5:9 Inhibition by a Lactobacillus of the growth of Brochothrix thermosphacta in mixed culture.

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Introduction

Whilst lactic acid bacteria dominate the flora of vacuum-packaged beef, Brochothrix thermosphacta may also be present (Pierson, Collins-Thompson and Ordal, 1970; Gill and Newton, 1978; Dainty et al., 1979). However there are a number of apparently conflicting reports concerning the ability of B.thermosphacta to grow on vacuum-packaged beef. These studies are discussed by Campbell et al. (1979) who showed that the amount of growth of this organism is controlled by a combination of two factors, the pH of the meat and the availability of oxygen. These results were obtained in pure culture.

In contrast, other studies have suggested that poor growth of B.thermosphacta in mixed culture is caused by the other bacteria present. Roth and Clark (1975) observed that the growth of B.thermosphacta on vacuum-packaged beef was restricted by lactobacilli and Newton and Gill (1978) showed that a Lactobacillus sp. inhibited the growth of B.thermosphacta on beef stored under anaerobic conditions.

Previously, Gill (1976) had shown that the amount of growth of a Lactobacillus sp. on beef was limited by the rate of diffusion of fermentable substrates from within the meat to the surface and this led Newton and Gill (1978) to suggest that Lactobacillus inhibited the growth of B.thermosphacta by competition for glucose. However when grown together under anaerobic conditiions in glucose-limited continuous culture B.thermosphacta displaced the Lactobacillus from the chemostat, presumably because it had a higher affinity for glucose. This result led those workers to conclude that the Lactobacillus sp. inhibited the growth of B.thermosphacta on meat by producing an antibacterial agent, however they were unable to demonstrate inhibition in liquid culture.

We have now examined in more detail the growth of B.thermosphacta and a Lactobacillus in pure and mixed culture both on beef of high pH and in glucose-limited continuous culture in a chemostat. Under aerobic conditions no significant interactions between the organisms were observed, but under anaerobic conditions the growth of B.thermosphacta was inhibited in the presence of the Lactobacillus. These results may be explained in terms of competition for glucose.

Materials and Methods

B.thermosphacta was maintained on nutrient agar slopes and Lactobacillus L13 (Shay and Egan, 1981) in cooked meat medium. Cultures were stored at 0°C. Growth of inocula, measurement of meat pH, preparation of meat samples, conditions of incubation and preparation of samples for viability determination have been described previously (Campbell et al., 1979). To determine the viable count of Lactobacillus L13 in pure culture, samples were plated on MRS Agar (Oxoid) and Tryptone Soya Agar (Oxoid) supplemented with 0.5% (w/v) yeast extract and 0.2% (w/v) glucose (TSYG agar). For B.thermosphacta in pure culture TSYG agar and STAA agar (Gardner, 1966) were used. For mixed cultures all three media were used.

A New Brunswick Bioflo C30 apparatus was used for continuous culture experiments as previously described (Hitchener, Egan and Rogers, 1979). The culture medium consisted of a mineral-salts basal medium (medium 56 of Monod, Cohen-Bazire and Cohn, 1951) used half-strength and supplemented with 0.3% (w/v) yeast extract and 0.1% (v/v) Tween 80. The medium was sterilized by autoclaving and after cooling sterile solutions of manganese sulphate (final concentration 0.3 mM) and glucose were added. The temperature for all continuous culture experiments was 25°C and that for the meat experiments was 5°C.

Results

Pieces of beef semitendinosus muscle, pH 6.5-6.7, inoculated with aerobically grown cells of B.thermosphacta and/or Lactobacillus L13, were incubated in Thunberg tubes at 5°C. Fig. 1a shows the results of a typical experiment where conditions of growth were aerobic. Starting from an initial population of ca. 10^4 cells/g of meat, B.thermosphacta and the Lactobacillus reached maximum populations in excess of 10^9/g and 10^8/g respectively. In all experiments, B.thermosphacta reached the higher population. In an experiment in which the initial population of B.thermosphacta was 10^5/g and that of the Lactobacillus only 10^4/g, there appeared to be a slight inhibition of the growth rate of the Lactobacillus but it still reached the same maximum population.

