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# INTERACTION BETWEEN *LACTOCOCCUS LACTIS* STRAINS AND *LISTERIA MONOCYTOGENES* IN MIXED CULTURES

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Competition between a bacteriocinogenic and a non-bacteriocinogenic *Lactococcus lactis* strain, respectively, and a *Listeria monocytogenes* strain was studied in two semi-synthetic liquid media at various temperatures. The media used for the study were ST I and modified ST I broth (ST I broth + 1 g $l^{-1}$ Tween 80). In both media, at 30 °C, a significant cell count reduction (5 log) of *L. monocytogenes* occurred only when the cell concentration of the bacteriocinogenic competitor reached the level of at least $10^7$ CFU $ml^{-1}$ required for the production of sufficient concentration of nisin-like bacteriocin. The same phenomenon was also observed when the initial level of the lactic acid bacteria (LAB) was one log higher or lower than that of the *Listeria*, however, the reduction of *Listeria* cell count occurred earlier with the higher initial concentration of the LAB.

Incubation of the mixed cultures at 20 °C gave similar results but the bacteriocinogenic activity resulted in only a three log decline of the cell count of *L. monocytogenes*.

At 10 °C *Lactococcus lactis* produced much less bacteriocin than at 30 °C, therefore, a drastic decrease of the *Listeria* cell count was not observed. Suppression of the *Listeria* growth was expressed in its decreased maximum population level (i.e. in an earlier appearance of the stationary phase). When the non-bacteriocinogenic *Lac. lactis* and *Listeria* were present at the same initial level (approx. $10^5$ CFU $ml^{-1}$), the *Lactococcus* did not affect the growth of *L. monocytogenes* at 30 °C in modified ST I broth.

**Keywords:** interaction, *Lactococcus lactis, Listeria monocytogenes*

In recent years, interest in the role of microbial competition in assuring food safety has increased. A number of studies have reported that growth of *Listeria monocytogenes* is suppressed in the presence of high levels of lactic acid bacteria (HARRIS et al., 1989; BUCHANAN & BAGI, 1997; NILSSON et al., 1999). Antagonistic effects of lactic acid bacteria have often been attributed to the production of acids (SCHILLINGER & LÜCKE, 1989) or hydrogen peroxide (TAGG et al., 1976; GILLILAND & SPECK, 1977) although others have shown that the inhibition is due to the production of

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antimicrobial substances called bacteriocins (KLAENHAMMER, 1988). A variety of bacteriocin-producing strains of lactic acid bacteria occurring commonly in foods have been identified and are being evaluated as potential means for controlling foodborne pathogens such as *Listeria* (BUCHANAN & BAGI, 1997). One of them is *Lactococcus lactis,* which produces nisin. Nisin has bactericidal activity against a broad range of Gram-positive microorganisms (SPELHAUG & HARLANDER, 1989; DELVES-BROUGTHON, 1990). Because of their antilisterial activity, nisin producer strains of lactococci may be suitable candidates as protective cultures to control *Listeria* in food systems. For effective commercial application their nisin production must be optimalized. The non-ionic surfactant Tween 80 seems to have an effect on bacteriocin synthesis by *Lactococcus* spp. HUOT and co-workers (1996) suggested that this surfactant is of importance since the bacteriocin activity sharply increased when the culture medium was supplemented with Tween 80. Furthermore, JUNG and co-workers (1992) reported that the addition of Tween 80 has been shown to increase significantly the activity of nisin against *L. monocytogenes* in milk regardless of fat content.

The aim of present work was to study the competition of a bacteriocinogenic and a non-bacteriocinogenic *Lactococcus lactis* strain, respectively, with a *Listeria monocytogenes* strain in two semi-synthetic liquid media at various temperatures. The effect of the non-ionic detergent Tween 80 on bacteriocin activity during the co-culture studies was investigated. The role of inoculum levels of the competing strains in the suppression of growth of *L. monocytogenes* was also studied.

## 1. Materials and methods

### *1.1. Microorganisms*

*Lactococcus lactis* BFE 920 (bact+) (Federal Research Centre for Nutrition, Karlsruhe, Germany) a bacteriocinogenic strain, *Lactococcus lactis* DSM 20384 (bact-), a non-bacteriocinogenic strain (Deutsche Sammlung von Mikroorganismen, Braunschweig, Germany) and *Listeria monocytogenes* SLCC 2540 (serotype 3b) (Federal Centre for Meat Research, Kulmbach, Germany) were used in the experiments. Stock cultures of *Lactococcus* strains and *Listeria monocytogenes* were kept in MRS broth (Merck, Germany) and in ST I broth (Merck, Germany) with 15% glycerol at –18 °C.

### *1.2. Inoculum*

Before the initiation of a co-culture trial, 100 µl of the stock cultures was inoculated into 9 ml of medium. Lactic acid bacteria were grown in MRS broth at 30 °C and *Listeria monocytogenes* in ST-I broth at 37 °C for 24 h. After 24 h 100 µl of these

cultures were inoculated into MRS and ST I broth. Tubes were incubated at the same temperature mentioned before. These 24-h cultures, which composed of stationary phase cells, were used for inoculation.

*1.3. Media*

The media used for the competitive studies were Standard I broth (Merck, Germany) and modified Standard I broth. The latter was prepared from Standard I broth supplemented with 1 g $l^{-1}$ Tween 80. The broth was divided into 100 ml portions in 250 ml screw capped Duran flasks and sterilised after closure by autoclaving for 20 min at 121 °C. The pH of the broth was not adjusted. It was approx. 7.5.

*1.4. Competitive growth studies*

The Duran flasks containing 100 ml of ST-I broth or modified ST-I broth were inoculated with 1 ml of appropriate dilutions of the 24-h cultures of *Lac. lactis* and/or *L. monocytogenes* to achieve the following initial viable cell counts:

– *Lac. lactis* (bact+): $10^5$ CFU $ml^{-1}$
– *L. monocytogenes*: $10^5$ CFU $ml^{-1}$
– *Lac. lactis* (bact+): $10^5$ CFU $ml^{-1}$ + *L. monocytogenes*: $10^5$ CFU $ml^{-1}$
– *Lac. lactis* (bact+): $10^5$ CFU $ml^{-1}$ + *L. monocytogenes*: $10^6$ CFU $ml^{-1}$
– *Lac. lactis* (bact+): $10^6$ CFU $ml^{-1}$ + *L. monocytogenes*: $10^5$ CFU $ml^{-1}$
– *Lac. lactis* (bact-): $10^5$ CFU $ml^{-1}$
– *Lac. lactis* (bact-): $10^5$ CFU $ml^{-1}$ + *L. monocytogenes*: $10^5$ CFU $ml^{-1}$

The co-cultures and the monocultures were incubated at 30 °C, 20 °C and 10 °C, respectively, for various time periods.

