GROWTH OF *MICROBACTERIUM THERMOSPHACTUM* ON FRESH BEEF

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SUMMARY

Studies were made on the growth rate of *Microbacterium thermosphactum* at 5°C on vacuum-packaged and unpackaged fresh beef stored in air or CO_2-enriched air, to determine the role of this organism in spoilage and the effect of CO_2 on its growth. Tests were made with samples contaminated in the processing plant during operation and with samples inoculated with a mixture of 10 strains of *M. thermosphactum*. The organism did not grow on vacuum-packaged beef in the presence of the usual incident bacteria but did grow in the absence of other organisms. CO_2 concentrations above 20% retarded the growth rate of *M. thermosphactum* but pure CO_2 was necessary to completely inhibit growth. The results show that *M. thermosphactum* is a major spoilage organism in aerobically stored beef (about 25% of the total count), but is not a spoilage organism in vacuum-packaged beef. Inhibition of its growth in vacuum-packaged meat is possibly due to competition from other organisms.

INTRODUCTION

Since its isolation from fresh pork sausage in 1953 (10) *Microbacterium thermosphactum* has been found fairly frequently on the surface of fresh and spoiled meat. It has been isolated from irradiated chickens (11), beef (1,8,12), pork (7) and lamb (2). Its role as a spoilage organism on meats, however, has not been clearly elucidated although it has been found in large numbers in some cases and is lipolytic (5). Its association with various kinds of meats and ability to compete with the recognized spoilage types (e.g. pseudomonads and achromobacter), in some instances at least, suggests a general significance that has perhaps been overlooked.

Of particular interest is the organism's varying growth behavior at low O_2 concentrations, which raises questions regarding its role in the spoilage of vacuum-packaged meats. For example, Pierson *et al.* (8) reported that *M. thermosphactum* did not grow on beef vacuum-packaged in oxygen-impermeable film while Barlow and Kitchell (2) found it in high numbers on

lamb similarly packaged and Weidmann (12) found it growing to the exclusion of all other types on beef stored in nitrogen. Clearly, further studies are required to determine the growth rates of *M. thermosphactum* on meat surfaces and the physical and chemical factors that affect its growth.

This paper reports the results of studies made to determine (a) the growth rate of *M. thermosphactum* on the surface of naturally-contaminated fresh beef stored aerobically of vacuum-packaged in oxygen impermeable film, (b) the growth rate on beef in the absence of other bacteria and (c) the effect of CO_2 on growth. Tests done for b and c were made with pure cultures. The CO_2 studies were included because it has been frequently reported that CO_2 build-up in vacuum-packaged fresh beef may explain the reduced growth rate of spoilage organisms in such a package.

MATERIALS AND METHODS

Naturally-contaminated beef

All tests were made with approximately 1-lb samples obtained from a local packing plant which processes and vacuum-packages primal and subprimal cuts of fresh beef. The samples were cut from hind quarters ("ham inside") and rolled on the cutting-line belt (Cryovac line) during normal operation, to contaminate the freshly-cut surfaces with the incident microflora. Most of the samples were placed in separate vinyldene chloride - vinyl chloride copolymer (VC) bags* which were then evacuated and heat shrunk (Cryovac packager: vacuum drawn, 15 in of Hg; shrink conditions, 198°F/15 sec). The remainder of the samples were overwrapped with a layer of a gas-permeable polyvinyl chloride (PVC) film** such that the entire top surface of the samples was in contact with the film. The samples were stored at 5°C for up to 32 days and analyzed at 2-4 day intervals. Some samples were re-wrapped in PVC (gas permeable) at three different periods during the first 18 days of storage to determine the growth of *M. thermosphactum* after the vacuum-package was opened. The re-wrapped samples were also stored at 5°C and plated at 2-4 day intervals.

For the bacteriological analyses, 12 cm^2 of the surface of each sample was washed with 0.1% (w/v) peptone water using the spray gun technique (4). The resultant suspension was diluted with 0.1% peptone as required and surface plated on

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* Cryovac S; 0.002 in thich; oxygen permeability 10-30 $cc/m^2/atm/24$ hr; W.R. Grace and Co., Duncan, S.C., U.S.A.

**Vynar MWI; 0.00068 in thick; oxygen permeability 2,600-3,400 $cc/m^2/atm/24$ hr; TCF of Canada Ltd., Cornwall, Ontario.

APT agar (Difco) to determine the total aerobes and on STAA agar (6) to determine the number of cells of *M. thermosphactum*. The plates were incubated at 25°C for 48 hr.

