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A Study of Psychrophilic Microorganisms Occuring on Pork

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Cold resistant microorganisms are gaining importance with the increased application of cold storage, quick freezing and refrigerated distribution. In order to be able to work out methods to prevent spoilage in foods caused by these organisms it is inevitable to investigate their characteristics /Ayres, 1960; Peterson and Gunderson, 1960/.

Hitherto comparatively little was known of these microorganisms capable of proliferation at lo w temperatures, which were termed cryophiles, psychrophiles etc., in related literature /Forster, 1887; Schmidt Nielson, 1902/. It is not easy to classify them and to distinguish them from mesophilic bacteria /Ingraham and Stokes, 1959; Witter, 1961/. Usually the temperature limits of growth serve as basis of differentiation. Frequently this group is defined as being restricted to microorganisms that have optimum growth temperature below 20° C. However investigations of a quantitative character proved /Zobel and Conn, 1950; Lawton and Nelson, 1954. Van der Zant and Moore, 1955; Ingraham, 1958; etc/that none of these organisms possesses an optimum growth temperature below 20-25° C.

Because of the uncertainity prevailing in the definition of these organisms it seemed desirable to investigate the growth characteristics of psychrophiles occuring on meat in Hungary.

The first object of the investigations was to determine the quantitative distribution of microorganisms occuring on frozen pork, beef and chichen, respectively, stored at -20° C and the frequency of occurrence of psychrophiles. Viable aerobic cell counts of samples taken after different storage periods from all three kinds of meat, kept in frozen storage, ranged between 10^4 and 10^7 per gram. Viable cell count was determined by the pour-plate method at various dilution levels on "universal culture medium" x incubated at 30° C. 5 parallels were applied at each dilution level. To determine the frequency of occurrence of psychrophilic bacteria, all colonies from a culture at a suitable dilution level were transferred to liquid universal medium and universal agar slant. 302 strains were isolated from pork, 275 from beef and 202 from chicken. The macromorphological properties of the colonies developed from the isolated strains, as well as microscopical, staining and growth tests were used to characterise the strains and to determine the composition of the microbial population.

x/ Composition of the universal culture medium was the following: meat extract, 4 g; whey, 200 ml; yeast water, 1:10 dilution, 100 ml; glucose, 10 g; peptone, 5 g; distilled water, 700 ml; pH adjusted at 7,2; containing 2 % agar.

This paper contains the results of a study into the microbial flora of pork.

Most authors /Haines, 1934; Brown and Weidemann, 1958; Halleck et al., 1958; Walker and Ayres, 1956/ ascribe the spoilage in meat to the presence of Gram negative rods. According to Árpai and Bánhegyi /1960/ the microbial flora consists mainly of cocci. The difference between the different literary data is probably due to the circumstance that the composition of the microbial population is dependent on slaughter house technology and changes during storage.

The distribution of the microbial flora of frozen stored pork between the main microbial strains is shown in Figure 1. The majority of the total aerobic cell count is provided by Gram positive cocci most of which were identified as belonging to the genus Micrococcus. The rest of the flora consisted of Gram negative and Gram positive non spore forming rods, some Gram positive spore forming microorganisms, yeasts, and of a very small number of various strains not belonging to either of the aforesaid groups. As may be seen in the frequency histogram, psychrophiles may be found only among the non spore forming bacteria. The taxonomic investigation of the great number of isolated strains will be described in another paper.

In the first investigations a strain was considered as psychrophilic when, cultured on universal nutrient medium at

temperatures between +3 and +5° C, the total cell count increased from the initial 10^5 to at least 10^7/ml in 7 days. Subsequent investigations aimed at clearing, wether any definite border line may be drawn between the bacteria proliferating rapidly between +3° C and +5° C and those considered mesophilic, not growing below 10° C and having an optimum growth temperature of 37° C.

20 strains, considered psychrophilic according to the above mentioned investigations, and a coliform strain of mesophilic character were selected. These were tested at the temperature levels -15, 0, +10, +25, +30 and +45° C, in 10 paralells each.

The growth of bacteria was followed at temperatures above 0° C by turbidimetric measurements with Vas's method /1955/, using a test tube adapter and a Pulfrich photometer. The statistical averages of the optical density values obtained by the measurement of 10 parallels were converted into cell counts with the aid of calibration curves plotted on the basis of direct cell counts, obtained by means of Helber's counting chamber. In samples incubated at -15° C and with strains not multiplyng at 0° C, the pour-plate viable cell counting method was applied.