Periodically, 1 ml sample of each co-culture and each monoculture were removed, diluted as needed in sterile 0.1% peptone water. Bacterial counts were determined by selective spread-plating on MRS agar (Merck 10660) for *Lactococcus lactis*, and on Standard I agar (Merck 7881) and Palcam agar (Merck 11755) for *Listeria monocytogenes* and bacteria were incubated at 30 °C and 37 °C for 24–48 h, respectively.

*1.5. Determination of spectrum of antagonistic activity*

Cell-free neutralised supernatant (CFNS) was used to determine the spectrum of antagonistic activity. For preparation of CFNS , bacteriocinogenic *Lac. lactis* was grown in MRS broth for 24 h at 30 °C. The culture was centrifuged using an Eppendorf-centrifuge (Biofuge 13, Heraeus Sepatech) at 10 000 r.p.m for 10 min, supernatant was adjusted to pH 6.5–7 using 1 M NaOH and heated at 100 °C for 5 min to inactivate remaining cells. The spectrum of antagonistic activity was tested against a wide range of

lactic acid bacteria (LAB) as well as foodborne pathoghens (Table 1) by spotting CFNS onto soft agar beds with indicator microorganism (ca. $10^8$ CFU $ml^{-1}$) according to previously described methods (SCHILLINGER et al., 1993). Antagonistic activity was manifested when a clear inhibition zone was observed after 24 h incubation on the indicator lawn.

*1.6. Determination of bacteriocin activity*

Bacteriocin activity assays were performed by the critical dilution method as described previously by SCHILLINGER and LÜCKE (1989). Cell-free supernatant of *Lac. lactis* was used to determine the bacteriocin activity against *L. monocytogenes*. Bacteriocinogenic *Lac. lactis* was grown in a pure culture and in a mixed culture with *L. monocytogenes* at different temperatures in two semi-synthetic media (ST-I or modified ST-I). At certain time intervals 1 ml samples were withdrawn, centrifuged at 10 000 r.p.m. for 10 min. The supernatants were transferred to a new tube and heated at 100 °C for 5 min to inactivate remaining cells. Ten µl-volumes of two-fold serial dilutions of the supernatants were spotted onto the surface of a Standard I agar plate overlaid with 7 ml of Standard I soft agar (0.7%) which had been inoculated with 0.2 ml of a 24-h culture of *L. monocytogenes*. The plates were incubated at 30 °C for 24 h. Bacteriocin activity in arbitrary activity units (AU) was defined as the reciprocal of the highest dilution yielding a clear zone of inhibition on the indicator lawn, and was multiplied by a factor of 100 to obtain the AU $ml^{-1}$ of the original sample. Unless stated otherwise, *Listeria monocytogenes* SLCC 2540 was used as indicator strain.

## 2. Results and discussion

The bacteriocinogenic *Lactococcus lactis* strain used in this work was isolated from mungbean by FRANZ and co-workers (1997). They suggested that *Lac. lactis* BFE 920 (bact+) produced a nisin-like bacteriocin. According to their finding it was active against a wide range of bacteria, including several lactic acid bacteria and some relevant foodborne pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus.*

Our preliminary studies gave similar results (Table 1). Furthermore, we realised that from all of the tested *Listeria* spp. *Listeria monocytogenes* SLCC 2540 proved to be the most sensitive strain to the nisin-like bacteriocin in the bacteriocin activity assay (data are not shown). Thus, *Listeria monocytogenes* SLCC 2540 was used during the competitive growth studies.

Table 1

*Inhibitory spectrum of cell free neutralised culture supernatants (CFNS) of bacteriocin producing* Lactococcus lactis *BFE 920*

| Indicator | Sensitivity to CFNS of *L. lactis* BFE 920 |
|---|---|
| *Lactobacillus sake* DSM 20017 | + |
| *Enterococcus faecium* DSM 20477 | + |
| *Pediococcus acidilactici* DSM 20333 | + |
| *Streptococcus mutans* DSM 6178 | – |
| *Salmonella enteritidis* 5271 | – |
| *Bacillus cereus* CCM 2010 | (+) |
| *Escherichia coli* DSM 116 | – |
| *Staphylococcus aureus* ST 11 | – |
| *Staphylococcus aureus* ST 13 | + |
| *Staphylococcus aureus* ATCC 14458 | + |
| *Staphylococcus aureus* DSM 1104 | – |
| *Listeria monocytogenes* SLCC 2540 | + |
| *Listeria monocytogenes* DSM 20600 | + |
| *Listeria monocytogenes* WS 2251 | + |
| *Listeria monocytogenes* Scott A | + |
| *Listeria ivanovii* WS 2254 | + |
| *Listeria seeligeri* WS 2253 | + |
| *Listeria welshimeri* WS 2254 | + |

– no sensitivity; + sensitivity; (+) small sensitivity

The results of the study with pure cultures in two semi-synthetic media showed that the growth of the bacteriocinogenic *Lac. lactis* and *L. monocytogenes* in their monocultures did not differ significantly in ST-I and modified ST-I broth at 30 °C. However, when *Lac. lactis* (bact+) was grown in ST-I broth bacteriocin activity was not detectable against *L. monocytogenes* (Figs 1, 2).

The competition studies demonstrated that the growth of *Lac. lactis* (bact+) and *L.monocytogenes* in a co-culture was similar to the aforementioned two growth media, when the initial cell numbers of the two bacteria were about equal (Figs 3a,b-4a,b). In both media, the inactivation of *L. monocytogenes* occurred only when the cell concentration of the bacteriocinogenic competitor reached the level of at least $10^7$ CFU $ml^{-1}$. Although bacteriocin activity was not detectable against *L. monocytogenes* when the co-culture was inoculated into ST I broth, the decrease of the viable cell count of *Listeria* seemed to follow the same manner as in modified ST I broth.

