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HEALTH EFFECTS OF PROJECT SHAD BIOLOGICAL AGENT:

BACILLUS GLOBIGII

[Bacillus licheniformis]
[Bacillus subtilis var. niger]
[Bacillus atrophaeus]

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SPECIAL NOTE ON PSYCHOGENIC SEQUELAE OF PERCEIVED EXPOSURE TO BIOCHEMICAL WARFARE AGENTS

This report deals primarily with the biological health challenges engendered by the agent that is the subject of the report. Nevertheless, this report also incorporates, by reference and attachment, a supplement entitled "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents".

The supplement addresses and describes a growing body of health effects research and interest centered upon the psychogenic sequelae of the stress experienced personally from actual or perceived exposure to chemical and biological weaponry. Because awareness of exposure to agents in Project SHAD logically includes the exposed person also possessing a perception of exposure to biochemical warfare agents, the psychogenic health consequences of perceived exposure may be regarded as additional health effects arising from the exposure to Project SHAD agents. This reasoning may also apply to simulants and tracers. Therefore, a general supplement has been created and submitted under this contract to address possible psychogenic effects of perceived exposure to biological and chemical weaponry.

Because such health effects are part of a recent and growing public concern, it is expected that the supplement may be revised and expanded over the course of this contract to reflect the actively evolving literature and interest in the issue.

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I. EXECUTIVE SUMMARY

Bacillus globigii (BG) has been called B. *subtilis* var *niger*, B *licheniformis* and, most recently, *B. atrophaeus*. It is a Gram-positive, spore-forming, facultative anaerobe is commonly found in dust, soil and water. It is widely used as a biological tracer and has been shown to produce substances that exhibit antimicrobial activity. In Project SHAD, *B.globigii* was used to simulate biological warfare agents, because it was then considered a contaminant with little health consequence to humans.

BG is now considered a pathogen for humans. Most infections are associated with the experience of invasive trauma (e.g. catheters, surgery) and/or a debilitates health state, thus it is often encountered as a nosocomial pathogen. BG is also a well-known cause of food poisoning, resulting in diarrhea and vomiting. Infections are rarely known to be fatal, although fatal food poisoning has been reported. Ocular infections, bacteremia, sepsis/septicemia, ventriculitis, peritonitis are the reported types of infection and they are usually treated with antibiotics. Cases of long-term persistence or recurrence, or of extended latency have not been found.

Psychogenic effects specifically of BG exposure are not reported. General psychogenic effects of perceived exposure to biological and chemical weapons are found in the supplement under this contract entitled, "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents." Prevention of exposure is conscientious hospital and food hygiene. Treatment involves various regimens of antibiotics; the literature provides inconsistent reports on resistance and efficacy of various anti-microbial agents.

II. BACKGROUND DATA

Bacteriology

Bacillus globigii (BG) is a Gram-positive, motile, facultatively anaerobic rod that makes endospores. It forms large, mucoid colonies which are opaque and have a rough, wrinkled surface. Characteristic biochemical reactions include positive reactions for catalase production and arginine hydrolase. Detailed biochemical characteristics are provided in Santini et al 1995 and Sugar & McCloskey, 1977.

Taxonomy

Bacillus globigii (BG) was named in 1900 by Migula. It was later renamed *Bacillus subtilis var. niger* and then *Bacillus licheniformis*. Most recently, *Bacillus atrophaeus* has become the standard term. *(*Strain DSM 675 was previously named *Bacillus globigii*, "red strain" or "*Bacillus niger*", or "*Bacillus subtilis var. niger*" Strain DSM 2277 was also previously named "*Bacillus globigii*" (Fritze and Pukall, 2001; Nakamura 1989)).

Ecology

Commonly found in soils, dust, air, water and wet surfaces, it is a saprophyte, to which decaying material is a feast (Hannah & Ender 1999). It has also been isolated from recycled paper products, which, if used for packaging foodstuffs, could result in contamination and possible food poisoning (Suihko, et al. 2003; Pirttijarvi, et al. 2000).

Uses

Bacillus globigii has been used as a tracer organism or biological indicator.

Specific examples of application purposes include spatial distribution of microorganisms in treated drinking water (Gale et al 2002); contamination characteristics of infected blood (Anonymous 1975); stability of aerosolized contaminants (Adams, et al. 1982); evaluating cleaning/disinfection agents (Penna, et al. 2001;Blakistone, et al. 1999); testing for sterilization (Christensen, et al. 1979; Fritze and Pukall, 2001).

BG may be best known as the source for the restriction endonuclease Bgl I (Johannssen et al.1979). BG also produces antibacterial compounds: bacitracin (an antibiotic metallopeptide) (Ming, et al. 2002); and a food-preserving bacteriocin (Martirani et al 2002).

In Project SHAD, *B. globigii* was one of the simulants for biological warfare agents. It was used to determine characteristics such as the behavior of biological aerosols such as downwind travel, dispersion, penetration, and the tenacity of its presence after washing equipment (Project 112 Autumn Gold 2001).

III. HEALTH EFFECTS

General

BG infections result mainly from invasive events, such as penetrating injuries, surgical procedures, catheters and intravenous lines; the latter are most noted for localized and systemic infections (Matsumoto, et al. 2000; Blue, et al. 1995). There are also reports of BG infections arising from sources such as heroin samples (McLauchlin, et al. 2002) and drain cleaner (Hannah, et al. 1999). It should be stressed that a compromised or debilitated health condition is also usually associated with infections by BG (Blue et al 1995; Bannerjee et al 1988; Matsumoto 2000).

Fever is a primary symptom in these infections, rather than the diarrhoea and vomiting associated with BG food-poisoning. The presence of BG is determined by Gram stain and by culturing body fluids (blood, peritoneal, cerebrospinal, meningeal fluid, etc.) Although infections from BG have a low mortality, they can cause significant morbidity from recurring bacteremia and potentially pathogenic in the immunocompromised (Blue et al. 1995)

Food Poisoning

B. globigii is an important organism in food safety, being a causative agent in food poisoning (Pirttijarvi, et al. 2000; Salkinoja-Salonen, et al. 1999; Mikkola, et al. 2000; Brown 2000). It can contaminate cooked meat, cooked vegetables, milk (Pacova, et al. 1996), infant formulae (Rowan, et al. 1997; Rowan, et al. 2001), and is a significant contaminant of bread, causing a characteristic type of spoilage called "ropy" (Sorokulova et al 2003).

With an onset of about 7-16 hours, the most prominent symptoms of food poisoning are diarrhea and vomiting. Other symptoms that may occur are: fever, sweating, abdominal pain and nausea. Recovery time ranges from 13 days to 6 weeks (Tabbara et al 1979; Tarabay 1979; Sugar & McCloskey 1977; Jones, et al. 1992; Santini et al 1995). A few cases of death have been reported (Mikkola et al 2000)

BG is also an etiologic infectious agent in other organ systems:

Cardiovascular/circulatory

Prosthetic valve endocarditis has been traced to BG (Santini, et al. 1995). Peritonitis has also been a consequence of BG infection. (Ryoo, et al. 2001; Sugar & McCloskey, 1977) Bacteremia and sepsis/septicemia have been noted consequences of BG infection. (Sugar & McCloskey, 1977; Hannah & Ender. 999; Blue et al 1995; Peloux et al 1976). These events are typically associated with debilitated conditions and/or invasive procedures.

Neurologic

Ventriculitis and brain abcesses induced by BG infection have been reported. These have been associated with surgical trauma (Jones, et al. 1992; Young, et al. 1982)

Ophthalmic

Endophthalmitis and other ocular infections associated usually with local trauma have been reported to arise from BG. (Maucour, et al. 1999; Tabbara, et al. 1979; Thurn, et al. 1988),

Long-Term Issues

Mortality is rare with BG infection, though it has occurred in cases of food poisoning (Mikkola et al. 2000; Matsumoto 2000). Modern treatment with antibiotics almost certainly reduces the mortality and morbidity risks (Tabbara et al 1979). No cases were found of long-term persistence, chronic recurrence of disease, or latency of the pathogen.

IV. PSYCHOGENIC EFFECTS

Reports of psychogenic effects specifically in response to BG have not been found. General psychogenic effects of perceived exposure to biological and chemical weapons are found in the supplement under this contract entitled, "Psychogenic Effects of Perceived Exposure to Biochemical Warfare Agents"

V. TREATMENT/PREVENTION

Infections caused by BG are usually treated with antibiotics, and, where appropriate, catheter removal and/or replacement (Blue, et al. 1995.). In some instances, the affected tissue requires debridement (Santini et al 1995; Hannah and Ender 1999).

Antibiotics used in treatment of BG infections include netilmycin and cefotiam (Ryoo et al); ciprofloxacin (Hannah & Ender 1999; Jones et al 1992); gentamicin and staphicillin (Tabbara et al 1979); cephazolin (Santini et al 1995; Sugar & McCloskey 1977).

There are inconsistent reports regarding the efficacy of some antibiotics. Some reports found BG to be susceptible to these antibiotics: nafcillin and gentamicin (Blue, et al. 1995); tetracycline (Tabbara et al 1979); chloramphenicol and gentamicin (Young, et al. 1982). Other reports found BG to be resistant to the same drugs: nafcillin and clindamycin (Sugar and McCloskey; Young, et al. 1982); tetracycline, (Santini et al 1995;Young, et al. 1982); chloramphenicol (Jones et al 1992).

BG has been reported to be resistant to these antibiotics: penicillin, (Santini et al (Tabbara et al 1979; Young, et al. 1982); erythromicin, (Santini et al (Tabbara et al 1979; Young, et al. 1982); clindamicin, ampicillin (Young, et al. 1982).

Illnesses from BG can be prevented by proper handling and storage of food at temperatures that either inhibit or prevent its growth. Infections from *Bacillus* spp., including BG, were reduced substantially in one hospital after implementing one-time use of the intravenous lines' stopcock caps, new skin disinfectant, and uninterrupted infusions (Matsumoto, et al. 2000).

VI. SECONDARY SOURCE INFORMATION

Project 112 Autumn Gold (2001) states:

> Harmless to humans, *Bacillus globigii* is ubiquitous and found easily in samplings of wind-borne dust. BG is safely used in biological studies as a stand-in for pathogenic bacteria. *Bacillus globigii* is used as a biological tracer for anthrax because its particle size and dispersal characteristics are similar to those of anthrax. A household bleach-and-water solution easily kills *Bacillus globigii*. The Centers for Disease Control and Prevention place this in BSL-1, suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans.

A report published on the National Library of Medicine website describes the Army's BG trials refers to BG as a "harmless simulant" (HSTAT 2002).

BG nonetheless can be under certain circumstances, as related in this report, a quite harmful pathogen.

It should also be noted that the changes in and multiplicity of the nomenclature for BG and its strains can render research and compilation of information more complex than might be initially supposed.

VII. BIBLIOGRAPHY WITH ABSTRACTS

{The following bibliography includes supplemental material not cited in the text, in addition to the text citations. Unless otherwise noted, the abstracts for the following references are rendered verbatim as provided by the original publication or as made available in a standard print or electronic catalogue, or database. Errors, omissions, or other defects of language, form, style, or substance are strictly those of the original source or its transmission.}

Anonymous 1975. Experimental Studies on Environmental Contamination with Infected Blood during Haemodialysis. *J.Hyg.(Lond).* Vol. 74(1) : 133-148.
To assess the relative importance of different postulated modes of spread of hepatitis B in dialysis units, blood charged with various tracer organisms was used in simulated haemodialysis runs in four laboratories, and the resulting contamination of equipment and environment was measured semi-quantitatively. Some airborne spread of the tracer organism occurred when tubing containing contaminated blood was needled as the "patient" went on and came off the dialyser. Virtually no small airborne particles could be demonstrated however in simulated emergencies in which a blood line was disconnected, or even when bottles of blood were dropped on to a hard floor from a height of 2 metres. *Bacillus globigii* spores from contaminated blood leaked in small numbers into the dialysing fluid through apparently intact coils. T3 phage, with a particle size of the same order as hepatitis B virus, passed in small quantities through the membrane of a Kiil dialyser from blood to dialysing fluid and also in the reverse direction when added to the header tank. A number of other dialysers were also permeable to phage. Visual assessment of the appropriate moment for inserting the venous line into the "patient" at the onset of dialysis was shown to be unreliable, as the displaced fluid from the end of the venous line was already contaminated before it contained visible red blood cells. Considerable contamination of exposed surfaces and of the buttons on the proportionating unit cabinet occurred. Minor visible splashing of blood was a common-place of the laboratory experiments and was shown to be also a common event during routine haemodialysis in two of the dialysis units taking part in the studies.

ADAMS, et al. 1982. Aerosol Stability of Infectious and Potentially Infectious Reovirus Particles. *Appl.Environ.Microbiol.* Vol. 44(4) : 903-908.
The aerosol stability of two particle forms, infectious and potentially infectious, of reovirus were examined under static conditions for a range of relative humidities at 21 and 24 degrees C. Virus aerosolization efficiency was determined for two methods of dissemination: Collison nebulizer and Chicago atomizer. Suspensions of *Bacillus subtilis*

var. niger spores were added to reovirus preparations that included both particle forms and disseminated into a dynamic aerosol toroid to estimate the physical decay of the aerosols. At 90 to 100% relative humidity, both reovirus particle forms showed less than 10-fold loss of infectivity after 12 h of aging. At lower relative humidities the aerosol decay curve showed rapid initial decay followed by a markedly lower decay rate. Our findings reveal that reovirus particles are relatively stable in the airborne state.

AGERHOLM, et al. 1995. Experimental Infection in Mice with *Bacillus Licheniformis*. *Zentralbl.Veterinarmed.B.* Vol. 42(4) : 247-256.
The pathogenicity of *Bacillus licheniformis* was assessed in normal and immunodepressed BALB/c mice. The animals were challenged intravenously with 4 x 10(7) colony forming units of *B. licheniformis* (ATCC 14580) and both normal and immunodepressed mice were susceptible. However, the infection was more severe in the immunosuppressed animals. In normal mice, lesions were restricted to the liver and kidneys, while lesions also occurred in other organs of immunodepressed mice. By crossed immunoelectrophoresis it was shown that antigens of *B. licheniformis* are potent immunogens, and the bacteria could be identified in tissue sections by immunostaining. Immunohistochemically, *B. licheniformis* was demonstrated in hepatic and pulmonic macrophages, and from some animals the bacteria were also reisolated.

AGERHOLM, et al. 1999. Experimental Infection of Pregnant Cows with *Bacillus Licheniformis* Bacteria. *Vet.Pathol.* Vol. 36(3) : 191-201.
To study the abortifacient potential and fetoplacental tropism of *Bacillus licheniformis* bacteria, eight cows in the sixth to eighth month of gestation were inoculated intravenously either once (n = 4) or on four successive days (n = 4) with *B. licheniformis* at doses ranging from 10(9) to 10(12) colony-forming units. Cows were euthanatized and necropsied prior to abortion (n = 2), at the time of abortion (n = 2), or at calving (n = 4). Live-born calves (n = 5) were euthanatized immediately after delivery and necropsied. *B. licheniformis* was reisolated from placentomes/endometrium in six of eight (75%) cows and from one fetus aborted 43 days after inoculation. Lesions associated with *B. licheniformis* were restricted to the pregnant uterus, with the exception of one cow, which developed pneumonia. Necrosis in the fetal compartment of the placenta were present in three of four (75%) cows of both inoculation groups. Lesions were mainly restricted to fetal membranes and especially to the fetal side of the placentomes. Necrosis and diffuse neutrophil infiltrations of both villi and intervillous areas occurred in the fetal part of the placenta, and the placentomal interface was distended by bacteria, neutrophils, erythrocytes, and debris. Within trophoblasts, bacteria were located both free in the cytoplasm and in cytoplasmatic vesicles. Inflammation was present in three of eight

(38%) calves. Placental and fetal lesions were similar to those found in cases of spontaneous abortions associated with *B. licheniformis*. The abortifacient potential of *B. licheniformis* and the tropism for the bovine placenta is demonstrated here for the first time.