The rate of growth was characterized by the reciprocal of the generation times /the number of generations per hour/ related to the logarithmic growth phase. Figure 2. shows the relative growth

rates of some of the strains investigated, as a function of the incubation temperature. The relative growth rate was characterized by the quetient of the generation times, measured at a given temperature /G_T/ and at the optimum growth temperature /G_{min}/. resp.

As may be seen in Figure 2. the rest of the strains plotted, occupy various transitional places between the most psychrophilic species, Micrococcus N 15/6 on one hand, and the mesophilic Escherichia 034/1 species on the other. Consequently no strict line may be drawn between psychrophiles and mesophiles in relation to the cardinal growth temperatures or the optimum growth temperature. However, the data, represented in the order of cold resistance, show, that although transition is gradual, the possibility of differentiation is given and the strains shown in Figure 2. fall into four groups.

Bacteria growing rapidly at 0° C, but unable to proliferate at 37° C, were termed obligate psychrophiles /for instance Micrococcus N 15/6 species/. The term psychrotolerant was applied to microorganisms reproducing equally well at 0° C and 37° C or at even higher temperatures. The organisms that are not capable of propagation at 0° C, but grow rapidly above 40° C, and differ from the obligate

mesophilic species, 034/1 Escherichia only by being able to proliferate in a wider range of temperatures, were termed psychrotrophic mesophiles. It may be mentioned here, that every strain capable of growing at or below +5° C, regardless of their optimum growth temperature, were termed psychrotrophic by Eddy /1960/. It is suggested to use the term psychrophilic only when the optimum growth temperature is at a low level.

The rate of growth of some characteristic strains as a function of incubation temperature is shown in Figure 3.

The investigation of growth and death characteristics of the isolated strains was extended to temperatures below 0° C. The viable cell count in cultures quick frozen and incubated at -15° C, as a function of storage time is shown in Figure 4. It may be seen that the viable cell count of minced liver did not change throughout a longer storage period. However the viable cell count of cold tolerant strains inoculated on universal culture medium, and frozen, was reduced by about two orders of magnitude during the 150 days incubation period. The Escherichia 034/1 strain, under similar circumstances, was totally killed within 1 to 2 days. On no occasion was increase of cell count observed.

By comparing the results of investigations on the kinetics of growth and death, no correlation was found between the cold

tolerance observed in relation to cell growth on one hand, and the cold tolerance related to death on the other. No correlation could be found between cold tolerance and cell size or shape, either.

The kinetic tests related to the logarithmic phases of growth were extended to the determination of the obtainable maximum cell yield. The maximum cell counts, obtained, as averages of 10 parallel samples grown on universal culture medium at an initial viable cell count of 10^5/ ml at given temperatures, are shown in Figure 5. It may be seen very clearly, that No. 306/13 psychrotolerant strain gave maximum yield in the large interval between +5° C to 30°C, whereas the obligate mesophilic strain No. 034/1 gave maximum cell yields only in the narrow interval between 25° C and 37° C. The larger extent of the maximum yield range was characteristic of psychrotolerant strains, however between obligate psychrophiles and obligate mesophiles every variation may occur. A strain showing these transitory characteristics is P 34/12 which gave maximum yield between 10°C and 37° C and slack growth at 0° C.

Summing up the aforesaid it may be concluded that because of the continuous and gradual transition no strict line may be drawn between psychrophilic and mesophilic microorganisms. The large changes in cell yields caused by small changes in temperature, prove that the biochemical properties related to the physiology of growth

have to be more throughly investigated.

Acknowledgment.

The author is indebted to É. Andrássy, I.Kiss, N.Kutz and I.Farkas, members of the Central Food Research Institute, for their valuable cooperation in performing the great number of tests.

