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# THE INFLUENCE OF CULTURE CONDITIONS ON ADHESION OF *LISTERIA MONOCYTOGENES* TO HEXADECANE

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## ABSTRACT

Results of various culture conditions on adhesion of *Listeria monocytogenes* strains to hexadecane are presented. *Listeria* strains cultured in optimal conditions adhered to this hydrophobic carbohydrate to a small extent. It reveals that their cell walls have hydrophilic properties. Application of low-nutrient medium, incubation at low temperature, acidification of medium to pH 5 and addition of 8% of NaCl caused transformation of strains into hydrophobic. Growing in vacuum conditions produced the opposite effect – all strains were extremely

hydrophilic. Changes of strain properties from hydrophilic into hydrophobic affected analysed strains to a different degree. One of tested strains continued to be hydrophilic regardless of culture conditions. The other – a standard strain isolated from patient – became hydrophobic most frequently.

**Key words:** hydrophobicity, *Listeria monocytogenes*

## INTRODUCTION

*Listeria* spp. are widely distributed in the environment. They are easily and frequently found in soil, water, bottom sediment, sewage, plants, animal digestive tracts and also in food products [14]. During a last decade *Listeria monocytogenes* together with enterohaemorrhagic strains of *Escherichia coli*, particularly of an O157H7 serotype, and some species of *Campylobacter* sp. were included into a group of serious and the most important food-borne pathogens. More frequently *L. monocytogenes* becomes a cause of sporadic and epidemic human listeriosis. Significant increase of listeriosis incidence appeared at the end of 1970s in many western European countries, the United States and Canada. Food-borne way of infection was verified then. Microbiological contamination of processing environments, raw and processed materials is based on adhesion abilities of micro-organisms to different surfaces. The first step of infection is also supported by adherence of bacteria to biological membranes what enables a colonisation, penetration and progress of disease [4]. Adhesion is determined by creating of hydrogen bonds, Van der Wall's forces, electric charges, hydrophobic properties of both surface and micro-organism produced by outer structures of its cell wall. Hydrophobicity of bacteria is particularly significant for this phenomenon [12]. Its correlation with pathogenicity has been already proved [10]. It has been also suggested that it enables adherence of micro-organisms to a waxy layer –a cuticle – that covers the aerial parts of a plant [16]. It also plays an important role in a sewage treatment and in binding micro-organisms with bottom sediments of water bodies. It probably accelerates contamination of food plants processing fat-containing materials.

Opinions on *L. monocytogenes* hydrophobicity are divergent. Strains are classified either to be hydrophilic [7] or hydrophobic [13]. Our previous research showed that such a divergence is caused by various culture conditions. In this paper we present results of the influence of pH, vacuum, medium composition and temperature on hydrophobicity of *L. monocytogenes* measured by its affinity to hexadecane.

## MATERIALS AND METHODS

11 strains of *L. monocytogenes* presented in Table 1, identified by API Listeria tests (bioMerieux), were analysed. Strains originated from a collection of Department of Food Microbiology, Agricultural University of Szczecin.

**Table 1. Strains of *L. monocytogenes* tested for hydrophobicity by MATH**

| Strain number | Source |
|---|---|
| L.m.1-IV | pork |
| L.m.2-XVI | pork |
| L.m.15 | pork |
| L.m.19 | pork |

| | |
|---|---|
| L.m.61 | pork |
| L.m.34-X | beef |
| L.m.3-VII | beef |
| L.m.4-XI | beef |
| L.m.4-IX | beef |
| L.m.3-V | beef |
| L.m.1577 | ATCC 19114 |

The influence of medium composition was tested on *Listeria* cultures on Brain Heart Infusion (BHI) agar (Oxoid), supplemented agar (Difco) and nutrient agar (BTL) incubated at 30°C.

The influence of pH was tested by BHI acidification to pH 5 by means of lactic, hydrochloric or acetic acid.

The influence of vacuum conditions was analysed on *Listeria* cultures on BHI growing in a vacuum jar.

The influence of culture temperature was tested on BHI cultures incubated at 30°C, 20°C and 5°C.

The influence of salt was tested on BHI supplemented with 8% of NaCl.