AGERHOLM, et al. 1997. A Preliminary Study on the Pathogenicity of *Bacillus Licheniformis* Bacteria in Immunodepressed Mice. *APMIS.* Vol. 105(1) : 48-54.
The pathogenicity of 13 strains of *Bacillus licheniformis* was studied in immunodepressed mice. The strains had been isolated from cases of bovine abortions (n = 5), bovine feedstuffs (n = 3), soil (n = 1), and grain products (n = 2). The origin of two strains was unknown. Groups of 10 mice were inoculated intravenously with *B. licheniformis* bacteria at doses from andlt; 10(6) to 10(10) colony-forming units. Following 7 days of infection, the animals were euthanized and examined bacteriologically, histologically, and immunohistochemically using a PAP technique based on primary polyclonal rabbit anti-*B. licheniformis* antibodies. *B. licheniformis* bacteria were reisolated from the liver, spleen or kidneys of mice in all groups. Inflammatory lesions were present in mice of all immunodepressed groups, but only brain and pulmonic lesions were definitely attributed to *B. licheniformis* infection, as strong immunostaining was found within these lesions. It is concluded that all strains of *B. licheniformis* examined were pathogenic for immunodepressed mice, and that spontaneous infections may be established by bacterial strains to which susceptible individuals are accidentally exposed.

AGERHOLM, et al. 1995. A Retrospective Study of Bovine Abortions Associated with *Bacillus Licheniformis*. *Zentralbl.Veterinarmed.B.* Vol. 42(4) : 225-234.
A retrospective study of bovine abortions associated with *Bacillus licheniformis* is described. The material consisted of 2445 bovine abortions submitted for diagnostics from 1986 through 1993. Initially, *B. licheniformis* had been isolated from 81 cases. Sections of these cases were re-examined microscopically and immunohistochemically by a PAP technique using a primary antibody against *B. licheniformis*. Of these abortions, 47 were most likely associated with *B. licheniformis* as tissue lesions with immunostained bacteria were present in these. In the remaining cases the diagnosis may not have been established due to the lack of sufficient materials, or the isolation of the bacterium was considered to be a result of contamination. In four cases concomitant infections with *B. licheniformis* and bovine virus diarrhoea virus were present. Abortions caused by *B. licheniformis* were predominantly seen during the winter months and in late pregnancy. The most common lesions were necrotizing placentitis followed by fetal multifocal suppurative bronchopneumonia. Immunohistochemically, *B. licheniformis* was

demonstrated in association with tissue lesions and intracellularly in trophoblasts. The pattern of bacterial isolations, especially from the placenta, lungs, and abomasal contents, combined with the histological findings points to *B. licheniformis* abortions as being of haematogenous origin with subsequent transplacental spread to the fetus.

AGERHOLM, et al. 1997. Diagnostic Studies of Abortion in Danish Dairy Herds. *Zentralbl.Veterinarmed.A.* Vol. 44(9-10) : 551-558.
Diagnostic findings in 218 aborted bovine foetuses are reported. The materials were examined in a matched case-control study of 69 Danish dairy herds with a sudden increase in the number of abortions and a corresponding 69 control herds. Foetuses aborted during the subsequent 6-month period were examined to identify the cause of abortion if possible. A total of 186 specimens were submitted from case herds and 32 from control herds. A likely cause of abortion was diagnosed in 73 foetuses. The most common cause was bovine viral diarrhoea virus (BVDV: 13%) followed by Neospora caninum infection (10%), mycosis (5%) and *Bacillus licheniformis* infection (4%). Foetal and/or placental lesions were found in a further 27 cases. Only BVDV infection and neosporosis were diagnosed in more than one foetus per herd and only protozoal associated abortions occurred significantly more frequently in the case, rather than in the control, herds.

BABB, et al. 1976. A Source Isolator for Infected Patients. *J.Hyg.(Lond).* Vol. 76(3) : 355-366.
A plastic, mechanically ventilated source isolator with filters in the air effluent was designed to enable infected patients to be nursed and treated in a general ward or to be transported without risk to staff or other contacts. Two models of isolator were developed. Their potential value was tested by the challenge of heavy dispersal, inside the isolator, of bacteria (a) from patients with burns, during the change of dressings, (b) from contaminated bedding during simulated bed-making, and (c) from the dispersal of a suspension of *Bacillus subtilis var. globigii*. Sampling of air by slit samplers outside the isolator and, in comparable control patients, from the air of the room in which dressings were changed, showed consistently lower counts of bacteria and of Staph. aureus during dressings when the isolator was used; on removal of the isolator canopy there was, in some experiments, a considerable increase in airborne bacteria, due to residual bacteria in the isolator of to the re-dispersal of bacteria which settled on the patient and his bedding during the dressing. Simultaneous sampling with slit samplers inside and outside the isolator during and after bed-making or dispersal of B. *subtilis var. globigii* showed an almost complete protection of the air outside the isolator against contamination by bacteria released inside the isolator.

BANERJEE, et al. 1988. *Bacillus* Infections in Patients with Cancer. *Arch.Intern.Med.* Vol. 148(8) : 1769-1774.
Eighteen febrile patients experienced 24 episodes of *Bacillus* bacteremias from January 1978 to June 1986. *Bacillus* species isolated included *Bacillus* cereus (eight cases), *Bacillus* circulans (three), *Bacillus subtilis* (two), *Bacillus* pumilus (two), *Bacillus licheniformis* (one), *Bacillus* sphaericus (one), *Bacillus* coagulans (one), and six that could not be speciated. Fifteen patients had lymphoma or leukemia and three had breast cancer. Nine patients were neutropenic (polymorphonuclear neutrophil count, less than 1.0 x 10(9)/L), seven patients had a Hickman catheter in place, and 14 had recently received chemotherapy. Twelve of the bacteremic episodes were clinically significant, and four of these 12 involved Hickman catheters. Catheter removal was ultimately necessary in all four patients. Scanning and transmission electron microscopy was performed on one of the removed Hickman catheters and showed *Bacillus* organisms embedded in a biofilm composed of gram-positive cocci and glycocalyx. *Bacillus* species were uniformly susceptible to vancomycin, imipenem, and aminoglycosides, with penicillin susceptibilities being variable. *Bacillus* appears to be another gram-positive organism now being recognized as a bacterial pathogen for compromised hosts. When such infections involve long-term indwelling venous access devices, treatment should include immediate catheter removal as well as antibiotic therapy.

BEATTIE, et al. 1999. Detection of Toxigenic Strains of *Bacillus* Cereus and Other *Bacillus* Spp. with an Improved Cytotoxicity Assay. *Lett.Appl.Microbiol.* Vol. 28(3) : 221-225.
An improved qualitative cell cytotoxicity assay for the detection of *Bacillus* cereus emetic and enterotoxin is described. The presence of toxin in culture supernatant fluids was detected by measurement with the tetrazolium salt MTT, as it adversely affects the metabolic status of cultured CHO cells. Psychrotrophic B. cereus isolates (65) were assessed for toxin production using the cytotoxicity assay, and 91% of culture supernatant fluids were cytotoxic. Toxin assessment using BCET-RPLA and ELISA immunoassays indicated that 51% and 85% of the cultures, respectively, were toxigenic. There were pronounced strain differences in the amount of toxin produced by the B. cereus isolates. Some isolates of B. circulans, *B. l*aterosporus/cereus, *B. l*entus, *B. licheniformis*, B. mycoides, B. *subtilis* and B. thuringiensis were also toxigenic.

BEDENIC, et al. 1995. Clinical and Laboratory Significance of Inducible Beta-Lactamases. *Lijec.Vjesn.* Vol. 117(9-10) : 249-253.
Many species of bacteria have inducible expression of beta-lactamase and the enzyme

production in these bacteria is normally held at a low level by a repressor mechanism, but in the presence of a beta-lactamase inducer this repression is lifted and enzyme production is greatly increased. Inducible synthesis of beta-lactamase was first described for the gram-positive organism *Bacillus licheniformis*. After that inducible expression of beta-lactamase was discovered in gram-negative bacteria like Enterobacter cloacae, Citrobacter freundii, Pseudomonas aeruginosa, Morganella morganii, Proteus vulgaris, Serratia spp and Aeromonas spp. Inducible beta-lactamases belong into class I according to Richmond and Sykes. They are chromosomally mediated cephalosporinases. beta-lactam antibiotics differ in their inducing power. Cefoxitin and imipenem are among the strongest inducers. Induction of beta-lactamase caused by these substances can lead to antagonism with other beta-lactam antibiotics if they are used in combination. The most important clinical problem connected with inducible beta-lactamases is the emergence of multiple resistant strains which are associated with therapeutic failures. It is important to distinguish induction from derepression. Induction is a temporary phenomenon in which an inducer interacts with a functional AmpD protein, which consequently prevents complexing of the AmpD and ampR proteins. In contrast, genetic derepression is permanent and results from synthesis of a defective AmpD protein unable to complex with the AmpR protein.

BLOCK. **2004.** The effect of Perasafe and sodium dichloroisocyanurate (NaDCC) against spores of Clostridium difficile and *Bacillus atrophaeus* on stainless steel and polyvinyl chloride surfaces. *J.Hosp.Infect.* Vol. 57(2): 144-148.
Clostridium difficile is an important cause of nosocomial diarrhoea. The aim of this study was to evaluate the potential for Perasafe, a recently introduced biocide, to contribute to control of C. difficile spores in the patient environment, in comparison with the chlorine-releasing agent sodium dichloroisocyanurate (NaDCC). These agents were evaluated against a water control, in a surface test on stainless steel and polyvinyl chloride (PVC) floor covering, materials commonly found in the hospital environment. The organisms studied were a toxigenic clinical isolate of C. difficile, and *Bacillus atrophaeus* (formerly B. *subtilis* var *niger*). The data indicate that in our in vitro system, Perasafe was significantly more active than NaDCC (1000 ppm available chlorine) against C. difficile spores dried on stainless steel surfaces, and against *B. atrophaeus* on PVC floor covering material, achieving mean log10 reduction factors in viable counts of 6 and 5.5, respectively, at 10 min exposures. Perasafe appeared to be less lethal in 10 min exposures to C. difficile spores fixed on PVC floor covering material. In general, 1000 ppm chlorine generated from NaDCC showed lower log10 reduction factors in viable counts at 10 min, ranging from 0.7 to 1.5, than Perasafe which ranged from 2.7 to 6.0. The potential efficacy of Perasafe in reducing the density of C. difficile spores in the patient environment in hospitals, nursing homes or other long-stay facilities should be evaluated in field studies.

BISSET, et al. 1978. The Isolation and Characters of L-Forms and Reversions of *Bacillus Licheniformis Var.* Endoparasiticus (Benedek) Associated with the Erythrocytes of Clinically Normal Persons. *J.Med.Microbiol.* Vol. 11(3) : 335-349.
Thirty-eight strains of the Gram-positive bacterium identified as *Bacillus licheniformis var.* endoparasiticus (Benedek), referred to as BLE, were isolated in various stages of reversion form the L-forms, from 28 out of 100 samples of whole blood or erythrocytes from normal healthy subjects, after prolonged incubation. Similar results were obtained from 100 samples from hospital patients with conditions not usually associated with blood infection. BLE was isolated from only one of 125 samples of plasma, including those separated from infected erythrocytes. Isolates from cultures incubated for up to 4 months were usually in the form of spheroplasts or diphtheroid bacilli; the fully reverted phase, resembling *B. licheniformis*, with the capacity to form endospores, was isolated occasionally from cultures aged 1--6 months, and it constituted about half the isolates recovered from cultures aged 6--25 months. BLE was isolated in subculture, and with the usual frequency, in previously unopened, primary cultures. It did not occur in 1200 subcultures of 150 control cultures made with autoclaved or irradiated blood cells; it was not detected in the environment of the laboratory or blood-sampling areas, or on the skin or in the respiratory passages of the operators and other persons associated with the laboratory, where typical, saprophytic *B. licheniformis* was very rare. It is concluded that this *Bacillus* species exists as an L-form, associated with the erythrocytes of a large proportion of normal persons, as previously recorded by several observers. Some of the morphological variants associated with the L-cycles have in the past been described as different organisms, for example L-forms of various bacteria or mycoplasmas, and the diphtheroid stage has been thought to belong to the genera Corynebacterium and Listeria. The sporogenous stage, although frequently described, has normally been discounted as a contaminant. These observations do not admit of any conclusion in respect of the claims that such bacteria may have a role in arthritis, cancer or other diseases.

BLAKISTONE, et al. 1999. Efficacy of Oxonia Active Against Selected Spore Formers. *J.Food Prot.* Vol. 62(3) : 262-267.
Alternatives to hydrogen peroxide are being sought for use in aseptic packaging systems because this sterilant is efficacious at temperatures higher than some of the newer packaging materials can tolerate. Earlier in this century, peracetic acid was known to be bactericidal, sporicidal, and virucidal but was not widely used because of handling, toxicity, and stability problems. Sanitizer suppliers have capitalized on the efficacy of hydrogen peroxide, acetic acid, and peracetic acid stabilized with a sequestering agent. Formulations have been improved and marketed as Oxonia Active, and its use as an alternative sterilant to hydrogen peroxide merits evaluation. Oxonia was assessed at a

concentration of 2% and a temperature of 40 degrees C against a number of spore-forming organisms, including foodborne pathogens. Spores tested in aqueous suspension showed an order of sensitivity (least to greatest) to Oxonia as follows: *Bacillus* cereus andgt; B. *subtilis* A andgt; B. stearothermophilus andgt; B. *subtilis var. globigii* andgt; B. coagulans andgt; Clostridium sporogenes (PA3679) andgt; C. butyricum andgt; C. botulinum type B (nonproteolytic) andgt; C. botulinum type B (proteolytic) = C. botulinum type A = C. botulinum type E. B. *subtilis* A and B. stearothermophilus spores tested in the dry state were less sensitive to Oxonia than when tested in aqueous suspension. B. cereus, a foodborne pathogen, proved to be markedly less sensitive to Oxonia under the described test conditions. The decreased sensitivity to Oxonia by the foodborne pathogen B. cereus raises concern about the efficacy of the sterilant for aseptic packaging of low-acid foods. Further work will be needed to determine if this decreased sensitivity is an inherent property of the organism that affords unusual protection against Oxonia or if the challenge parameters selected were at the minimum conditions for efficacy.

