pH-elevating *Bacillus* spp. in acid pasteurised foods

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Introduction

Trends in consumer and industry processing are influencing packaging formats for acid foods, which in turn impact upon in-pack oxygen availability. For example, microwave cooking is influencing the growing popularity of (oxygen permeable) polymer containers over traditional (oxygen impermeable) glass jars and cans. Single-serve packages that boast a higher surface-area-to-volume ratio than larger (e.g. family-serve) packs are similarly growing in popularity.

Some strains of aciduric *Bacillus* spp. have been reported to elevate the pH of acid foods (usual pH <4.6)[1]. The change in pH due to growth of these organisms increases the likelihood of growth of *Clostridium botulinum*[2], which would not normally be regarded as a pathogen in acid foods.

In the past, concern has focused on facultative *Bacillus* spp. and pH elevation of (essentially anaerobic) canned foods[3]. Although growth of pH-elevating *Bacillus* spp. has been observed in acid foods under aerobic conditions[1, 4], the importance of these observations has been largely disregarded up to now, presumably as a consequence of the strong traditional association of *Cl. botulinum* with canned foods.

However, it has been demonstrated that storage in oxygen permeable packaging is inadequate to prevent toxin production by *Cl. botulinum* in low acid foods[5]. A concern then is that the increased partial pressure of oxygen at the seams or food-polymer interface of contemporary oxygen permeable packs might allow for the growth of some of the pH-elevating *Bacillus* spp. that have previously been dismissed as of scant importance in canned foods[6].

In turn, growth of these organisms in acid foods may result in an unanticipated safety hazard through metabiotic interaction with toxin-producing *Cl. botulinum.*

The metabiotic effect

Metabiosis is the phenomena whereby the growth of one organism has a favourable impact on the growth of another. Studies focussing on the metabiotic relationship between *Bacillus* spp. and *Cl. botulinum* in laboratory media and acid foods have proposed two major mechanisms to explain the favourable impact of the growth of *Bacillus* spp. on the growth of *Cl. botulinum*; these are:

- Elevation of pH by *Bacillus* spp., during growth, to a pH at which *Cl. botulinum* may grow[3].
- Removal of residual oxygen and lowering of redox potential by *Bacillus* spp., thus favouring the growth of (anaerobic) *Cl. botulinum*, and possibly even at pH values much less than 4[6, 7].

A case of mistaken identity

Bacillus licheniformis is most often cited in the literature as the species of greatest concern with respect to pH elevation of acid foods; however, this connection has been strongly influenced by an early study conducted by Fields *et al.*[3]. In this study, 21 isolates identified as *B. licheniformis* were shown to elevate the pH of tomato juice serum above 4[8]. However Hoffman[6], re-examining Fields' *B. licheniformis* isolates, suggested that the isolates were actually *Bacillus subtilis*, on the basis of biochemical studies, in particular propionate utilisation and anaerobic growth. The initial misidentification of Fields' isolates has subsequently been confirmed by the original research group[6], and independently by a third group of researchers[4].

While *B. licheniformis* is a facultative anaerobe, *B. subtilis* is generally regarded as an aerobe. However, growth of *B. subtilis* at low oxygen partial pressures has been demonstrated in in-pack inoculation studies, employing glass jars sealed at one end with various types of polymer with different oxygen permeabilities[6]. In these studies, *B. subtilis* demonstrated a superior ability over *B. licheniformis* strains to grow and elevate the pH of tomato juice (initially pH 4.4) at the food-polymer interface, at lower oxygen partial pressures[6].

Growth of Field's (now correctly identified) *B. subtilis* strains has also been compared with *B. licheniformis* in tomato juice[1]. Neither *B. subtilis* nor *B. licheniformis* strains could germinate and outgrow anaerobically in tomato juice (pH 4.4). However, *B. licheniformis* could do so under aerobic conditions when elevated inoculum levels (10^4 spores/mL) were used, while *B. subtilis* could do so, even from very low inoculum levels (1 spore/mL).

In all cases, an increase in pH of the tomato juice, as high as pH 8-9 after 60 days' growth at 35°C, was observed[1]. In liquid laboratory media, these same *B. subtilis* strains showed confluent surface growth, under which a zone of increased pH formed. In one medium, formation of an anaerobic zone directly beneath *Bacillus* surface growth allowed outgrowth of spores of co-inoculated *Cl. botulinum* and toxin production[2].