For similar experiments using anaerobic conditions, cells for use as inocula were also grown anaerobically. Under such conditions the Lactobacillus reached populations of 2 x 10^8 to 10^9/g in both pure and mixed culture (Fig. 1b). In contrast whilst the population of B.thermosphacta exceeded 10^8/g in pure culture its' growth in mixed culture was inhibited. Starting from an initial population of ca. 10^4/g it reached a maximum of ca. 6 x 10^6/g and then tended to decline on further storage. In an experiment where the Lactobacillus started at an initial count of 10^4/g and B.thermosphacta at 10^5/g, less inhibition was observed. In this experiment the Lactobacillus reached a maximum population of 2-3 x 10^8/g and B.thermosphacta reached ca. 5 x 10^7/g.

Lactobacillus L13 was grown in continuous culture in the chemostat at 25°C. With 3.7 mM glucose in the input medium the culture was glucose limited over a range of dilution rates from 0.1 to 0.5 h^{-1} and the population of cells was between 10^8 and 10^9/ml. Similar results were obtained under both aerobic and anaerobic conditions of growth, however most pure-culture experiments were done using anaerobic conditions.

When B.thermosphacta was grown in glucose-limited continuous culture in the chemostat at 25°C the growth characteristics were similar to those observed previously in a more detailed study (Hitchener, Egan and Rogers, 1979). In the present experiments it reached a maximum population in excess of 10^8/ml under both aerobic and anaerobic conditions.

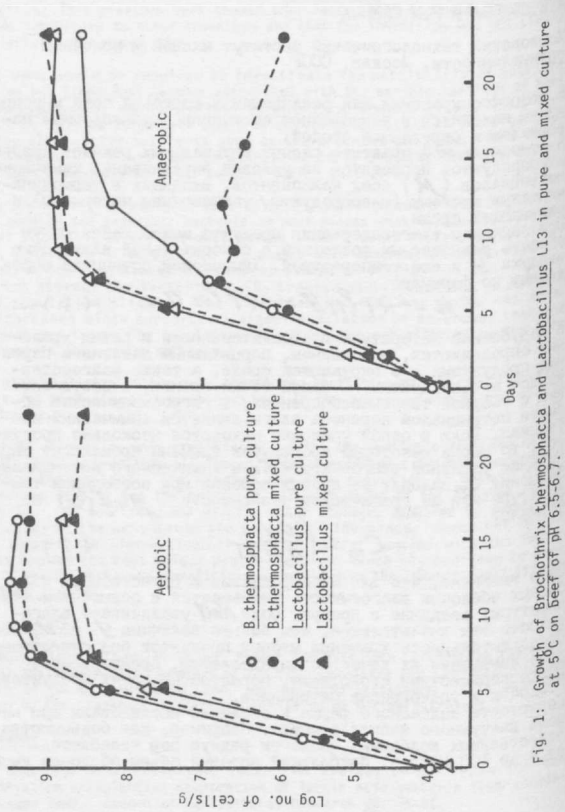

Fig. 1: Growth of Brochothrix thermosphacta and Lactobacillus L13 in pure and mixed culture at 5°C on beef of pH 6.5-6.7.

The results of a typical experiment in which the two organisms were grown together in mixed culture in the chemostat under conditions of glucose limitation are shown in Fig. 2. Under aerobic conditions, B.thermosphacta grew from an initial inoculum of ca. 10^6 cells/ml to a population in excess of 10^8/ml, in spite of the presence of a high population (ca. 2 x 10^8/ml) of the Lactobacillus. However when the culture was made anaerobic, the population of B.thermosphacta declined to between 10^6 and 10^7/ml. Re-establishment of aerobic conditions resulted in its' population increasing to the previous level. Under anaerobic conditions, B.thermosphacta was never completely eliminated from the chemostat but it continued to co-exist with a much greater population of the Lactobacilllus.

