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Effect of meat starter cultures and some ingredients on the growth of *Salmonella anatum* in thai fermented meat (Nham) [an in-vitro study]

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Introduction : From our early report [1] which implied the advantage of adding garlic in Nham (thai fermented meat product) on stimulating the growth of lactic starter cultures and their lactic acid productive activities in both of Nham samples and an in-vitro created Nham model broth. Since many publications reported an inhibitory effects of garlic on various food poisoning bacteria [2, 3, 4] and an inhibitory effect of nitrite on contaminated pathogens in meat products [5, 6], and both of garlic and nitrite are also the ingredients in the production of Nham. Then, we realized that these ingredients must present an inhibitory effect on *Salmonella anatum* ,which is mostly found contaminated in Nham [7, 8]. Besides, this serotype of was also found to be the most resistant either to acidity or other bactericidal substances produced by lactic acid bacteria [7]. Thus, this preliminary study was planned to ascertain the effects of garlic, commercial meat starter cultures (*Lactobacillus curvatus, L. sakei* and *Pediococcus acidilactici*) and nitrite on the growth of *S. anatum* in the created model broth [1]. Due to the spread of using nitrate in the production of this typical thai fermented meat product, an inhibitory effects of using nitrate as a food additive in Nham on *S. anatum* was also compared to nitrite in this study.

Materials and Methods

Starter cultures : Three strains, *L. curvatus, L. sakei* and *P. acidilactici*, of commercially available starter cultures (from Gewürzmüller GmbH, Stuttgart, Federal Republic of Germany) and the strain of *S. anatum* (LTH 4545, from Genera Food Technology and Microbiology, University of Hohenheim, Stuttgart) were used for this study.

Liquid medium : MRS broth - medium modified [9] was used as cultivation medium for the study LAB.

: Trypticase soy broth (TSB)- the medium [10] was used as cultivation medium for *S. anatum*. Pure cultures from trypticase soy agar (TSA) slant was transferred to TSB and incubated at 37° C for 24 h.

: The Nham model broth (NMB)- the medium simulated the conditions of Nham production (aw 0.970, pH 6.3 , microaerophilic condition with paraffin oil) [1], was used as a model instead of Nham product. The combination of using nitrite (125 ppm) and nitrate (500 ppm) as food additives by filter-sterilization [11], together with various studied starter cultures at a level of 10^6 cfu/ml and 5 % fresh sterilized garlic, were used for the study of their inhibitory effect on *S. anatum* at the level of 10^4 cfu/ml in NMB. The NMB without glucose and curing salts was used for study the effect of LAB on the growth of *S. anatum*.

The samples of each studied condition in NMB were left to ferment at 30° C for 2-3 days after LAB and *S. anatum* inoculation. Duplicate samples were determined on pH, LAB growth for every 12 h and *S. anatum* growth for every 6 h.

Preparation of fresh sterilized garlic : Local unpeeled garlic bulbs were used to prepare fresh sterilized garlic. The cloves were gently peeled, washed thoroughly with sterile distilled water, soaked in 70% ethanol for 30 minutes and washed thoroughly with sterile distilled water. Five percent of sterilized garlic was aseptically cut in small pieces and transfered into the sterilized NMB.

Microbiological and chemical analysis : The samples of Nham model broth were examined daily for enumerating LAB growth on MRS agar and *S. anatum* existence on TSA agar [12], and were determinated for pH [7].

Results and Discussion

Growth of LAB starter cultures in Nham model broth (NMB)

The results revealed that all studied LAB could grow in the model broth up to nearly 2 log cycle after one day of fermentation in both of NMB with and without glucose. Use of nitrate (500 ppm) and nitrite (125 ppm) as a curing salt alone, and in combination with 5 % sterile fresh garlic (Table 1), have no effect on the growth of all studied LAB when compared to NMB without curing salts and garlic. In contrary, an effect of garlic on the pH was measurable[1].

