

Untersuchungen über die antimikrobiellen Eigenschaften von aus Dauerwurst isolierten Stämmen *L. plantarum*.

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In vorliegender Arbeit wird die antagonistische Aktivität von 30 Stämmen *L. plantarum*, die aus Dauerwurst isoliert wurden, in Bezug auf 8 Arten Testmikroorganismen - *Str. faecalis*, *E. coli*, *Proteus vulgaris*, *B. subtilis*, *B. mesentericus*, *B. cereus*, *B. idosus*, *Staph. aureus* - bestimmt. Die Versuche wurden bei pH des Nährmediums 5.0, 5.5, 6.0 und 6.5 und einer Kulturtemperatur von 15, 26 und 32°C durchgeführt.

Bei diesen Versuchen wurde festgestellt, dass die antagonistische Wirkung der Laktobazillen von den pH-Werten des Nährbodens, der Kulturtemperatur und der Art der Testmikroorganismen beeinflusst wird. Der stärkste Antagonismus wird gegenüber *B. cereus* und den anderen einbezogenen sporenbildenden Mikroorganismen, sowie auch gegenüber *S. aureus*, beobachtet. Gegenüber den Enterobakterien ist diese Wirkung wesentlich schwächer. Die schwächste Aktivität ist gegenüber *Str. faecalis* festzustellen.

A study of the antimicrobial properties of *L. plantarum* strains isolated from raw-dried sausages

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A determination was made of the antagonistic activity of 30 *L. plantarum* strains, isolated from raw-dried sausages, towards 8 test microorganisms: *Str. faecalis*, *E. coli*, *Proteus vulgaris*, *B. subtilis*, *B. mesentericus*, *B. cereus*, *B. idosus*, *Staph. aureus*. The experiments were carried out at medium pH values of 5.0, 5.5, 6.0, and 6.5, and cultivation temperatures of 15, 26, and 32°C.

The antagonistic action of the lactobacilli was found to be affected by the pH values of culture media, cultivation temperature and the test microorganisms. The most powerful antagonism was observed in respect to *B. cereus* and other included spore-forming microorganisms, and also towards *S. aureus*. In respect of enterobacteria, the action was considerably weaker. The activity was weakest with relation to *Str. faecalis*.

Etude des propriétés antimicrobiales des souches *L. plantarum*, isolées des saucissons secs

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On a déterminé l'activité antagoniste de 30 souches de *L. plantarum*, isolées de saucissons secs, à l'égard de 8 espèces de microorganismes-tests: *Str. faecalis*, *E. coli*, *Proteus vulgaris*, *B. subtilis*, *B. mesentericus*, *B. cereus*, *B. idosus*, *Staph. aureus*. Les expériences ont été effectuées à un pH de 5,0, 5,5, 6,0 et 6,5 et à une température d'étuvage de 15, 26 et 32°C.

On a établi que l'action antagoniste des lactobacilles était influencée des valeurs du pH des milieux culturels, de la température d'étuvage et de l'espèce des microorganismes-tests. Le plus fort antagonisme a été observé à l'égard de *B. cereus* et des autres microorganismes sporulants, ainsi qu'à l'égard de *S. aureus*. Cette action a été beaucoup moins fortement exprimée à l'égard des entérobactéries. Pour les *Str. faecalis* l'activité était la plus faible.

Исследование антимикробных свойств штаммов *L. plantarum*, изолированных из сыровяленных колбас

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Определена антагонистическая активность 30 штаммов *L. plantarum*, изолированных из сыровяленных колбас, в отношении 8 видов тест-микроорганизмов - *Str. faecalis*, *E. coli*, *Proteus vulgaris*, *B. subtilis*, *B. mesentericus*, *B. cereus*, *B. idosus*, *Staph. aureus*. Опыты проводены при pH среды 5.0, 5.5, 6.0, и 6.5 и температуре выращивания 15, 26 и 32°C.

Установлено, что на антагонистическое действие лактобацилл оказывают влияние значения pH питательных сред, температура выращивания и вид тест-микроорганизмов. Наиболее сильный антагонизм наблюдается в отношении *B. cereus* и других включенных спорообразующих микроорганизмов, а также в отношении *S. aureus*. По отношению к энтеробактериям действие значительно слабее. Наиболее слабой является активность в отношении *Str. faecalis*.

A study of the antimicrobial properties of *L. plantarum* strains isolated from raw-dried sausages

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Lactic acid bacteria are one of the reasons for the selection of microorganisms in the raw sausage during ageing (2, 3). Their antagonistic action towards different bacteria has been studied by different authors (3, 7). The inhibitory effect on the growth and enterotoxin production of *Staph. aureus* has been proved (4, 6).

The interrelations between the lactobacilli and the remaining microflora depend on many factors and change during the growth of microorganisms (1).