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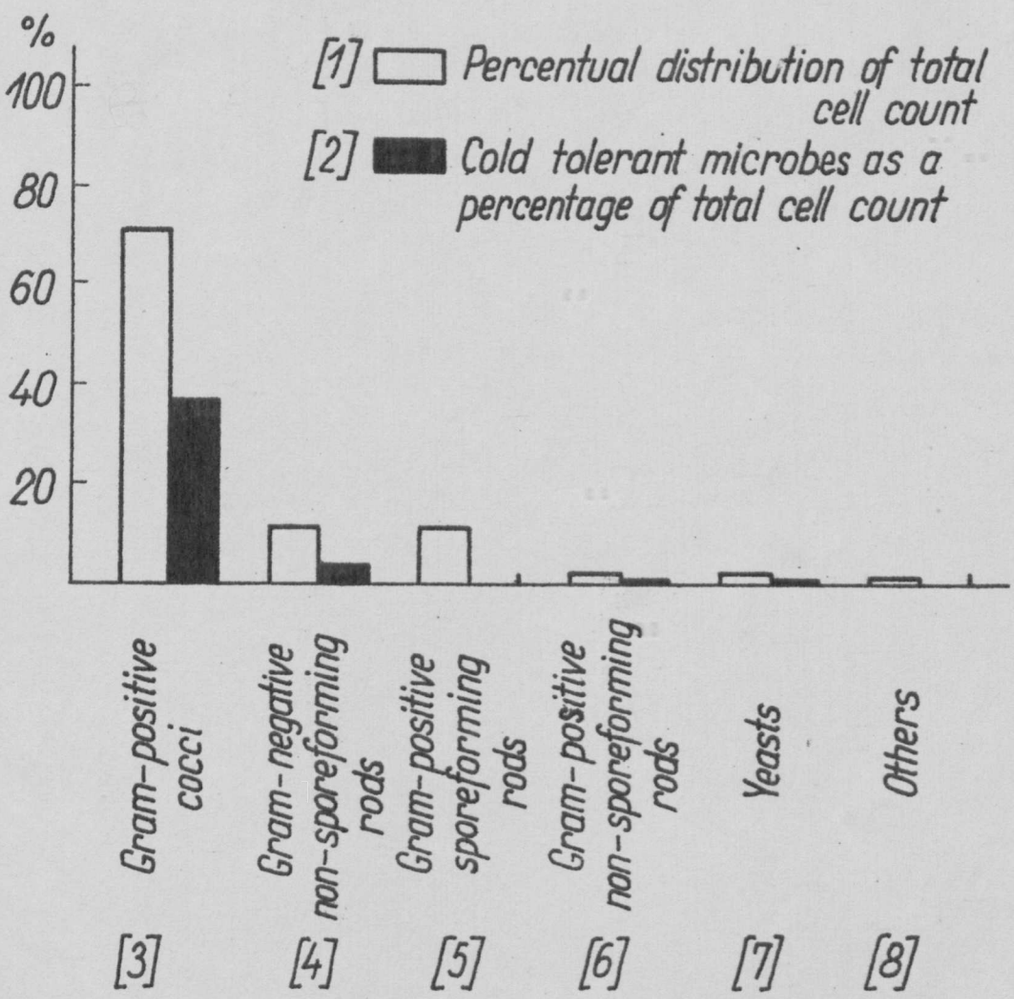
%
100
80
60
40
20
[1] Percentual distribution of total cell count
[2] Cold tolerant microbes as a percentage of total cell count
Gram-positive cocci
Gram-negative non-sporeforming rods
Gram-positive sporeforming rods
Gram-positive non-sporeforming rods
Yeasts
Others
[3]
[4]
[5]
[6]
[7]
[8]

Fig. 1

Signs [1]		[2] Temperature (T) 0°C	10°C	25°C	30°C	37°C	45°C	
N15/6	Micrococcus sp.	●	✳	✳	●	○	○	Obligate psychrophiles
308/17	Micrococcus sp.	●	✳	✳	✳	●	○	Psychrotolerants
306/13	Achromobacter sp.	●	●	✳	●	●	○	
306/4	Streptococcus sp.	●	●	✳	✳	●	○	
306/14	Flavobacter sp.	●	●	✳	✳	✳	◐	
306/2	Micrococcus sp.	●	●	✳	✳	✳	◐	
N15/4	Micrococcus sp.	◐	●	✳	✳	✳	●	
308/31	Micrococcus sp.	◐	●	✳	✳	✳	●	
P34/12	Aerobacter sp.	◐	●	●	●	✳	◐	
308/18	Micrococcus sp.	○	●	✳	✳	✳	◐	Psychrotrophic mesophiles
P34/19	Aerobacter sp.	○	●	✳	✳	✳	●	
306/27	Micrococcus sp.	○	●	✳	✳	✳	●	
308/11	Micrococcus sp.	○	●	✳	✳	✳	●	
034/1	Escherichia sp.	○	◐	●	✳	✳	●	Obligate mesophiles