Effect of curing salts, fresh garlic and LAB starter on the growth of *S. anatum* in NMB

The treatment of *S. anatum* at the amount of 10^4 cells/ml with various starter cultures (*L. curvatus, L. sakei* and *P. acidilactici*) and 5 % sterile fresh garlic was first done in NMB without glucose and curing salts. Therefore, influence of pH and curing salts on *S. anatum* could be excluded. The results (Figure. 1) showed that LAB starter cultures did not retard the growth of *S. anatum,* while the model broth with 5 % sterile fresh garlic could hinder the growth of the studied pathogen during the first day of incubation. The positive effect was also observed in NMB with 1 % glucose and 5 % sterile fresh garlic (Figure. 2). This can be explained that the lower of pH in the medium from converting of glucose to lactic acid by all LAB and the presence of allicin from disrupted garlic which was known as the principal antimicrobial component [13] could exhibit an inhibitory effect on *S. anatum* during the first day of incubation. All studied LAB could reduce the cell number of *S. anatum* in NMB with glucose after one day of fermentation. The broth with *L. sakei* revealed a better result in reduction the cell number of *S. anatum* than those of NMB with *P. acidilactici* and *L. curvatus* did. The presence of nitrate at 500 ppm in NMB gave a little effect on the growth of *S. anatum* when compared to control broth without curing salts (Figure 3), while the broth with 125 ppm sodium nitrite exhibited much more inhibitory effect on the growth of *S. anatum* during the first 30 hours of fermentation. The inhibitory activity of the added nitrite in this model broth might be diminished after 30 hours of fermentation as the result of chemical reactions with some ingredients in NMB, especially in the presence of ascorbate in NMB could rapidly convert nitrous acid to nitric oxide [14]. Synergistic effect of nitrite and 5 % disrupted garlic show the best results in diminishing *S. anatum* from the broth within 48 hours of fermentation, while nitrate and the same amount of disrupted garlic could only retard the growth of this pathogen (Figure 3).

Conclusions : This preliminary study revealed that disrupted garlic, nitrite and nitrate had no effect on the growth and productivity of all studied meat starter cultures. In contrary, the disrupted garlic could retard the growth of *S. anatum* during one day of fermentation. Use of nitrite as curing salt exhibited the better results in inhibiting the growth of *S. anatum* in the model broth than use of nitrate as a curing salt. All studied LAB could be used as the starter culture to control the growth of *S. anatum* in Nham. Among these three strains of LAB, the commercial *L. sakei* revealed the best results in reduction the cell number of *S. anatum*.

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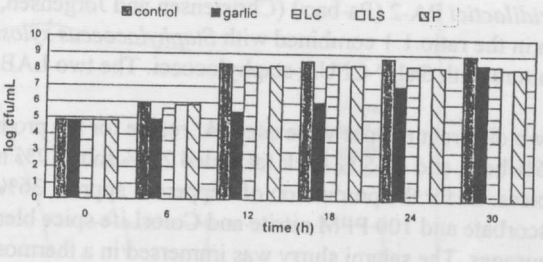
Table 1 : Growth of LAB starter cultures and pH change during fermentation in various conditions of NMB