The present work studies the changes in the antagonistic activity of lactobacilli isolated from raw-dried sausages, due to changes in pH values and cultivation temperature.

Materials and methods

The studies made use of 30 lactobacilli strains defined as *L. plantarum* and isolated from "Panagyurska Loukanka" during ripening. *Str. faecalis*, *E. coli*, *Proteus vulgaris*, *B. subtilis*, *B. mesentericus*, *B. cereus*, *B. idosus*, and *Staph. aureus* (museum strains from the collection of the Institute of Hygiene and Nutrition) were used as test microorganisms.

The antagonistic activity was determined using the method shown by Kafel et al. (8), in terms of mm of sterile zone between the different *L. plantarum* strains and the test microorganisms studied. Cultivation was done at a temperature of 16°C, close to the one employed in the ripening and drying of raw sausages, and at 26 and 32°C, fermentation temperatures for different fast-ripening sausages with bacterial starters. The experiments were conducted on nutrient media of a pH of 5,0, 5,5, 6,0 or 6,5, values close to those of the sausage meat during the ripening of the sausages. Meat-infusion agar of a neutral pH (7,0) and a cultivation temperature of 30°C was used as a control of the antimicrobial activity of the lactobacilli.

Results and Discussion

Under the experimental conditions, the inhibitory action of the lactobacilli studied towards *Str. faecalis* was found to be very weak, if any. The strongest antagonism was observed in respect of *B. cereus*. This fact is of great importance since this microorganism is considered capable of causing food poisonings (5). The strains studied have the best manifested antagonistic properties towards *B. cereus* at a pH of 5,5 and a cultivation temperature of 16°C. This is consequently the explanation why spore-forming microorganisms are rarely isolated during the ripening and drying of raw sausages when the temperature and pH values are close to the above-mentioned ones.

The results obtained on the antagonistic activity of the *L. plantarum* strains studied, are close in values, as far as the remaining test microorganisms are concerned. This enabled their mathematical processing and graphical presentation as mean values. The figures are built on the basis of the  $\bar{X}$  quantities. Table 1 presents also the m values (square error of mean value) for each individual test microorganism with a statistical confidence for 99% of the strains studied.

The data on antagonistic activity at a cultivation temperature of 26°C and pH values above 5,0 are not presented because of the great differences existing in the results and precluding mathematical processing. In isolated cases, no antagonistic activity was found in some *L. plantarum* strains towards some definite test microorganism, for which reason the degree of probability of positive results was calculated, with an interval from 0 (all negative) to 1 (all po-

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Table 1. Antagonistic action of *L. plantarum* towards different test microorganisms under different conditions

n = 30

Temp.	pH	$\bar{X}$	m	$\bar{X}$	m	$\bar{X}$	m	$\bar{X}$	m	$\bar{X}$	m	$\bar{X}$	m
		1. <i>B. subtilis</i>		2. <i>B. mesentericus</i>		3. <i>B. idosus</i>		4. <i>Staph. aureus</i>		5. <i>Proteus vulgaris</i>		6. <i>Esch. coli</i>	
16°C	5,0	6,6	0,4	5,0	0,4	14,4	0,4	10,9	0,7	4,0	0,5	4,2	0,6
	5,5	6,2	0,5			11,3	0,5	8,9	0,7	3,5	0,5	3,0	0,4
	6,0	10,0	0,3	8,7	0,2	10,2	0,4	12,2	0,8	6,2	0,4	10,5	0,3
	6,5	9,0	0,3	8,0	0,4	9,0	0,2	10,0	0,6	5,9	0,3	8,7	0,3
32°C	5,0	8,8	0,2	7,8	0,3	3,7	0,3	7,4	0,3	2,7	0,3	1,9	0,3
	5,5	7,4	0,3	6,2	0,3	3,5	0,2	5,6	0,4	2,4	0,3	1,8	0,3
	6,0	6,8	0,3	5,1	0,3	6,0	0,3	8,4	0,3	7,0	0,3	7,4	0,2
	6,5	6,3	0,2	4,9	0,2	5,4	0,2	7,9	0,2	6,3	0,3	7,2	0,2
26°C	5,0	9,6	0,6	9,0	0,5	4,9	0,5	9,0	0,8	3,3	0,6	1,7	0,4
30°C	7,0	5,8	0,2			5,2	0,2	6,7	0,2	7,6	0,2	4,8	0,2

sitive) /Table 2/. It is obvious that 78% of the analyses give results with a degree of probability of 1.

The data on the inhibitory action of *L. plantarum* towards the bacilli of the subtilis-mesentericus group are presented in Fig. 1. A less manifested antimicrobial activity is observed in respect to *B. mesentericus*, compared to *B. subtilis* (with the exception of that at a pH of 5,0 and a cultivation temperature of 26°C). It is characteristic of both bacilli species that, at the lower cultivation temperature, their sensitivity is greater at higher pH values. This could be explained by the growth intensity, under the given conditions, of both the antagonist and the test microorganism. The lactobacillus growth is weaker at the lower pH values and its inhibitory action is weaker accordingly.