[3] ✳ Highly vigorous growth, $1{,}0 \leq \frac{G_T}{G_{min}} < 2{,}5$

[4] ● Vigorous growth, $2{,}5 \leq \frac{G_T}{G_{min}} < 10{,}0$

[5] ◐ Slack growth, $10{,}0 \leq \frac{G_T}{G_{min}}$

[6] ○ No growth in 7 days

Fig. 2

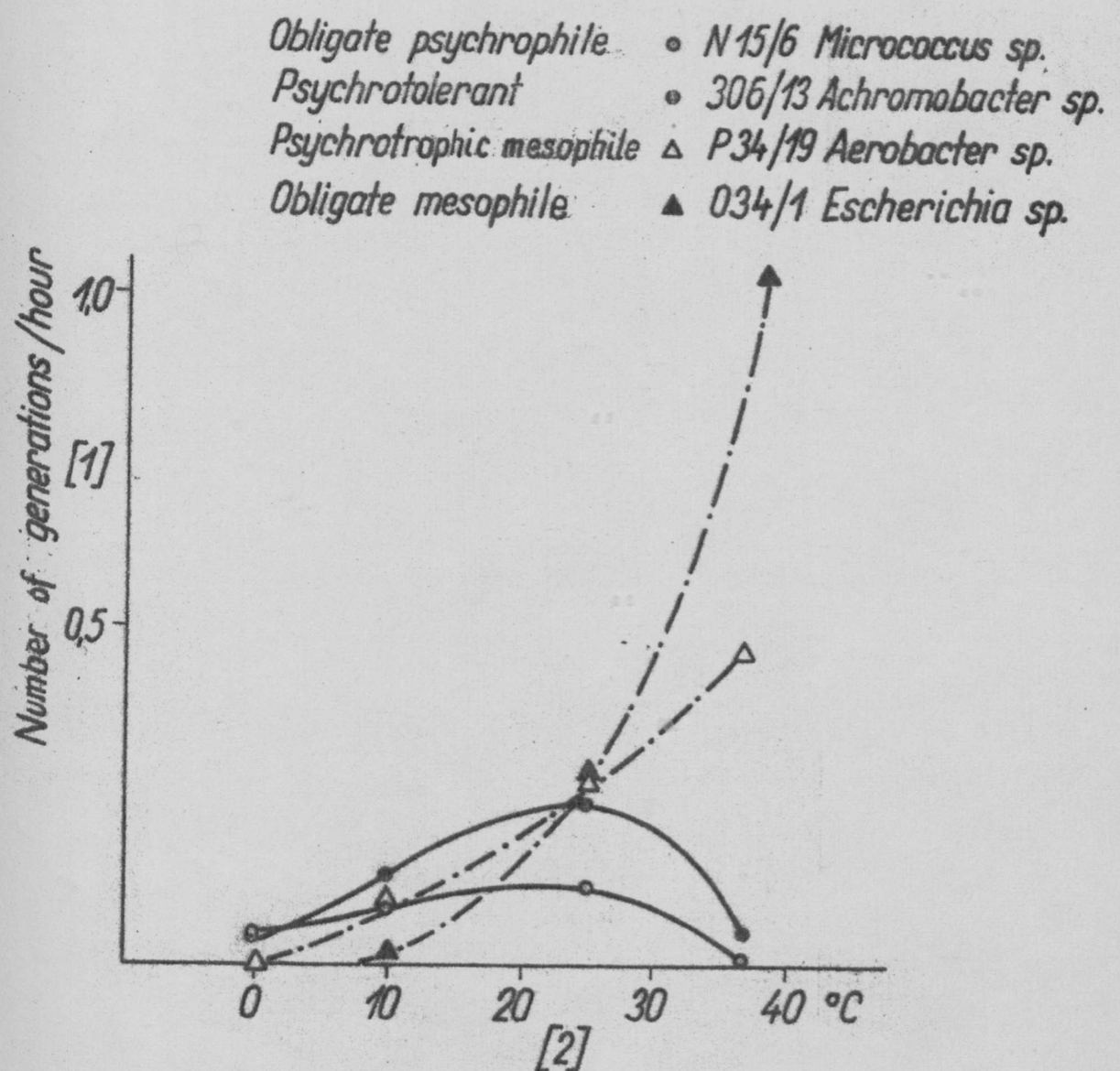
Obligate psychrophile ○ N 15/6 Micrococcus sp.
Psychrotolerant ● 306/13 Achromobacter sp.
Psychrotrophic mesophile △ P34/19 Aerobacter sp.
Obligate mesophile ▲ 034/1 Escherichia sp.
Number of generations/hour
1,0
[1]
0,5
0
10
20
30
40 °C
[2]

