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EFFECTS OF SOME TECHNOLOGICAL PROCESSES ON THE SURVIVAL OF PSEUDOMONAS AERUGINOSA IN FOODS

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Abstract

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Experiments on the effects of NaCl, nitrite curing mixture, pasteurization, freezing and lactic acid fermentation upon the survival of Pseudomonas aeruginosa yielded the following results: /1/ 3% NaCl inhibited the growth of P. aeruginosa more than the other salts employed. /2/ Pasteurization temperatures used for dairy milk treatment were fully effective in killing P. aeruginosa organisms. /3/ Freezing at -18°C reduced P. aeruginosa counts, but did not result in complete devitalization of the organisms. /4/ Lactic acid fermentation reduced P. aeruginosa counts depending on the environmental acidity.

Pseudomonas aeruginosa, NaCl, nitrite curing mixture, pasteurization, freezing, lactic acid fermentation.

Viewed from the standpoint of food industry, Pseudomonas aeruginosa is a potentially pathogenic microorganism that can give rise to alimentary disease if conditions favouring its multiplication prevail. Data on food poisoning due to P. aeruginosa are scarce, with most of them being reported for newborn babies and sucklings (Burzynska et al. 1974). Ormay et al. (1980) reported that P. aeruginosa was responsible for 0.1% of cases of alimentary disease.

P. aeruginosa has been isolated from various foods and raw material, maily raw milk and meat. Kielwein (1968) isolated 69 (6.7%) strains of P. aeruginosa from 1022 samples of raw milk. Otte et al. (1978) found P. aeruginosa in 34.7% of milk samples examined. Katona and Lányi (1982) reported on the occurrence of P. aeruginosa in raw milk and milking equipment with reference to the hygiene of the environment. P. aeruginosa findings in meat were reported by Ormay et al. (1980).

Moreover, reports are also available on P. aeruginosa isolations from other foods such as pasteurized milk (Haladová and Lacová 1979), frozen egg-containing products (Popa et al. 1974) and frozen ready-to-cook products containing flour and potatoes (Burzynska 1978).

The present study was designed to assess the effects of some technological procedures on the growth and survival of P. aeruginosa in foods.

Materials and Methods

Five strains of P. aeruginosa with properties identifying them as typical representatives of this species (Bergey's Manual 1984) were included in the experiment. The procedures studied for their effects on the strains were treatments with NaCl and nitrite curing mixture, pasteurization, freezing and lactic acid fermentation.

Treatment with NaCl and Nitrite Curing Mixture. The strains were inoculated into meat-peptone broth containing 2% NaCl, 3% NaCl or 2.5% nitrite curing mixture (0.6% $NaNO_2$ + 94.0% NaCl + + 5.4% other ingredients). Tubes without the salts were inoculated at the same time to serve as controls. P. aeruginosa counts were determined after incubation for 1, 2 and 7 days at 37°C.

Pasteurization. Dried semi-skim milk was reconstituted and used to grow the strains. After being heated to 65°C for 30 minutes, 75°C for 2 minutes and 85°C for 2 seconds, the samples were cooled quickly and P. aeruginosa counts were determined.

Freezing. The strains were inoculated into freshly pasteurized egg melange and stored at -18°C for 1, 2 and 4 weeks. P. aeruginosa counts were determined after the samples were allowed to thaw at room temperature.

Lactic Acid Fermentation. Ten per cent skim milk powder was reconstituted, dispensed in 50 ml volumes, heated to 85°C for 10 minutes and then cooled to 45°C. To each sample, 2 ml of yoghourt culture J 22 (diluted 1:1 with milk) and a suspension of P. aeruginosa strains were added. After being incubated at 43°C for 3 hours, the samples were examined for actual acidity potentiometrically and for titratable acidity using NaOH c (0.25 $mol.l^{-1}$) and P. aeruginosa counts were determined. The samples were subsequently stored at a refrigerator temperature for 24 and 48 hours and examined in the same way as described above.

P. aeruginosa counts were determined quantitatively on agar plates using Pseudomonas F agar. The initial concentrations of the inoculated strains were determined in all the samples. The substrates used were checked to ensure the absence of other P. aeruginosa organisms.