Hydrophobicity of strains was tested by a MATH (microbial adhesion to hydrocarbons) method according to Van der Mei et al. [17] based on a degree of micro-organism adhesion to a non-polar dissolvent – hexadecane. After incubation bacteria were washed off with 0.85% NaCl and a suspension containing $6x10^8$ $–1.8×10^9$ CFU/ml in 0.1 M phosphatic buffer (extinction 0.4 measured at 600 nm) was prepared. Then 4 ml of suspension were supplemented with 1.5 ml of hexadecane, vortexed with 10 seconds and left intact for 10 min. After 10 min extinction of a buffer phase was measured. Procedure was repeated until 60 s of vortexing was obtained. Function log ($At/A_0x100$, $A_0$ – an initial extinction of suspension, $A_t$ – extinction measured after particular time of vortexing in relation to blank sample) was a determinant of micro-organism affinity to a hydrophobic liquid. Strains were considered hydrophilic when the decrease of suspension optical density after a 60-second vortexing was less than 30%. In case of decrease between 30 to 70% strains were considered medium hydrophobic. The decrease higher than 70% suggested highly hydrophobic strains.

## RESULTS

Ten strains of *L. monocytogenes* (serotype 1) isolated from food and 1 ATCC, clinical strain (serotype 4a) were analysed. Only the most significant changes obtained during particular experiments were presented graphically. Changes of hydrophilicity of strains caused by various culture conditions, i.e. temperature, vacuum, using low-pH or supplemented by NaCl media as well as adapting combinations of such conditions are presented in Figures 1-8. Comprehensive results of experiments on *Listeria* hydrophobic transformations are shown in Table 2. Collected data point out that culture conditions are a factor, which determines hydrophobic properties of *L. monocytogenes*.