BLOCK. **2004.** The effect of Perasafe and sodium dichloroisocyanurate (NaDCC) against spores of Clostridium difficile and *Bacillus atrophaeus* on stainless steel and polyvinyl chloride surfaces. *J.Hosp.Infect.* Vol. 57(2): 144-148.
Clostridium difficile is an important cause of nosocomial diarrhoea. The aim of this study was to evaluate the potential for Perasafe, a recently introduced biocide, to contribute to control of C. difficile spores in the patient environment, in comparison with the chlorine-releasing agent sodium dichloroisocyanurate (NaDCC). These agents were evaluated against a water control, in a surface test on stainless steel and polyvinyl chloride (PVC) floor covering, materials commonly found in the hospital environment. The organisms studied were a toxigenic clinical isolate of C. difficile, and *Bacillus atrophaeus* (formerly B. *subtilis* var *niger*). The data indicate that in our in vitro system, Perasafe was significantly more active than NaDCC (1000 ppm available chlorine) against C. difficile spores dried on stainless steel surfaces, and against *B. atrophaeus* on PVC floor covering material, achieving mean log10 reduction factors in viable counts of 6 and 5.5, respectively, at 10 min exposures. Perasafe appeared to be less lethal in 10 min exposures to C. difficile spores fixed on PVC floor covering material. In general, 1000 ppm chlorine generated from NaDCC showed lower log10 reduction factors in viable counts at 10 min, ranging from 0.7 to 1.5, than Perasafe which ranged from 2.7 to 6.0. The potential efficacy of Perasafe in reducing the density of C. difficile spores in the patient environment in hospitals, nursing homes or other long-stay facilities should be evaluated in field studies.

BLUE, ET AL. 1995. *Bacillus Licheniformis* Bacteremia: Five Cases Associated with Indwelling Central Venous Catheters. *Clin.Infect.Dis.* Vol. 20(3) : 629-633.

Bacillus species are being more frequently recognized as pathogens in immunocompromised hosts or in patients with cancer and central venous catheters. Only nine cases of *Bacillus licheniformis* infection have been reported in the English-language literature since 1966. In a retrospective study we describe six patients and 17 episodes of *B. licheniformis* bacteremia over a 5-year span. All six patients had either a Hickman or a Broviac catheter in place for more than 3 months. Five of the six patients had multiple clinically significant episodes of bacteremia due to *B. licheniformis*. The six patients ranged in age from 4 years to 62 years. Two patients had leukemia or lymphoma and three patients had solid tumors, but only one patient was neutropenic. No deaths were related to *B. licheniformis* bacteremia. *B. licheniformis* should be considered as a potential pathogen in immunocompromised patients, especially when bacteremia is associated with the presence of long-term central venous catheters. Mortality due to *B. licheniformis* bacteremia is low, but recurrent bacteremia due to this organism causes significant morbidity and usually necessitates removal of the catheter.

BROWN. 2000. Control of Bacterial Spores. *Br.Med.Bull.* Vol. 56(1) : 158-171.
Bacterial spores are much more resistant than their vegetative counterparts. The most dangerous spore-former is Clostridium botulinum which produces a potent neurotoxin that can prove fatal. The most common food poisoning from a spore-former is caused by C. perfringens. Other food poisoning spore-formers include *Bacillus* cereus, B. *subtilis* and *B. licheniformis*. There are a number of non-pathogenic spore-formers including butyric and thermophilic anaerobes that cause significant economic losses to food producers. Some unusual spoilage complaints have been reported, for example, B. sporothermodurans in UHT milk, Alicyclo*bacillus* acidoterrestris in apple and orange juice and Desulfotomaculum nigrificans in hot vending machines. Control of spore-formers requires an understanding of both the resistance and outgrowth characteristics of the spores.

BURKE, ET AL. 2004. Detection of molecular diversity in *Bacillus atrophaeus* by amplified fragment length polymorphism analysis. *Appl.Environ.Microbiol.* Vol. 70(5): 2786-2790.
Phenotypically, *Bacillus atrophaeus* is indistinguishable from the type strain of *Bacillus subtilis* except by virtue of pigment production on certain media. Several pigmented variants of B. *subtilis* have been reclassified as *B. atrophaeus*, but several remain ambiguous in regard to their taxonomic placement. In this study, we examined strains within the American Type Culture Collection originally deposited as *Bacillus globigii*, B. *subtilis var. niger*, or *Bacillus niger* using 16S rRNA gene sequencing and amplified fragment length polymorphism (AFLP) analysis to determine the level of molecular diversity among these strains and their relationship with closely related taxa. The 16S rRNA gene sequences revealed little variation with one base substitution between the *B. atrophaeus* type strain ATCC 49337 and the other pigmented bacilli. AFLP analysis

produced high-quality DNA fingerprints with sufficient polymorphism to reveal strain-level variation. Cluster analysis of Dice similarity coefficients revealed that three strains, ATCC 31028, ATCC 49760, and ATCC 49822, are much more closely related to *B. atrophaeus* than to B. *subtilis* and should be reclassified as *B. atrophaeus*. A very closely related cluster of *B. atrophaeus* strains was also observed; this cluster was genetically distinct from the type strain. The level of variation between the two groups was approximately the same as the level of variation observed between members of the two B. *subtilis* subspecies, *subtilis* and spizizenii. It is proposed that the cluster of strains typified by ATCC 9372 be designated a new subspecies, *B. atrophaeus* subsp. *globigii*.

BUTTNER, ET AL. 2004. Determination of the efficacy of two building decontamination strategies by surface sampling with culture and quantitative PCR analysis. *Appl.Environ.Microbiol.* Vol. 70(8): 4740-4747.
The efficacy of currently available decontamination strategies for the treatment of indoor furnishings contaminated with bioterrorism agents is poorly understood. Efficacy testing of decontamination products in a controlled environment is needed to ensure that effective methods are used to decontaminate domestic and workplace settings. An experimental room supplied with materials used in office furnishings (i.e., wood laminate, painted metal, and vinyl tile) was used with controlled dry aerosol releases of endospores of *Bacillus atrophaeus* ("*Bacillus subtilis* subsp. *niger*," also referred to as BG), a *Bacillus* anthracis surrogate. Studies were performed using two test products, a foam decontaminant and chlorine dioxide gas. Surface samples were collected pre- and posttreatment with three sampling methods and analyzed by culture and quantitative PCR (QPCR). Additional aerosol releases with environmental background present on the surface materials were also conducted to determine if there was any interference with decontamination or sample analysis. Culture results indicated that 10(5) to 10(6) CFU per sample were present on surfaces before decontamination. After decontamination with the foam, no culturable *B. atrophaeus* spores were detected. After decontamination with chlorine dioxide gas, no culturable *B. atrophaeus* was detected in 24 of 27 samples (89%). However, QPCR analysis showed that *B. atrophaeus* DNA was still present after decontamination with both methods. Environmental background material had no apparent effect on decontamination, but inhibition of the QPCR assay was observed. These results demonstrate the effectiveness of two decontamination methods and illustrate the utility of surface sampling and QPCR analysis for the evaluation of decontamination strategies.

CASTAGNOLA, et al. 1997. Broviac Catheter-Related Bacteraemias due to Unusual Pathogens in Children with Cancer: Case Reports with Literature Review. *J.Infect.* Vol. 34(3) : 215-218.
Among 102 episodes of intravenous catheter related bacteraemias documented between January 1989 and July 1996 in children receiving antineoplastic chemotherapy or bone marrow transplantation at G. Gaslini Children's Hospital, Genoa, Italy, were identified seven episodes due to unusual pathogens: *Bacillus* circulans, *Bacillus licheniformis*, Brevibacterium casei, Flavimonas oryzihabitans, Porphyromonas asaccharolytica,

Comamonas acidovorans and Agrobacterium radiobacter. Susceptibility to different antibiotics of all strains are reported. In all cases catheter removal was required for culture negativization. All episodes were diagnosed in absence of granulocytopenia.

CHRISTENSEN, et al. 1979. Biological Indicators for the Control of Ethylene Oxide Sterilization. *Acta Pathol.Microbiol.Scand.[B].* Vol. 87B(3) : 147-154.
A new biological indicator has been developed for the control of ethylene oxide sterilization, particularly for large scale sterilization of disposable medical equipment. The aim has been to provide the new indicator with the same resistance to the combined effect of ethylene oxide and water vapour as the biological indicator referred to by the health authorities in Scandinavia. The reference indicator contains spores of a Danish test strain, *Bacillus subtilis*, in sand. The new one contains spores of a test strain used extensively for biological indicators, viz. B. *subtilis var. niger* (*B. globigii*). The spores in the new preparation are dried in pieces of cotton yarn. The two indicators were exposed to ethylene oxide and water vapour in five different series of experiments and almost the same resistance was found. In simulated routine sterilization procedures, the new indicator was placed at locations not easily accessible for the gas and water vapour, and the results reflected the blockage of diffusion. The experiments included samples of household dust. The resistance of the microorganisms in the dust was compared with that of the biological indicators. Based on these comparisons, it is concluded that the resistance of the two biological indicators to ethylene oxide is in accordance with the official Scandinavian standard for sterilized medical equipment when used in the control of sterilization of products with low microbial contamination.

CHUN, ET AL. 2000. Phylogenetic analysis of *Bacillus subtilis* and related taxa based on partial gyrA gene sequences. *Antonie Van Leeuwenhoek.* Vol. 78(2): 123-127.
Partial gyrA sequences were determined for twelve strains belonging to *Bacillus* amyloliquefaciens, *B. atrophaeus*, *B. licheniformis*, B. mojavensis, B. *subtilis* subsp. *subtilis*, B. *subtilis* subsp. spizizenii and B. vallismortis. The average nucleotide and translated amino acid similarities for the seven type strains were 83.7 and 95.1%, respectively, whereas the corresponding value for the 16S rRNA sequences was 99.1%. All of the type strains were sharply separated; the closest relationship was found between *B. atrophaeus* and B. mojavensis which shared a nucleotide similarity of 95.8%. Phylogenetic trees were inferred from gyrA nucleotide sequences using the neighbor-joining, Fitch-Margoliash and maximum parsimony algorithms. The test strains were divided into four groups, which generally reflected results previously reported in restriction digest and DNA-DNA hybridization studies. It is concluded from the comparative sequence analysis that the gyrA sequences provide a firm framework for the rapid and accurate classification and identification of *Bacillus subtilis* and related taxa.

CID, et al. 1988. The Relationship between the Structures of Four Beta-Lactamases obtained from *Bacillus* Cereus. *Arch.Biol.Med.Exp.(Santiago).* Vol. 21(1) : 101-107.
Bacillus cereus has proved to be one of the most interesting microorganisms in the study of beta-lactamases. It secrets these enzymes very efficiently and, frequently, in multiple forms. Three different forms are produced by strain 569/H; mutant 5/B of the same microorganism is constitutive for the secretion of beta-lactamases I and II. The present study, based on secondary structure prediction by two independent methods, states the relationship among the structures of beta-lactamases I, II and III produced by B. cereus 569/H and beta-lactamase I from the strain 5/B of this microorganism. A strong similarity is also established for the enzyme type III of B. cereus and the enzyme type I produced by *B. licheniformis* which could have an evolutionary explanation. A structural analysis of the leader peptide regions of these enzymes by the method of Mohana and Argos is also reported.

DAVID, et al. 1990. Infected Foreign Body due to *Bacillus Licheniformis* in Rheumatoid Arthritis. *Br.J.Rheumatol.* Vol. 29(4) : 320.

DUNCAN, et al. 1978. Biochemical and Genetic Properties of Site-Specific Restriction Endonucleases in *Bacillus Globigii*. *J.Bacteriol.* Vol. 134(1) : 338-344.
Bacillus globigii contains two site-specific endonucleases, BPGLI AND BglI. A rapid technique for selection of mutants deficient in each of these enzymes was developed using sensitivity to infection by bacteriophage SP50 as an indication of the levels of enzyme. Mutants defective in BglI, BglII, and both BglI and BglII retained the wild-type modification phenotype. Genetic and biochemical studies have established that these enzymes are involved in restriction in vivo. Simplified purification procedures for BglI and BglII using these mutants are described.

FEDORENKO, ET AL. 1985. [Genetic control of Actinomycetes resistance to antibiotics**].** *Mol.Gen.Mikrobiol.Virusol.* (3)(3): 3-14.
The results of studies on genetic control of resistance to antibiotics in Streptomyces strains are discussed. Cloning and sequence analysis of resistance genes yield information concerning their expression in homo- and heterologous systems, allow analysis of signal sequences responsible for initiation of transcription and translation. Cloning of genes coding for resistance to neomycin,viomycin, thiostrepton in Streptomyces and Bac. *licheniformis* ermD gene made them convenient selective markers for constructing vector molecules, useful for identification of homology regions in S. fradiae aph gene and TnS of E. coli; the site homologous to ermD gene has been thus revealed in S. erythreus chromosome. Possibilities of the studies aimed at elucidation of instability of many actinomycete characters using determinants of natural multiple resistance to antibiotics as a model are demonstrated. It has been shown that genetic instability is not related to the loss of plasmids and is associated with genes having chromosomal location.

Simultaneous high frequency loss of a number of resistance characters determined by non-linked genes suggests the participation in gene activity regulation of actinomycete genome rearrangements. This is confirmed by evidence for such rearrangements found in strains with mutant phenotypes, including deletions in tyrosinase and streptomycin phosphotransferase genes in Mel- and StrS strains of S. reticuli and S. glaucescens.

FERGENSON, ET AL. 2004. Reagentless detection and classification of individual bioaerosol particles in seconds. *Anal.Chem.* Vol. 76(2): 373-378.
The rapid chemical analysis of individual cells is an analytical capability that will profoundly impact many fields including bioaerosol detection for biodefense and cellular diagnostics for clinical medicine. This article describes a mass spectrometry-based analytical technique for the real-time and reagentless characterization of individual airborne cells without sample preparation. We characterize the mass spectral signature of individual *Bacillus* spores and demonstrate the ability to distinguish two *Bacillus* spore species, B. thuringiensis and B.*atrophaeus*, from one another very accurately and from the other biological and nonbiological background materials tested with no false positives at a sensitivity of 92%. This example demonstrates that the chemical differences between these two *Bacillus* spore species are consistently and easily detected within single cells in seconds.

FOSTER, ET AL. 2004. Identification of sporulated and vegetative bacteria using statistical analysis of fourier transform mid-infrared transmission data. *Appl.Spectrosc.* Vol. 58(2): 203-211.
A combined mid-infrared spectroscopic/statistical modeling approach for the discrimination and identification, at the strain level, of both sporulated and vegetative bacterial samples is presented. Transmission mode spectra of bacteria dried on ZnSe windows were collected using a Fourier transform mid-infrared (FT-IR) spectrometer. Five *Bacillus* bacterial strains (*B. atrophaeus* 49337, *B. globigii* Dugway, B. thuringiensis spp. kurstaki 35866, B. *subtilis* 49760, and B. *subtilis* 6051) were used to construct a reference spectral library and to parameterize a four-step statistical model for the systematic identification of bacteria. The statistical methods used in this initial feasibility study included principal component analysis (PCA), classification and regression trees (CART), and Mahalanobis distance calculations. Internal cross-validation studies successfully classified 100% of the samples into their correct physiological state (sporulated or vegetative) and identified 67% of the samples correctly as to their bacterial strain. Analysis of thirteen blind samples, which included reference and other bacteria, nonbiological materials, and mixtures of both nonbiological and bacterial samples, yielded comparable accuracy. The primary advantage of this approach is the accurate identification of unknown bacteria, including spores, in a matter of minutes.

