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INFLUENCE OF SOME FOOD INDUSTRY TECHNOLOGIES ON THE CHARACTERISTICS OF PSEUDOMONAS AERUGINOSA STRAINS

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Abstract

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An investigation was made into the effects of 2 and 3% concentration of NaCl,2.5% nitrite salting mixture, freezing and milk fermentation on the characteristics of 5 typical Pseudomonas aeruginosa strains isolated from water (1), bull semen (2) and bovine mastitis (2).

The results were as follows:

1. On examination of 75 re-isolated P. aeruginosa strains qualitative changes were found 12 times, being recorded in a total of 10 (13.3%) strains. They concerned only dubious to negative attempts at gelatin hydrolysis (9 times), pyoverdine loss (twice) and absence of visible gas from KNO_2 (once). Another frequent finding was the loss of blue-green pigmentation on King´s agar A, but the production of pyocyanin in Gessard´s medium was unaffected.

2. A total of 55 minor quantitative changes were found in 4 strains of the foregoing group and in 41 (54.7% other strains. They concerned delayed growth at 41°C (35 times), production of visible gas from KNO_2 only after adaptation passage in nitrate broth (16 times), delayed oxidase reaction (twice), decreased motility (once) and delayed nitrate reduction (once). They occurred mainly in cases where 2 or 3% NaCl or nitrite salting mixture was added.

3. In 16 (21.3%) strains, decreased mortality of mice inoculated i.p. with 0.2 ml of 18- to 24 h culture was recorded. Almost half of these strains came from meat-peptone broth containing 2% NaCl. Involved in this phenomenon were 12 (23.5%) out of 51 aberrant strains and 4 (16.6%) out of 24 typical strains.

4. Out of 5 virtually unchanged re-isolates, 1 strain from meat-peptone broth with 3% NaCl (1 week) and 2 strains from yoghurt (48 h) showed a decrease in LD_{50}, on average, by 29%, nitrite salting mixture (2 weeks) the shifts of LD_{50} were only -13.1% and +1%, respectively.

Pseudomonas aeruginosa, foodstuffs.

Food industry tecnnologies are directed towards prolonged keeping possibility, acquisition of a new quality or flavour and, in meat industry, also towards fresh appearance of the product. Undesired bacteria are looked upon as a whole and should be destroyed or at least stopped in their replication during the processing.

Numerous reports on isolation of Pseudomonas aeruginosa from raw milk (K i e l w e i n 1968; O t t e et al. 1978; K a t o n a and L á n y i 1982) or meat (B u r z y n s k a and M a c i e j s k a 1974; O r m a y et al. 1980 are, no doubt, of interest but offer no solution to this special problem. Similarly, little help in this respect can be derived from positive findings of P. aeruginosa in baby food (B u r z y n s k a and M a c i e j s k a 1974) or pasterurized milk (H a l a d o v á and L a c o v á 1979) which rather indicate additional contamination.

In other words, few data are available on the influence of technological factors on the characteristics of microbial species in general. These aspects also remained unnoticed in a monograph (J e d l i č k o v á 1981) and recent food industry manuals (A r p a i and B a r t l 1977; Š i l h á n k o v á 1983) published in our country.

Materials and Methods

The following strains of P. aeruginosa were included in the experiments:

No. 39 (CCEB[+] 775)	isolated from water
No. 73 (SVÚP 3/82)	isolated from bull semen
No. 85 (SVÚP 20/82)	isolated from bull semen
No. 105 (VŠÚPT 37069/83)	isolated from bovine mastitis
No. 118 (VŠÚPT 37066/83)	isolated from bovine mastitis

Viewed from the standpoint of systematic bacteriology (K r i e g and H o l t 1984), all the 5 strains can be regarded as typical (Table 1). The technological factors under study, their specification and the lengths of exposure are shown in the following survey:

a) NaCl (in meat-peptone broth):
   2% concentration: 24 h, 48 h, 1 week
   3% concentration: 24 h, 48 h, 1 week

b) Nitrite salting mixture (in meat-peptone broth):
   2.5% concentration: 24 h, 1 week, 2 weeks

c) Freezing (freshly pasteurized egg melange):
   exposure to - 18° C: 1 week, 2 weeks, 4 weeks

d) Lactic acid fermentation (preparation of yoghurt and its storage):
   incubation at 43° C: 3 h
   storage at 10° C: 24 h, 48 h.