Artifically inoculated beef

Ten strains of *M. thermosphactum* were selected at random from STAA plates used in the studies on naturally-contaminated beef. Positive identification was made by comparison studies with known strains. Tests were made with approximately 1-lb samples of lean meat cut from rump muscle ("round") of red-brand fresh beef (aged 4-5 days) obtained from a local packing plant. To minimize contamination the meat was trimmed with a sterile knife before excising samples and the knife was swabbed with ethyl alcohol and wiped dry with a sterile cloth after each cut. The top surfaces of the samples were inoculated evenly in a spray-type inoculating chamber (3) with a uniform mixture of the 10 strains of *M. thermosphactum*. The inoculum was prepared from 4-day-old cultures grown at 5°C on tryptone glucose extract agar (Difco). The inoculated samples were vacuum-packaged in VC using the Cryovac packager as described above and then stored at 5°C. For the bacteriological analyses 6 cm^2 of the inoculated surfaces were washed with 0.1% peptone water using the spray gun, and plated on tryptone glucose extract agar. The plates were incubated at 25°C for 48 hr.

Tests with CO_2

Beef samples were prepared and inoculated as described above and incubated unwrapped for up to 30 days at 5°C in a saturated atmosphere containing various concentrations of CO_2 (0 [air], 20, 50, 75, 100%). The incubators consisted of 8-liter desiccators. The gas mixtures were obtained by continuous mixing of metered flows of air and commercial grade CO_2 (Union Carbide, 99.8% pure). The mixtures were sterilized by filtering through cotton, humidified by bubbling through vertical 6-in columns of sterile water, and continually passed through the desiccators containing the meat samples. The concentrations of CO_2 were measured using a gas chromatograph (poropack T column) with a thermal conductivity detector. Bacteriological analyses were carried out as described above for the inoculated beef.

RESULTS AND DISCUSSION

Naturally-contaminated beef

M. thermosphactum constituted about 25% of the total bacterial count of the fresh beef obtained from the packing plant. During storage at 5°C, under aerobic conditions

(packaged in PVC film), the organism grew rapidly resulting in a final count of $10^8/cm^2$ (Fig. 1). However, when samples were vacuum-packaged in a gas-impermeable film (VC), *M. thermosphactum* failed to grow and even showed a slight decline in numbers. After repackaging from VC to PVC film, the organism began to grow but only slowly; its ultimate population was indirectly proportional to the length of time the meat sample was stored in the vacuum-package. For example, repackaging after 4 days of vacuum-packaged storage limited the ultimate population to 10% of that observed on samples held continuously under aerobic conditions.

Artifically inoculated beef

While *M. thermosphactum* did not grow on vacuum-packaged beef in the presence of other naturally occurring microorganisms, it did grow on vacuum-packaged meat when only strains of this organism were present (Fig. 2). However, the growth rate, as shown by the generation time during the log phase, was slower than that of the same mixture growing on beef under aerobic conditions (Table 1). Also, the maximum population was less than 10% of the maximum count observed under aerobic conditions, either as part of the natural flora or as pure cultures.

The relative importance of *M. thermosphactum* as a spoilage organism of unpackaged and vacuum-packaged fresh beef can be seen from the data on generation times and population densities in Table 1. While it appears to play an important role in the aerobic spoilage of beef, this organism does not appear to present a spoilage threat to beef vacuum-packaged in a gas-impermeable film.

Effect of CO_2

The results of the CO_2 storage tests indicated that CO_2 was not the cause of growth inhibition in the vacuum-packaged naturally-contaminated samples (Fig. 3). A 20% concentration of CO_2, which inhibits the growth of most aerobic meat spoilage organisms, had very little effect on the log phase and had little or no effect on the growth rate during the logarithmic phase. Even at 75% CO_2 the organism grew well reaching a count of $1 \times 10^8/cm^2$ within 20 days. Since published evidence indicates that the CO_2 content of a gas-impermeable meat pack does not increase above 30% (7) it appears that some factor other than CO_2 concentration inhibits the growth of *M. thermosphactum* on vacuum-packaged meat.

As a matter of general interest, a 100% CO_2 atmosphere had a definite inhibitory effect. There was an initial decline in numbers for the 1st 10 days followed by a very slow increase during the remaining 15 days of storage, but the count did not exceed the original inoculum level (3 x

$10^4/cm^2$). Poor growth in 100% CO_2 appeared to be due to an effect of CO_2 *per se* and not a lack of O_2 since it was observed in other tests that this mixture of strains grew rapidly in a pure nitrogen atmosphere.