FRITZE AND PUKALL. 2001. Reclassification of bioindicator strains *Bacillus subtilis* DSM 675 and *Bacillus subtilis* DSM 2277 as *Bacillus atrophaeus*.
Int.J.Syst.Evol.Microbiol. Vol. 51(Pt 1): 35-37.
On the basis of high DNA-DNA reassociation values and confirmatory automated RiboPrint analysis, two aerobic spore-forming strains hitherto allocated to *Bacillus*

subtilis and used as bioindicators (DSM 675, hot-air sterilization control; DSM 2277, ethylene oxide sterilization control) are reclassified as *Bacillus atrophaeus*.

GALE, ET AL. 2002. The Effect of Drinking Water Treatment on the Spatial Heterogeneity of Micro-Organisms: Implications for Assessment of Treatment Efficiency and Health Risk. *Water Res.* Vol. 36(6) : 1640-1648.
The effect of drinking water treatment (ferric coagulation, floc blanket clarification, rapid sand filtration) on the spatial heterogeneity of five species of micro-organism was studied at pilot scale. It was found that the spatial heterogeneity of vegetative bacteria (namely total coliform and heterotrophic (22 degrees C; 3 d) bacteria) was little affected by treatment. Indeed, counts of total coliform bacteria within 500 l volumes of treated water were Poisson distributed (i.e. showed minimum variation). In contrast, treatment appeared to increase the spatial heterogeneity (or clustering) of both aerobic spores indigenous to the raw water and *Bacillus subtilis* var *niger* spores added to the raw water. Furthermore, B. *subtilis* var *niger* spores added to the raw water were detected in the treated water 25 h after termination of spiking to the raw water. The effect on C. parvum oocysts added to the raw water could not be determined because few oocysts broke through treatment into the treated water. Indeed oocyst removals of 5-6 logs were apparent. andquot;Species-specific" differences in the removal ratios were also demonstrated. It is concluded that audits for treatment processes based on single 100 ml "spot" samples for spores will tend to over-estimate the net spore removal and hence underestimate the public health risk. Spatial heterogeneity of counts in treated water contributes to explaining why no "ideal" surrogate has been identified for treatment plant performance.

GALIERO, et al. 1998. Abortion in Water Buffalo (Bubalus Bubalis) Associated with *Bacillus Licheniformis*. *Vet.Rec.* Vol. 143(23) : 640.

GRIFFITHS, ET AL. 1998. Replication terminator protein-based replication fork-arrest systems in various *Bacillus* species. *J.Bacteriol.* Vol. 180(13): 3360-3367.
The replication terminator protein (RTP) of *Bacillus subtilis* interacts with its cognate DNA terminators to cause replication fork arrest, thereby ensuring that the forks approaching one another at the conclusion of a round of replication meet within a restricted terminus region. A similar situation exists in Escherichia coli, but it appears that the fork-arrest systems in these two organisms have evolved independently of one another. In the present work, RTP homologs in four species closely related to B. *subtilis* (*B. atrophaeus*, *B. a*myloliquefaciens, B. mojavensis, and B. vallismortis) have been identified and characterized. An RTP homolog could not be identified in another closely related species, *B. licheniformis*. The nucleotide and amino acid changes from B. *subtilis* among the four homologs are consistent with the recently established phylogenetic tree for these species. The GC contents of the rtp genes raise the possibility that these

organisms arose within this branch of the tree by horizontal transfer into a common ancestor after their divergence from *B. licheniformis*. Only 5 amino acid residue positions were changed among the four homologs, despite an up to 17.2% change in the nucleotide sequence, a finding that highlights the importance of the precise folded structure to the functioning of RTP. The absence of any significant change in the proposed DNA-binding region of RTP emphasizes the importance of its high affinity for the DNA terminator in its functioning. By coincidence, the single change (E30K) found in the B. mojavensis RTP corresponds exactly to that purposefully introduced by others into B. *subtilis* RTP to implicate a crucial role for E30 in the fork-arrest mechanism. The natural occurrence of this variant is difficult to reconcile with such an implication, and it was shown directly that RTP.E30K functions normally in fork arrest in B. *subtilis* in vivo. Additional DNA terminators were identified in the new RTP homolog-containing strains, allowing the definition of a *Bacillus* terminator consensus and identification of two more terminators in the B. *subtilis* 168 genome sequence to bring the total to nine.

HANNAH & ENDER 1999. Persistent *Bacillus Licheniformis* Bacteremia Associated with an Intentional Injection of Organic Drain Cleaner. *Clin.Infect.Dis.* Vol. 29(3) : 659-661.

In recent years manufacturers have developed several products containing saprophytic bacteria, previously believed to be of minimal pathogenicity. We describe the first case of persistent *Bacillus licheniformis* bacteremia occurring after intentional injection of a consumer product that includes *B. licheniformis* spores. We postulate that these spores remained in the tissue, unaffected by antimicrobials, ultimately necessitating soft-tissue debridement of the area surrounding the injection site. On the basis of this case and a review of the literature, we submit that some consumer products contain bacteria with demonstrated pathogenicity. Manufacturers should study these bacteria in detail in order to rapidly provide information such as bacteriologic data and antimicrobial susceptibility data to clinicians.

HSTAT (Health Services/Technology Assessment Text) AHRQ Evidence Reports 2002. Number 59. Bioterrorism Preparedness and Response: Use of Information Technologies and Decision Support Systems.
[http://www.ncbi.nlm.nih.gov/books/bv.fcgi?rid=hstat1.section.78786]

HUANG, et al. 1970. Characterization of Inducible Bacteriophages in *Bacillus Licheniformis*. *J.Virol.* Vol. 5(2) : 237-246.

IZUI. 1980. Mechanism of Enzyme Secretion in Bacteria. Studies on Penicillinase of *Bacillus Licheniformis* and Alkaline Phosphatase of Escherichia Coli (Author's Transl). *Seikagaku.* Vol. 52(5) : 285-304.

JARMAN, et al. **2000.** An algorithm for automated bacterial identification using matrix-assisted laser desorption/ionization mass spectrometry. *Anal.Chem.* Vol. 72(6): 1217-1223.
An algorithm for bacterial identification using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry is being developed. This mass spectral fingerprint comparison algorithm is fully automated and statistically based, providing objective analysis of samples to be identified. Based on extraction of reference fingerprint ions from test spectra, this approach should lend itself well to real-world applications where samples are likely to be impure. This algorithm is illustrated using a blind study. In the study, MALDI-MS fingerprints for *Bacillus atrophaeus* ATCC 49337, *Bacillus* cereus ATCC 14579T, Escherichia coli ATCC 33694, Pantoea agglomerans ATCC 33243, and Pseudomonas putida F1 are collected and form a reference library. The identification of test samples containing one or more reference bacteria, potentially mixed with one species not in the library (Shewanella alga BrY), is performed by comparison to the reference library with a calculated degree of association. Out of 60 samples, no false positives are present, and the correct identification rate is 75%. Missed identifications are largely due to a weak B. cereus signal in the bacterial mixtures. Potential modifications to the algorithm are presented and result in a higher than 90% correct identification rate for the blind study data, suggesting that this approach has the potential for reliable and accurate automated data analysis of MALDI-MS.

JARMAN, ET AL. **1999.** Extracting and visualizing matrix-assisted laser desorption/ionization time-of-flight mass spectral fingerprints. *Rapid Commun.Mass Spectrom.* Vol. 13(15): 1586-1594.
We have developed a method for constructing and extracting matrix-assisted laser desorption/ionization (MALDI) fingerprints. This method is fully automated and statistically based, allowing a large number of spectra to be analyzed at a time in an objective manner. This method can be used to extract the fingerprint of a particular analyte from a spectrum containing multiple analytes. Therefore, this method lends itself well to real-world applications where samples to be analyzed are likely to be impure. We illustrate this method on experimental results from a series of studies of E. coli and *B. atrophaeus* MALDI time-of-flight mass spectrometry (TOFMS) fingerprints.

JERICHO, et al. 1974. Deposition in the Respiratory Tract of Cattle of Spores of *Bacillus Subtilis* Var *Niger* by Inhalation and by Nasal Instillation. *Can.J.Comp.Med.* Vol. 38(3) : 260-265.

JOHANNSSEN ET AL.1979 Quaternary structure of the isolated restriction endonuclease EndoR.Bgl I from *Bacillus globigii* as revealed by electron microscopy.J Mol Biol. 134(4):707-26.

JOHNSON, ET AL. **2000.** Precise molecular weight determination of PCR products of the rRNA intergenic spacer region using electrospray quadrupole mass spectrometry for differentiation of B. *subtilis* and *B. atrophaeus*, closely related species of bacilli. *J.Microbiol.Methods.* Vol. 40(3): 241-254.
Assessment of 16S-23S rRNA intergenic spacer region (ISR) sequence variability is an

important supplement to 16S rRNA sequencing for differentiating closely related bacterial species. Species differentiation can also be achieved by determination of approximate size of PCR (polymerase chain reaction) products of ISRs, based on their relative electrophoretic mobility on agarose gels. Closely-related species can have ISR PCR products that are similar in size. More precise molecular weight (M.W.) determination of these products might allow improved discrimination of such species. Electrospray quadrupole mass spectrometry (ESI-Q-MS) has the potential to provide such precision. For ESI-Q-MS analysis, size limitation of PCR products is currently limited to around 130 base pairs (bp). *Bacillus subtilis* and *Bacillus atrophaeus* are two closely related species with few distinguishing phenotypic characteristics. B. *subtilis* has recently been sub-divided into two subgroups, W23 (type strain, W23) and 168 (type strain, 168). PCR products amplified from the ISR including the 5' terminal end of the 23S rRNA and a conserved portion of the ISR were analyzed by ESI-Q-MS. A 119 or 120 bp PCR product was produced for *B. atrophaeus* strains. However, strains of B. *subtilis* subgroups W23 and 168 each produced 114 bp products. In summary, a mass spectrometry method was developed for differentiation of B. *subtilis* and *B. atrophaeus*. Also, the genetic similarity of B. *subtilis* subgroups W23 and 168 was confirmed. Accurate determination of the molecular weight of PCR products from the 16S-23S rRNA intergenic spacer region using electrospray quadrupole mass spectrometry has great potential as a general technique for characterizing closely related bacterial species.

JONES, et al. 1992. Isolation of *Bacillus Licheniformis* from a Brain Abscess Following a Penetrating Orbital Injury. *J.Infect.* Vol. 24(1) : 103-104.

KELLER, ET AL. 2003. Effect of microorganism characteristics on leak size critical to predicting package sterility. *J.Food Prot.* Vol. 66(9): 1716-1719.
The effects of microorganism size and motility on the leak size critical to the sterility of a package, along with the imposed pressure required to initiate liquid flow for the critical leak size, were measured. Pseudomonas fragi Lacy-1052, *Bacillus atrophaeus* ATCC 49337, and Enterobacter aerogenes ATCC 29007 were employed to assess package sterility. One hundred twenty-six 7-mm-long microtubes with interior diameters of 5, 10, and 20 microm were used to simulate package defects. Forty-two solid microtubes were used as controls. No significant differences were found between sizes or motility statuses of test organisms with respect to loss of sterility as a result of microbial ingress into test cells with microtube interior diameters of 5, 10, and 20 microm (P andgt; 0.05). Interactions between the initiation of liquid flow as a result of applied threshold pressures and sterility loss for test cells were significant (P andlt; 0.05).

KYRIAKIS, et al. 1999. The Effect of Probiotic LSP 122 on the Control of Post-Weaning Diarrhoea Syndrome of Piglets. *Res.Vet.Sci.* Vol. 67(3) : 223-228.
Post-weaning diarrhoea syndrome (PWDS) of piglets is caused mainly by enterotoxigenic Escherichia coli (ETEC) strains. A new in-feed probiotic, LSP 122 (Alpharma), containing viable spores of *Bacillus licheniformis* was tested for its efficacy to control PWDS in piglets in a low health-status farm, using four groups with a total of 256

weaned piglets for a 28-day period. One group (negative control) was offered antimicrobial-free and probiotics-free fed, one group was offered feed supplemented with 10(6)viable spores of *Bacillus* toyoi (Toyocerin(R)) per gram of feed and two groups were offered feed supplemented with 10(6)and 10(7)viable spores of *B. licheniformis* per gram of feed, respectively, and were compared with regard to the appearance of clinical signs, mortality, weight gain and feed conversion. The results showed that all groups supplemented with probiotics exhibited a reduced incidence and severity of diarrhoea. Mortality in all probiotic supplemented pigs was significantly lower compared with the negative control group (Pandlt;0.05). The evaluation of the weight gain data, as well as feed conversion ratio, indicated that the three treated groups performed remarkably better than the negative control group (Pandlt;0.05) and the group receiving the high inclusion of LSP 122 performed better than the two other groups receiving probiotics (Pandlt;0.05). No ETEC strains were detected on day 22 in the high inclusion of LSP 122 and Toyocerin groups as compared with the untreated control. It was concluded that the high dosage schedule of LSP 122, providing 10(7)viable spores of *B. licheniformis* per g of feed, is a very useful agent for the control of PWDS due to ETEC.

LEO, ET AL. **2004.** Evaluation of Blow/Fill/Seal extrusion through processing polymer contaminated with bacterial spores and endotoxin. *PDA J.Pharm.Sci.Technol.* Vol. 58(3): 147-158.
A study has been carried out to further the understanding of the extrusion process and its impact upon the quality of Blow/Fill/Seal product. Controlled challenges to the extrusion system, comprising low-density polyethylene granulate contaminated with characterized levels of *Bacillus atrophaeus* (ATCC 9372) endospores and Escherichia coli 055:B5 bacterial endotoxin, have been conducted. Batches of spore contaminated polymer, at challenge levels varying from 10(3) to 10(6) spores g(-1) polymer with derived D160 values ranging from 1.22 to 2.07 minutes, and endotoxin contaminated polymer, at challenge levels varying from 10(2) to 10(4) EU g(-1) polymer, were processed through a Blow/Fill/Seal machine employing Tryptone Soya Broth and Water for Injection as the fill mediums, respectively. Relationships have been established between the levels of challenge for each of spores and endotoxin and the extent of product contamination. The relationships allow for prediction of microbiological and pyrogenic quality based upon the microbiological and pyrogenic attributes of unprocessed polymeric granulate and for rationalized choices of polymeric granulate acceptance limits. It is stressed that the findings apply only to the particular Blow/Fill/Seal machine and to the specific conditions of machine operation.

LONGO, et al. 2003. About a Case of Parotid Gland Abscess by *Bacillus Licheniformis*. *Br.J.Plast.Surg.* Vol. 56(4) : 424-426.