**Fig. 1. Hydrophobicity of *L.monocytogenes* strains incubated on BHI for 48 h at 30°C**

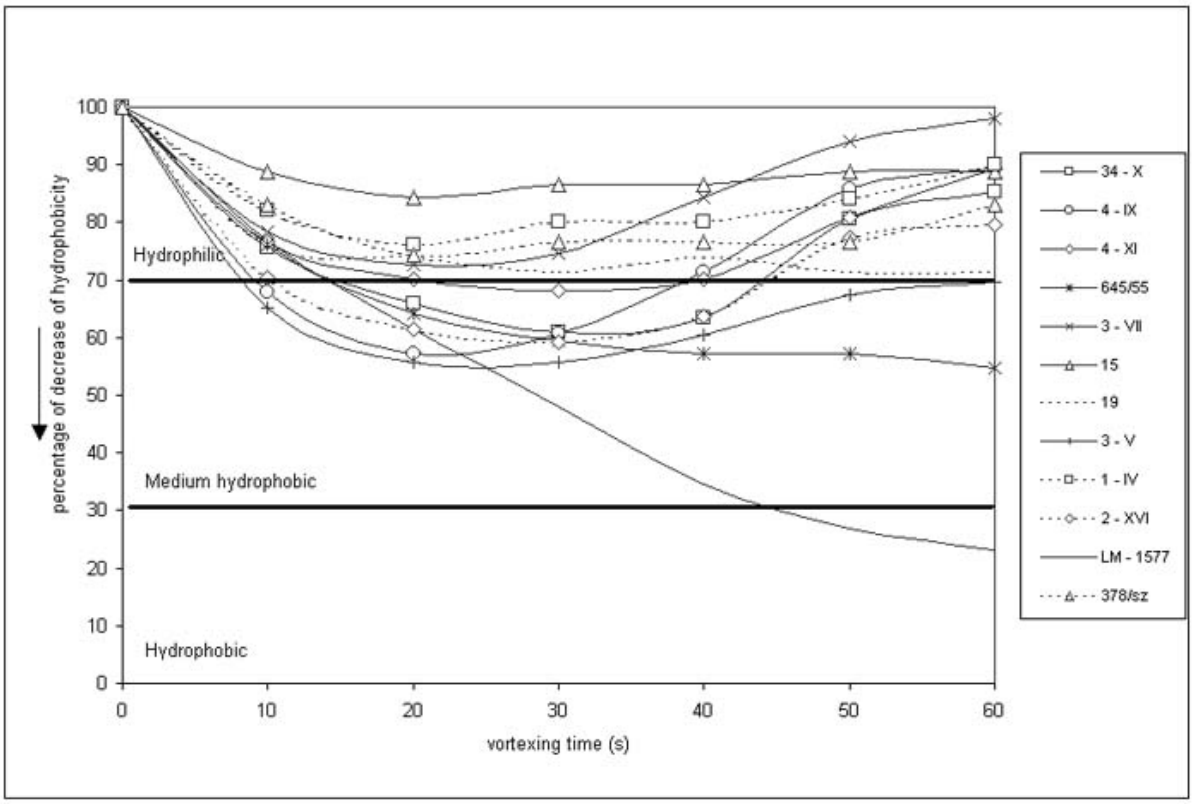


**Fig. 2. Hydrophobicity of *L. monocytogenes* strains incubated on BHI for 45 days at 5°C**

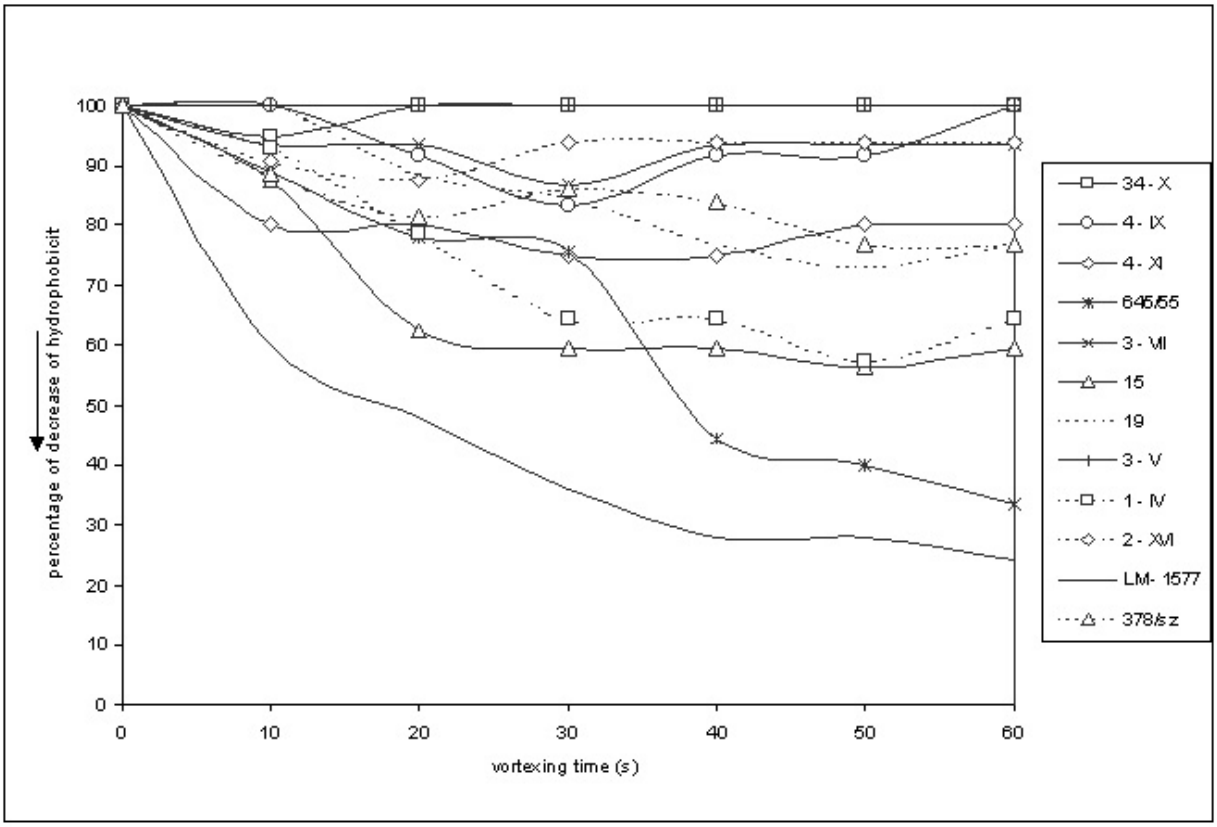

**Fig. 3. Hydrophobicity of *L. monocytogenes* strains incubated on supplemented agar (pH 5, HCL applied) for 48 h at 30°C**

