A C T A I C H T H Y O L O G I C A E T P I S C A T O R I A
Vol. 1 1970

Stanisław ZALESKI, Edward CERONIK and Krystyna SOBOLEWSKA-CERONIK

Microbiology

THERMAL RESISTANCE OF *BACILLUS SUBTILIS* SPORES STUDIED IN THE MEAT EXTRACTS OF THE VARIOUS SEA AND FRESH-WATER FISHES

CIEPŁOOPORNOŚĆ ZARODNIKÓW *BACILLUS SUBTILIS* BADANA W WYCIĄGACH Z MIĘSA RÓŻNYCH GATUNKÓW RYB MORSKICH I SŁODKOWODNYCH

From Department of Fish Microbiology
Head: Prof. Dr. Stanisław Zaleski

Subject of the studies, was a thermal resistance of the spores Bacillus subtilis suspended in the meat extracts of a sea and fresh-water fish. Depending on the spore suspending environment, the thermal resistance proved to be different.

INTRODUCTION

The thermobacterial investigations in certain fields of the food preservation play a dominating part. As the more important, may be considered the investigations performed on the dependence of a bacteria thermal resistance in relation to the specific physical and chemical characteristics of an environment in which the lethal heat affects the bacteria. An influence of the carbohydrates, fats and proteins as the elements of heating medium, on a thermal resistance of bacteria was defined in the course of such investigations.

A considerably extensive literature variously deals with an influence of carbohydrates and fats on a differentiation of the bacteria thermal resistance. However, any data relating in this subject to proteins, deals mainly with their protective influence during an action of lethal heat.

Already Murray and Headlee (1931) proved, that the pepton protects the spores Cl. tetani. Amaha and Sakaguchi (1954) ascertained, that such nitro-compounds as albumin, pepton and nucleic acids have the protective influence on the spores PA 3679; however such influence was declining as a concentration of the compounds was decreased from 2,5% to 0.25%. The same authors simultaneously proved, that a mixture of 15 amino-acids caused slight decrease of the thermal dead point at PA 3679 in relation

to the control-test performed in a heating range of phosphate buffer.

Also H a n s e n (1963) reported, that the proteins contained in a heating environment raise the thermal resistance of bacteria.

The above-mentioned works indicate, that basically the proteins act protectively on the bacteria during the thermal treatments. However, no comparative values are concluded for the various natural environments, in which the natural protein is a basic component. Therefore, the presented studies are to define comparatively the thermal resistance level of Bac. subtilis in the extracts prepared from a meat of various fish species.

MATERIALS AND METHODS

Materials

The spores Bacillus subtilis No. 32 obtained from the strains museum of Institute of Hygiene at Warsaw, were used for the studies.

The strain was cultured on the constant medium composed acc. to R o t h and co-authors (1955): tryptose (Difco) - 2.0 per cent, glucose - 1.0 per cent, NaCl - 0.5 per cent, agar - 2.0 per cent. Final pH of medium - 7.0.

The meat extracts were prepared acc. to B u r b i a n k a and P l i s z k a (1963) from the following species of a sea and fresh-water fish:

1. cod-fish (Gadus morrhua calarias),
2. Norway haddock (Sebastes marinus),
3. mackerel (Scomber scombrus),
4. herring (Clupea harengus),
5. tench (Tinca tinca),
6. lavaret (Coregonus lavaretus),
7. whitefish (Coregonus albula),
8. pike (Esox lucius).

The pH of extracts after sterilization amounted to 7.0.

Methods

The Bac. subtilis had been cultured in temperature of 34°C with the 24 hr incubation time. The comparative specimen with safranine and malachite-green pigments were prepared from the culture. The microscopic examination of the specimen proved, that under the applied culturing conditions, the strains produces 80% of spores.

The stock suspension, in distilled water of a concentration 50 x 10^6/ml, was prepared on the 24-hr culture of Bac. subtilis. For destruction of the vegetative cells, the suspension was heated for 30 min. on a water-bath of temp. 65°C, and thenafter stored upto 72 hrs in temp. of 1-2°C.

The meat-extracts were prepared fat-free by cooling down to temperature of ab. 1°C and by careful lowering of the bottom layers free of fat.

The Bac. subtilis were twice washed with an extract of cod-fish; a suspension, of concentration 100 000 spores/ml, was prepared in such environment. A suspension of the same concentration was also prepared of the cod-fish meat extracts on the non-washed spores.