MARTIRANI et al 2002. Purification and partial characterization of bacillocin 490, a novel bacteriocin produced by a thermophilic strain of Bacillus licheniformis. *Microb Cell Fact*. 1(1):1. (Apr. 18).
BACKGROUND: Applications of bacteriocins as food preservatives have been so far limited, principally because of their low antimicrobial activity in foods. Nisin is the only bacteriocin of significant use, but applications are restricted principally because of its very low activity at neutral or alkaline pH. Thus the isolation of new bacteriocins active in foods is desirable. RESULTS: We isolated a Bacillus licheniformis thermophilic strain producing a bacteriocin with some novel features, named here bacillocin 490. This bacteriocin was inactivated by pronase E and proteinase K and was active against closely related Bacillus spp. both in aerobic and in anaerobic conditions. Bactericidal activity was kept during storage at 4 degrees C and was remarkably stable in a wide pH range. The bacteriocin was partially purified by elution after adhesion to cells of the food-isolated strain Bacillus smithii and had a rather low mass (2 KDa). Antimicrobial activity against B. smithii was observed also when this organism was grown in water buffalo milk. CONCLUSIONS: Bacillocin 490 is a novel candidate as a food anti-microbial agent since it displays its activity in milk, is stable to heat treatment and during storage, is active in a wide pH range and has bactericidal activity also at high temperature. These features may allow the use of bacillocin 490 during processes performed at high temperature and as a complementary antimicrobial agent of nisin against some Bacillus spp. in non-acidic foods. The small size suggests its use on solid foods

MATSUMOTO, et al. 2000. Management of Suspected Nosocomial Infection: An Audit of 19 Hospitalized Patients with Septicemia Caused by *Bacillus* Species. *Jpn.J.Infect.Dis.* Vol. 53(5): 196-202.
From April to August of 2000, *Bacillus* spp. were detected in the blood culture of 29 patients in a hospital in Japan. Of these patients, 19 had clinical signs of septicemia; positive culture in the remaining 10 patients was attributed to contamination with skin flora at the site of puncture. Of the 18 strains evaluated, 15 were *Bacillus* cereus, 2 were *Bacillus subtilis*, and one was *Bacillus licheniformis*. The only hospital death observed was that of a patient who had no clinical signs of septicemia at the time of blood sampling. That death is now considered attributable to the underlying neoplasm. The hospital committee for prevention of nosocomial infection concluded after a critical review of the patient records that the cause of septicemia in most cases had been contaminated intravenous lines. To control the situation, the committee recommended the use of a new skin disinfectant, and medical personnel were advised to avoid infusion pauses with interruption of intravenous lines and to replace the caps for the stopcocks with new ones each time the caps were removed. These measures were rigorously

observed in addition to the conventional measures for preventing catheter sepsis, and the incidence of septicemia due to the *Bacillus* spp. declined dramatically thereafter.

MAUCOUR, et al. 1999. Bacillary Endophthalmitis. Four Case Reports. *J.Fr.Ophtalmol.* Vol. 22(3): 371-376.
PURPOSE: Bacillary endophthalmitis occurring after penetrating ocular trauma with an intraocular foreign body is always associated with poor visual outcome. Bacilli cause fulminant infection associated with tissue damage in the intraocular structures. CASE REPORTS: Our series consisted of four patients with penetrating ocular trauma and endophthalmitis caused by B. cereus or *B. licheniformis*. Three eyes ultimately developed phtisis. Only on eye recovered good vision (2.5/10 P4). DISCUSSION: Recommended early treatment includes topical, subconjunctival, parenteral antibiotics. A review of the literature indicates that intravitreal antibiotic infusion is crucial for sufficient concentration to control infection. Early vitrectomy is recommended in the management of endophthalmitis. Vitreous and intraocular foreign bodies should be cultured to identify pathogens and ascertain antibiotic susceptibilities.

MCLAUCHLIN, et al. 2002. An Investigation into the Microflora of Heroin. *J.Med.Microbiol.* Vol. 51(11) : 1001-1008.
In 2000, an unusual increase of morbidity and mortality among illegal injecting drug users in the UK and Ireland was reported and Clostridium novyi was identified as the likely source of the serious infection, although infections due to C. botulinum and *Bacillus* cereus were also reported. Because heroin was a possibile source of infection, this study investigated the microflora of heroin samples seized in England during 2000 and 2002. Two methods were developed for the examination of the microflora of heroin. The first consisted of suspension of the drug in maximum recovery diluent (MRD) which was inoculated directly into Clostridium Botulinum Isolation Cooked Meat Broth (CBI). The second method rendered the heroin soluble in citric acid, concentrated particulate material (and bacterial cells) by filtration and removed heroin residues by washing with citric acid and phosphate-buffered saline before placing the filter in CBI broth. Duplicate CBI broths from both methods were incubated without heating and after heating at 60 degrees C for 30 min. Subcultures were made after incubation for 7 and 14 days on to eight different solid media. The methods were evaluated with heroin samples spiked with either C. botulinum or C. novyi spore suspensions; recovery of 10 spores in the original sample was demonstrated. Fifty-eight heroin samples were tested by citric acid solubilisation and 34 by the MRD suspension technique. Fifteen different gram-positive species of four genera were recognised. No fungi were isolated. Aerobic endospore-forming bacteria (*Bacillus* spp. and Paeni*bacillus* macerans) were the predominant

microflora isolated and at least one species was isolated from each sample. B. cereus was the most common species and was isolated from 95% of all samples, with *B. licheniformis* isolated from 40%. Between one and five samples yielded cultures of B. coagulans, *B. l*aterosporus, B. pumilus, B. *subtilis* and P. macerans. Staphylococcus spp. were isolated from 23 (40%) samples; S. warneri and S. epidermidis were the most common and were cultured from 13 (22%) and 6 (10%) samples respectively. One or two samples yielded cultures of S. aureus, S. capitis and S. haemolyticus. The remainder of the flora detected comprised two samples contaminated with C. perfringens and two samples with either C. sordellii or C. tertium. Multiple bacterial species were isolated from 43 (74%) samples, a single species from the remaining 15. In 13 samples B. cereus alone was isolated, in one B. *subtilis* alone and in one sample B. pumilus alone. C. botulinum and C. novyi were not isolated from any of the heroin samples. Recommendations for the optimal examination of the microflora of heroin are given.

MIKKOLA, et al. 2000. Toxic Lactonic Lipopeptide from Food Poisoning Isolates of *Bacillus Licheniformis*. *Eur.J.Biochem.* Vol. 267(13) : 4068-4074.
Toxins from three *Bacillus licheniformis* strains connected to a fatal food poisoning were isolated and their structures elucidated. Toxins were purified from methanol extracts of the *B. licheniformis* biomass using boar sperm cells as the toxicity indicator. The HPLC purified toxins showed protonated masses m/z 1007, 1021 and 1035 in MALDI-TOF-MS. The toxins isolated from the strains of different origins contained the same three components of which and each had a same amino-acid residues L-Gln, L-Leu, D-Leu, L-Val, L-Asp, D-Leu and L-Ile in that order. Toxins were identified as lichenysin A, a cyclic lactonic heptalipopeptide in which the main 3-hydroxy fatty acids are 13-15 carbons in length. We showed that the toxins from food and food poisoning isolates of *B. licheniformis* were identical to lichenysin A both in the structure and in the toxic symptoms induced to boar spermatozoa. Confocal laser scanning microscopy showed that the acrosome and the plasma membrane of boar spermatozoa were the targets of lichenysin A toxicity.

MING, et al. 2002. Metal Binding and Structure-Activity Relationship of the Metalloantibiotic Peptide Bacitracin. *J.Inorg.Biochem.* Vol. 91(1) : 46-58.
Bacitracin is a widely used metallopeptide antibiotic produced by *Bacillus subtilis* and *Bacillus licheniformis* with a potent bactericidal activity directed primarily against Gram-positive organisms. This antibiotic requires a divalent metal ion such as Zn(2+) for its biological activity, and has been reported to bind several other transition metal ions, including Mn(2+), Co(2+), Ni(2+), and Cu(2+). Despite the widespread use of bacitracin since its discovery in the early 1940s, the structure-activity relationship of this drug has

not been established and the coordination chemistry of its metal complexes was not fully determined until recently. This antibiotic has been suggested to influence cell functioning through more than one route. Since bacterial resistance against bacitracin is still rare despite several decades of widespread use, this antibiotic can serve as an ideal lead for the design of potent peptidyl antibiotics lacking bacterial resistance. In this review, the results of physical (including NMR, EPR, and EXAFS) and molecular biological studies regarding the synthesis and structure of bacitracin, the coordination chemistry of its metal derivatives, the mechanism of its antibiotic actions, its influence on membrane function, and its structure and function relationship are discussed.

MODDERMAN, et al. 1995. Safety Evaluation of Pullulanase Enzyme Preparation Derived from *Bacillus Licheniformis* Containing the Pullulanase Gene from *Bacillus* Deramificans. *Regul.Toxicol.Pharmacol.* Vol. 21(3) : 375-381.
Pullulanase enzyme is an amylopectin debranching enzyme used in starch hydrolysis. This article describes studies conducted to investigate the safety of a pullulanase enzyme preparation produced by a strain of *Bacillus licheniformis* that has been transformed by introduction of genetic material from another *Bacillus* species, B. deramificans. A 4-week dietary toxicity study in rats was conducted in which test animals received pullulanase in the feed at concentrations of 0.2, 1.0, and 5.0%. No adverse treatment-related effects were observed. Lack of genetic toxicity potential was demonstrated by the results of a bacterial mutation assay in Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, and TA1538, in an in vitro histidine forward mutation study in mouse lymphoma cells, and in in vivo mouse bone marrow chromosome aberration and micronucleus assays. The enzyme preparation also has been shown to be a nonirritant in eye and primary dermal irritation tests in rabbits and is nontoxic by inhalation exposure. Finally, the genetically altered *B. licheniformis* has been demonstrated to be nonpathogenic upon single intraperitoneal injection to rats of both live and killed cells at doses up to 10(11) cells/kg. The results of these studies demonstrate that the enzyme preparation may be considered safe when employed in starch processing.

MONTVILLE. 1982. Metabiotic Effect of *Bacillus Licheniformis* on Clostridium Botulinum: Implications for Home-Canned Tomatoes. *Appl.Environ.Microbiol.* Vol. 44(2) : 334-338.
The metabiotic effect of *Bacillus licheniformis* on Clostridium botulinum was examined. *B. licheniformis* elevated the pH of a model system with an initial pH of 4.4 so that C. botulinum grew and produced toxin. Toxin production was observed when spores from both species were coinoculated at levels as low as 10 spores per ml. When pint jars of tomatoes were used, canner size contributed to a 10,000-fold difference in the lethality of

a boiling water bath process on *B. licheniformis* spores. Botulinal toxin was not detected in pH-elevated jars of tomatoes containing C. botulinum spores.

NAKAMURA 1989. Taxonomic relationship of black-pigmented *Bacillus subtilis* strains and a proposal for *Bacillus atrophaeus* sp. nov. *Int. J. Syst. Bacteriol.*, 39**:** 295-300.

NANDI, et al. 1998. Microbial Production of Hydrogen: An Overview. *Crit.Rev.Microbiol.* Vol. 24(1) : 61-84.
Production of hydrogen by anaerobes, facultative anaerobes, aerobes, methylotrophs, and photosynthetic bacteria is possible. Anaerobic Clostridia are potential producers and immobilized C. butyricum produces 2 mol H2/mol glucose at 50% efficiency. Spontaneous production of H2 from formate and glucose by immobilized Escherichia coli showed 100% and 60% efficiencies, respectively. Enterobactericiae produces H2 at similar efficiency from different monosaccharides during growth. Among methylotrophs, methanogenes, rumen bacteria, and thermophilic archae, Ruminococcus albus, is promising (2.37 mol/mol glucose). Immobilized aerobic *Bacillus licheniformis* optimally produces 0.7 mol H2/mol glucose. Photosynthetic Rhodospirillum rubrum produces 4, 7, and 6 mol of H2 from acetate, succinate, and malate, respectively. Excellent productivity (6.2 mol H2/mol glucose) by co-cultures of Cellulomonas with a hydrogenase uptake (Hup) mutant of R. capsulata on cellulose was found. Cyanobacteria, viz., Anabaena, Synechococcus, and Oscillatoria sp., have been studied for photoproduction of H2. Immobilized A. cylindrica produces H2 (20 ml/g dry wt/h) continually for 1 year. Increased H2 productivity was found for Hup mutant of A. variabilis. Synechococcus sp. has a high potential for H2 production in fermentors and outdoor cultures. Simultaneous productions of oxychemicals and H2 by Klebseilla sp. and by enzymatic methods were also attempted. The fate of H2 biotechnology is presumed to be dictated by the stock of fossil fuel and state of pollution in future.

PACOVA, et al. 1996. Identification of Aerobic and Facultatively Anaerobic Sporulating Bacteria Isolated during the Primary Milk Collection. *Vet.Med.(Praha).* Vol. 41(1) : 19-23.
Aerobic and/or facultatively anaerobic sporulating Gram-positive bacteria of the genus *Bacillus* influence nutritive and sensory properties of pasteurized milk by their proteolytic and lipolytic activity. Since particularly raw milk is a source of pasteurized milk contamination by spore-producing bacteria, our investigations were focused on identification of bacilli from operations of milk agricultural primary production. The species *B. licheniformis* and B. cereus (Crielly et al., 1994) are the most frequently isolated ones in the process of milk production. While *B. licheniformis* as well as B.

subtilis and B. pumilus are mesophilic species, B. cereus is rather psychrotrophic, and as to their health impacts they cause diseases from foods (Griffiths, 1990; Christiansson, 1992; Becker et al., 1994). Sixty-six strains were isolated and identified from operations of milk agricultural primary production (Tab. I). *B. licheniformis* occurrence (58 strains) was most frequent in the set of samples, followed by B. *subtilis* (5 strains), B. pumilus (one strain) and B. cereus (one strain), i.e. these are species classified to morphological group I (oval, cell-nonswelling spores). Only one strain Paeni*bacillus* amylolyticus (formerly *Bacillus* amylolyticus) was isolated from morphological group II (oval, cell-swelling spores). Species representation of isolated strains is in agreement with literacy data (Phillips and Griffiths, 1986; Sutherland and Murdock, 1994; Crielly et al., 1994;). Our results did not confirm the seasonal occurrence (winter-summer) of mesophilic and psychrotrophic bacilli species as reported in literature (McKinnon and Pettipher, 1983; Sutherland and Murdock, 1994; Crielly et al., 1994). Biochemical and physiological characteristics of 66 isolates (Tab. I) are in agreement with literary data (Gordon et al., 1973; Parry et al., 1983; Priest et al., Ash et al., 1993). Strong proteolytic, amylolytic or lipolytic activities of the tested strains could influence the nutritive value of milk as a raw material. Taking into account the dominant representation of bacilli of morphological group I in raw and pasteurized milk (Sutherland and Murdock, 1994; Crielly et al., 1994) their review with basic phenotypic characteristics is shown in Tab. II. As follows from our results mesophilic species from so called “B. *subtilis* group” (96.9%) were isolated from agricultural primary production of milk most frequently. This is the reason why in addition to B. cereus it is also necessary to identify these species: seven tests shown in Tab III are recommended for their rapid differentiation.

