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THE EFFECT OF CULTURE CONDITIONS ON HYDROPHOBICITY OF STRAINS OF *LISTERIA MONOCYTOGENES* ISOLATED FROM FOOD

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ABSTRACT

The effect of incubation temperature on hydrophobic properties of *Listeria* spp. using ammonium sulphate aggregation test was analyzed. 29 strains of *Listeria* spp. isolated from beef, pork and smoked fish were tested. Results show that reducing the incubation temperature causes transformation of hydrophilic *Listeria* spp. into hydrophobic. At 30°C 3% of strains was hydrophobic, 3% medium hydrophobic, 20% low hydrophobic and 74% of strains were found to be hydrophilic. At 20°C there were 30% of hydrophobic strains, 10% of medium hydrophobic, 23% of low hydrophobic and 37% of strains were regarded as hydrophilic. Having reduced the incubation temperature to 5°C no increase of hydrophobic strains was detected. The greatest changes were observed in *L. monocytogenes*: 50% of hydrophobic, 19% of low hydrophobic were detected and 31% remained hydrophilic. Significant changes were observed among strains of *L. welshimeri*: 35% became hydrophobic, 15% medium hydrophobic, 15% low hydrophobic and 35% remained hydrophilic. The correlation between hydrophobicity and source of isolates was also analyzed. Incubated at 30°C the hydrophilic strains came mainly from pork (33%) whereas only 16,6% of such strains was isolated from smoked fish. At 5°C most strains which transformed into hydrophobic came from pork isolates.

Key words: *Listeria monocytogenes*, hydrophobicity

INTRODUCTION

Listeria spp. are widely distributed in the environment. They are present in water, soil, vegetation and animal gastrointestinal tracts. *Listeria monocytogenes* and occasionally *L. ivanovii*, *L. seligeri*, *L. grayi* are considered to be human pathogens. In favorable circumstances they can proliferate in an organism and lead to life-threatening disease called listeriosis which generally affects compromised groups of people. Until quite recently cases of human listeriosis were rare and not recognized as a serious epidemiological problem. The significant increase of incidence of listeriosis has been observed in Western Europe, United States and Canada since the 1980s. Epidemiological data point to transmission to human through the food ingestion. Food of the animal origin is considered to be the main source of infection [9]. Attachment of bacteria to surfaces is the initial step of either human infection or contamination of raw material and processing environment. Not only physical and chemical parameters of surface but also external cell wall structures and their properties account for successful attachment of cells to surface. One of the most significant properties which play an important role in such process is hydrophobicity of cell wall. Conditions for optimal growth of bacteria deteriorate during production, processing, retail and storage of food. It causes changes in surface structures of cells and may influence their hydrophobic properties. There are few data concerning this subject in the literature. It may be the reason why the results reported by various authors are inconsistent; *L. monocytogenes* is regarded either as hydrophilic [7, 11], or as hydrophobic [1, 12, 3]. In this paper we present results of studies on the effect of incubation temperature on hydrophobicity of cultured strains of *L. monocytogenes* isolated from food.

MATERIALS AND METHODS

Listeria spp. isolated from beef, pork and smoked fish were analyzed. Identification of strains was preformed using API Listeria tests and PCR technique (detection of hly gene) as described previously [6]. Before the experiment the strains had been cultured on Listeria Selective Agar (LSA) + Listeria Selective Supplement (Oxoid) and incubated at 30°C for 48 h. Then, they were streaked onto (BTL) and incubated at 30°C, 20°C and 5°C. Samples at 30°C and 20°C were incubated for 48 h whereas samples at 5°C were stored for 2 weeks. Characteristic of strains is presented in Table 1.

Tab. 1. Strains of *Listeria* spp. used in the studies

Strains	Identification	Source
1. 1-II	*L.monocytogenes*	pork
2. 1-IV	*L.monocytogenes*	pork
3. 1-XV	*L.monocytogenes*	pork
4. 1-V	*L.monocytogenes*	pork
5. 2-II	*L.monocytogenes*	pork
6. 15	*L.monocytogenes*	pork
7. 19	*L.monocytogenes*	pork
8. 2-XI	*L.monocytogenes*	pork
9. 2-XVI	*L.monocytogenes*	pork
10. 21	*L.monocytogenes*	pork
11. 3-1	*L.monocytogenes*	beef
12. 3-V	*L.monocytogenes*	beef
13. 3-VII	*L.monocytogenes*	beef
14. 3-X	*L.monocytogenes*	beef
15. 4-XI	*L.monocytogenes*	beef
16. 4-IX	*L.monocytogenes*	beef
17. 34-X	*L.monocytogenes*	beef
18. 35	*L.monocytogenes*	smoked fish
19. 61	*L.welshimerii*	pork
20. M-9	*L.welshimerii*	pork
21. 2-VI	*L.welshimerii*	pork
22. 30	*L.welshimerii*	pork
23. 34	*L.welshimerii*	smoked fish
24. 36	*L.welshimerii*	smoked fish

Table 1 cont.