The T.D.T. of the washed and non-washed spores were estimated acc. to technics described by B a l l (1957). The estimations were taken in five temperatures - 95, 98, 101, 104 and 107°C with the heating times of 0.25-100,0 min.

A thermal resistance estimations of the Bac. subtilis in the extracts of the remaining species of fish, were performed identically to the non-washed spores. A stock suspension of the Bac. subtilis in distilled water, to give their concentration of 100 000/ml, had been added to the fat-free meat extracts of the particular fish species.

The samples were heated on a glycerine-bath with an accuracy of ±0,1°C; a correction of the time amounting to 1.5 min. required for heat penetration into the geometrical centre of test tubes, was considered as per S o g n e f e s t and B e n j a m i n (1944).

Nine test-tubes of 10 x 100 mm containing jointly 9.0 ml of the spores suspension, were simultaneously subjected to thermal treatment in each of the adapted time-temperature scheme. The tubes were sealed with the rubber plugs in the stands prepared acc. to P u t and W y b i n g a (1963). Upon heating time, the samples were immediately subjected to cooling-down in a running water and to incubation for 72 hrs at temp. of 34°C. After such time of reanimation in the heating environment, the readings were taken.

RESULTS

The points representing an average of the time-logarithms of survival or destruction of the Bac. subtilis for each time-temperature scheme, are entered in the semi-logarithmis arrangement of co-ordinates.

The T.D.T. curves of the spores were drawn on the basis of these points. According to B a l l (1957), for each curve the value "z" was taken, and the value "F" was calculated.

Diagram No.1 represents the comparative value of parameters "z" and "F" for the particular environments.

It appears from diagram No.1, that the highest "z" value amounting to 9,4°C, was noted in the case of the Bac. subtilis being heated in an environment of the meat extract of a lavaret (Coregonus lavaretus). In the meat extracts of the remaining species of a fish, the value "z" was gradually decreasing and amounted: for Norway haddock z = 8,0°C, pike z = 7,4°C, mackerel z = 7.3°C, tench z = 7.0°C, cod-fish and herring z = 6.7°C. The lowest "z" = 6.0°C was obtained from the extract of a whitefish.

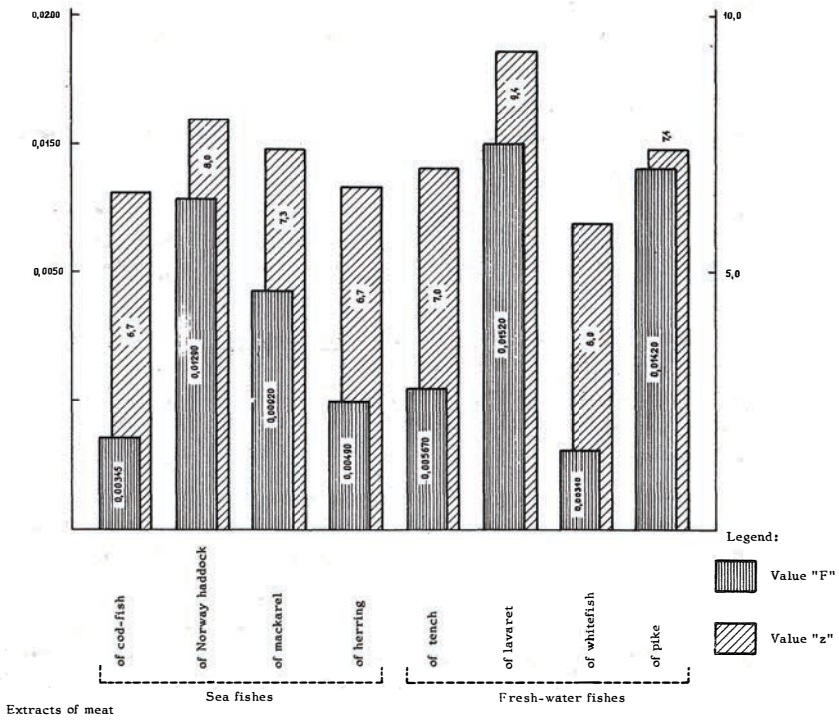

Fig.1. Values "z" and "F" obtained from the T.D.T. curves of the Bacillus subtilis No. 32 heated in an environment of the meat extracts of the various fish species

The highest value "F" = 0.01520 min. revealed the suspension of the Bac. subtilis in the environment of a meat extract of lavaret ; further, a pike - 0.01420 min. and Norway haddock - 0.01 290 min. In extracts of the remaining fish species, the value "F" was decreasing from 0,00567 for tench extract to be at minimum 0.00310 min. for a whitefish meat extract.