PEDERSEN, et al. 2002. Cytotoxic Potential of Industrial Strains of *Bacillus* Sp. *Regul.Toxicol.Pharmacol.* Vol. 36(2) : 155-161.
The cytotoxic potential of selected strains of *Bacillus licheniformis*, *Bacillus* amyloliquefaciens, and *Bacillus subtilis*, used in the production of industrial enzyme products, has been assessed. Cytotoxicity was determined in Chinese hamster ovary (CHO-K1) cells by measuring total cellular metabolic activity using the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Initially the MTT assay was validated against toxigenic strains of *Bacillus* cereus, to define the exact criteria for a toxigenic versus a nontoxigenic response. The assay proved sensitive to culture broths of both a diarrheagenic strain and an emetic strain of B. cereus. The enzyme-producing strains tested were nontoxic to CHO-K1 cells. Additionally it was demonstrated that our industrial strains did not react with antibodies against B. cereus enterotoxins by use of commercial antibody-based kits from Oxoid and Tecra. A short survey of the literature concerning the toxigenic potential of species within the *subtilis*

group is included, as is a database search of known B. cereus enterotoxins against B. *subtilis* and *B. licheniformis* DNA sequences.

PELOUX, et al. 1976. New Opportunist Infection due to a *Bacillus*. A Case of Bacteremia with *Bacillus Licheniformis*. *Pathol.Biol.(Paris).* Vol. 24(2) : 97-98.
The authors report a case of bacteriemia with *Bacillus licheniformis* in a pregnant woman with coagulation disorders (acute fibrinolysis). Eight blood cultures were positive. Recovery rapidly occurred. The origin of this affection is discussed : a venous mode of entry is possible, either by the use of contaminated infusion fluid, or following several catheterizations.

PENNA, et al. 2001. The Efficacy of Chemical Agents in Cleaning and Disinfection Programs. *BMC Infect.Dis.* Vol. 1(1) : 16.
BACKGROUND: Due to the growing number of outbreaks of infection in hospital nurseries, it becomes essential to set up a sanitation program that indicates that the appropriate chemical agent was chosen for application in the most effective way. METHOD: For the purpose of evaluating the efficacy of a chemical agent, the minimum inhibitory concentration (MIC) was reached by the classic method of successive broth dilutions. The reference bacteria utilized were *Bacillus subtilis var. globigii* ATCC 9372, *Bacillus* stearothermophilus ATCC 7953, Escherichia coli ATCC 25922, Staphylococcus aureus ATCC 25923. The strains of Enterobacter cloacae IAL 1976 (Adolfo Lutz Institute), Serratia marcescens IAL 1478 and Acinetobactev calcoaceticus IAL 124 (ATCC 19606), were isolated from material collected from babies involved in outbreaks of infection in hospital nurseries. RESULTS: The MIC intervals, which reduced bacteria populations over 08 log10, were: 59 to 156 mg/L of quaternarium ammonium compounds (QACs); 63 to 10000 mg/L of chlorhexidine digluconate; 1375 to 3250 mg/L of glutaraldehyde; 39 to 246 mg/L of formaldehyde; 43750 to 87500 mg/L of isopropanol or ethanol; 1250 to 6250 mg/L of iodine in polyvinyl-pyrolidone complexes, 150 to 4491 mg/L of chlorine-releasing-agents (CRAs); 469 to 2500 mg/L of hydrogen peroxide; and, 2310 to 18500 mg/L of peracetic acid. CONCLUSIONS: Chlorhexidine showed non inhibitory activity over germinating spores. A. calcoaceticus, was observed to show resistance to the majority of the agents tested, followed by E. cloacae and S. marcescens.

PIRTTIJARVI, et al. 2000. Properties of *Bacillus* Cereus and Other Bacilli Contaminating Biomaterial-Based Industrial Processes. *Int.J.Food Microbiol.* Vol. 60(2-3) : 231-239.
This paper is an overview on bacilli in industrial processes, with focus on food grade paper and paperboard production. Paperboards mainly contain sporeforming bacteria

belonging to the genera *Bacillus*, Paeni*bacillus* and Brevi*bacillus*, usually found in quantities from andlt; 50 to 250 cfu g(-1) homogenized paperboard. Of those frequently found, *Bacillus* cereus group, *B. licheniformis*, B. *subtilis* and Brevi*bacillus* brevis are important for food hygiene because of their hydrolytic activities on food components and the ability of some strains to produce food poisoning toxins or to grow at refrigerated temperatures. We found that the phenotypic properties (lecithinase activity, nitrate reduction) used in standard methods (e.g., ISO, FDA, IDF) to recognize B. cereus, were unreliable for industrial isolates. Whole cell fatty acid composition of a group of the industrial isolates deviated so much from those in a widely used commercial database that the strains were not or only poorly recognized as B. cereus. Industrial isolates, including toxigenic ones, often missed one or more of these characters, even in cases where 100% 16S rDNA identity was found with B. cereus or with B. thuringiensis. 11-Methyldodecanoic acid and trans-9-hexadecenoic acid were found without exception in over 200 industrial B. cereus group isolates and in over 30 culture collection strains. The detection of these fatty acids is a secure method for the identification of B. cereus. Negative reaction for starch hydrolysis and for BCET-RPLA test and a specific ribotype were found in all B. cereus strains producing the emetic toxin.

PROBANZA, et al. 2001. Effects of Inoculation with PGPR *Bacillus* and Pisolithus Tinctorius on Pinus Pinea L. Growth, Bacterial Rhizosphere Colonization, and Mycorrhizal Infection. *Microb.Ecol.* Vol. 41(2) : 140-148.
The effect of co-inoculation with Pisolithus tinctorius and a PGPR belonging to the genus *Bacillus* (*Bacillus licheniformis* CECT 5106 and *Bacillus* pumilus CECT 5105) in enhancing growth of Pinus pinea plants and the changes that occurred in rhizosphere microbial communities and the degree of mycorrhization were evaluated. Both bacterial strains of *Bacillus* promote the growth of Pinus pinea seedlings, but this biological effect does not imply a synergic effect with mycorrhizal infection. However, the positive response to mycorrhiza in a longer-term experiment it could be expected. The introduction of both inocula causes an alteration in the microbial rhizosphere composition, despite the low levels of inocula that were found at the end of the assay.

PROJECT 112 Autumn Gold 2001. *DeploymentLink.*
[http://deploymentlink.osd.mil/pdfs/autumn_gold.pdf]

ROBERTS, et al. **1994.** *Bacillus* mojavensis sp. nov., distinguishable from *Bacillus subtilis* by sexual isolation, divergence in DNA sequence, and differences in fatty acid composition. *Int.J.Syst.Bacteriol.* Vol. 44(2): 256-264.

A number of *Bacillus* strains isolated from desert soil samples were shown to belong to a previously unidentified species, for which we propose the name *Bacillus* mojavensis. The type strain is RO-H-1 (= NRRL B-14698). On the basis of restriction digest data, B. mojavensis is most closely related to *Bacillus* amyloliquefaciens, *Bacillus atrophaeus*, and *Bacillus subtilis*. So far, B. mojavensis can be distinguished from B. *subtilis* only by differences in whole-cell fatty acid composition, divergence in DNA sequence, and resistance to genetic transformation between taxa (in addition to reduced genome relatedness values). Sequence divergence and sexual isolation may prove to be more useful than metabolic characteristics for delimiting cryptic *Bacillus* species.

ROWAN, et al. 1997. The Bacteriological Quality of Hospital-Prepared Infant Feeds. *J.Hosp.Infect.* Vol. 35(4) : 259-267.
Twenty-four pasteurized infant feeds, prepared in a Glasgow hospital, were examined microbiologically. All produced a satisfactory total aerobic mesophilic count of andlt; or = 1.0 x 10(4) cfu/g (mean 6.3 x 10(1) cfu/g) within 1 h of preparation. *Bacillus* cereus was detected in two infant feeds immediately after preparation and one of these had a B. cereus count of 1.4 x 10(3) cfu/g exceeding the recommended safety limit of andlt; or = 1.0 x 10(3) cfu/g. Subsequent storage over a 14 h period at 25 degrees C or greater resulted in the appearance of B. cereus in a further eight feeds, the majority of which exceeded the safety limit of 10(3) cfu/g. The microbiological quality of each infant feed depended on the type and number of organisms initially present, and on the temperature and duration of storage. Incubation of feeds at andlt; or = 10 degrees C for 14 h did not alter the microbiological quality (P = 0.05). While *Bacillus licheniformis* and *Bacillus subtilis* were the predominant organisms isolated within 8 h of incubation (45.8 and 20.8% of feeds, respectively), additional storage resulted in the emergence of B.cereus I (25%) and II (20.8%) as dominant *Bacillus* spp. The addition of glucose polymers and other supplements to infant formulae did not affect the type and number of organisms present (P = 0.05). Diarrhoeal enterotoxin was detected in three of the five formulations which supported the growth of B. cereus II via the B. cereus enterotoxin reverse phase latex agglutination test BCET-RPLA system. Although the infant feeds were of similar microbiological quality (P = 0.05), the majority of *Bacillus* spp. isolated have been previously implicated in either foodborne illnesses and/or opportunist infections.

ROWAN, et al. 2003. Production of Diarrheal Enterotoxins and Other Potential Virulence Factors by Veterinary Isolates of *Bacillus* Species Associated with Nongastrointestinal Infections. *Appl.Environ.Microbiol.* Vol. 69(4) : 2372-2376.
With the exceptions of *Bacillus* cereus and *Bacillus* anthracis, *Bacillus* species are

generally perceived to be inconsequential. However, the relevance of other *Bacillus* species as food poisoning organisms and etiological agents in nongastrointestinal infections is being increasingly recognized. Eleven *Bacillus* species isolated from veterinary samples associated with severe nongastrointestinal infections were assessed for the presence and expression of diarrheagenic enterotoxins and other potential virulence factors. PCR studies revealed the presence of DNA sequences encoding hemolysin BL (HBL) enterotoxin complex and B. cereus enterotoxin T (BceT) in five B. cereus strains and in *Bacillus* coagulans NB11. Enterotoxin HBL was also harbored by *Bacillus* polymyxa NB6. After 18 h of growth in brain heart infusion broth, all seven *Bacillus* isolates carrying genes encoding enterotoxin HBL produced this toxin. Cell-free supernatant fluids from all 11 *Bacillus* isolates demonstrated cytotoxicity toward human HEp-2 cells; only one *Bacillus licheniformis* strain adhered to this test cell line, and none of the *Bacillus* isolates were invasive. This study constitutes the first demonstration that *Bacillus* spp. associated with serious nongastrointestinal infections in animals may harbor and express diarrheagenic enterotoxins traditionally linked to toxigenic B. cereus.

ROWAN, et al. 2001. Putative Virulence Factor Expression by Clinical and Food Isolates of *Bacillus* Spp. After Growth in Reconstituted Infant Milk Formulae. *Appl.Environ.Microbiol.* Vol. 67(9) : 3873-3881.
Forty-seven strains representing 14 different *Bacillus* species isolated from clinical and food samples were grown in reconstituted infant milk formulae (IMF) and subsequently assessed for adherence to, invasion of, and cytotoxicity toward HEp-2 and Caco-2 cells. Cell-free supernatant fluids from 38 strains (81%) were shown to be cytotoxic, 43 strains (91%) adhered to the test cell lines, and 23 strains (49%) demonstrated various levels of invasion. Of the 21 *Bacillus* cereus strains examined, 5 (24%) were invasive. A larger percentage of clinically derived *Bacillus* species (20%) than of similar species tested from the food environment were invasive. Increased invasion occurred after growth of selected *Bacillus* species in reconstituted IMF containing glucose. While PCR primer studies revealed that many different *Bacillus* species contained DNA sequences encoding the hemolysin BL (HBL) enterotoxin complex and B. cereus enterotoxin T, not all of these isolates expressed these diarrheagenic genes after growth in reconstituted IMF. Of the 47 *Bacillus* isolates examined, 3 isolates of B. cereus and 1 isolate of B. *subtilis* produced the HBL enterotoxin after 18 h of growth in brain heart infusion broth. However, eight isolates belonging to the species B. cereus, *B. licheniformis*, B. circulans, and B. megaterium were found to produce this enterotoxin after growth in reconstituted IMF when assessed with the B. cereus enterotoxin (diarrheal type) reversed passive latex agglutination (RPLA) kit. It is concluded that several *Bacillus* species occurring

occasionally in clinical specimens and food samples are of potential medical significance due to the expression of putative virulence factors.

RYAN. 1970. Abortion in Cattle Associated with *Bacillus Licheniformis*. *Vet.Rec.* Vol. 86(22): 650-651.

RYOO, et al. 2001. *Bacillus Licheniformis* Peritonitis in a CAPD Patient. *Perit.Dial.Int.* Vol. 21(1): 97.

SAENZ, et al. **1999.** Reproducibility of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry for replicate bacterial culture analysis. *Rapid Commun.Mass Spectrom.* Vol. 13(15): 1580-1585.
Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOFMS) was used to demonstrate the reproducibility of bacterial spectra collected on different days. The reproducibility of analysis by MALDI-MS of intact Escherichia coli and *Bacillus atrophaeus* is presented as a replicate culture study in which spectra were collected on ten different occasions over a three-month period and by two different operators. The analysis resulted in the detection of specific biomarkers in the m/z 2000-20 000 range. Some of the peaks in the Escherichia coli spectra are identified by comparison with other published work. All of the spectra obtained are reproducible over the course of the experiment, but operator variability does exist. The Escherichia coli spectra show operator variability while the *Bacillus atrophaeus* spectra do not. This work demonstrates the utility of MALDI in obtaining consistent spectra from bacteria over a period of time.

SALKINOJA-SALONEN, et al. 1999. Toxigenic Strains of *Bacillus Licheniformis* Related to Food Poisoning. *Appl.Environ.Microbiol.* Vol. 65(10) : 4637-4645.
Toxin-producing isolates of *Bacillus licheniformis* were obtained from foods involved in food poisoning incidents, from raw milk, and from industrially produced baby food. The toxin detection method, based on the inhibition of boar spermatozoan motility, has been shown previously to be a sensitive assay for the emetic toxin of *Bacillus* cereus, cereulide. Cell extracts of the toxigenic *B. licheniformis* isolates inhibited sperm motility, damaged cell membrane integrity, depleted cellular ATP, and swelled the acrosome, but no mitochondrial damage was observed. The responsible agent from the *B. licheniformis* isolates was partially purified. It showed physicochemical properties similar to those of cereulide, despite having very different biological activity. The toxic agent was nonproteinaceous; soluble in 50 and 100% methanol; and insensitive to heat, protease, and acid or alkali and of a molecular mass smaller than 10,000 g mol(-1). The toxic *B. licheniformis* isolates inhibited growth of Corynebacterium renale DSM 20688(T), but

not all inhibitory isolates were sperm toxic. The food poisoning-related isolates were beta-hemolytic, grew anaerobically and at 55 degrees C but not at 10 degrees C, and were nondistinguishable from the type strain of *B. licheniformis*, DSM 13(T), by a broad spectrum of biochemical tests. Ribotyping revealed more diversity; the toxin producers were divided among four ribotypes when cut with PvuII and among six when cut with EcoRI, but many of the ribotypes also contained nontoxigenic isolates. When ribotyped with PvuII, most toxin-producing isolates shared bands at 2.8 +/- 0.2, 4.9 +/- 0.3, and 11.7 +/- 0.5 or 13.1 +/- 0.8 kb.