Fig.3

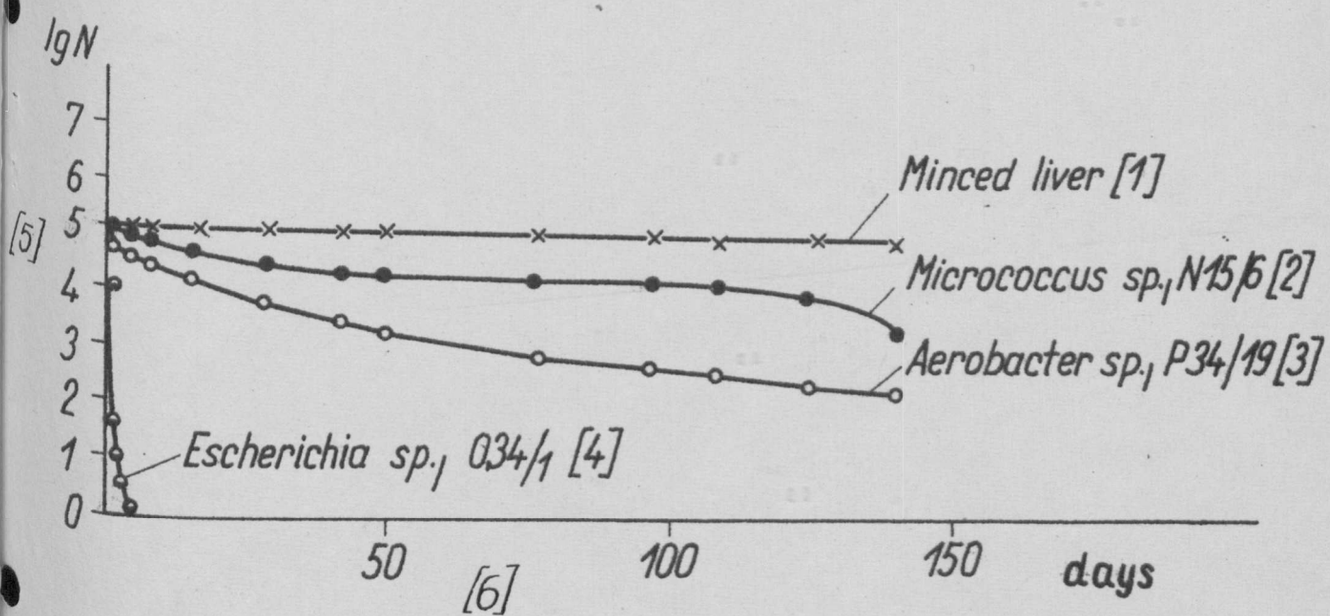
lg N
7
6
5
4
3
2
1
0
[5]
Minced liver [1]
Micrococcus sp.1 N15/6 [2]
Aerobacter sp.1 P34/19 [3]
Escherichia sp.1 034/1 [4]
50
100
150
days
[6]