1	2	3
25. 4-VII	*L.innocua*	beef
26. 37	*L.innocua*	smoked fish
27. 38	*L.innocua*	smoked fish
28. 39	*L.innocua*	smoked fish
29. 40	*L.innocua*	smoked fish

Analyses of hydrophobicity of strains used in the studies (salting test with ammonium sulphate) were preformed according to modified method described by Rozgonyi et al. [14].

Cultures were washed off from the solid medium and suspended in saline to obtain McFarland density 6°. 20 µl of suspension was mixed with 20 µl of ammonium sulphate at concentrations from 0.1 to 3.2 M on Micro Test II plates (Falcon). Negative control was included with ammonium sulphate substituted by saline. The plate was rocked gently for 1 min and the aggregation effect was observed by a magnifying glass. Strains which aggregated at 0.1-0.2 M ammonium sulphate were considered to be hydrophobic.

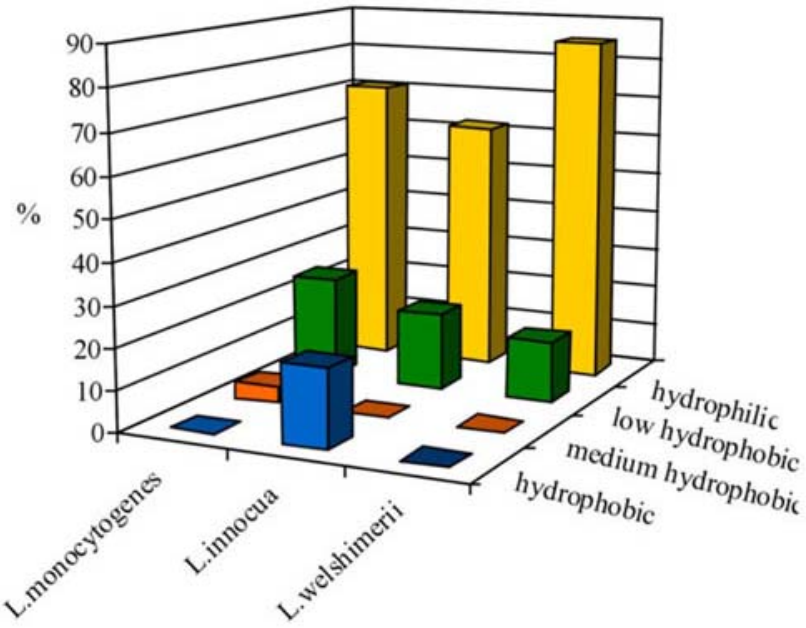
Aggregation at 0.4-1.0 M was characteristic of medium hydrophobicity whereas strains aggregating at 1.2-1.6 M were regarded as hydrophilic. Aggregation or its lack at 1.8 M or higher concentrations referred to hydrophilic strains.

RESULTS

The effect of incubation temperature on hydrophobic properties of strains of *Listeria* spp. tested with ammonium sulphate was analyzed. Results showed that reducing the incubation temperature causes transformation of hydrophilic strains into hydrophobic. Transformation depended on *Listeria* species and did not affect all tested strains in the same level.

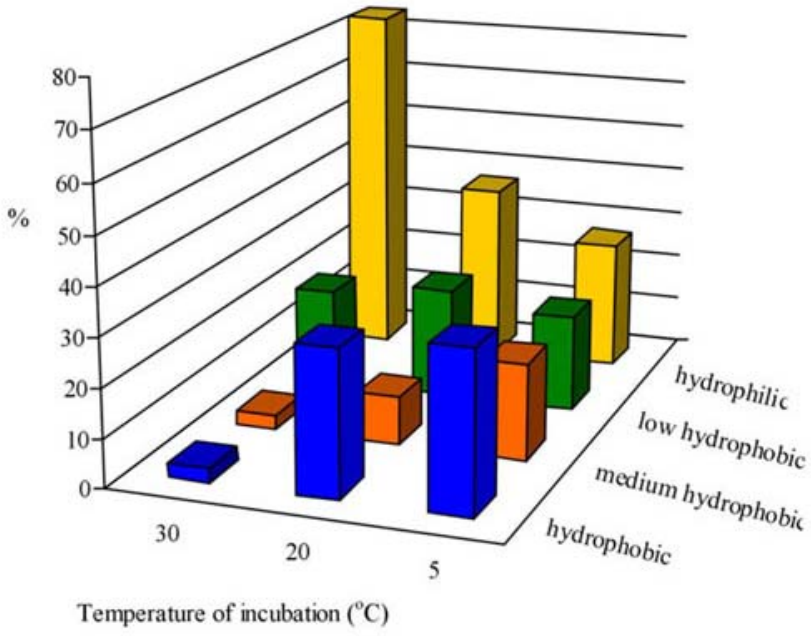
At 30°C (Fig. 1.), the highest rate of hydrophobic strains was found in *L. innocua* (38%). There were 19% of each hydrophobic and low hydrophobic strains. Among *L. welshimerii* only 15% strains was low hydrophobic and 85% of hydrophilic.