Bacillus subtilis – a threat to acid pasteurised foods in O_2 permeable packs?

Botulism arising from acid foods is a rare event, but 4.7% of outbreaks of botulism between 1899 and 1975 involved consumption of acid foods, and metabiotic interaction has been suggested as the cause of 8.6% of these[8]. The impact of the growing popularity of oxygen permeable packs on incidence of botulism from acid foods is unknown. Taking the collected literature on the topic, the most likely organism of concern for metabiotic interaction with *Cl. botulinum* in acid foods appears to be pH-elevating *B. subtilis*.

Recently in our laboratory, 25 aciduric pH-elevating *Bacillus* isolates were recovered from 10 of 18 soil and compost samples, using a modification of the method by Al Dujaili and Anderson[9]. Eight of these pH-elevating isolates were selected for further characterisation, and results of biochemical tests are compared with *Bacillus* species (Table 1).

One isolate, grown aerobically in tomato juice (pH 4.2), was able to raise the pH above 4.6 within 2 weeks, and above pH 8 within 5 weeks (Figure 1). 16sRNA sequencing indicates the isolate is most closely related to *B. subtilis.* The isolate has demonstrated ability to grow microaerophilically on laboratory media, but its ability to elevate pH in foods under low oxygen conditions has yet to be determined.

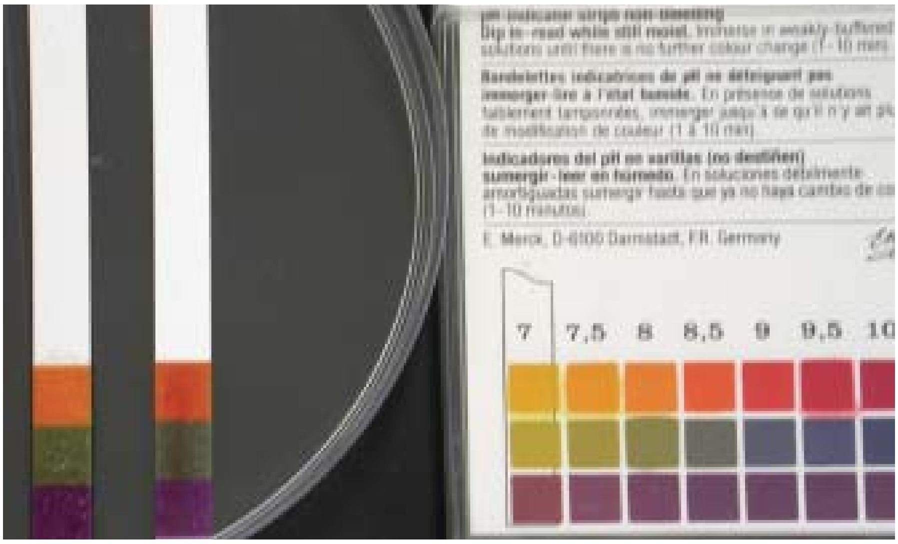

Figure 1. pH elevation of inoculated tomato juice indicated by change in colour of pH test strips (initial pH 4.2; results for duplicate samples shown after 5 weeks' incubation).

The mechanism by which aciduric *Bacillus* spp. elevate the pH of a food is unclear, but is likely to involve production of biogenic amines. pH-elevation will likely depend on factors including available growth substrates, initial pH and acidulant type, as well as prevailing oxygen and redox conditions. Improved understanding of the mechanism by which pH-elevation occurs could help to predict foods at risk, and may facilitate intelligent design of food preservation systems, including packaging selection.

References

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Table 1. Comparative biochemical characteristics of *Bacillus* spp. and pH-elevating isolates from compost and soil.

Biochemical tests	*B. coagulans*	*B. licheniformis*	*B. subtilis*	pH-elevating isolates
Anaerobic growth	Positive	Positive	Negative	Negative
Propionate utilisation	Negative	Positive	Negative	Negative (4 strains tested)
Glucose utilisation	Positive	Positive	Positive	Negative
Lysine decarboxylation	Negative	Negative	Negative	Negative
Proteolysis in litmus milk	Negative	Positive	Positive	Positive