After experimental contamination of the individual substrates (10^6 to 10^{10} microbial cells . ml^{-1}) and subsequent incubation the inoculations

[+] CCEB = Czechoslovak Collection of Entomogenous Bacteria, Prague
SVÚP = State Veterinary Institute, Pardubice
VŠÚP = Plant Breeding Research Institute, Troubsko

Table 1

Characteristics of the used strains of Pseudomonas aeruginosa

Diagnostic features:	Strains: 39	73	85	105	118
Rods	+	+	+	+	+
Motility	+	+	+	+	+
Gram	-	-	-	-	-
Relation to O_2	aerobe	aerobe	aerobe	aerobe	aerobe
Growth at 41° C	+	+	+	+	+
Formation of:					
Catalase	+	+	+	+	+
Oxidase	+	+	+	+	+
Pyocyanine	+	+	+	+	+
Pyoverdine	+	+	+	+	+
Nitrate reduction	+	+	+	+	+
Denitrification	+	+	+	+	+
Haemolysis (ovine r. c)	+	+	+	+	+
Milk peptonization	+	+	+	+	+
O-F test in:					
Glucose	0	0	0	0	0
Maltose	-	-	-	-	-
Hydrolysis of:					
Acetamide	+	+	+	+	+
Arginine	+	+	+	+	+
Gelatin	+	+	+	+	+
Starch	-	-	-	-	-
Tween 80	+	+	+	+	+
O-antigen	4	1	11	3	6
Pathogenicity for:					
White mouse: per os	-	-	-	-	-
i. p.	+	+	+	+	+

Table 2

Frequency of changes in characteristics of P. aeruginoss strains after the experiments carried out

Technologic factor:	Exposition:	Delayed growth at 41° C	Decreased motility	Delayed nitrate reduction	Gas from KNO_2 not before 24hrs passage in KNO_3	Absence of gas from KNO_2	Dubious to negative results in gelatin	Delayed oxidase reaction	Absence of pyoverdine	Decreased virulence for white mouse
2 % of NaCl (broth) 37° C	24 hrs	5								3
	48 hrs	5			1					3
	1 week	5			1		1			1
3 % of NaCl (broth) 37° C	24 hrs	4			1					
	48 hrs	3			2					2
	1 week	5			2					1
2.5 % of nitrite salting mixture (broth) 37° C	24 hrs	2			2			1		1
	1 week	2			1		2		1	1
	2 weeks			1		1	1		1	1
Freezing (egg melange) -18° C	1 week				1		1	1		2
	2 weeks	2			2		1			1
	4 weeks						1			
Fermentation of milk (youghurt)	3 hrs/43°C				1					
	24 hrs/10°C	2	1		2		1			
	48 hrs/10°C						1			
Changes on the whole		35	1	1	16	1	9	2	2	16

were made on Pseudomonas agar F IMUNA. For evaluation of 24- to 48-h cultures grown at 30° C, an UV lamp was used so that one suspect colony each could also be taken for bacteriological examination.

The re-isolated strains were examined for 20 diagnostic features as specified in Table 1. In addition to this, antigenic structure (O-serovar) and virulence for white mice were retested. The techniques used in our study are in keeping with those described in standard manuals (C a r t e r 1973; C o w a n and S t e e l 1974; S t a r r et al. 1981; Krieg and H o l t 1984) and recent relevant reports (A r a i et al. 1970). They were cited in full in a previous study by M r á z (1987) who also suggested some improvements:

1) Relation to molecular oxygen is tested by inoculation into 0.3% meat--peptone agar containing 0.5 g sodium thioglycolate and 0.15 ml of 1% aqueous solution of resazurin per 1 litre medium.

2) Motility is assessed on the basis of diffuse growth after inoculation into semi-solid agar ad 1) supplemented with 0.1% KNO_3. In negative and dubious cases the hanging drop technique is used in addition.

3) Denitrification ability is examined in anaerobic meat-peptone broth with 0.1% KNO_2 and with an inverted Durham gas tube. Incubation at 37 °C is carried out for no longer than 10 days. Possible negative (defective or anaerogenic) strains can be completed by 24-h passage in nitrate broth (P a l l e r o n i and D o u d o r o f f 1972).