Fig. 1. Hydrophobicity of strains *Listeria* spp. incubated at 30°C



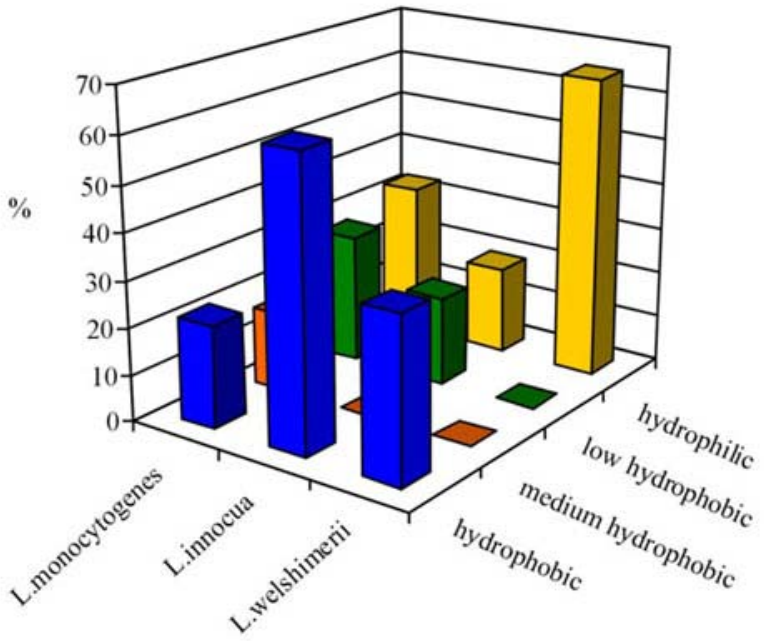
No hydrophobic strains were found within *L. monocytogenes* incubated at 30°C whereas 4% of medium hydrophobic and 25% of low hydrophobic isolates were observed. 71% of hydrophilic strains of *L. monocytogenes* was detected. 3% of hydrophobic, 3% of medium hydrophobic, 20% of low hydrophobic and 74% of hydrophilic strains were found among all *Listeria* spp. tested (Fig. 2).

Fig. 2. Hydrophobic properties of strains of *Listeria* spp. incubated at different temperatures



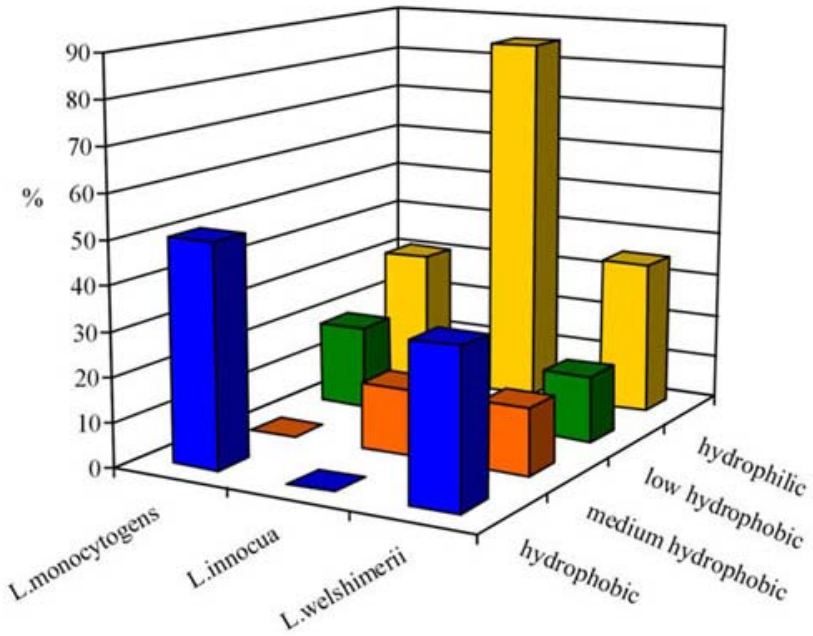
At 20°C the significant differences in hydrophobicity were detected (Fig. 3). Among isolates of *L. innocua* 62% of hydrophobic, 19% of low hydrophobic and only 19% of hydrophilic strains was detected. *Listeria welshimeri* was represented by 35% of hydrophobic and 65% of hydrophilic strains. Within isolates of *L. monocytogenes* 22% of strains was hydrophobic, 17% of medium hydrophobic, 28% of low hydrophobic and 33% of hydrophilic. Concluding, the effect of 20°C on *Listeria* spp. 30% of strains became hydrophobic, 10% medium hydrophobic, 23% low hydrophobic and 37% remained hydrophilic (Fig. 2).