Fig. 4

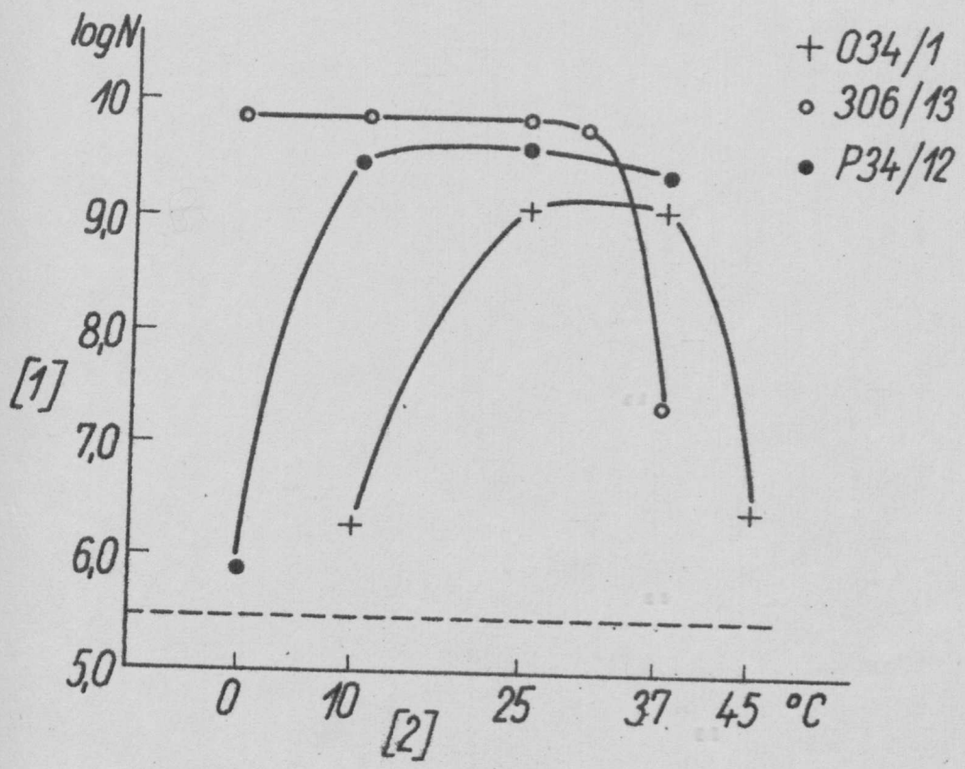

Fig. 5

Untersuchungen über die im Schweinefleisch vorkommenden psichrophylen Mikroorganismen

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Es wurde die qualitative Zusammensetzung der Mikroflora des in gefrorenem Zustand gelegerten Schweinefleisches und die Häufigkeit der kälteresistenten Mikroorganismen untersucht. Von 302 isolierten Stämmen erwiesen sich 75 % als grampositive /grösstenteils zum Genus Micrococcus gehörende/ Kokken. Etwa 40% der isolierten Stämme waren kälteresistent /bei einer Inkubationstemperatur von +3 - +5° C zeigten diese Stämme in flüssigem Nährboden in 7 Tagen eine Kemzahlerhöhung von wenigstens 2 Grössenordnungen/.

Es wurden 20 Stämme von den kälteresistenten Mikroben, ferner ein mesophyle Eigenschaften aufweisender coliform Mikroorganismus bezüglich Vermehrungs- und Abtötungseigenschaften bei -15, 0, +10, +25, +30 und + 45°C untersucht. Die Untersuchungsergebnisse deuten darauf hin, dass auch Übergangseigenschaften aufweisende Spezies neben den bei 0° C gut gedeihenden, doch bei 37° C zur Vermehrung unfähigen Bakterien und neben den bei 0° C sich nicht mehr vermeherenden doch über 40° C wachsenden Stämmen in grosser Menge vorhanden sind. Deshalb kann zwischen den psichrophylen und mesophylen Mikroorganismen nach den kardinalen Vermehrungstemperaturen bzw. dem Temperaturoptimum keine scharfe Grenze gezogen werden.

Aufschrift der Abbildungen:

Abbildung 1.: Qualitative Zusammensetzung der Mikroflora vom Schweinefleisch

Bezeichnungen:

/1/ Prozentuale Verteilung der Gesamtkeimzahl

/2/ Kälteresistente Mikroben in Prozenten der Gesamtkeimzahl ausgedrückt

/3/ Grampositive Kokken

/4/ Gramnegative Stäbchen ohne Sporenbildung

/5/ Grampositive sporenbildende Stäbchen

/6/ Grampositive Stäbchen ohne Sporenbildung

/7/ Hefen

/8/ Andere Mikroorganismen

Abbildung 2.: Relative Vermehrungsfähigkeit $/\frac{G_T}{G_{min}}/$ einiger Bakterienstämme von der Inkubationstemperatur abhängig