4) Pyocyanin production is tested using a modification of G e s s a r d (1981) medium (1% glycerol and 2% Bacto-Protone or Neoptone Difco in distilled water) incubated at 30° C for 5 days.

The virulence of the strains was tested in groups of 2 mice each inoculated i.p. with 0.2 ml of 18- to 24-h broth culture and in separate experiments per os using contaminated feed tablets. Moreover, the LD_{50} (R e e d and M u e n c h 1938) was determined in all 5 starting strains and in 5 virulent re-isolates.

Results

The effects of technological factors were found in 51 (68%) out of 75 P. aeruginosa re-isolates (Table 2). A total of 67 changes shown by the strains can be divided into two groups:

a) Qualitative changes in the diagnostic sense were recorded 12 times, being found in 10 (13.3%) strains. They concerned only dubious to negative attempts at gelatin hydrolysis (9 times), loss of pyoverdine (twice) and absence of visible gas from KNO_2 (once). Another frequent phenomenon was the loss of blue-green pigmentation on King´s agar A, but the production of pyocyanin in the modified Gessard medium remained unaffected.

b) A total of 55 minor quantitative changes were found in 4 strains of the foregoing group and in 41 (54.7%) other strains. They concerned delayed growth at 41° C (35 times),

production of visible gas from KNO_2 only after adaptation passage in nitrate broth (16 times), delayed oxidase reaction (twice), decreased motility (once) and delayed nitrate reduction (once). They occured mainly in meat-peptone broth containing 2 or 3% NaCl or nitrite salting mixture.

In certain agreement with the aforementioned data was the finding of 16 (21.3%) cases of decreased mortality in i.p. inoculated mice. Involved in this phenomenon were 12 (23.5%) out of 51 aberrant strains and 4 (16.6%) out of typical strains. The highest frequency (7 times) was recorded for meat-peptone broth containing 2% NaCl from which also the majority (3) of entirely innocuous strains came. All 75 mouse pairs fed P. aeruginosa cultures survived.

The antigenic structure (O-serovar) showed no changes during the experiments.

Table 3 shows the results obtained in 15 re-isolates each of the 5 starting strains. It can be seen that the number of changes ranged from 10 to 15, averaging 13.4 (0.9 per re-isolate). Decreased mortality of mice was observed mainly in re-isolates of strain No. 39 from water and strain No. 85 from bull semen.

Bioassays for determination of the LD_{50} in the 5 starting strains and 5 virulent re-isolates were carried out almost concurrently and in immediate continuation of the density determination of their 18- to 24-h broth cultures (Table 4). Comparison of the results shows that the LD_{50} of re-isolates of strains Nos. 73, 85 and 105 was 17.9 to 43.1% lower, whereas in the re-isolates of strains Nos. 39 and 118 the shifts of LD_{50} were only -13.1 and +1%, respectively.

Discussion

It is well-known that changes in the environment may produce more or less pronounced changes in the characteristics of bacteria. The extent and intensity of this variability are of many-sided importance, being of value, among other things, to bacteriological diagnostics. This question was therefore considered by us concurrently with our investigation into the effects of some technological processes on the survival of P. aeruginosa in foods (L u k á š o v á and M r á z 1986).

Table 3

Frequency of changes in re-isolates of P. aeruginosa according to the starting strains

Re-isolates (15 each) of starting strain No:	Delayed growth at 41° C	Decreased motility	Delayed nitrate reduction	Gas from KNO_2 not before 24hrs passage in KNO_3	Absence of gas from KNO_2	Dubious to negative results in gelatin	Delayed oxidase reaction	Absence of pyoverdine	Changes on the whole	Decreased virulence for white mouse
39	6			3			1		10	5
73	8			5		2		2	17	3
85	7	1		3			1		12	5
105	9		1	3	1	1			15	2
118	5			2		6			13	1
Altogether	35	1	1	16	1	9	2	2	67	16

The changes in the biochemical characteristics were mainly quantitative. In gelatin, however, where the proportion of dubious to negative cases was 12%, a qualitative aberration is indicated. In practice it means that this characteristic should be assigned the sign of d or v.