Figure 2 shows the data on the antagonistic action towards *B. idosus* and *Staph. aureus*. In relation to *B. idosus*, a considerably greater growth inhibition is observed at 16°C. This indicates that, with the decrease in pH values during sausage ripening and drying, an enhanced inhibitory action of *L. plantarum* could be expected towards that microbial species. A strong antagonistic action is also observed in respect to *Staph. aureus*. There are quite a lot of data in literature on the antagonistic activity of lactic acid microorganisms towards that

Table 2. Antagonism of *L. plantarum*: degree of probability of positive results

Temp.	pH	Bac. idosus	Bac. cereus	Bac. subtilis	Bac. mesentericus	Staph. aureus	Str. faecalis	Proteus vulgaris
16°C	5,0	1	1	1	1	1	0,32	0,80
	5,5	1	1	1	0,85	1	0,26	0,70
	6,0	1	1	1	1	1	-	1
	6,5	1	1	1	1	1	-	1
32°C	5,0	0,92	1	1	1	1	0,54	0,77
	5,5	0,96	1	1	1	0,93	0,52	0,67
	6,0	1	1	1	1	1	-	1
	6,5	1	1	1	1	1	-	1
26°C	5,0	1	1	1	1	1	0,58	0,75
	5,5	1	1	1	1	1	0,50	0,75
30°C	7,0	1	1	1	1	1	-	1

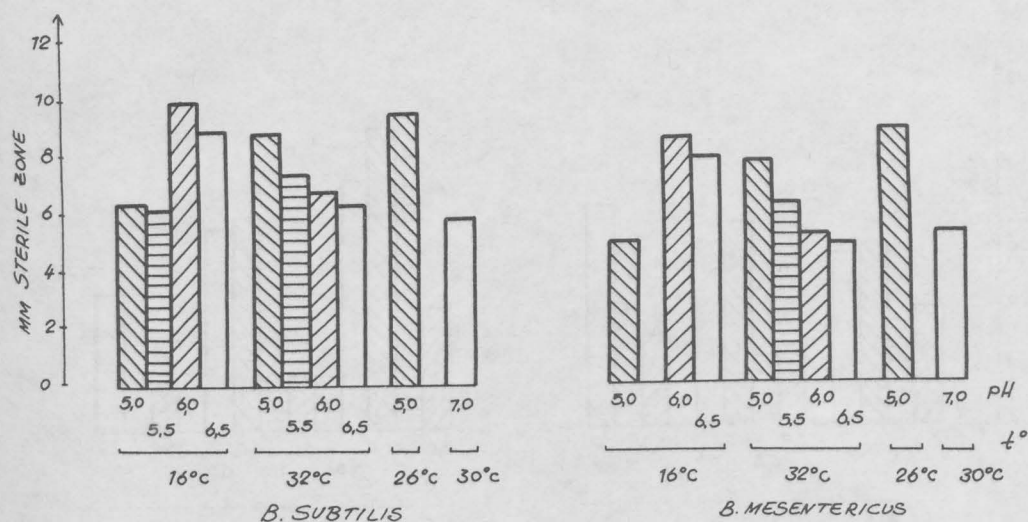


FIG. 1

test microorganism. The results obtained give a further demonstration of that. It is obvious that conditions close to those in industrial production, help to enhance that action, especially the ripening and drying temperature of 16°C. *Staph. aureus* growth is weaker under those conditions and its susceptibility to injury is greater.

The antagonistic action of the *L. plantarum* strains studied towards the enterobacteria used in the experiment is not very strong under the experimental conditions (Fig. 3). With *Proteus vulgaris* as a test microorganism, a decrease in activity is observed in the experimental conditions compared to the control. Since that microorganism is not isolated from sausages in advanced ageing, one may suppose that its disappearance already in the first days is due to the action

