, and DNA mutations increased. This indicates that for dry inactivation, the SASP proteins play a crucial role in protecting DNA inside the spore core.

* (SAL6) – Sterility Assurance Level 10^6 is a standard for medical devices. It is the probability of 1 non-sterile item in 1 million.

Current $scCO_2$ applications

The NovaSterilis technology is being utilized by both US and International Tissue Banks to produce sterile allograft tissue in final packaging ready for transplantation. To date over 90,000 $scCO_2$ sterilized allograft transplants have been performed with no adverse reports. This sterilization technology is currently employed for sterilization of 2 medical devices with regulatory approval. NovaSterilis is now offering a larger 80 liter $scCO_2$ sterilization unit, the Nova8800, to meet the high throughput needs of larger tissue processors and medical device manufacturers. NovaSterilis' $scCO_2$ process provides the medical materials industry with a safe and effective, in house, low cost terminal sterilization alternative.

About NovaSterilis

NovaSterilis currently markets terminal sterilization technology and equipment related to their supercritical carbon dioxide platform. The supercritical or fluid phase of CO_2, occurs at low pressure (72.9 atm) and moderate temperatures (31.1 °C). $ScCO_2$ retains advantageous properties of the gas and liquid phases of carbon dioxide making it an ideal fluid for manufacturing processes. The company currently markets the Nova 2200 (20 liter) and Nova8800 (80 liter) fully automated $scCO_2$ terminal sterilization equipment. NovaSterilis is a privately held biotechnology company located in Lansing, New York. NovaSterilis is the recipient of a 2007 Presidential Green Chemistry Challenge Award presented by the Environmental Protection Agency.

For more information on NovaSterilis and supercritical carbon dioxide visit www.novasterilis.com or call 607-330-2771.