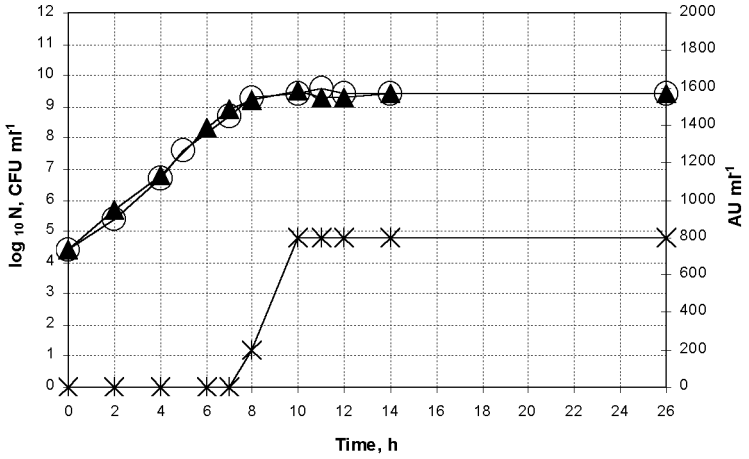


Fig. 1. Growth and bacteriocin production of *Lactococcus lactis* (bact+) in ST-I broth and in modified ST-I broth at 30 °C. s: *Lac. lactis* (bact+) ST-I; ○: *Lac. lactis* (bact+) modified ST-I; s: bacteriocin activity modified ST-I

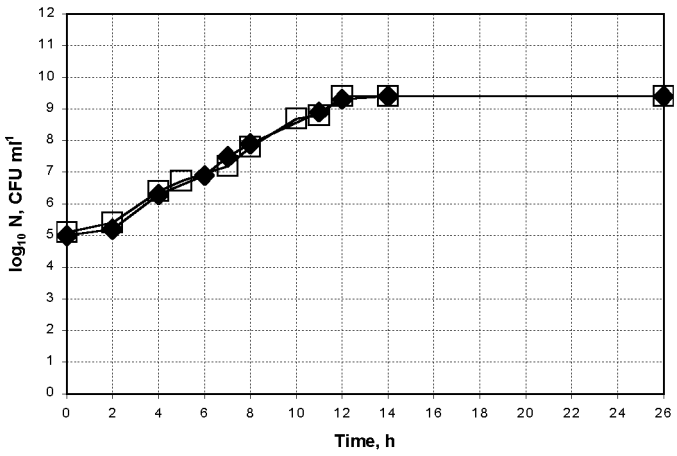


Fig. 2. Growth of *Listeria monocytogenes* in ST-I broth and in modified ST-I broth at 30 °C. u: *L. monocytogenes* ST-I; □: *L. monocytogenes* modified ST-I

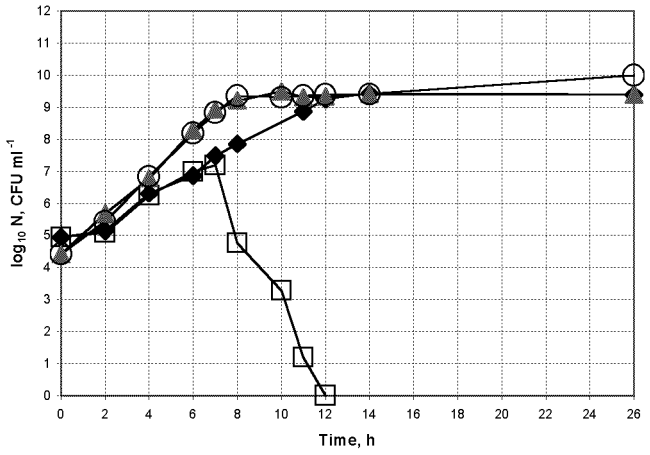


Fig. 3a. Growth of *Listeria monocytogenes* in the presence of the bacteriocinogenic *Lactococcus lactis* (bact+) strain in ST-I broth. Culture conditions, 30 °C, pH 7.5. u: *L. monocytogenes*; □: *L. monocytogenes* (co-culture); s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+) (co-culture)

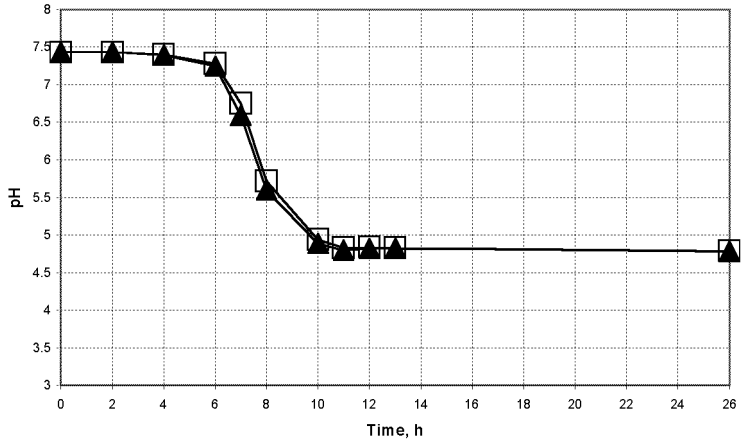


Fig. 3b. pH-drop in the monoculture and in the co-culture. □: *Lac. lactis* (bact+) (pH); s: co-culture (pH)

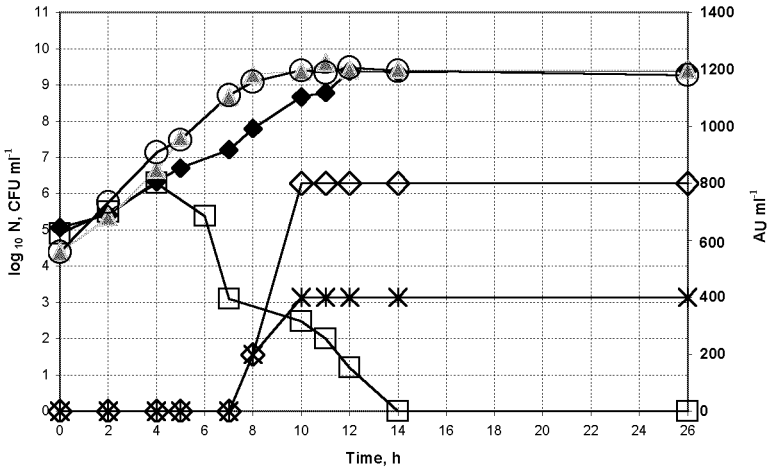


Fig. 4a. Effect of *Lactococcus lactis* (bact+) on the growth of *Listeria monocytogenes* in modified ST-I broth. Bacteriocin production of *Lactococcus lactis* BFE 920. Culture conditions, 30 °C, pH 7.5. u: *L. monocytogenes*; s: *Lac. lactis* (bact+); ◊: bacteriocin activity (monoculture); □: *L. monocytogenes* (co-culture); ○: *Lac. lactis* (bact+) (co-culture); S: bacteriocin activity (co-culture)