SANTINI, et al. 1995. *Bacillus Licheniformis* Prosthetic Aortic Valve Endocarditis. *J.Clin.Microbiol.* Vol. 33(11) : 3070-3073.
A 73-year old man developed an acute prosthetic aortic valve dehiscence for which emergent operation was undertaken. The intraoperative evidence of an aortic annular disruption and of a subannular abscess led to the hypothesis that an endocarditis process was involved. The aortic valve was replaced with a stentless porcine bioprosthesis. Cultures taken intraoperatively from the aortic area had a pure growth of aerobic, spore-forming, gram-positive bacilli identified as *Bacillus licheniformis*. The patient responded to specific antibiotic therapy with no relapse at a 20-month follow-up. The potentiality of *B. licheniformis* as a pathogen should be reconsidered.

SETLOW, ET AL. 2004. Mechanism of the hydrolysis of 4-methylumbelliferyl-beta-D-glucoside by germinating and outgrowing spores of *Bacillus* species. *J.Appl.Microbiol.* Vol. 96(6): 1245-1255.
AIMS: To determine the mechanism of the hydrolysis of 4-methylumbelliferyl-beta-D-glucopyranoside (beta-MUG) by germinating and outgrowing spores of *Bacillus* species. METHODS AND RESULTS: Spores of *B. atrophaeus* (formerly B. *subtilis var. niger*, Fritze and Pukall 2001) are used as biological indicators of the efficacy of ethylene oxide sterilization by measurement of beta-MUG hydrolysis during spore germination and outgrowth. It was previously shown that beta-MUG is hydrolysed to 4-methylumbelliferone (MU) during the germination and outgrowth of *B. atrophaeus* spores (Chandrapati and Woodson 2003), and this was also the case with spores of B. *subtilis* 168. Germination of spores of either *B. atrophaeus* or B. *subtilis* with chloramphenicol reduced beta-MUG hydrolysis by almost 99%, indicating that proteins needed for rapid beta-MUG hydrolysis are synthesized during spore outgrowth. However, the residual beta-MUG hydrolysis during spore germination with chloramphenicol indicated that dormant spores contain low levels of proteins needed for beta-MUG uptake and hydrolysis. With B. *subtilis* 168 spores that lacked several general proteins of the phosphotransferase system (PTS) for sugar uptake, beta-MUG hydrolysis during spore germination and outgrowth was decreased andgt;99.9%. This indicated that beta-MUG is taken up by the PTS, resulting in the intracellular accumulation of the phosphorylated form of beta-MUG, beta-MUG-6-phosphate (beta-MUG-P). This was further demonstrated by the lack of detectable glucosidase activity on beta-MUG in dormant,

germinated and outgrowing spore extracts, while phosphoglucosidase active on beta-MUG-P was readily detected. Dormant B. *subtilis* 168 spores had low levels of at least four phosphoglucosidases active on beta-MUG-P: BglA, BglH, BglC (originally called YckE) and BglD (originally called YdhP). These enzymes were also detected in spores germinating and outgrowing with beta-MUG, but levels of BglH were the highest, as this enzyme's synthesis was induced ca 100-fold during spore outgrowth in the presence of beta-MUG. Deletion of the genes coding for BglA, BglH, BglC and BglD reduced beta-MUG hydrolysis by germinating and outgrowing spores of B. *subtilis* 168 at least 99.7%. Assay of glucosidases active on beta-MUG or beta-MUG-P in extracts of dormant and outgrowing spores of *B. atrophaeus* revealed no enzyme active on beta-MUG and one enzyme that comprised andgt; or =90% of the phosphoglucosidase active on beta-MUG-P. Partial purification and amino-terminal sequence analysis of this phosphoglucosidase identified this enzyme as BglH. CONCLUSIONS: Generation of MU from beta-MUG by germinating and outgrowing spores of *B. atrophaeus* and B. *subtilis* is mediated by the PTS-driven uptake and phosphorylation of beta-MUG, followed by phosphoglucosidase action on the intracellular beta-MUG-P. The major phosphoglucosidase catalyzing MU generation from beta-MUG-P in spores of both species is probably BglH. SIGNIFICANCE AND IMPACT OF THE STUDY: This work provides new insight into the mechanism of uptake and hydrolysis of beta-MUG by germinating and outgrowing spores of *Bacillus* species, in particular *B. atrophaeus*. The research reported here provides a biological basis for a Rapid Readout Biological Indicator that is used to monitor the efficacy of ethylene oxide sterilization.

SHAVER, ET AL. 2002. Restriction fragment length polymorphism of rRNA operons for discrimination and intergenic spacer sequences for cataloging of *Bacillus subtilis* sub-groups. *J.Microbiol.Methods.* Vol. 50(2): 215-223.
Restriction fragment length polymorphism of rRNA operons (RFLP) and 16S-23S rRNA intergenic region (ISR) sequences of *Bacillus subtilis* subsp. *subtilis*, B. *subtilis* subsp. spizizenii, and *B. atrophaeus* were compared. ISR sequences of the B. *subtilis* subspecies were extremely similar (W23 versus 168 rrn H, J, G,W; 96.8%; rrn D, E; 98.4%; rrnB; 97.9%) and, therefore, not useful for their differentiation. However, RFLP of rRNA operons of the B. *subtilis* subspecies were distinct in terms of numbers and organization within the genome (e.g. the 168 sub-group generally contained 8.3- and 8.0-kb fragments absent in the W23 sub-group). The more distantly related *B. atrophaeus* was distinct from both B. *subtilis* subspecies in terms of ISR sequence and rRNA operon number and organization. RFLP of rRNA operons discriminates the two sub-groups of *Bacillus subtilis* that are indistinguishable by ISR sequence. However, ISR sequence defines the relatedness of B. *subtilis* to other species (e.g. *B. atrophaeus*) within the genus *Bacillus*.

SHAW, et al. 1999. Protein Engineering of Alpha-Amylase for Low pH Performance. *Curr.Opin.Biotechnol.* Vol. 10(4) : 349-352.
Industrial-scale starch liquefaction is currently constrained to operating at pH 6.0 and above, as the enzyme used in the process, *Bacillus licheniformis* alpha-amylase, is

unstable at lower pH under the conditions used. There is a need to develop an enzyme that can operate at lower pH. Recent progress has been made in engineering the *B. licheniformis* enzyme for improved industrial performance. The availability of crystal structures and subsequent analysis of improved variants, in a structural context, is revealing common factors and a rationale to make further improvements.

SOROKULOVA et al. 2003. Genetic diversity and involvement in bread spoilage of Bacillus strains isolated from flour and ropy bread. *Lett Appl Microbiol*. 37(2):169-73. AIMS: To study Bacillus contamination of wheat flour and ropy bread, to analyse genetic diversity of isolated strains and to evaluate the ability of these strains to produce ropy bread. METHODS AND RESULTS: Classical and molecular methods [16S rDNA sequencing and random amplified polymorphic DNA (RAPD)-PCR] were used to identify and type-isolated strains. The predominant species isolated were *Bacillus subtilis* and *B. licheniformis*. RAPD analysis demonstrated that the same sample may harbor different strains. Ten of 15 strains of B. subtilis and four of six strains of B. licheniformis were able to cause rope spoilage of the laboratory-baked bread. CONCLUSION: RAPD typing can be useful in the tracking of Bacillus strains during bakery processing and in the understanding of the role of different Bacillus strains in the rope spoilage of bread. SIGNIFICANCE AND IMPACT OF THE STUDY: The results indicate the variability of Bacillus strains isolated from flour and responsible for rope spoilage of bread.

STEIN, ET AL. 2004. Subtilosin production by two *Bacillus subtilis* subspecies and variance of the sbo-alb cluster. *Appl.Environ.Microbiol.* Vol. 70(4): 2349-2353. Eight different *Bacillus subtilis* strains and *Bacillus atrophaeus* were found to produce the bacteriocin subtilosin A. On the basis of the subtilosin gene (sbo) sequences two distinct classes of B. *subtilis* strains were distinguished, and they fell into the two B. *subtilis* subspecies (B. *subtilis* subsp. *subtilis* and B. *subtilis* subsp. spizizenii). The entire sequence of the subtilosin gene cluster of a B. *subtilis* subsp. spizizenii strain, B. *subtilis* ATCC 6633, was determined. This sequence exhibited a high level of homology to the sequence of the sbo-alb gene locus of B. *subtilis* 168. By using primer extension analysis the transcriptional start sites of sbo in B. *subtilis* strains ATCC 6633 and 168 were found to be 47 and 45 bp upstream of the sbo start codon, respectively. Our results provide insight into the incipient evolutionary divergence of the two B. *subtilis* subspecies.

SUGAR & MCCLOSKEY 1977. *Bacillus Licheniformis* Sepsis. *JAMA.* Vol. 238(11) : 1180-1181.

SUIHKO, et al. 2003. Identification of Aerobic Mesophilic Bacilli Isolated from Board and Paper Products Containing Recycled Fibres. *J.Appl.Microbiol.* Vol. 94(1) : 25-34. AIMS: To identify aerobic mesophilic bacteria isolated from coreboard, kitchen roll paper and food packaging boards containing recycled fibres and to create a rapid

fingerprint-based database for their identification. METHODS AND RESULTS: A total of 197 isolates and 20 relevant type strains were characterized by automated ribotyping and as far as possible identified by the similarities of their riboprints to the relevant type strains. One strain from each unidentified ribotype, a total of 87 strains, was subjected to partial 16S rDNA sequencing and in most cases also to fatty acid analysis and physiological tests. From the isolates 113 and seven different ribotypes were generated belonging to the genera *Bacillus* and Paeni*bacillus*, respectively. The dominating species, or closest related to them, were B. simplex (22.8% of isolates), *B. licheniformis* (18.3%) and *B. a*myloliquefaciens (12.7%); 5.1% of the isolates were identified as B. cereus, a potential food-borne pathogen. In particular, this species was present in one food packaging board (26.3% of isolates). Based on these results, 40.1% of the isolates and 45.0% of ribotypes were so different from the relevant type strains that they may represent novel species. CONCLUSIONS: All isolates were aerobic spore-formers, indicating that all non-spore-formers were eliminated during the drying stage of the processes. Although many isolates could be affiliated to described species of *Bacillus* or Paeni*bacillus*, a significant proportion of the isolates could not be identified unambiguously as members of a described species. SIGNIFICANCE AND IMPACT OF THE STUDY: A RiboPrint identification database, composed of 120 composite patters, was established for bacteria originating from the pulp and paper industry. Considering the discrimination power of ribotyping, this database will be extremely useful in future for the reliable and rapid identification of bacteria isolated from pulp and paper industrial sources.

SUOMINEN, et al. 2001. Toxic *Bacillus* Pumilus from Indoor Air, Recycled Paper Pulp, Norway Spruce, Food Poisoning Outbreaks and Clinical Samples. *Syst.Appl.Microbiol.* Vol. 24(2) : 267-276.

Forty-four B. pumilus isolates of food poisoning, clinical, environmental and industrial origins were investigated for toxin production using the boar spermatozoan motility assay, previously shown to be a sensitive method for detecting non-protein toxins from B. cereus and *B. licheniformis*. The three toxic isolates originated from live tree, indoor air and recycled paper pulp and were more toxic than the previously described food poisoning isolates of *B. licheniformis*, whereas the B. pumilus food poisoning and clinical isolates were lower in toxicity. The type strain also produced inhibitory substances. The toxic substances were insensitive to heat (100 degrees C, 20 min), to pH 2 or pH 10 and to digestion with pronase. The substances were readily soluble in methanol and chloroform, but less soluble in toluene. Exposure of boar spermatozoa to 1-10 microg ml(-1) (EC50) of methanol soluble substance from the four strains disrupted the plasma membrane permeability barrier, induced abnormalities in the postacrosomal sheath,

collapsed the mitochondrial and suppressed cytoplasmic NAD reduction. No change was observed in human peripheral blood lymphocytes exposed to concentrations of B. pumilus extract that affected spermatozoa. The toxin producing isolates were 99.4 to 99.6% similar in 16SrDNA (500 bp) to the type strain and could not be distinguished from the 41 non-toxic isolates by biochemical properties or whole cell fatty acid composition.

TABBARA, et al. 1979. *Bacillus Licheniformis* Corneal Ulcer. *Am.J.Ophthalmol.* Vol. 87(5) : 717-719.
A 46-year-old woman developed a corneal ulcer after an injury to the right eye. Cultures were positive for *Bacillus licheniformis*. Although resistant to some antibiotics, the corneal ulcer resolved after treatment with topical, subconjunctival, and intramuscular gentamicin sulfate.

TAM, ET AL. 1998. Genes encoding thymidylate synthases A and B in the genus *Bacillus* are members of two distinct families. *Mol.Gen.Genet.* Vol. 258(4): 427-430.
Bacillus subtilis strain 168 is known to possess two genes that encode thymidylate synthases, thyA and thyB. We have identified genes similar to the thyA and thyB genes in several *Bacillus* strains by Southern hybridization and by DNA amplification with sequence-specific primers. Analysis of thyA genes cloned from B. *subtilis* W23 strain 2A6, B. *subtilis* ATCC6633, *B. a*myloliquefaciens S18 and *B. atrophaeus* S223 reveals that they are very similar to the thyA genes from B. *subtilis* 168 and its phage phi3T, but differ considerably from the majority of known prokaryotic and eukaryotic thymidylate synthases.

THOMPSON, et al. 2003. Identification of Bacterial Spores using Statistical Analysis of Fourier Transform Infrared Photoacoustic Spectroscopy Data. *Appl.Spectrosc.* Vol. 57(8) : 893-899.
Fourier transform infrared photoacoustic spectroscopy (FTIR-PAS) has been applied for the first time to the identification and speciation of bacterial spores. A total of forty specimens representing five strains of *Bacillus* spores (*Bacillus subtilis* ATCC 49760, *Bacillus atrophaeus* ATCC 49337, *Bacillus subtilis* 6051, *Bacillus* thuringiensis subsp. kurstaki, and *Bacillus globigii* Dugway) were analyzed. Spores were deposited, with minimal preparation, into the photoacoustic sample cup and their spectra recorded. Principal component analysis (PCA), classification and regression trees (CART), and Mahalanobis distance calculations were used on this spectral library to develop algorithms for step-wise classification at three levels: (1) bacterial/nonbacterial, (2) membership within the spore library, and (3) bacterial strain. Internal cross-validation studies on library spectra yielded classification success rates of 87% or better at each of these three levels. Analysis of fifteen blind samples, which included five samples of

spores already in the spectral library, two samples of closely related *Bacillus globigii* 01 spores not in the library, and eight samples of nonbacterial materials, yielded 100% accuracy in distinguishing among bacterial/nonbacterial samples, membership in the library, and bacterial strains within the library.

THURN, et al. 1988. Post-Traumatic Ophthalmitis due to *Bacillus Licheniformis*. *Am.J.Med.* Vol. 85(5) : 708-710.