CONCLUSIONS

The results show that *M. thermosphactum* comprised a substantial proportion (25%) of the total bacterial population that developed on aerobically-stored fresh beef but that the organism did not grow on vacuum-packaged beef in the presence of competing organisms. This ability to grow rapidly under aerobic conditions coupled with possession of lipolytic properties (5) indicates that *M. thermosphactum* can be an important spoilage organism of beef stored in air. Its inhibition in a vacuum package does not appear to be the result of a CO_2 build-up since *M. thermosphactum* tolerates high concentrations of CO_2 and grows on vacuum-packaged beef in pure culture. It seems plausible that growth inhibition in a vacuum-package is the result of antagonism or competition from other organisms. Lactobacilli may be the organisms responsible for this effect since they have been found to predominate the flora of vacuum-packaged beef (8,9). Work is underway in this laboratory to investigate this possibility.

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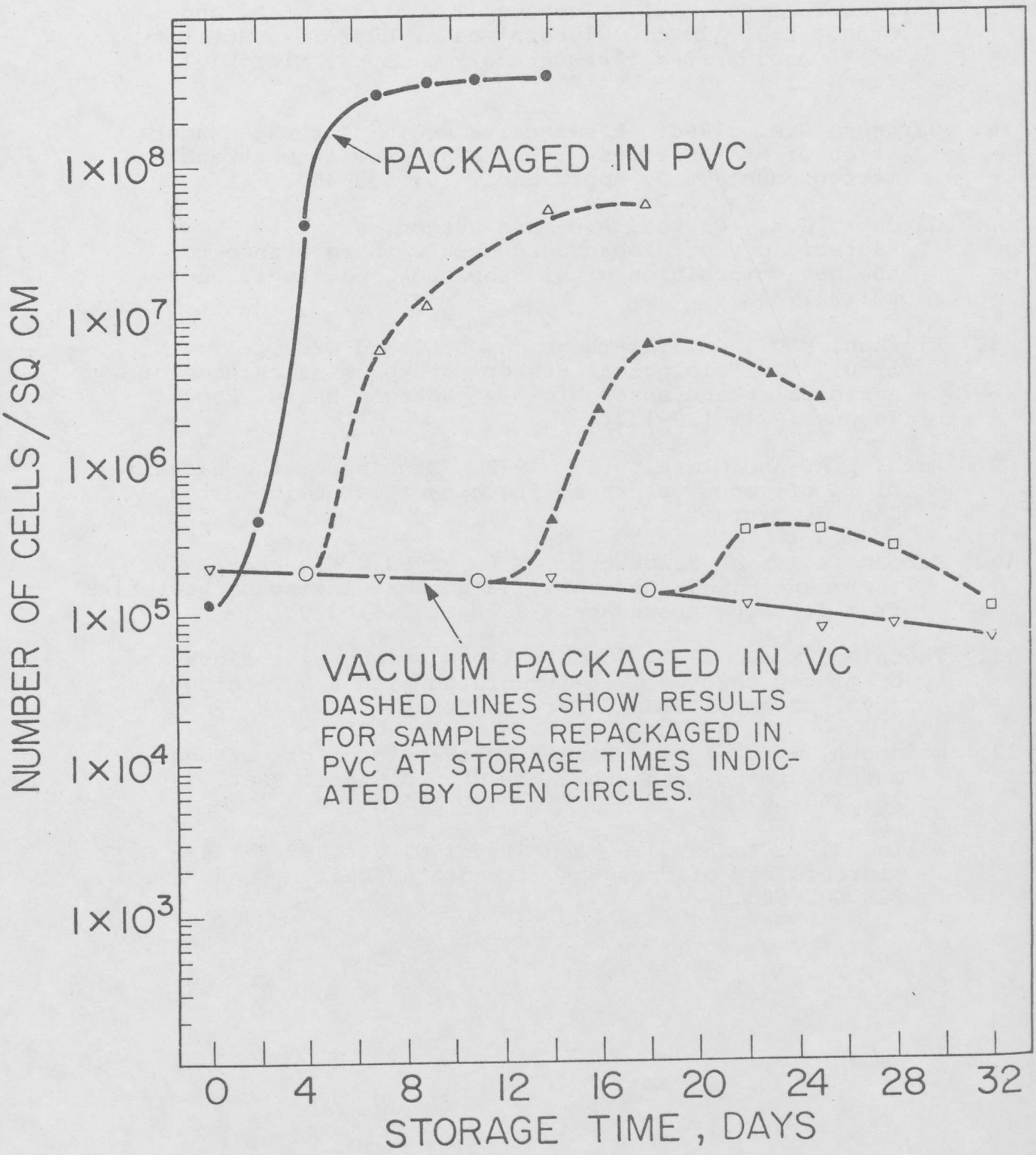

Fig. 1. Changes in the counts of *Microbacterium thermosphactum* on naturally-contaminated PVC- and VC-packaged beef during storage at 5°C. (PVC = polyvinyl chloride, gas-permeable; VC = vinyldene chloride - vinyl chloride copolymer, gas-impermeable).