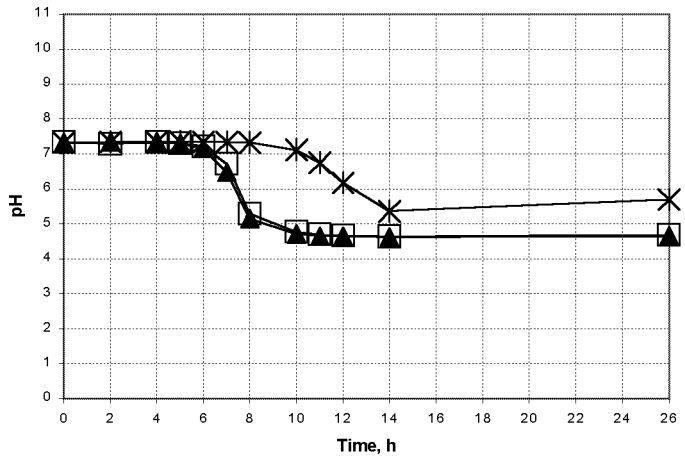


Fig. 4b. pH-drop in the monoculture and in the co-culture. □: *Lac. lactis* (bact+) (pH); s: *Lac. lactis* (bact+) (co-culture) (pH); S: *L. monocytogenes* (pH)

When the non-bacteriocinogenic *Lac. lactis* and *Listeria* as competitors were present in a co-culture in the same initial level (approx. $10^5$ CFU $ml^{-1}$), the *Lactococcus* did not affect the growth of *L. monocytogenes* at 30 °C in modified ST I broth. This is shown in Figs 5a, b.

From the competition studies with different initial cell numbers of the bacteriocinogenic strain and *L. monocytogenes* at 30 °C, 20 °C and 10 °C in the modified medium the following conclusions can be drawn: The two bacteria grew in a mixed culture in the same way as their monocultures at 30 °C, until nisin-like bacteriocin reached the detectable concentration, when a five log decrease of the viable count of *L. monocytogenes* occurred. The same phenomenon was also observed when the initial level of the lactic acid bacteria was one log higher or lower than that of the *Listeria*. Differences were noticed only between the time periods needed for the bacteriocinogenic competitor to reach the cell concentration required for the sufficient bacteriocin production needed for the *Listeria* inactivation. When the *Lac. lactis* initial cell number was higher or equal to its competitor the reduction of *Listeria* cell number was observed earlier than by lower initial LAB concentration (Figs 6a, b, c, d).

Incubation of the mixed cultures at 20 °C gave similar results but the bacteriocinogenic activity resulted in only a three log decline of the cell count of *L. monocytogenes*. Furthermore, the suppression of the pathogen was induced much later at this temperature than at 30 °C (Figs 7a, b, c, d).

At 10 °C *Lactococcus lactis* produced much less bacteriocin than at 30 °C, therefore, a drastic decrease of the *Listeria* cell count was not observed. Suppression of the *Listeria* growth was expressed in its decreased maximum population level (i.e. in an earlier appearance of the stationary phase). The results of the co-culture study at 10 °C are shown in Figs 8a, b, c, d.

No significant difference could be observed at each temperature between the bacteriocin production of *Lactococcus lactis* in co-cultures with *Listeria* as compared to its monoculture. However, at lower temperature, *Lac. lactis* produced much less nisin both in the monoculture and the mixed culture.

The results of this study showed that Tween 80 did not affect the growth of *Lactococcus lactis* and *Listeria monocytogenes* in their monocultures as well as in their co-cultures, however, in the present work it is supposed that the addition of Tween 80 to the culture media influenced the detection of bacteriocin production beneficially.

The role of Tween 80 in bacteriocin production is not totally clear yet. GRAVER and MURIANA (1994) noted that attempts to produce curvaticin FS47 in MRS broth without Tween 80 were unsuccessful, and although *Lactobacillus curvatus* FS47 grew luxuriously, bacteriocin activity was not detectable. HUOT and co-workers (1996) reported that at pH 5.5, and in the presence of 1% (v/v) Tween 80, bacteriocin

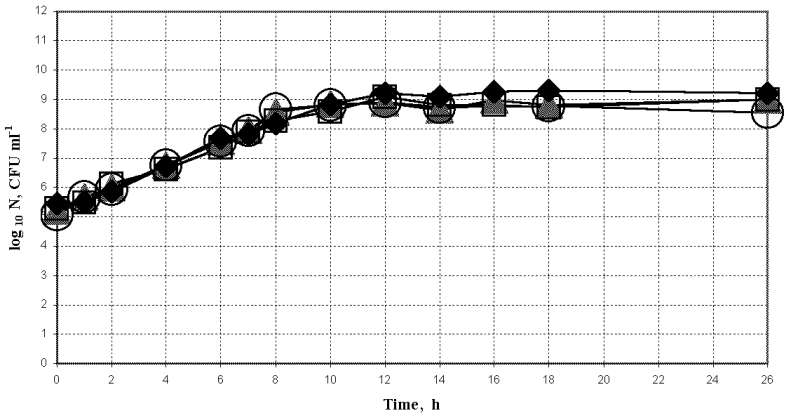


Fig. 5a. Effect of the non-bacteriocinogenic *Lactococcus lactis* (bact-) strain on the growth of *Listeria monocytogenes* in modified ST-I broth. Culture conditions, 30 °C, pH 7.5. s: *Lac. lactis* (bact-); ○: *Lac. lactis* (bact-)(co-culture); u: *L. monocytogenes*; □: *L. monocytogenes* (co-culture)