TRAN, et al. 1999. Phage Abortive Infection of *Bacillus Licheniformis* ATCC 9800; Identification of the abiBL11 Gene and Localisation and Sequencing of its Promoter Region. *Appl.Microbiol.Biotechnol.* Vol. 52(6) : 845-852.
The virulent bacteriophage BL11 infects almost all *Bacillus licheniformis* strains tested, including the industrial bacitracin-producing *B. licheniformis* 19. *B. licheniformis* ATCC 9800, however, was virtually insensitive to phage BL11 infection, and all of the few surviving progeny phages proved to be mutants. The phage-resistance mechanism was neither inhibition of adsorption, nor restriction or exclusion provided by a resident prophage, but was, instead, of another type. Phage BL11 adsorbed well on to ATCC 9800 cells, its DNA was injected, but replication of phage DNA was inhibited and the infected cells died. Thus, the mechanism of phage resistance was identified as abortive infection (AbiBL11). The so-called abiBL11 gene was identified on the chromosome of strain ATCC 9800 by Tn917PF1 transposon mutagenesis. Part of the abiBL11 gene from the phage-sensitive ATCC 9800::Tn917PFI was cloned. Gene-disruption analysis, based on Campbell-type integration, showed that a 0.3-kb EcoRI fragment contained the 5' end of abiBL11. The promoter region of abiBL11 was identified using promoter- and terminator-probe plasmids. The deduced sequence (206 amino acids) of the N-terminal part of abiBL11 showed no significant homology to known abortive-infection genes, but did show homology to a Saccharomyces cerevisiae gene coding for a serine/threonine protein kinase (RCK1).

TURNBOUGH. 2003. Discovery of Phage Display Peptide Ligands for Species-Specific Detection of *Bacillus* Spores. *J.Microbiol.Methods.* Vol. 53(2) : 263-271.
Short peptides are capable of tight and specific binding to physiological or fortuitous receptors on the surface of cells. These peptides can be used to tag or capture target cells in an assortment of detector platforms. As part of an effort to identify small-molecule ligands for advanced detectors for spores of *Bacillus* anthracis, the causative agent of anthrax, we are screening (or biopanning) commercial phage display peptide libraries for peptides that bind tightly and selectively to spores of several *Bacillus* species. In addition to *B. a*nthracis, these species include B. cereus, B. *subtilis*, and *B. globigii*. This review

summarizes the methods used in our studies, the results from the biopanning experiments, and the characterization of the spore-binding peptides identified to date. Briefly, several unique families of peptides, with consensus sequencesandlt; or = seven-amino-acids long, were identified that exhibit preferential binding to spores (but not vegetative cells) of either one or only a few *Bacillus* species. At least one peptide family binds well to spores of multiple strains of *B. a*nthracis, while binding poorly or not at all to spores of phylogenetically similar species. This review also discusses other points of interest regarding the use of peptide ligands for spore detection and for the detection of other types of cells.

VARDAXIS, et al. 1997. Sporicidal Activity of Chemical and Physical Tissue Fixation Methods. *J.Clin.Pathol.* Vol. 50(5) : 429-433.
AIMS: The effects of alcohol based fixation and microwave stimulated alcohol fixation were investigated on spores of *Bacillus* stearothermophilus and *Bacillus subtilis* (*var. niger*). METHODS: Spores were exposed to 10% formalin, or different concentrations of various alcohol containing fixatives (Kryofix/Spuitfix). Adequate controls were also set up in conjunction with the test solutions. The spores were immersed with and without adjunctive microwave stimulation in the various solutions tested. Possible surviving spores were recovered in revival broth and after incubation, and Gram staining viable counts were performed. RESULTS: Alcohol based fixatives did not have a sporicidal effect on B stearothermophilus or B *subtilis* (*var. niger*) spores, and microwave stimulated alcohol fixation at 450 W and up to 75 degrees C did not have a sporicidal effect. CONCLUSIONS: When alcohol based fixatives are used for fixation, precautions should be taken with the material thus treated, as it may contain viable spores or other pathogens, which are destroyed after 24 hours of formalin treatment. Of the physicochemical methods tested involving microwaving, none was successful in eliminating viable spores from the test material.

WAINWRIGHT, et al. 2003. Is this the Historical 'Cancer Germ'? *Med.Hypotheses.* Vol. 60(2) : 290-292.
A highly pleomorphic bacterium, isolated from a canine mammary tumour was found to share many characteristics with recently described cancer-related bacteria and with the Glover organism, a historically important 'cancer germ'. We suggest that both the Glover organism, and possibly other cancer-related bacteria, are likely to be a strain of the highly pleomorphic bacterium, *Bacillus licheniformis*.

WATTIAU, ET AL. 2001. A PCR test to identify *bacillus subtilis* and closely related species and its application to the monitoring of wastewater biotreatment.

Appl.Microbiol.Biotechnol. Vol. 56(5-6): 816-819.
A PCR test based on the 16S rRNA gene was set up that could identify any of the five species of the '*Bacillus subtilis* group' (B. *subtilis*, B. pumilus, *B. atrophaeus*, *B. l*ichenijormis and *B. a*myloliquefaciens). The test was directly applicable to single colonies and showed excellent specificity. In the mixed population context of wastewater analysis, direct detection of the target *Bacillus* species by PCR on either crude or purified DNA extracts had poor sensitivity. When assayed on cell suspensions derived from enriched wastewater samples, sensitivity was increased. Using a simple calibration method, it was possible to estimate the proportion of the target organisms. This method was found suitable for easy monitoring of a wastewater bioaugmentation experiment carried out with a mixture of sporulated *Bacillus* strains.

WEBER, ET AL. 2003. Efficacy of selected hand hygiene agents used to remove *Bacillus atrophaeus* (a surrogate of *Bacillus* anthracis) from contaminated hands. *JAMA.* Vol. 289(10): 1274-1277.
CONTEXT: The intentional use of *Bacillus* anthracis transmitted via the US mail in October-November 2001 resulted in 22 people developing inhalation or cutaneous anthrax. Glove use with handwashing prior to and after contact with potential contaminated environmental surfaces and cutaneous lesions has been recommended. However, only limited data are available on the susceptibility of B anthracis to antiseptics. OBJECTIVE: To evaluate the efficacy of several hand antiseptics (interventions) and soap and water (control) against *Bacillus atrophaeus*, a surrogate of B anthracis. DESIGN, SETTING, AND PARTICIPANTS: Challenge study conducted among healthy adult volunteers, using the Standard Test Method for Evaluation of the Effectiveness of Health Care Professional Handwash Formulations (American Society for Testing and Materials E 1174-94) to determine the efficacy of various hand hygiene products at wash times of 10, 30, and 60 seconds. Volunteers were excluded if they had eczema, psoriasis, or other chronic skin conditions; nonintact skin; or allergies to any study agent. Study agents were a waterless rub containing 61% ethyl alcohol, a 2% chlorhexidine gluconate preparation, and an antibacterial microfiber towel that releases hypochlorite. A nonantimicrobial soap was used as a control. MAIN OUTCOME MEASURE: Reduction of B *atrophaeus* spores (log10 CFU/mL) on contaminated hands. RESULTS: Washes of 10, 30, and 60 seconds with either soap and water or 2% chlorhexidine gluconate eliminated 1.5 to 2.0 log10 CFUs/mL of B *atrophaeus* spores at wash 3. Mean reductions (95% confidence intervals) with 10-, 30-, and 60-second washes with soap and water were 2.4 (2.2-2.5), 2.3 (2.2-2.4), and 2.1 (1.9-2.4) log(10) CFUs/mL, respectively; and with 2% chlorhexidine gluconate, 2.1 (2.0-2.3), 1.8 (1.5-2.0), and 1.7 (1.5-1.9) log10 CFUs/mL, respectively. Handwashing with chlorine-containing towels was increasingly effective as the wipe time increased; reductions at 10, 30, and 60 seconds were 1.3 (1.1-1.5), 1.6 (1.2-2.0), and 2.2 (2.1-2.2) log10 CFUs/mL, respectively. A waterless rub containing 61% ethyl alcohol was ineffective in eliminating B *atrophaeus* spores at all times tested (0 [-0.1 to 0.1], -0.2 [-0.3 to -0.1], and 0 [-0.2 to 0.2] log10 CFUs/mL). CONCLUSIONS: In this evaluation of hand hygiene agents, handwashing with soap and water, 2% chlorhexidine gluconate, or chlorine-containing

towels reduced the amount of B *atrophaeus* spore contamination, whereas use of a waterless rub containing ethyl alcohol was not effective in removing spores.

WEBER, et al. 1988. In Vitro Susceptibility of *Bacillus* Spp. to Selected Antimicrobial Agents. *Antimicrob.Agents Chemother.* Vol. 32(5) : 642-645.
Although often dismissed as contaminants when isolated from blood cultures, *Bacillus* spp. are increasingly recognized as capable of causing serious systemic infections. As part of a clinical-microbiological study, 89 strains of *Bacillus* spp. isolated from clinical blood cultures between 1981 and 1985 had their species determined and were tested for antimicrobial agent susceptibility to 18 antibiotics. Species of isolates were determined by the API 50CH and API 20E systems. *Bacillus* cereus (54 strains) was the most common species isolated, followed by B. megaterium (13 strains), B. polymyxa (5 strains), B. pumilus (4 strains), B. *subtilis* (4 strains), B. circulans (3 strains), *B. a*myloliquefaciens (2 strains), *B. licheniformis* (1 strain), and *Bacillus* spp. (3 strains). Microdilution MIC susceptibility tests revealed all B. cereus strains to be susceptible to imipenem, vancomycin, chloramphenicol, gentamicin, and ciprofloxacin. Non-B. cereus strains were most susceptible to imipenem, vancomycin, LY146032, and ciprofloxacin. Disk susceptibility testing suggested that B. cereus was rarely susceptible to penicillins, semisynthetic penicillins, or cephalosporins with the exception of mezlocillin. In contrast, many non-B. cereus strains were susceptible to penicillins, semisynthetic penicillins, and cephalosporins, but marked variability was noted among species.

WRIGHT, et al. 1978. Water-Borne *Bacillus Licheniformis* Infection in Mice. *Lab.Anim.* Vol. 12(3) : 149-150.
A water-borne *Bacillus licheniformis* infection was associated with depressed haemoglobin content, white cell and platelet count. The epidemic was resolved by changing from tanked to mains water supply.

XU, ET AL. **2003.** Phylogenetic relationships between *Bacillus* species and related genera inferred from comparison of 3' end 16S rDNA and 5' end 16S-23S ITS nucleotide sequences. *Int.J.Syst.Evol.Microbiol.* Vol. 53(Pt 3): 695-704.
The nucleotide sequences of the 3' end of the 16S rDNA and the 16S-23S internal transcribed spacer (ITS) of 40 Bacillaceae species were determined. These included 21 *Bacillus*, 9 Paeni*bacillus*, 6 Brevi*bacillus*, 2 Geo*bacillus*, 1 Marini*bacillus* and 1 Virgi*bacillus* species. Comparative sequence analysis of a 220 bp region covering a highly conserved 150 bp sequence located at the 3' end of the 16S rRNA coding region and a conserved 70 bp sequence located at the 5' end of the 16S-23S ITS of the 40 species and six sequences available in GenBank were used to infer the phylogenetic relationships between all 46 taxa. When a maximal distance (D(max), where D refers to the number of nucleotide substitutions per site) of 0.31 was introduced as a threshold to

determine groupings, 10 phylogenetically distinct clusters were revealed. Twenty-six *Bacillus* species were separated in seven groups (I, II, III, IV, V, VI and X), but *Bacillus* circulans remained ungrouped. All six Brevi*bacillus* species under study were in Group VII. The nine Paeni*bacillus* species fell into two distinct groups (VIII and IX). Species with D(max) values within 0.05 were considered to be very closely related. These were *Bacillus* psychrophilus and *Bacillus* psychrosaccharolyticus in Group II; '*Bacillus* maroccanus' and *Bacillus* simplex in Group II; *Bacillus* amyloliquefaciens, *Bacillus atrophaeus*, *Bacillus* mojavensis and *Bacillus subtilis* in Group VI; *Bacillus* fusiformis and *Bacillus* sphaericus in Group VI; Brevi*bacillus* brevis and Brevi*bacillus* formosus in Group VII; Paeni*bacillus* gordonae and Paeni*bacillus* validus in Group VIII; and *Bacillus* anthracis, *Bacillus* cereus, *Bacillus* mycoides and *Bacillus* thuringiensis in Group X. The phylogenetic classification presented here is, in general, in agreement with current classifications based on phenotypic and molecular data. Our findings suggest, however, that in some cases, further divisions or, conversely, further groupings might be warranted. Should current classifications be re-examined in the light of our results, D(max) values of 0.31 and 0.05, as exemplified here, may prove useful threshold values for the grouping of Bacillaceae into taxa akin to genera and species, respectively. These D(max) thresholds may also reveal, in a different way, bacterial species for which further characterization might be warranted for proper classification and/or reassignment.

YOUNG, et al. 1982. Postoperative Neurosurgical Infections due to *Bacillus* Species. *Surg.Neurol.* Vol. 18(4) : 271-273.

The cases of 2 patients with postoperative ventriculitis due to *Bacillus* species bacteria are presented. *Bacillus licheniformis* was isolated from one patient following removal of an intraventricular meningioma, and *Bacillus* cereus from another patient following placement of a ventriculoperitoneal shunt. Both isolates were resistant to a variety of antibiotics, but both were sensitive to gentamicin and chloramphenicol. These cases emphasize several points; (a) *Bacillus* species, usually thought to be nonpathogenic, may produce intracranial infections; (2) species identification is important for epidemiological purposes and for the selection of appropriate chemotherapeutic agents; and (3) in cases of suspected ventriculitis, chloramphenicol or gentamicin should be considered for Gram's staining revealing gram-positive bacilli. In addition, we recommend that when planning antibiotic prophylactic regimens, consideration should be given to including one of these agents to assure coverage of *Bacillus* species.

ZHOU, et al. 2002. Human Antibodies Against Spores of the Genus *Bacillus*: A Model Study for Detection of and Protection Against Anthrax and the Bioterrorist Threat. *Proc.Natl.Acad.Sci.U.S.A.* Vol. 99(8) : 5241-5246.

A naive, human single-chain Fv (scFv) phage-display library was used in bio-panning against live, native spores of *Bacillus subtilis* IFO 3336 suspended in solution. A direct in vitro panning and enzyme-linked immunosorbent assay-based selection afforded a panel

of nine scFv-phage clones of which two, 5B and 7E, were chosen for further study. These two clones differed in their relative specificity and affinity for spores of B. *subtilis* IFO 3336 vs. a panel of spores from 11 other *Bacillus* species/strains. A variety of enzyme-linked immunosorbent assay protocols indicated these scFv-phage clones recognized different spore epitopes. Notably, some spore epitopes markedly changed between the free and microtiter-plate immobilized state as revealed by antibody-phage binding. An additional library selection procedure also was examined by constructing a Fab chain-shuffled sublibrary from the nine positive clones and by using a subtractive panning strategy to remove crossreactivity with *B. licheniformis* 5A24. The Fab-phage clone 52 was improved compared with 5B and was comparable to 7E in binding B. *subtilis* IFO 3336 vs. *B. licheniformis* 5A24, yet showed a distinctive crossreactivity pattern with other spores. We also developed a method to directly detect individual spores by using fluorescently labeled antibody-phage. Finally, a variety of "powders" that might be used in deploying spores of *B. a*nthracis were examined for antibody-phage binding. The strategies described provide a foundation to discover human antibodies specific for native spores of *B. a*nthracis that can be developed as diagnostic and therapeutic reagents.