The results discussed relate to thermal resistance of the non-washed spores Bac. subtilis suspended in the meat-extracts of various fish species. By comparison, a thermal resistance of the washed and non-washed spores in an environment of a cod-fish meat extract, proved to be identical.

DISCUSSION

B a l l (1938, 1943) ascertains, that in the processes of thermal treatment, the thermal resistance of bacteria is among other things, dependent on the kind of food and that the value "z" of the time curves of bacteria thermal--death, is changing.

T h o m a s et al.(1966), while investigating a thermal resistance of Salmonella senftenberg and of two staphylococci strains, in the environment of

5 per cent NaCl, green-pease pudding, in a meat-soup and in free of fat pasteurized milk, noted, that the value "z" varied within 4.52-6.38°C.

Also, S e c r i s t and S t u m b o (1958) proved, that while in distilled water the value "z" for the strain PA 3679 amounted to 10.55°C, in environment of a green-pease, the "z" value was 9.83°C for the same strain.

The investigations in question, proved, that when the suspension of the Bac. subtilis had been heated in an environment of the meat extracts of various fish species, the value "z" of the corresponding time T.D.T. curves was changing. As the extracts were fat-free, their most important components left were probably the proteins peculiar for the particular species of a fish. The only variable parameters in these investigations, were the extracts prepared from various species of fish; thus, a changeability of "z" allows to conclud their individual effects on the thermal resistance of the Bac. subtilis.

An uniform and exact comparison of a thermal resistance of the Bac. subtilis in the meat extract of the particular fish species, was possible due to calculation of the value "F" obtained by equation of B a l l:

$$F = \tau \cdot 10^{\frac{T-121,1}{z}} \quad \text{(min.)}$$

When comparing an order of the quantities obtained in these investigations on value "F", a suggestion may be apparent, that the comparison of thermal resistance of the investigated Bac. subtilis should be performed at the lower temperature level, e.g. 100°C. To make, however, the obtained results comparative with data of the other thermobacteriologic investigations, it was advisable to relate them to one common level i.e. 121,1°C.

The results obtained in these investigations, allow to conclude, that an individual influence of the various protein environments on the thermal resistance of bacteria at temperatures below 100°C, may be of a higher practical meaning.

In fish processing, the temperatures not exceeding 100°C, may take place in a raw-fish preparations, such as frying, boiling and roasting, i.e. in production of "the ready to serve" foods. The surfacial temperature in such production may be as high as 180-200°C, e.g. at frying or roasting. Yet, the heating of a raw material is then effected nearly entirely by conduction and therefore the temperature at the centre, may even not exceed 60°C considering the short processing time.

Distinctly low lethal dose of a heat and the individual characteristics of a raw material, may favour the survival of the microorganisms, and in some cases, may even promote the thermal activity of germination and reproduction of a bacteria.

Considering an initially increased bacterial contamination of a fish, which may be used for production as a raw material of the improper quality, the probability of survival of the pathogenic and saprogenic microorganisms is creased. This, in turn, may cause a serious internal spoilage of a product and potentially lead to the food-poisoning of a consumer.

CONCLUSIONS

1. The thermal resistance differences of the Bac. subtilis in the meat extract of various species of a sea and fresh-water fish prove, that the thermal resistance of bacteria is functioned by the smallest changes in a chemical composition of heating environment.

2. In the processing temperatures below 100°C, an influence of the protein environments on a thermal resistance of bacteria, may be of a great practical importance.

3. The food-preserving process should be based on the first-class raw material, considering that the processing conditions may not always guarantee a distruction of the pathogenic and saprogenic microorganisms.

REFERENCES

Amaha M., Sakaguchi K.J., 1954: Effect of carbohydrates, proteins and bacterial cells on the heat resistance of Clostridium sporogenes. - J.Bact. 68: 338-345.

Ball C.O., 1938: Advancements in sterilization methods for canned foods. - Food.Res. 3:13-55.

Ball C.O., 1943: Short time pasteurization of milk. Ind.Ing.Chem. 35,1: 71-84.

Ball C.O., Olson F.C.W., 1957: Sterilization in Food Technology. New York, Mc Graw-Hill Book Co.Inc.

Burbianka M., Pliszka A., 1963: Mikrobiologiczne badanie produktów żywnościowych. Warszawa, PZWL.