When the order of inoculation was reversed, similar results were obtained. The Lactobacillus grew from an initial populatin of ca. 10^6/ml to greater than 10^8/ml in spite of the presence of a high population of B.thermosphacta. Under aerobic conditions the increase in population of the Lactobacillus had no significant effect on the pre-existing population of B.thermosphacta and high populations (>10^8/ml) of each organism co-existed together. However if conditions were anaerobic at the time of introduction of the Lactobacillus then the population of B.thermosphacta declined to ca. 10^6/ml concomitant with the increase in the population of the Lactobacillus.

In further experiments the affinity of each organism for the growth-limiting substrate (glucose) was determined. The steady-state glucose concentration was measured over a range of dilution rates for pure cultures of each organism. The residual glucose concentration increased as the dilution rate was increased, until the maximum growth rate was reached at which point the culture commenced to "wash-out". The data so obtained was plotted in double reciprocal form (Lineweaver-Burk plot) in order to determine K_s for glucose. For B.thermosphacta it was found to be ca. 0.1 mM under aerobic conditions and 0.6 mM under anaerobic conditions. For Lactobacillus L13 it was ca. 0.1 mM in both cases.

Discussion

The continuous culture experiments show that the compositon of a mixed culture of the B.thermosphacta and Lactobacillus strains used was dependent upon the availability of oxygen. These results are consistent with competition for glucose being the major factor controlling the composition of the population. Under aerobic conditions, K_s for glucose was the same for both bacteria, but under anaerobic conditions the affinity of the facultative B.thermosphacta for glucose was reduced, allowing the Lactobacillus to dominate in mixed culture. B.thermosphacta was not completely eliminated from the culture under anaerobic conditions presumably because it was able to utilize a component of the yeast extract.

Our continuous culture experiments gave different results from those of Newton and Gill (1978). The reason for this is not known as those workers did not determine K_s values.

The fact that the population dynamics of the two organisms growing in mixed culture on meat was similar to those in continuous culture, suggests that competition for substrate may have been a controlling factor in that situation also. Although there is considerable evidence that lactobacilli inhibit the

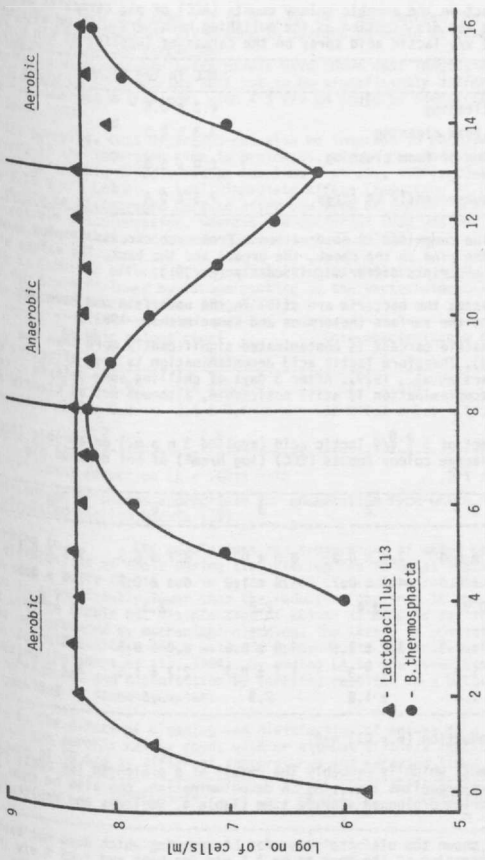

Fig. 2: Growth of Brochothrix thermosphacta and Lactobacillus L13 at 25°C in glucose-limited continuous culture in the chemostat.

growth of B.thermosphacta on both fresh (Roth and Clark, 1975; Newton and Gill, 1978) and processed meats (Collins-Thompson and Lopez, 1982), there appears to be no direct evidence for antibiotic production in these situations. In some studies it appears that environmental factors may not have been sufficiently closely controlled (discussed by Campbell et al., 1979) and the results presented here demonstrate that competition for substrate must also be considered as a mechanism of inhibition.

Further studies of substrate utilization by bacteria growing on meats are needed as part of wider studies on the interactions between organisms growing in mixed populations on meats. It seems likely that certain lactic acid bacteria may produce bacteriocins during growth on meat. The isolation of such strains would be of considerable importance in view of their potential for future use.

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