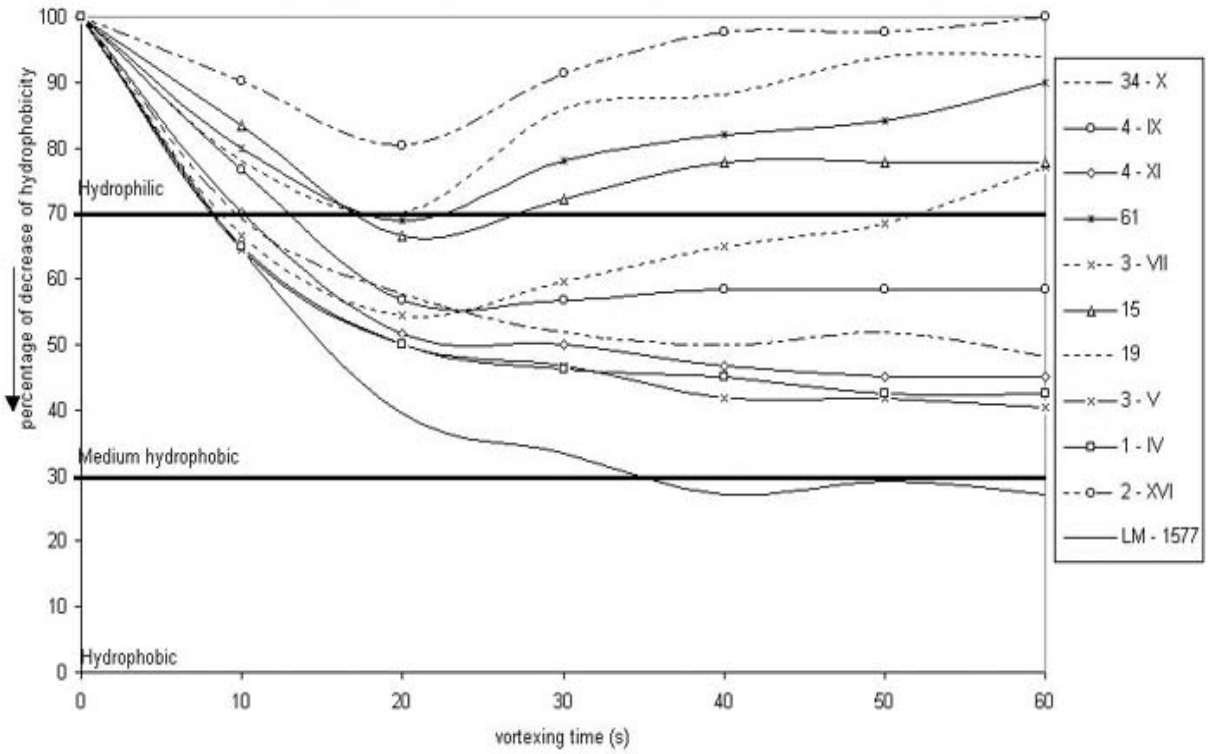


**Fig. 4. Hydrophobicity of *L. monocytogenes* strains incubated on supplemented agar (pH 5, lactic acid applied) for 48 h at 30°C**