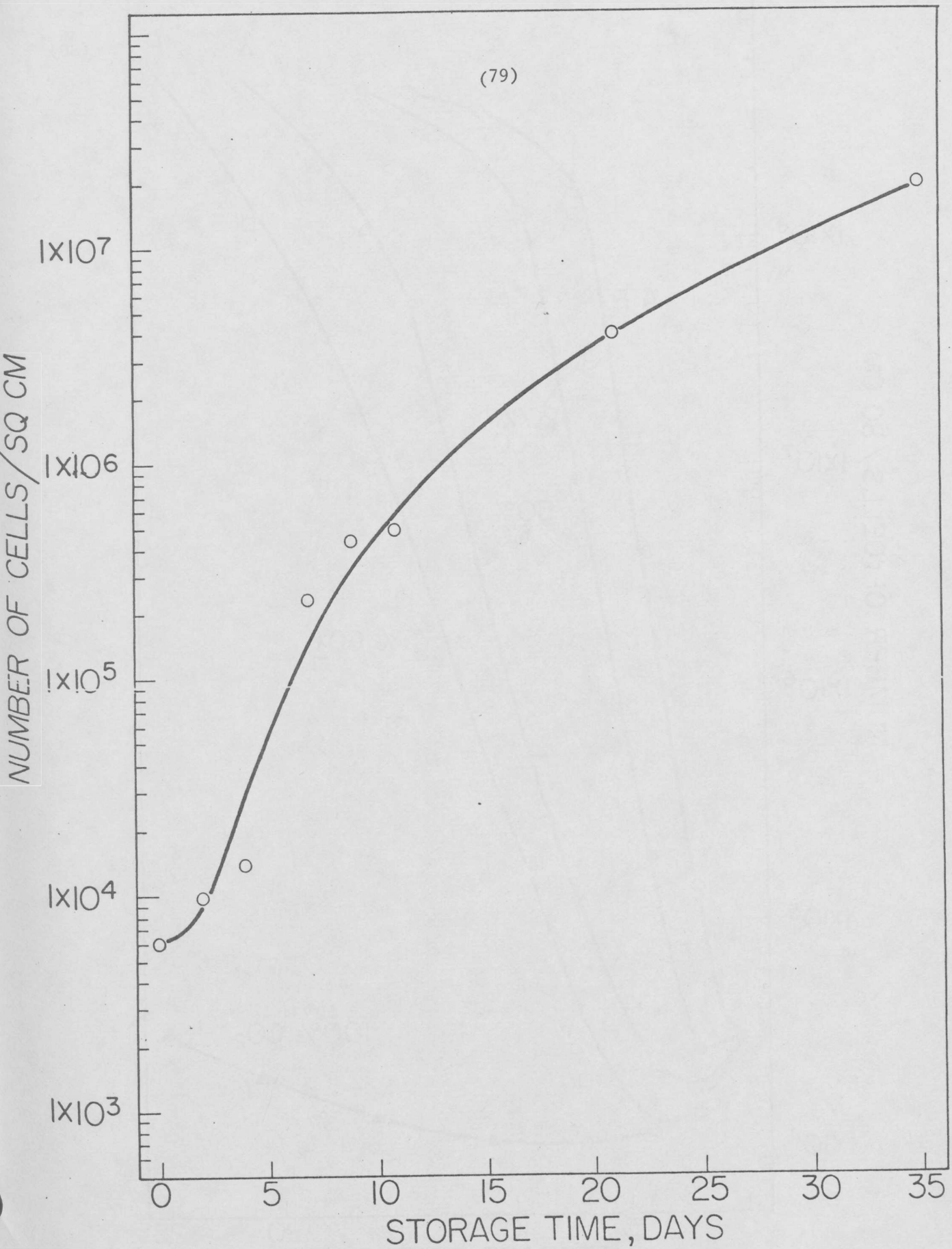

Fig. 2. Growth of *Microbacterium thermosphactum* on inoculated VC-packaged beef during storage at 5°C. (VC = vinyldene chloride - vinyl chloride copolymer, gas-impermeable).