Results

NaCl and Nitrite Curing Mixture. The addition of 2% NaCl to the nutrient medium did not exert a marked effect on the growth of the strains, particularly after 24-hour incubation. A slow--down of the growth or reduced P. aeruginosa counts were observed after 48 hours. Incubation for 7 days resulted in a major reduction in P. aeruginosa counts, compared with controls.

The addition of 3% NaCl did not substantially inhibit the growth of P. aeruginosa strains. Generally, in this environment too, the growth was reduced compared with controls.

The 2.5% nitrite curing mixture exerted no substantial effect on the growth of P. aeruginosa strains (Fig. 1).

Pasteurization. The temperatures used for dairy milk treatment caused complete devitalization of P. aeruginosa strains.

Freezing. Freezing and storage of egg mélangé samples inoculated with P. aeruginosa strains produced a reduction of P. aeruginosa counts by approximately 2 orders by the end of the first week. In the next weeks the reduction of P. aeruginosa counts continued at a slower rate (Fig. 2).

Lactic Acid Fermentation. The activity of the yoghourt culture in milk samples was manifested by a rise in titratable acidity from 7 ml to 26 ml NaOH c (0.25 mol.l) after 3-hour incubation and to 40 ml and 45 - 50 ml after storage for 24 and 48 hours, respectively. With the increasing acidity a decrease in P. aeruginosa counts was observed (Fig. 3). In two strains where the yoghourt acidity exceeded 47 ml NaOH and the pH was lower than 4.3, P. aeruginosa counts reached zero values.

Discussion

Up to now, P. aeruginosa has received attention mainly from the clinical point of view. In food microbiology, P. aeruginosa findings have been accidental, drawing systematic attention only when incriminated as causes of alimentary disease. Little is known about possible P. aeruginosa survival in foods with respect to various technological treatments, environmental effects and various food additives used in food industry.

From our results it appears that the pasteurization temperatures used for dairy milk treatment are fully effective in killing P. aeruginosa. The findings of P. aeruginosa in pasteurized milk reported by Haladová et al. (1979) were apparently due to subsequent contamination. In keeping with our results are the observations of Otte et al. (1978) who investigated the effects of a temperature range of 55°C to 80°C.

The temperatures used in freezing plants reduce P. aeruginosa counts, but do not kill the organisms completely. With increasing length of the storage a slow-down in the devitalization of P. aeruginosa occurs. The lack of a complete kill of P. aeruginosa organisms involves the risk of their renewed multiplication after the contaminated food is thawed. Pogorelska (1979) in her experiments with minced meat inoculated with a P. aeruginosa strain found that storage at -23°C for 6 months reduced P. aeruginosa counts by 60%. According to Popa and Vasilescu (1974) 10% of the original P. aeruginosa counts in egg products survive freezing and 6-month storage at -18°C.

Very few data are available on survival of P. aeruginosa in fermented milk products. It remains undisclosed whether P. aeruginosa counts are reduced by a high degree of acidity or antimicrobial activity of lactic bacteria. Polugani et al. (1979) assessing antimicrobial activity of various lactic cultures found that the growth of P. aeruginosa was inhibited by L. bulgaricus, L. acidophilus and Str. thermophilus. Kafel et al. (1981), on the other hand, studied the effect of the pH on P. aeruginosa survival in milk under various storage temperatures. They found that P. aeruginosa did not multiply when the pH of the milk was 4.5. The orga-