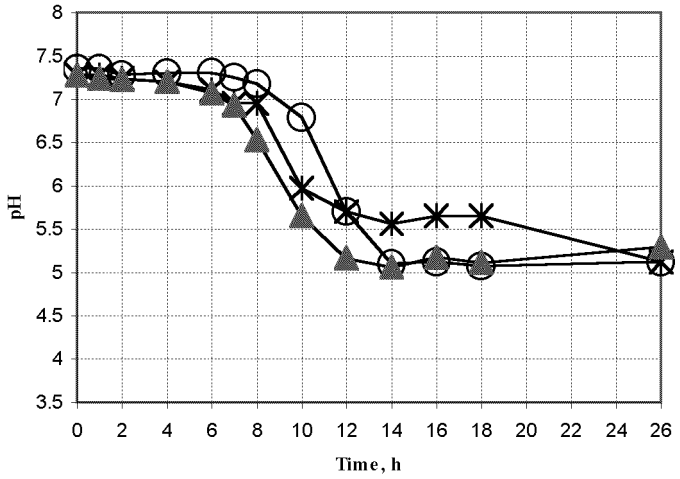


Fig. 5b. pH-drop in the monoculture and in the co-culture. S: *Lac. lactis* (bact-) (pH); ○: *L. monocytogenes* (pH); s: co-culture (pH)

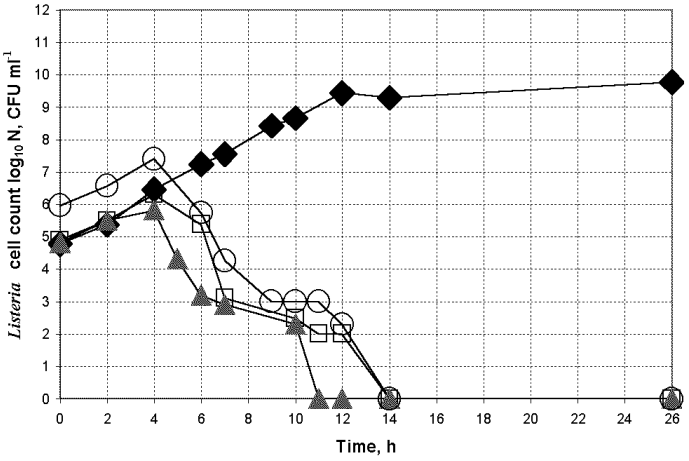


Fig. 6a. Effect of the initial cell number of the competing strains (the bacteriocinogenic *Lac. lactis* (bact+) and *L. monocytogenes*) in the inactivation of *Listeria monocytogenes* in modified ST I broth. Culture conditions, 30 °C, pH 7.5. u: *L. monocytogenes;* □: *L. monocytogenes : Lac. lactis* (bact+) (1:1); s: *L. monocytogenes : Lac. lactis* (bact+) (1:10); ○: *L. monocytogenes : Lac. lactis* (bact+) (10:1)

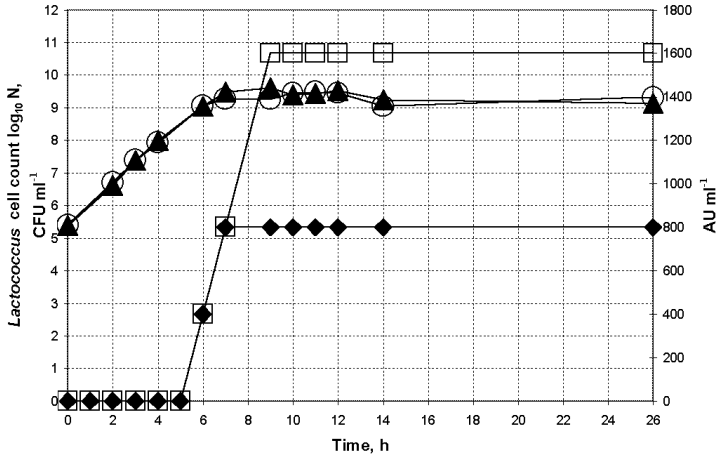


Fig. 6b. Growth and bacteriocin production of *Lactococcus lactis* in its monoculture and in a co-culture with *Listeria monocytogenes* (1:1) in modified ST I broth. Culture conditions, 30 °C, pH 7.5. s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+) (1:1); □: bacteriocin activity of *Lac. lactis* (bact+); u: bacteriocin activity of *Lac. lactis* (bact+) (1:1)

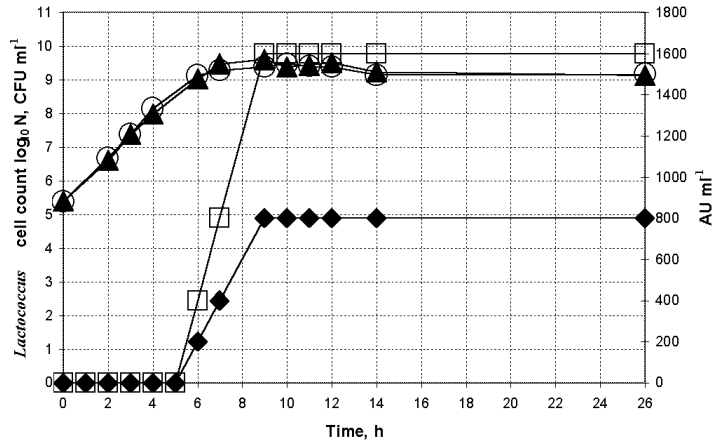


Fig. 6c. Growth and bacteriocin production of *Lactococcus lactis* in its monoculture and in a co-culture with *Listeria monocytogenes* (10:1) in modified ST I broth. Culture conditions, 30 °C, pH 7.5. s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+) : *L. monocytogenes* (1:10); □: bacteriocin activity of *Lac. lactis* (bact+); u: bacteriocin activity of *Lac. lactis* (bact+) (1:10)