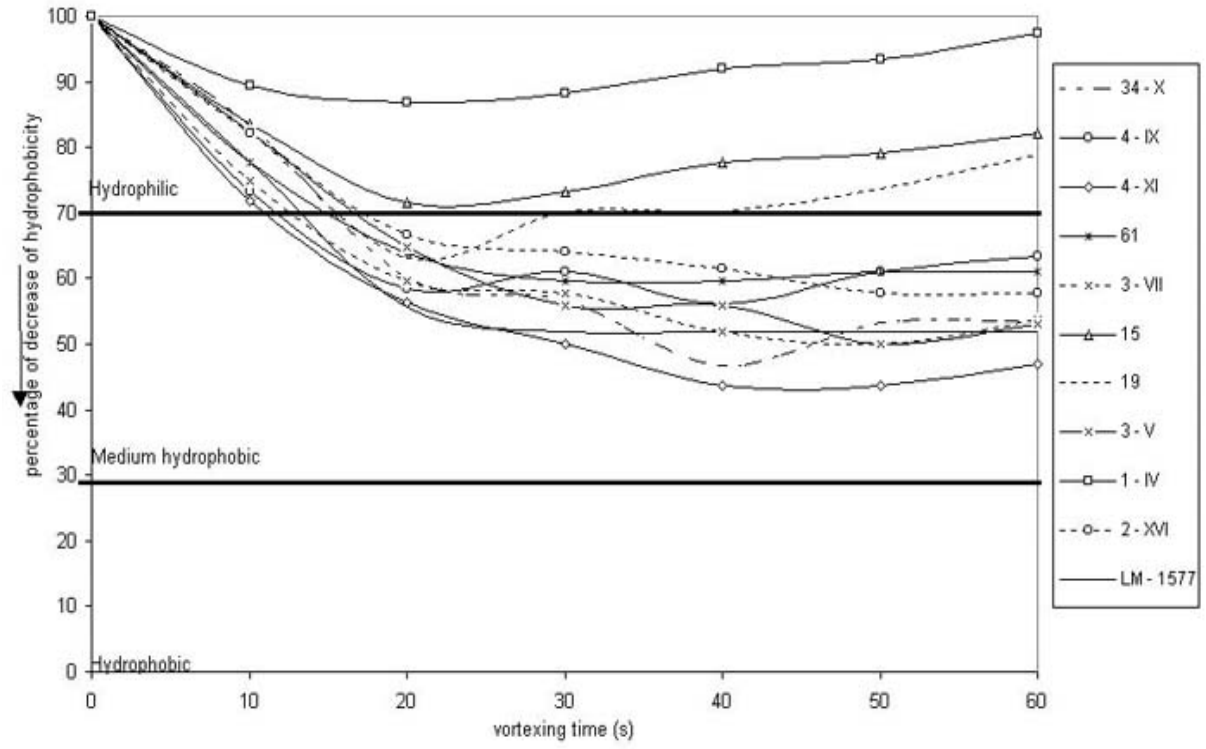

**Fig. 5. Hydrophobicity of *L. monocytogenes* strains incubated on supplemented agar (pH 5, acetic acid applied) for 48 h at 30°C**