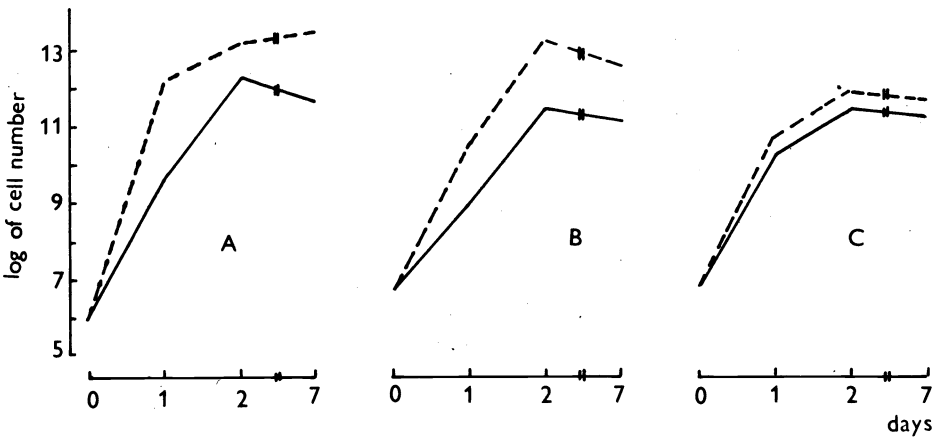

Fig. 1. Effects of various salts on the growth of P. aeruginosa
A = 2% NaCl; B = 3% NaCl; C = 2.5% nitrite curing mixture.
_____ experimental samples; ----- control samples.

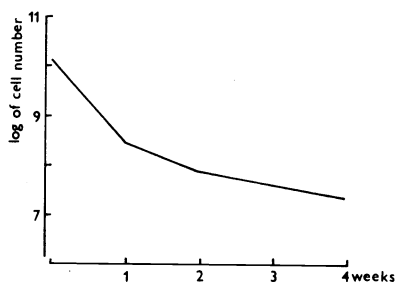

Fig. 2. Effect of freezing (-18°C) on the survival of P. aeruginosa.

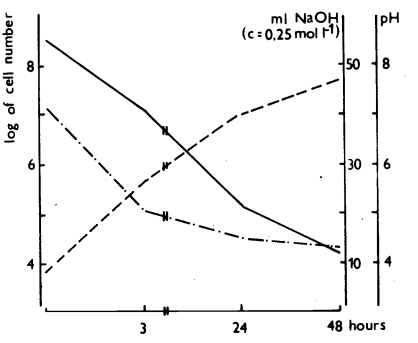

Fig. 3. Effect of lactic acid fermentation on the survival of P. aeruginosa
_____ P. aeruginosa counts;
----- titratable acidity;
-.-.- pH.

nisms died gradually, the rate of their devitalization being dependent on environmental temperatures: more P. aeruginosa organisms survived at 4.5°C than at 37°C. In the present study P. aeruginosa counts in milk incubated with a yoghourt culture for

3 hours were reduced. When the pH values were lower than 4.5 and the titratable acidity exceeded 47 ml NaOH c ($0.25\ mol.l^{-1}$) P. aeruginosa strains were completely devitalized. In the opposite case they survived.

The addition of NaCl inhibited P. aeruginosa growth. However, no substantial differences occurred between the effects of 2 and 3% addition. The effect on P. aeruginosa was also studied by Harris et al. (1974) who found that concentrations between 0.14 M and 4.5 M NaCl caused "osmotic shock" dependent on temperature.

NaCl in combination with $NaNO_2$ in nitrite curing mixture had no inhibitory effect on the growth of P. aeruginosa.

Vliv některých technologií na přežívání Pseudomonas aeruginosa v potravinách

Byl studován vliv NaCl, dusitanové solící směsi, pasteračních teplot, mražení a mléčného kysání na přežívání P. aeruginosa. Bylo zjištěno, že 1/ z použitých solí má největší inhibiční účinek 3% NaCl,

2/ pasteračních teploty, používané k ošetření mléka, ničí P. aeruginosa,

3/ mražení při $-18^{o}C$ způsobuje redukci počtu buněk, ale nemá za následek úplnou devitalizaci těchto mikrobů,

4/ mléčné kysání redukuje počty P. aeruginosa v závislosti na kyselosti prostředí.

Влияние некоторых технологий на выживаемость Pseudomonas aeruginosa в пищевых продуктах

Проводилиць исследования NaCl, азотистой соляной смеси, температуры пастеризации, замораживания и молочного квашения, их влияния на выживаемость *P. aeruginosa*. Было установлено, что

1) из числа используемых солей самое большое ингибирующее влияние оказывает 3% NaCl;
2) температура пастеризации, применяемая для обработки молока, уничтожает *P. aeruginosa*;
3) замораживание при температуре $-18^{\circ}C$ вызывает редукцию числа клеток, однако не выливается в полное уничтожение данных микробов;
4) молочная закваска редуцирует численность *P. aeruginosa* в зависимости от кислотности окружающей среди.

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