Hansen N.H., Riemann H., 1963: Factors affecting the heat resistance of non sporing organisms. - J.Appl.Bact.26:314-333.

Murray T.J., Headlee M.J., 1931 - cited from Amaha M., Sakaguchi K.J., 1954.

Put H.M.C., Wybinga S.J., 1963: The occurence of Bacillus coagulans with high heat resistance. - J.Appl.Bact.26: 428-434.

Roth N.G., Lively D.H., Hodge H.M., 1955: Influence of oxygen uptake and age of culture on sporulation of Bacillus anthracis and Bacillus globigii. - J.Bact. 69: 455-459.

Secrist J.L., Stumbo C.R., 1958: Some factors influencing thermal resistance values obtained by the thermoresistometer method. - Food Res.23: 51-60.

Sognefest P., Benjamin H.A., 1944: Heating lag in Thermal Death Time Cans and Tubes. - Food Res.9: 234-243.

Thomas C.T., White J.C., Longree K., 1966: Thermal resistance of salmonellae and staphylococci in foods. - Appl.Microbiol. 14: 815-820.

CIEPŁOOPORNOŚĆ ZARODNIKÓW BACILLUS SUBTILIS BADANA W WYCIĄGACH Z MIĘSA RÓŻNYCH GATUNKÓW RYB MORSKICH I SŁODKOWODNYCH

Streszczenie

Badano ciepłooporność zarodników szczepu Bacillus subtilis Nr 32 otrzymanego z Państwowego Zakładu Higieny w Warszawie. Jako środowisko grzejne w badaniach zastosowano wyciągi z mięsa następujących gatunków ryb morskich i słodkowodnych: dorsz, karmazyn, makrela, śledź, lin, sieja, sielawa i szczupak. Koncentracja zarodników wynosiła 100 000/ml.

Otrzymano zróżnicowane wartości „z" i „F". Najwyższą wartość z = 9,4°C wykazała krzywa czasu śmierci cieplnej otrzymana w przypadku ogrzewania zarodników w środowisku wyciągu z mięsa siei, zaś najniższą wartość z = = 6,0°C wykazała krzywa dla tychże zarodników badanych w środowisku wyciągu z mięsa sielawy. Dokonane wyliczenia wartości „F" wg wzoru Balla wykazały, że najwyższe F = 0,01520 min. odpowiadało ogrzewaniu zarodników z wyciągu z mięsa siei, zaś najniższe F = 0,00310 min. odpowiadało ogrzewaniu ich w środowisku wyciągu z mięsa sielawy.

Uzyskane w badaniach wyniki świadczą o tym, że na zróżnicowanie ciepłooporności tego samego szczepu bakterii wpływać mogą niewielkie nawet zmiany właściwości środowiska, w którym podda się je działaniu ciepła letalnego.

ТЕПЛОУСТОЙЧИВОСТЬ СПОР BACILLUS SUBTILIS ИЗУЧАЕМАЯ В ВЫТЯЖКАХ ИЗ МЯСА РАЗНЫХ ВИДОВ МОРСКИХ И ПРЕСНОВОДНЫХ РЫБ

Резюме

Изучалась теплоустойчивость спор штамма Bacillus subtilis Но 32 полученного из Государственного института гигиены в Варшаве. В качестве подогревающей среды в опытах применялись вытяжки из мяса морских и пресноводных рыб: трески, морского окуня, макрелы, сельдя, линя, сига, ряпушки и щуки. Концентрация спор - 100 000/мл.

Получены разные величины „z" и „F" . Найвысшую величину z = 9,5°C показала кривая времени тепловой смерти полученная в результате подогревания спор в среде вытяжки из мяса сига и самую низкую величину F = 6,0°C - кривая для спор помещённых в среде вытяжки из мяса ряпушки. Проводённые вычисления величины „F" по формуле Балла показали, что самое высокое F - 0,01520 мин. отвечало подогреву спор в вытяжке из мяса сига, а самое низкое F = 0,00310 мин. подогреву их в среде вытяжки из мяса ряпушки.

Полученные в опытах результаты свидетельствуют о том,что на дифференцировку теплоустойчивости того самого штамма бактерий могут оказывать влияние даже невеликие изменения свойств среды, в которой поддаётся он действию летального тепла.

Address:

Prof.dr Stanisław Zaleski
Katedra Mikrobiologii Rybackiej WSR

Szczecin, ul.Kazimierza Królewicza 4
Polska - Poland

Received 17.II.1969