/1/ Zeichen des Bakterienstammes

/2/ Inkubationstemperatur /T/

/3/ Reichliche Vermehrung, $1{,}0 \leq \frac{G_T}{G_{min}} < 2{,}5$

/4/ Mittelmässige Vermehrung, $2{,}5 \leq \frac{G_T}{G_{min}} \leq 10{,}0$

/5/ Schwache Vermehrung, $10{,}0 \leq \frac{G_T}{G_{min}}$

/6/ Keine Vermehrung

G_T Generationszeit bei Temperatur T

G_{min} minimale Generationszeit gemessen beim Temperaturoptimum

Abbildung 3.: Zahl der Generationen /1/ in einer Stunde bei manchen spezifischen Stämmen abhängig von der Inkubationstemperatur /2/

Abbildung 5.: Logarithmus der in flüssigem Nährboden erreichten maximalen Keimdichte /1/ abhängig von der Inkubationstemperatur /2/

Abbildung 4.: Gestaltung der Zahl der lebenden Keime /5/ in schnellgefrorenem gehacktem Leber /1/ bzw. in tiefgefrorenem flüssigen Kulturen einiger Stämme /2,3,4/ abhängig von der Lagerungszeit /Tage/ /6/ bei -15° C.

ИССЛЕДОВАНИЕ НА СВИНИНЕ ВСТРЕЧАЕМЫХ ПСИХРОФИЛЬНЫХ МИКРООРГАНИЗМОВ

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Проводили исследования качественного состава микрофлоры свинины храненного в замороженном виде и частость холодостойких микроорганизмов. Из выделенных 502 штаммов приблизительно 75 % составляли грамположительные /в большинстве принадлежащие к Micrococcus genus -ам/ коккусы. Из выделенных штаммов приблизительно 40 % оказались холодостойкими /при температуре инкубации +3 - +5°C в жидкой питательной среде в течении 7 суток показали по крайней мере двойной порядок увеличения числа зародышей/.

На 20 психрофильных штаммах и на одном штамме показывающий мезофильные особенности, исследовали свойства размножения и отмирания колиформных микроорганизмов при температурах -I5, 0, +I0, +25, +30 и +45°C.

Результаты исследований показали, что кроме бактерий размножающихся хорошо при 0°C, а не способных размножаться при температуре 37°C, а также кроме штаммов не размножающихся уже при 0°C, а размножающихся еще выше 40°C, имеются в большом количестве также и рассы распоряжающиеся временными особенностями.

Поэтому нельзя установить строгую границу между кардинальными температурами размножения, или по оптимальной темпера -

туре психрофильных и мезофильных микроорганизмов.

Надписи рисунков:

Рис. I.: Качественный состав микрофлоры свинины.

Обозначения:

/I/ Процентное разделение общего числа зародышей.

/2/ Психрофильные микробы в процентах общего числа зародышей.

/3/ Грамположительные коккусы.

/4/ Грамотрицательные, неспоровые палочки.

/5/ Грамположительные, споровые палочки.

/6/ Грамположительные, неспоровые палочки.

/7/ Дрожжи.

/8/ Прочие микроорганизмы.

Рис. 2.: Относительная способность размножения некоторых штаммов бактерий в зависимости от температуры инкубации $\frac{G_T}{G_{min}}$

/I/ Обозначение штамма бактерии.

/2/ Температура инкубации /T/.

/3/ Очень хорошо размножается $1{,}0 \leq \frac{GT}{G_{min}} < 2{,}5$

/4/ Хорошо размножается $2{,}5 \leq \frac{GT}{G_{min}} <$

/5/ Слабо размножается $10{,}0 \leq \frac{GT}{G_{min}}$

/6/ Не размножается

G_T = Время генерации измеряемое при температуре T.
G_{min} = Время /минимальной/ генерации измеряемое при оптимальной температуре.

Рис. 3.: У одиночных типичных штаммах число генерации по часам /I/ в зависимости от температуры инкубации /2/

Рис. 5.: Логарифм максимальной клеточной плотности достигнутой в жидкой питательной среде /I/ в зависимости от температуры инкубации /2/

Рис. 4.: Образование числа живых зародышей /6/ быстро-замороженной рубленной печени /I/, или быстро-замороженных жидких культур /2/, /3/, /4/ в зависимости от срока /дня/ хранения /6/, при температуре -I5°C.