November 23, 2021 Sporistatic and Sporicidal Effects of Food Additives

Request: We need a literature review on the mechanisms for sporistatic and sporicidal effects of food additives.

Response: While there are several reviews the examine general mechanisms of pharmaceutical sporistatic and sporicidal agents such as Russell (1983), fewer papers specifically looked at the sporicidal or sporistatic mechanisms of food additives.

***Bacillus* spp.:**

A recent review (Cho and Chung, 2020) contains a discussion of some of the food additives that have sporicidal activity against *Bacillus* spp., with some discussion of potential mechanisms related to surfactant-like molecules and the spore structures targeted.

***Bacillus anthracis*:**

Nisin "utilizes lipid II [an essential intermediate for cell wall biosynthesis] as the germinated spore target during outgrowth inhibition, and nisin-mediated membrane disruption is essential to inhibit spore development into vegetative cells" for *B. anthracis* (Gut et al., 2011).

***Bacillus cereus*:**

Chitosan, lactic acid, fermented pollen, and grapefruit extract did not inactivate *B. cereus* spores on their own. However, together with heat at 85°C for 30 minutes, these antimicrobials inactivated *B. cererus* spores more than 1 log beyond the inactivation achieved with heat treatment alone. Higher concentrations of lactic acid and thamine dilaurylsulfate for longer periods of time were able to inactivate *B. cereus* spores; 2.5% thiamine dilaurylsulfate was the only antimicrobial with significant activity against *B. cereus* spores at room temperature (~1 log reduction in spores numbers following 24 hour incubation at room temperature). The mechanism of the inactivation may have involved spore germination by the antimicrobials, followed by inactivation by heat or by the remaining antimicrobials (Shin et al., 2008).

Bacillus subtilis:

The sporicidal activity of essential oils against *B. subtilis* was tested, with cardamom, tea tree, and juniper leaf showing the most efficacy. Efficacy was enhanced at higher temperatures or for longer incubations. Scanning EM was used to examine the spores after essential oil treatment, and the authors concluded the oils damaged the spore coat, with "leakage of spore contents" the likely mechanism of sporicidal action (Lawrence and Palombo, 2009).

Ethanolic extracts of various herbs demonstrated sporicidal activity against *B. subtilis* spores. The researchers proposed that the herbal extracts acted as hydrophobic surfactants, damaging the protective spore coat and "allow the antimicrobial components to attack the genetic material inside" (Cho and Chung, 2017).

The same study looked at the sporicidal activity of lactic acid at pH 4 and 5. The spore count was reduced by 99.99% at pH 4, according to the authors, but only 92% at pH 5. The authors concluded that "the killing effect against intact spores on low pH condition was considered to be caused by the unstable growth environment due to changes in the ion composition, and basic mechanism was similar to the inactivation of bacteria" (Cho and Chung, 2017).

The same study looked at both the herbal extract, acidified with lactic acid to a pH of ~4-5. Spore values were reduced 3-4 log, greater than either the herb or acid alone. The authors speculated that the surfactant-like components of the essential oils (beta-pinene and para-cymene) bound to the outer spore coat, and destroyed it, allowing the acid to penetrate to the interior of the spore and "generating dissipation of proton-motive force and inability to maintain internal pH followed by denaturation of acid-sensitive proteins and DNA" (Cho and Chung, 2017).

***Clostridium botulinum*:**

Sorbate inhibits the emergence of vegetative cells from spores during outgrowth, possibly by inhibiting metabolic activity through actions on enzymes or transports systems. It also inhibits germination of both *Clostridium* spp. and *Bacillus* spores. Early studies suggested that potassium sorbate may have acted as a competitive inhibitor of amino acid-induced bacterial spore germination. Another study concluded that sorbate inhibits "a post-germinant binding reaction(s) in the process of bacterial spore germination." These studies are all discussed in detail from a mechanistic point of view in Sofos (1986) (Sofos et al., 1986).

Nitrite affects germination and outgrowth of *C. botulinum* spores, with potential mechanisms reviewed in (BENEDICT, 1980).

Extracts (aqueous and ethanolic) of plant extracts with sodium nitrite were shown to prevent spore germination, with the more hydrophobic extracts more likely to be effective (Cui et al., 2010).

Clostridium perfringens

(Talukdar et al., 2017) is an excellent review that discusses various inactivation strategies against *Clostridium perfringens* spores, although it does not focus on mechanistic details.

Vinegar with citrus extract and lemon juice are effective at inhibiting spore germination; the discussion section of one paper in the literature discusses the possible synergistic effects of organic acids and limonene on preventing *C. perfringens* spore germination (Smith et al., 2021).

Polyphosphates have been shown to inhibit *C. perfringens* growth, sporulation, and outgrowth. Researchers in one study demonstrated that while 1% STPP did not inhibit spore germination, it did significantly inhibit spore outgrowth. (Akhtar et al., 2008).

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