A rather surprising finding was the 29.3% absence of blue-green pigmentation on King´s agar A which is generally regarded as specific and very sensitive. Even though it is probable that the proportion of positive results might be increased by cutting the cultures and shaking out possible pyocyanin into chloroform, it seems more useful to choose Gessard´s modified medium.

Further biochemical examinations demonstrated a considerable value of the test for acetamide hydrolysis (A r a i et al. 1970) and the usefulness of the employed denitrification technique in pseudomonads.

Table 4

LD_{50} of P. aeruginosa strain No 39 re-isolated from egg melange (4 weeks of freezing)

Dilution	dead/whole	dead	alive	dead	alive	dead/whole	% of dying
1 : 1	3/4	3	1	7	1	7/8	87,5
1 : 2	3/4	3	1	4	2	4/6	66,6
1 : 4	1/4	1	3	1	5	1/6	16,6
1 : 8	0/4	0	4	0	9	0/9	0

$$x = 0.332$$

$$LD_{50} = 2 \cdot 2^{0.332}$$

$$= 2 \cdot 1.285$$

$$= 0.2 \text{ ml of culture diluted } 1 : 2.57$$

$$\text{Density of culture} = 750 \cdot 10^6 \text{ bacterial cells} \cdot \text{ml}^{-1}$$

$$LD_{50} = 58.366 \cdot 10^6 \text{ bacterial cells}$$

$$\text{Initial } LD_{50} = 67.185 \cdot 10^6 \text{ bacterial cells}$$

$$\text{Shift of } LD_{50} \text{ in \%} = -13.1$$

Table 4a (continued)

LD_{50} of further 4 re-isolated strains of P. aeruginosa

Dilution	Strain No: / Technology: / Exposition:	73 MPB + 3 % NaCl 1 week	85 youghurt 48 hrs	105 youghurt 48 hrs	118 MPB + 2,5 % KNO_2 2 weeks
1 : 1		4/4	3/4	4/4	4/4
1 : 2		2/4	2/4	4/4	3/4
1 : 4		1/4	1/4	2/4	4/4
1 : 8		0/4	0/4	1/4	1/4
x =		0.230	0	0.286	0.523
LD_{50} =		$2 . 2^{0.230}$	$2 . 2^{0}$	$4 . 2^{0.286}$	$4 . 2^{0.523}$
=		2 . 1.172	2 . 1	4 . 1.219	4 . 1.436
= 0.2 ml of culture diluted		1 : 2.344	1 : 2	1 : 4.876	1 : 5.744
Density of culture . ml^{-1}	=	$665 . 10^6$	$700 . 10^6$	$850 . 10^6$	$870 . 10^6$
LD_{50} (bacterial cells)	=	$56.74 . 10^6$	$70 . 10^6$	$34.864 . 10^6$	$30.292 . 10^6$
Initial LD_{50} (bact. cells)	=	$99.7 . 10^6$	$95.2 . 10^6$	$42.5 . 10^6$	$30 . 10^6$
Shift of LD_{50} in %	=	-43.1	-26.5	-17.9	+1

The evidence from the bioassays on mice suggests that involved in the 16 dubious to negative results was not only the nutrient medium (mainly meat-peptone broth containing 2% NaCl) but also the biochemical lability of some re-isolates. In this connexion it should be noted that decreased mortality to complete innocuity was found mainly in aberrant strains (cca by 7%).

The determinations of LD_{50} suggest that the virulence of pseudomonads can more or less increase in certain environments (particularly in yoghurt). From Table 4 it also appears that in routine bioassays on mice an i.p. dose of 0.2 ml of 18- to 24-h broth culture is preferable to the 0.1 ml dose used hitherto.

Of certain value is also the fact that all the re-isolates of P. aeruginosa were innocuous for mice when administered per os. In our view, this observation might be used for more favourable evaluation of microbial findings in feeds and some foods.

Vliv některých potravinářských technologií na vlastnosti kmenů Pseudomonas aeruginosa

V práci byl zkoumán vliv 2 a 3 % koncentrace NaCl, 2,5 % dusitanové solicí směsi, mražení a mléčného kysání na vlastnosti 5 typických kmenů P. aeruginosa z vody (1), býčího spermatu (2) a bovinní mastitidy (2).