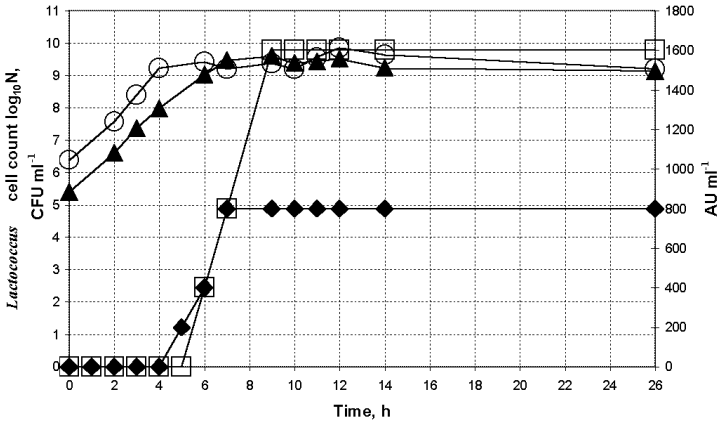


Fig. 6d. Growth and bacteriocin production of *Lactococcus lactis* in its monoculture and in a co-culture with *Listeria monocytogenes* (10:1) in modified ST I broth. Culture conditions, 30 °C, pH 7.5. s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+): *L. monocytogenes* (10:1); □: bacteriocin activity of *Lac. lactis* (bact+); u: bacteriocin activity of *Lac. lactis* (bact+) (10:1)

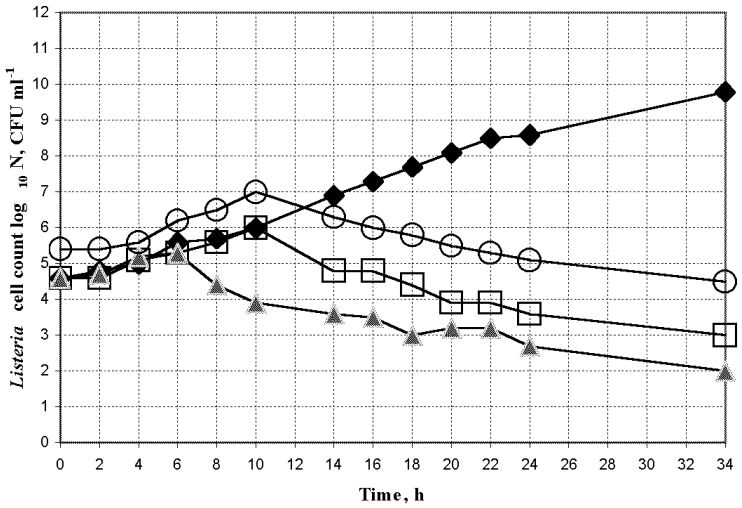


Fig. 7a. Effect of the initial cell number of the competing strains (the bacteriocinogenic *Lac. lactis* (bact+) and *L. monocytogenes*) in the suppression of growth of *Listeria monocytogenes* in modified ST I broth. Culture conditions, 20 °C, pH 7.5. u: *Listeria monocytogenes;* □: *L. monocytogenes : Lac. lactis* (bact+) (1:1); s: *L. monocytogenes : Lac. lactis* (bact+) (1:10); ○: *L. monocytogenes : Lac. lactis* (bact+) (10:1)

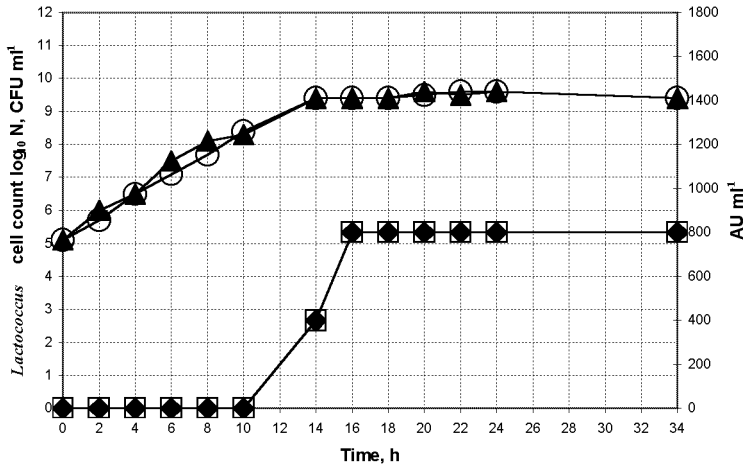


Fig. 7b. Growth and bacteriocin production of *Lactococcus lactis* in its monoculture and in a co-culture with *Listeria monocytogenes* (1:1) in modified ST I broth. Culture conditions, 20 °C, pH 7.5. s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+) : *L. monocytogenes* (1:1); □: bacteriocin activity of *Lac. lactis* (bact+); u: bacteriocin activity of *Lac. lactis* (bact+) (co-culture) (1:1)

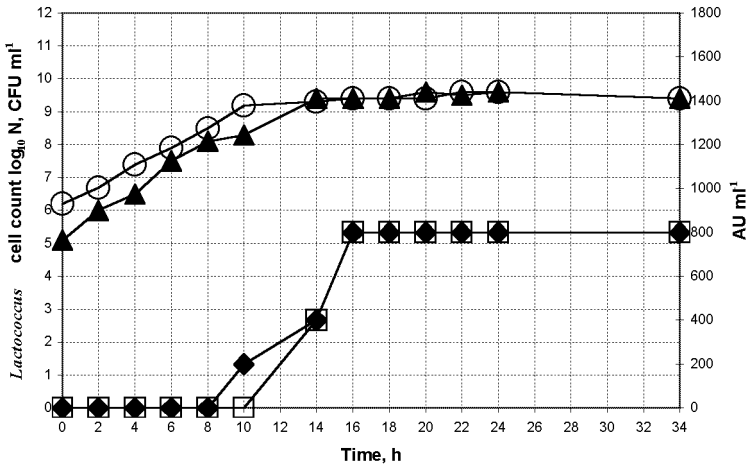


Fig. 7c. Growth and bacteriocin production of *Lactococcus lactis* in its monoculture and in a co-culture with *Listeria monocytogenes* (10:1) in modified ST I broth. Culture conditions, 20 °C, pH 7.5. s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+) : *L. monocytogenes* (10:1); □: bacteriocin activity of *Lac. lactis* (bact+); u: bacteriocin activity of *Lac. lactis* (bact+) (co-culture) (10:1)