starter culture	cfu/ml (day)				pH (day)			
	0	1	2	3	0	1	2	3
P-glu	2.0×10^6	1.2×10^8	1.0×10^8	9.6×10^7	6.05	6.04	6.09	6.08
P+glu	2.0×10^6	1.1×10^8	5.3×10^7	4.9×10^7	6.05	5.43	4.81	4.65
LC-glu	4.0×10^6	1.1×10^8	1.1×10^8	9.8×10^7	6.05	6.00	5.95	6.01
LC+glu	4.0×10^6	1.0×10^8	6.5×10^7	1.5×10^7	6.05	5.49	4.90	4.71
LS-glu	5.0×10^6	9.5×10^7	6.6×10^7	5.4×10^7	6.05	5.99	5.95	6.01
LS+glu	5.0×10^6	8.6×10^7	4.1×10^7	9.5×10^6	6.05	5.28	4.66	4.44
P+glu+NO_3	2.0×10^6	3.1×10^8	1.6×10^8	1.0×10^8	6.10	5.21	4.95	4.71
P+glu+NO_2	2.0×10^6	2.3×10^8	1.4×10^8	9.6×10^7	6.10	5.35	5.00	4.78
LC+glu+NO_3	4.0×10^6	9.5×10^7	6.1×10^7	3.4×10^7	6.10	5.41	5.06	4.66
LC+glu+NO_2	4.0×10^6	8.1×10^7	3.4×10^7	1.3×10^7	6.10	5.53	5.12	4.73
LS+glu+NO_3	5.0×10^6	1.0×10^8	9.5×10^7	8.8×10^7	6.10	5.19	4.82	4.58
LS+glu+NO_2	5.0×10^6	9.7×10^7	8.9×10^7	8.1×10^7	6.10	5.26	4.91	4.65

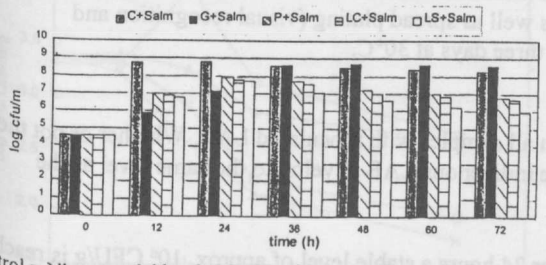
P = *P. acidilactici*, LC = *L. curvatus*, LS = *L. sake*, -glu = without glucose, +glu = with glucose, NO_3 = with 500 ppm sodium nitrate, NO_2 = with 125 ppm sodium nitrite

Figure 1 : Effect of fresh garlic and LAB on the growth of *S. anatum* in NMB without glucose and curing salt at 30^0 C



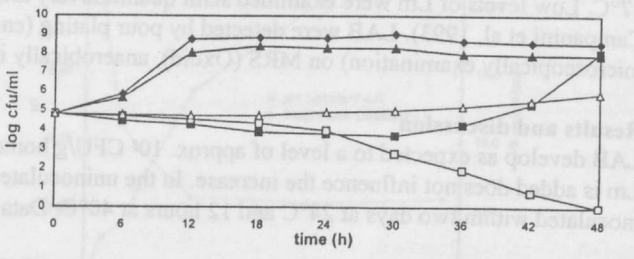
Contrrol = Nham model broth (NMB) without garlic and LAB starter culture, garlic = NMB with 5 % steriled fresh garlic, LC = NMB with 10^6 cells/ml of *L. curvatus*, LS = NMB with 10^6 cells/ml of *L. sake*, P = NMB with 10^6 cells/ml of *P. acidilactici*

Figure 2 : Inhibitory effect of steriled fresh garlic and LAB on *S. anatum* in NMB with 1 % glucose (without curing salt) at 30^0 C



control = Nham model broth (NMB) without garlic and LAB starter culture, garlic = NMB with 5 % steriled fresh garlic, LC = NMB with 10^6 cells/ml of *L. curvatus*, LS = NMB with 10^6 cells/ml of *L. sake*, P = NMB with 10^6 cells/ml of *P. acidilactici*, +Salm = added with 10^4 cells/ml of *S. anatum*

Figure 3 : Effect of nitrate or nitrite alone and in combination with garlic on the growth of *S. anatum* in NMB at 30^0 C



-◆- = NM[C], -▲- = NM+NO_3 (500), -■- = NM+NO_2 (125), -□- = NM+NO_2 (125)+G, -△- = NM+NO_3 (500)+G, NM [C] = NMB without curing salts, +G = with 5 % steriled gresh garlic, NM+Ni (125) = NMB with 125 ppm of sodium nitrite, NM+Na (500) = NMB with 500 ppm of sodium nitrate