Fig. 3. Hydrophobicity of strains of *Listeria* spp. incubated at 20°C



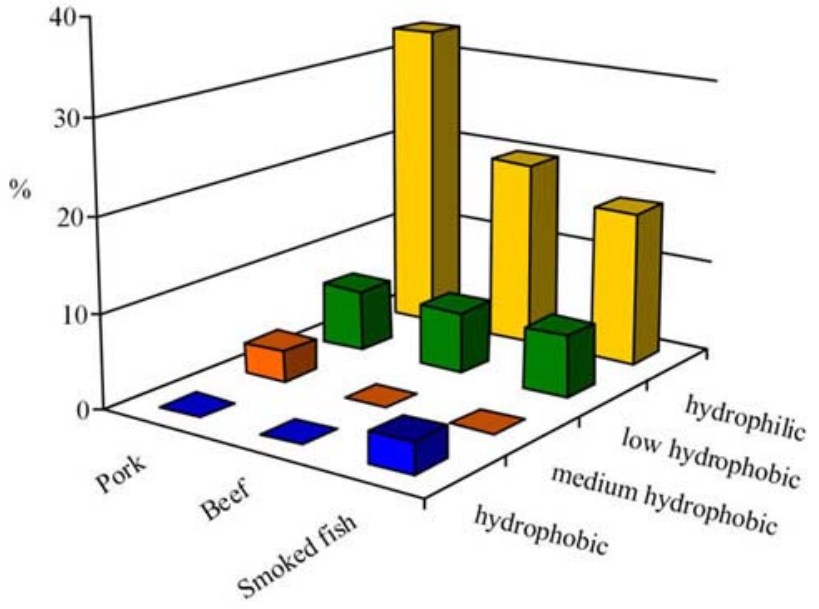
Incubation temperature lowered to 5°C did not increase transformation of strains of *Listeria* spp. into hydrophobic (Fig. 4). The greatest changes were observed among strains of *L. monocytogenes* tested: 50% of them were hydrophobic, 19% low hydrophobic and 31% remained hydrophilic. Significant changes were also observed in *L. welshimerii*: 35% of strains became hydrophobic 15% medium hydrophobic and 35% remained hydrophilic.

Fig. 4. Hydrophobicity of strains of *Listeria* spp. incubated at 5°C



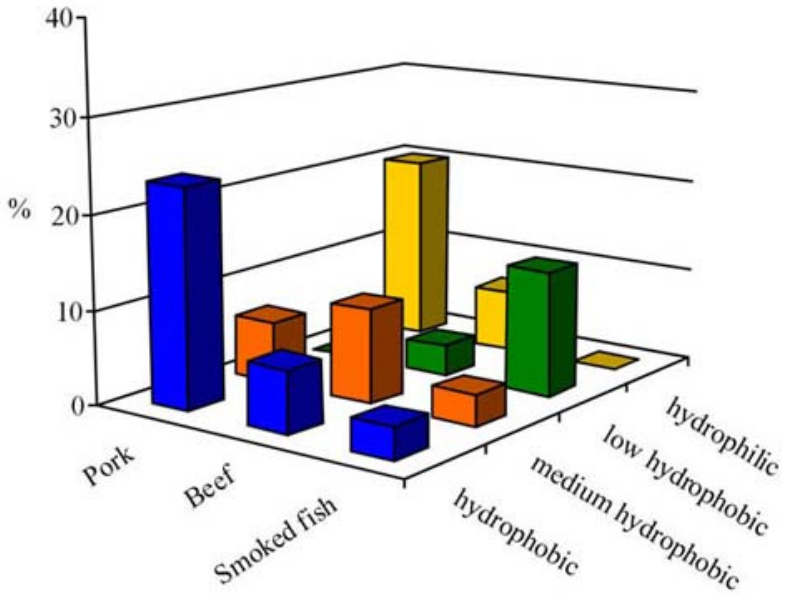
The correlation between hydrophobicity and source of isolates was also analyzed. At 30°C the hydrophilic strains came mainly from pork (33%) whereas only 16.6% was isolated from smoked fish (Fig. 5.)