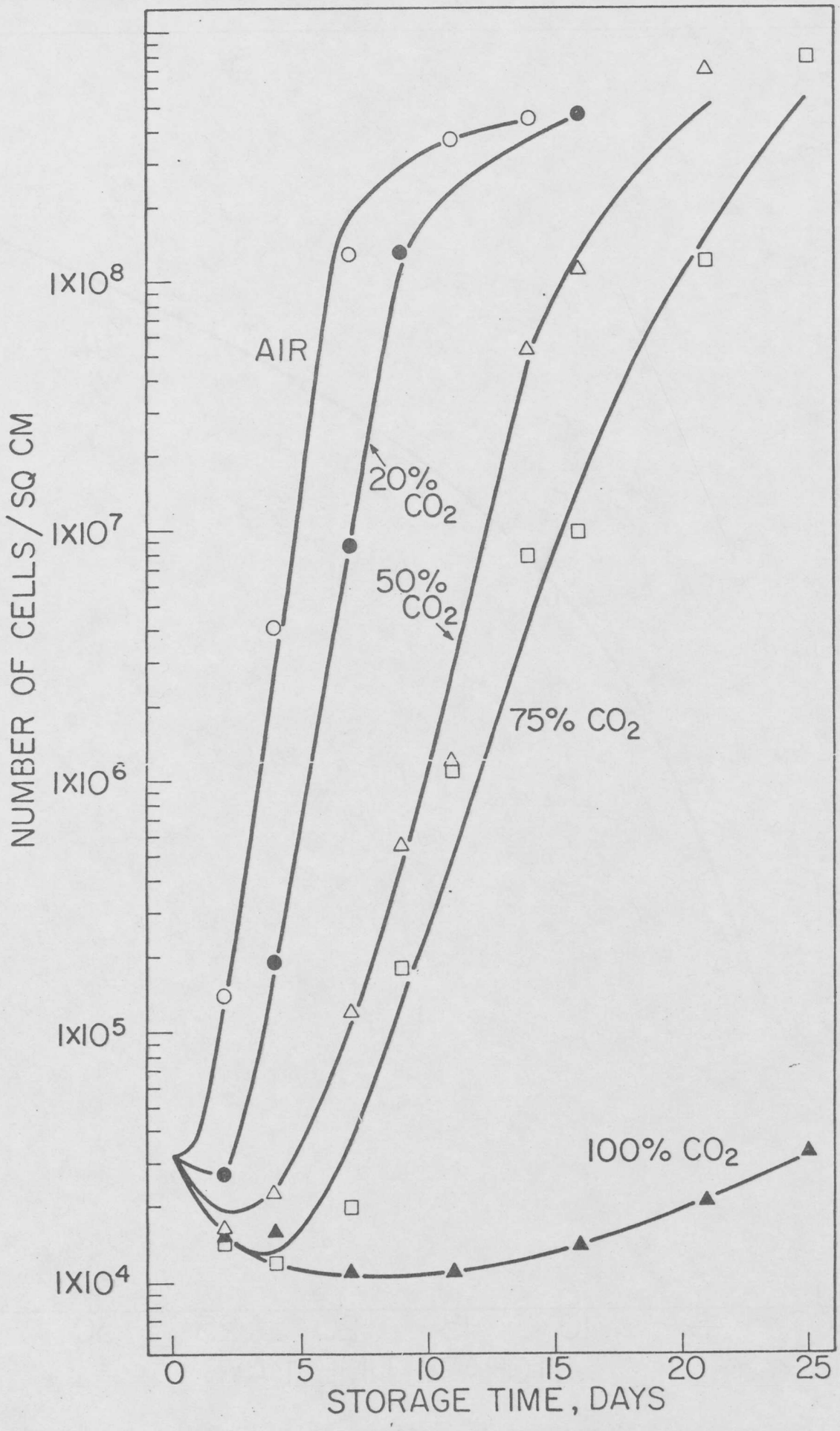

Fig. 3. Effect of CO_2 concentration on the growth rate of *Microbacterium thermosphactum* on beef at 5°C.

Table 1. The effect of vacuum-packaging on the logarithmic generation times and maximum population of *Microbacterium thermosphactum* on naturally-contaminated and inoculated beef.

Contamination	Packaging	Generation time (days)	Maximum population / cm^2
Natural Flora	unpackaged	0.35	4.0×10^8
	Vacuum packaged	—	—
Inoculated (Mixture of 10 strains)	unpackaged	0.44	4.5×10^8
	Vacuum packaged	1.3	2.0×10^7