Dosažené výsledky:

1) Z 75 reizolovaných kmenů P. aeruginosa byly kvalitativní změny zjištěny celkem 12x, a to u 10 (13,3 %) kmenů. Týkaly se jen dubiózních až negativních pokusů o hydrolýzu želatiny (9x), ztráty pyoverdinu (2x) a absence viditelného plynu z KNO_2 (1x). Častým úkazem byla i ztráta modrozelené pigmentace na Kingově agaru A, ale tvorba pyocyaninu v modifikovaném Gessardově médiu zůstala zachována.

2) Drobných kvantitativních změn bylo zjištěno celkem 55, a to u 4 kmenů z předchozí skupiny, a zbytek u 41 (54,7 %) dalších kmenů. Týkaly se opožděného růstu při 41 °C (35x), tvorby viditelného plynu z KNO_2 až po adaptační pasáži v nitrátovém bujónu (16x), opožděné reakce

na oxidázu (2x), snížené pohyblivosti (1x) a opožděné redukce nitrátu (1x). Vyskytovaly se hlavně za přídavku 2 a 3 % NaCl a dusitanové směsi.

3) U 16 (21,3 %) kmenů došlo ke snížení úhynu u naočkovaných myší (0,2 ml 18 - 24hod. bujónové kultury i.p.), z toho téměř v polovině případů (u 7 kmenů) ve spojitosti s 2 % NaCl v masopeptonovém bujónu. Z jiného pohledu lze říci, že se na tomto počtu podílelo 12 (23,5 %) z 51 kmenů aberantních a 4 (16,6 %) z 24 kmenů typických.

4) Z 5 vesměs nezměněných reizolant se LD_{50} u 1 kmene z masopeptonového bujónu s 3 % NaCl (1 týden) a 2 kmenů z jogurtu (48 hodin) v průměru o 29 % snížila, zatímco u 1 kmene z mražené vaječné melanže (4 týdny) a 1 kmene z pasopeptonového bujónu s 2,5 % dusitanové směsi (2 týdny) činil zjištěný posun LD_{50} jen -13,1 a +1 %.

Влияние некоторых пищевых технологий на свойства штаммов Pseudomonas aeruginosa

В работе проводились исследования влияния 2 и 3% концентрации хлористого натрия, 2,5% нитритного рассола, замораживания и молочной закваски на свойства 5 типичных штаммов P. aeruginosa из воды (1), бычей спермы (2) и воспаления вымени коров (2).

Достигнутые результаты:

1) Из 75 реизолированных штаммов P. aeruginosa качественные изменения были установлены в итоге в 12 раз, а именно у 10 (13,3%) штаммов. Они касались сомнительных даже негативных попыток гидролиза желатина (9 раз), потери пиовердина (2 раза) и отсутствия видимого газа из KNO_2 (1 раз). Часто встречающимся явлением стало исчезновение сине-зеленой пигментации на агаре Кинга А, однако образование пиоцианина в модифицированной среде Гессарда оставалось.

2) Незначительных количественных изменений было выявлено в итоге 55, в частности, у 4 штаммов предыдущей группы, остаток у 41 (54,7%) других штаммов. Они касались запоздалого роста при 41 °C (35 раз) образования видимого газа из KNO_2 только после адап-

тивного прохождения в нитратном бульоне (16 раз), запоздалой реакции на оксудазу (2 раза), пониженной подвижности (1 раз) и запоздалой редукции нитрата (1 раз). Они встречались, главным образом, при добавке 2 и 3% хлористого натрия и нитритной смеси.

3) В случае 16 (21,3%) штаммов произошло понижение отхода у привитых мышей (0,2 мл бульонной культуры i.p.), из этого почти в половине случаев (7 штаммов) в связи с 2% хлористым натрием в мясопептоновом бульоне. С другой точки следует отметить, что из 51 отличающегося штамма в данную численность входило 12 (23,5%) штаммов и из 24 типичных штаммов - 4 (16,6%) штамма).

4) Из 5 большей частью не измененных реизолянтов LD_{50} у 1 штамма из масопептонового бульона с 3% хлористого натрия (1 неделя) и 2 штаммов из югурта (48 часов) в среднем на 29% понизилась, между тем как у 1 штамма из замороженной яичной смеси (4 недели) и 1 штамма из масопептонового бульона с 2,5% нитритной смеси (2 недели) установленный сдвиг LD_{50} достигал лишь -13,1 и +1 %.

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