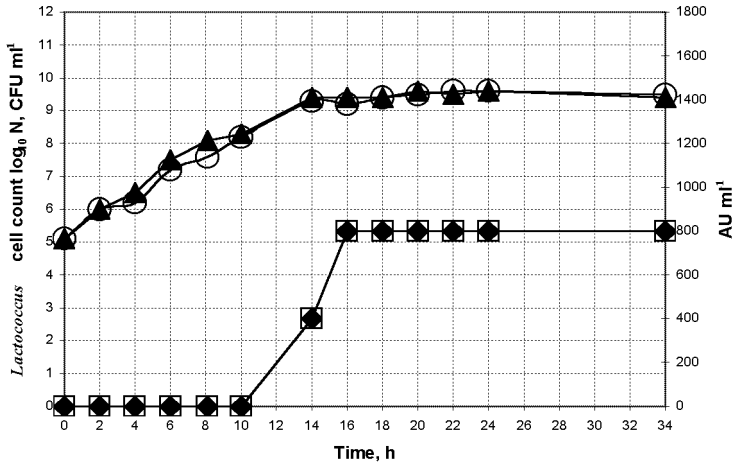


Fig. 7d. Growth and bacteriocin production of *Lactococcus lactis* in its monoculture and in a co-culture with *Listeria monocytogenes* (1:10) in modified ST I broth. Culture conditions, 20 °C, pH 7.5. s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+) : *L. monocytogenes* (1:10); □: bacteriocin activity of *Lac. lactis* (bact+); u: bacteriocin activity of *Lac. lactis* (bact+) (co-culture) (1:10)

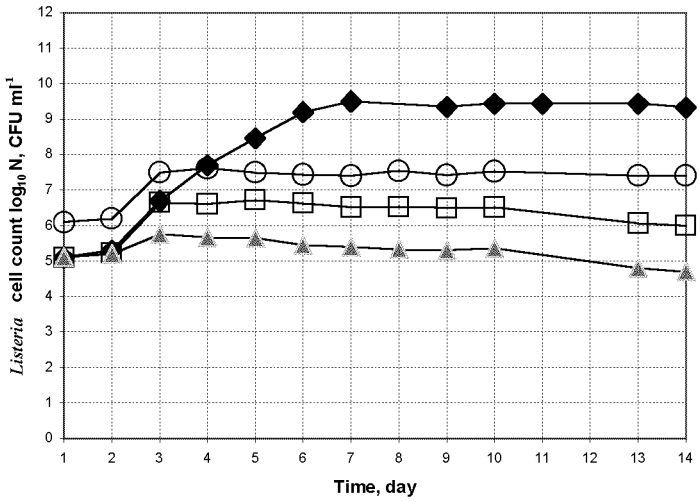


Fig. 8a. Effect of the initial cell number of the competing strains (the bacteriocinogenic *Lac. lactis* (bact+) and *L. monocytogenes*) in the suppression of growth of *Listeria monocytogenes* in modified ST I broth. Culture conditions, 10 °C, pH 7.5. u: *L. monocytogenes;* □: *L. monocytogenes : Lac. lactis* (bact+) (1:1); s: *L. monocytogenes : Lac. lactis* (bact+) (1:10); ○: *L. monocytogenes : Lac. lactis* (bact+) (10:1)

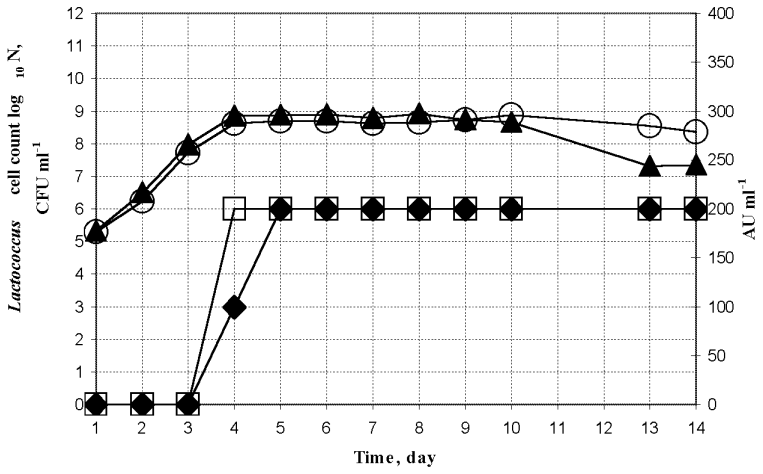


Fig. 8b. Growth and bacteriocin production of *Lactococcus lactis* in its monoculture and in a co-culture with *Listeria monocytogenes* (1:1) in modified ST I broth. Culture conditions, 10 °C, pH 7.5. s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+) : *L. monocytogenes* (1:1); □: bacteriocin activity of *Lac. lactis* (bact+); u: bacteriocin activity of *Lac. lactis* (bact+) (co-culture) (1:1)

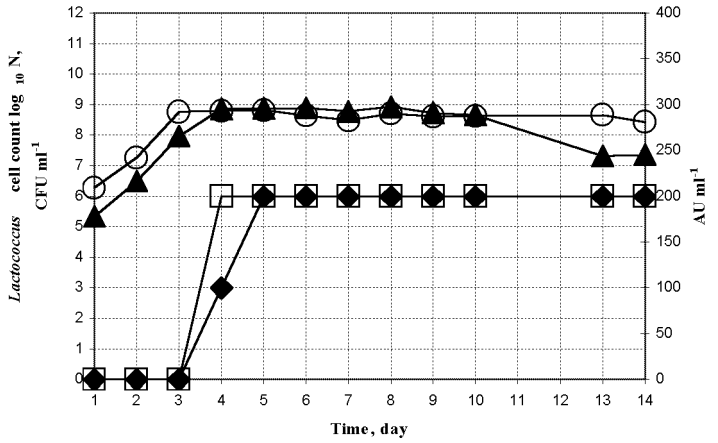


Fig. 8c. Growth and bacteriocin production of *Lactococcus lactis* in its monoculture and in a co-culture with *Listeria monocytogenes* (10:1) in modified ST I broth. Culture conditions, 10 °C, pH 7.5. s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+) : *L. monocytogenes* (10:1); □: bacteriocin activity of *Lac. lactis* (bact+); u: bacteriocin activity of *Lac. lactis* (bact+) (co-culture) (10:1)