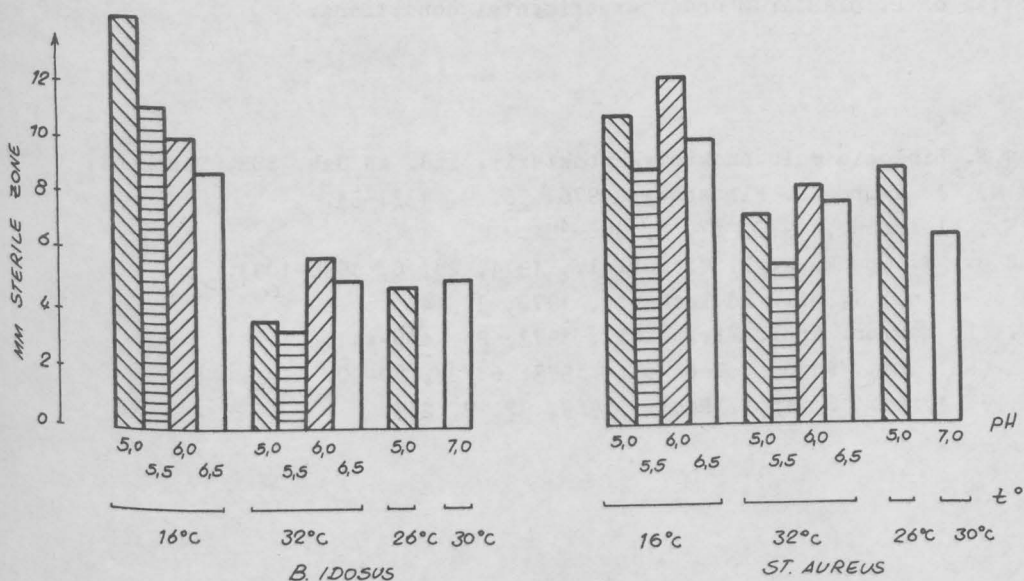


FIG. 2

of changed conditions in sausage meat: reduced water content, increased salt concentration, the presence of spices and the antagonistic action of other microorganisms. One of the problems in the manufacture of meat products is the presence of coliforms in them. The activity of *L. plantarum* with relation to *E. coli* at the experimental cultivation temperatures is enhanced at higher pH values. This indicates that the inhibitory action can be strong in the beginning, before product pH values started decreasing, and in the end of the technological process. It is very rarely that coliforms are found in a ready product.

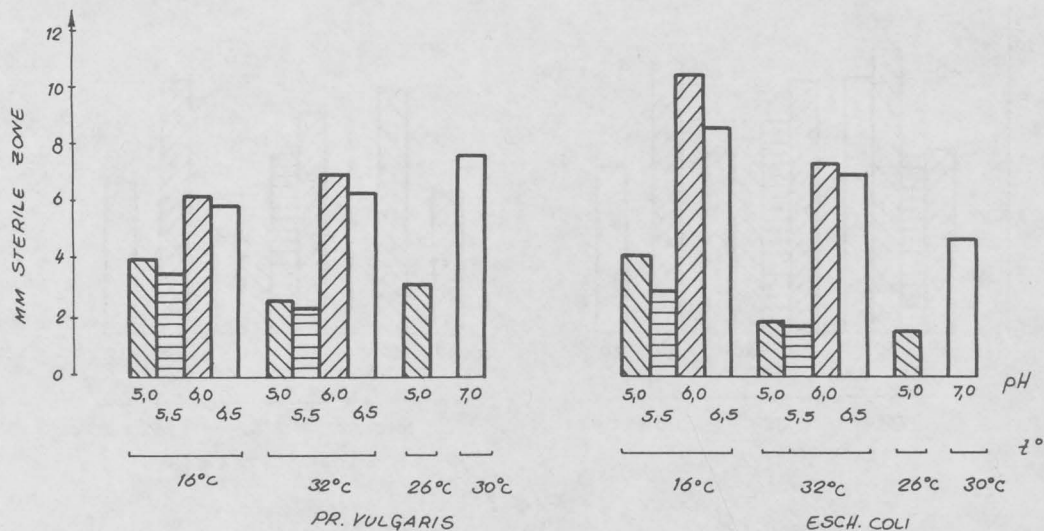


FIG. 3

In most of the studies, it was found that the activity of *L. plantarum* strains is weaker at lower temperatures and pH values. This could be explained by the more unfavourable conditions for their growth.

#### Conclusions

1. *L. plantarum* was found to show a stronger antimicrobial activity towards Gram-positive spore-forming and nonspore-forming microorganisms and a weaker activity towards Gram-negative bacteria.
2. Cultivation temperature and the medium pH values influence the intensity of the antimicrobial properties of *L. plantarum* under experimental conditions.

#### References

1. Kvasnikov E. *Biologia molochnokislykh bakterii*. Izd. AN Uzb. SSR, Tashkent, 1960.
2. Ayroulet M., J. Fournaud. *Fleischw.*, 1976, 56, 9, 1331-34.
3. Coretti K., *Fleischw.*, 1977, 57, 3, 386-394.
4. Gilliland S., M. Speck. *Appl. Microbiol.*, 1974, 28, 6, 1090-1093.
5. Goepfert J. et al. *J. Milk Fd Technol.*, 1972, 35, 4.
6. Haines W., L. Harmon. *Appl. Microbiol.*, 1973, 25, 436-441.
7. Hurst A. *Can. Inst. Fd Sci. Technol.*, 1973, 6 /2/, 80-90.
8. Kafel S., J. Ayres. *J. Appl. Bact.*, 1969, 32, 2, 217.