Fig. 5. Changes of hydrophobicity of *Listeria* spp. correlated with the origin of isolates. Incubation at 30°C



At 5°C most strains transforming into hydrophobic came from pork isolates (Fig. 6).

Fig. 6. Changes of hydrophobicity of *Listeria* spp. correlated with the origin of isolates. Incubation at 5°C



DISCUSSION

Listeria monocytogenes is often isolated from food products. As yet the reason of listeriosis which has been recorded since 1985 is not explained satisfactorily. It may be caused by changes in production, processing, packaging, retail and storage of food products. Introduction of products with extended shelf-life promotes multiplying of microorganisms during storage of food in conditions different than optimal e.g. in low temperature, vacuum atmosphere which may affect their surface structures. Among other things such changes influence hydrophobicity. In our studies we analyzed the effect of incubation temperature on this particular property of microorganisms isolated from pork, beef and smoked fish. Three incubation temperatures were applied: 30°C-the optimum for growth of *L. monocytogenes*, 20°C-an equivalent of ambient temperature and 5°C-temperature of food storage. We stated that lowering of incubation temperature results in decrease of hydrophilic strains and increase of hydrophobic strains, what is presented in Figure 2. Surprisingly, even a slight change of incubation temperature from 30°C to 20°C alters surface properties diametrically. It is essential that this change affects particular strains to a different degree. The intensity may be different and depends on hydrophobic transforming degree. Different degrees of hydrophobicity were detected among isolates tested whereas some strains remained hydrophilic. As already mentioned, the opinions on hydrophobicity of *L. monocytogenes* are divergent. *L. monocytogenes* is considered either hydrophilic or hydrophobic. It is probably result of different culture conditions and various strains tested. Briandet demonstrated on a frequently tested Scott strain of *L. monocytogenes* that even a slight modification of a medium composition and lowering the incubation time changed it into hydrophobic [5].

It is in agreement with our results but we additionally provide evidence that not all strains subject to hydrophobic changes to the same level and some of them may remain hydrophilic. Such transformation can have serious clinical and practical implications. It was proved that pathogenic strains of bacteria and fungi isolated from clinical cases and the environment are hydrophobic [4]. It is of crucial importance for pathogenic microorganisms. Tissue or cell surfaces including enterocytes in gastrointestinal tracts are hydrophobic [13]. First stage of infection is adhesion of bacteria to cells. Studies on *L. monocytogenes* show that hydrophobic strains demonstrate specific adhesion [5]. Unfavorable conditions in processing plants and during storage of food promote changes of strains into hydrophobic and may facilitate an infection process. Evidently the strains changing into high-hydrophobic will be promoted.

In our studies we observed the correlation among the environment of which strains originated, their hydrophobicity and ability to modify. Pork strains were able to change into hydrophobic exceedingly. It suggests

that the fat-rich environment promotes and selects such strains. Selection of strains occurring in food processing plants and their colonization by one strain, characteristic and analogous to intrahospital strains is described by Autio et al. [2].

The practical aspect of studies on hydrophobicity of *Listeria* spp. and the other food-borne human pathogens is clearly seen considering that spores of bacteria and fungi, cocci and *Salmonella* spp. are hydrophobic [8, 15, 10]. It enables to aim the lookout for hydrophilic food packages which could prevent microorganisms from colonizing them.

The correlation between hydrophobicity and *Listeria* species was analyzed. Incubated at 30°C, 38% strains of *L. innocua* were found to be hydrophobic, *L. monocytogenes* and *L. welshimeri* had 29% and 15% of hydrophobic strains, respectively. At 20°C *L. innocua* is more frequently than the others high hydrophobic. Results explain why *L. innocua* is the most often found in fat-rich products (milk, fish, cheese) whereas *L. monocytogenes* is isolated rarely and *L. welshimeri* is the rarest in such products.

CONCLUSIONS

1. Hydrophobic properties of cell surface of *Listeria* spp. were significantly affected by incubation temperature.
2. A vast majority of strains of *Listeria* spp. was hydrophilic at 30°C.
3. The increase of hydrophobic strains was observed at 5°C and 20°C.
4. At 5°C hydrophobic strains were dominant for *L. monocytogenes*.
5. Pork isolates were found to present the greatest ability to transform into hydrophobic strains.

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