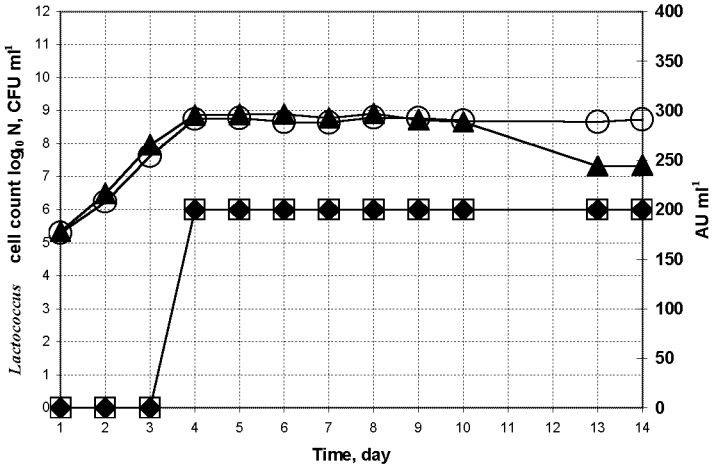


Fig. 8d. Growth and bacteriocin production of *Lactococcus lactis* in its monoculture and in a co-culture with *Listeria monocytogenes* (1:10) in modified ST I broth. Culture conditions, 10 °C, pH 7.5. s: *Lac. lactis* (bact+); ○: *Lac. lactis* (bact+) : *L. monocytogenes* (1:10); □: bacteriocin activity of *Lac. lactis* (bact+); u: bacteriocin activity of *Lac. lactis* (bact+) (co-culture) (1:10)

production by *Lac. cremoris* was about fourfold higher than in its absence. However, further increase in Tween 80 concentration did not result in a significant modification of the bacteriocin titre. NIESSEN-MEYER and co-workers (1992) showed that the addition of this surfactant to the microplate wells in the bacteriocin activity tests increased the sensitivity of the assays 2- to 10-fold. The effect of Tween 80 may be due to a reduction of the binding of bacteriocin to the plastic microplate wells. On the other hand, when Tween was added to the full grown *Lactococcus* culture of a 0% Tween broth, no stimulation of the antibacterial activity was observed, whatever the concentration added in range 0–3% (HUOT et al., 1996).

Since the reduction of *Listeria* cell number in a co-culture with *Lac. lactis* (bact+) in ST-I broth and modified ST-I broth succeeded in similar ways at 30 °C, it is supposed that the bacteriocinogenic *Lac. lactis* also produced nisin in ST I broth, although this activity was not detectable by the critical dilution method. This hypothesis seems to confirm the fact that the non-bacteriocinogenic *Lac. lactis* did not suppress the growth of *Listeria* in a co-culture of the two bacteria in modified ST-I broth at 30 °C. Furthermore, one can conclude from this latter observation that the considerable decline of the viable cell counts of *L. monocytogenes* found in the presence of the bacteriocinogenic *Lac. lactis* was not caused alone by lactic acid production by the lactic acid bacteria. Inhibition of the target organism can be explained by (i) nutrient depletion (BUCHANAN & BAGI, 1997), (ii) excretion of antilisterial compounds in laboratory broth at concentrations below the detection level when assayed in an agar diffusion test of antagonism (SCHILLINGER & HOLZAPFEL, 1990) or (iii) excretion of antilisterial compounds, such as bacteriocins (HARRIS et al., 1989). Results of the present study with bacteriocinogenic and non-bacteriocinogenic lactococci indicated that the antilisterial effect was primarily due to production of nisin-like bacteriocin. However, nutrient depletion and production of other antilisterial compounds could also contribute to the *Listeria* inactivation. BUCHANAN and BAGI (1997), who have investigated the interaction between *L. monocytogenes* and antilisterial strains of *C. piscicola*, found that suppression of *Listeria* was not always attributed to the production of antilisterial compounds but could be partially due to nutrient depletion. DEGNAN and co-workers (1992) suggested that the inhibitory effect of *Pediococcus acidilactici* LB42 against *L. monocytogenes* was caused by competitive antagonism.

The results of the competitive studies at different temperatures in the modified medium indicate that the production of the bacteriocin is regulated depending on environmental conditions. GEISEN and co-workers (1993) reported that bacteriocin production of *Leuconostoc carnosum* LA54A showed no obvious correlation between the cell number of the culture and bacteriocin production. Furthermore, cultures with slow growth produced more than one order of magnitude less bacteriocin, than cultures with higher growth rates, despite the fact that maximum cell numbers are reached after

extended incubation. This tendency was also observed in the case of *Lactoccoccus lactis*, however the maximum population density reached by the strain was also a little bit less at 10 °C than at 30 °C (Table 2) In spite of this observation the bacteriocin production of *Lac. lactis* seems to be tightly coupled to the growth rate.

Table 2

*Comparison of the determined specific growth rate of* Lactococcus lactis *BFE 920 to maximum population density (MPD) attained by its monoculture and to the maximum amount of bacteriocin (MAB) produced at different temperatures in modified ST I broth at a pH level 7.5*

| | MPD $Log_{10}$ CFU $ml^{-1}$ | MAB AU $ml^{-1}$ | Growth rate (μ) 1/h |
|---|---|---|---|
| 30 °C | 9.6 | 1600 | 1.61 |
| 20 °C | 9.6 | 800 | 0.63 |
| 10 °C | 8.92 | 200 | 0.12 |

## 3. Conclusions

Summarised, the results of the current study suggest that the bacteriocinogenic *Lactococcus lactis* BFE 920 is able to inactivate *Listeria monocytogenes* under environmental conditions that are beneficial for its bacteriocin production. The effect of Tween 80 on the bacteriocin production is not clear yet. It will require additional research, however supplementation of the culture media with this surfactant proved to enhance the detectable bacteriocin activity. At low temperature the bacteriocinogenic strain grew slowly and the low level of the produced nisin-like bacteriocin was not able to inhibit the growth of *Listeria monocytogenes* sufficiently. Since this pathogen microbe is commonly associated with refrigerated food products, it should be considered that *Lactococcus lactis* BFE 920 might not be able to control alone the growth of *Listeria* at low temperature. Thus additional controlling factors have to be used under those conditions.

*

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