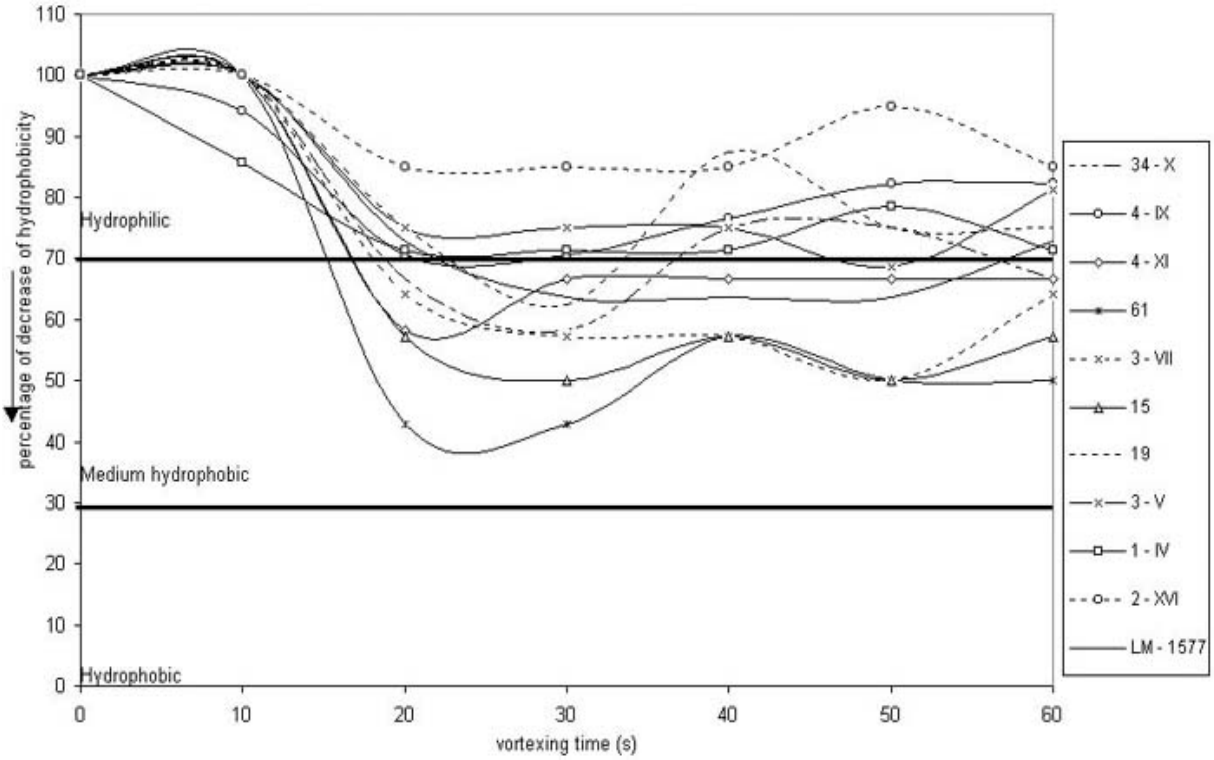


**Fig. 6. Hydrophobicity of *L. monocytogenes* strains incubated on supplemented agar with 8% NaCl added for 48 h at 30°C**

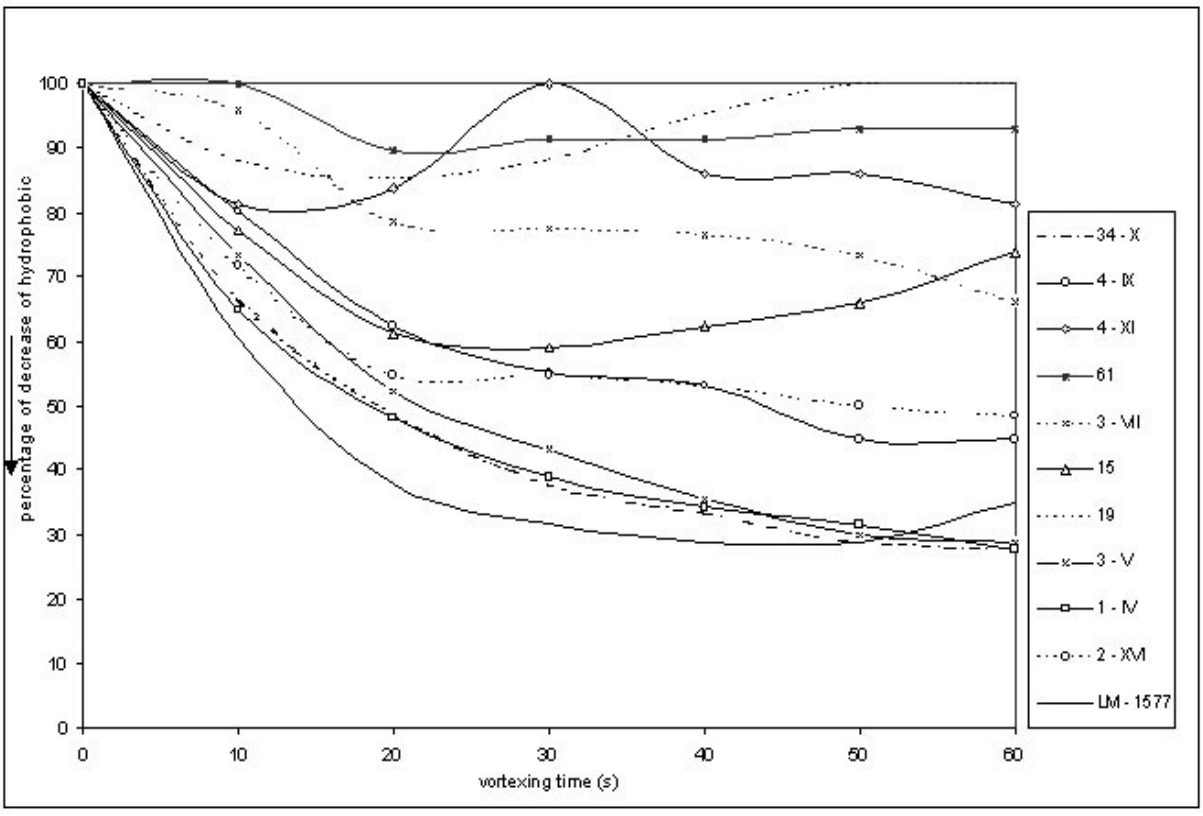

**Fig. 7. Hydrophobicity of *L. monocytogenes* strains incubated on BHI in vaccum contitions for 45 days at 5°C**

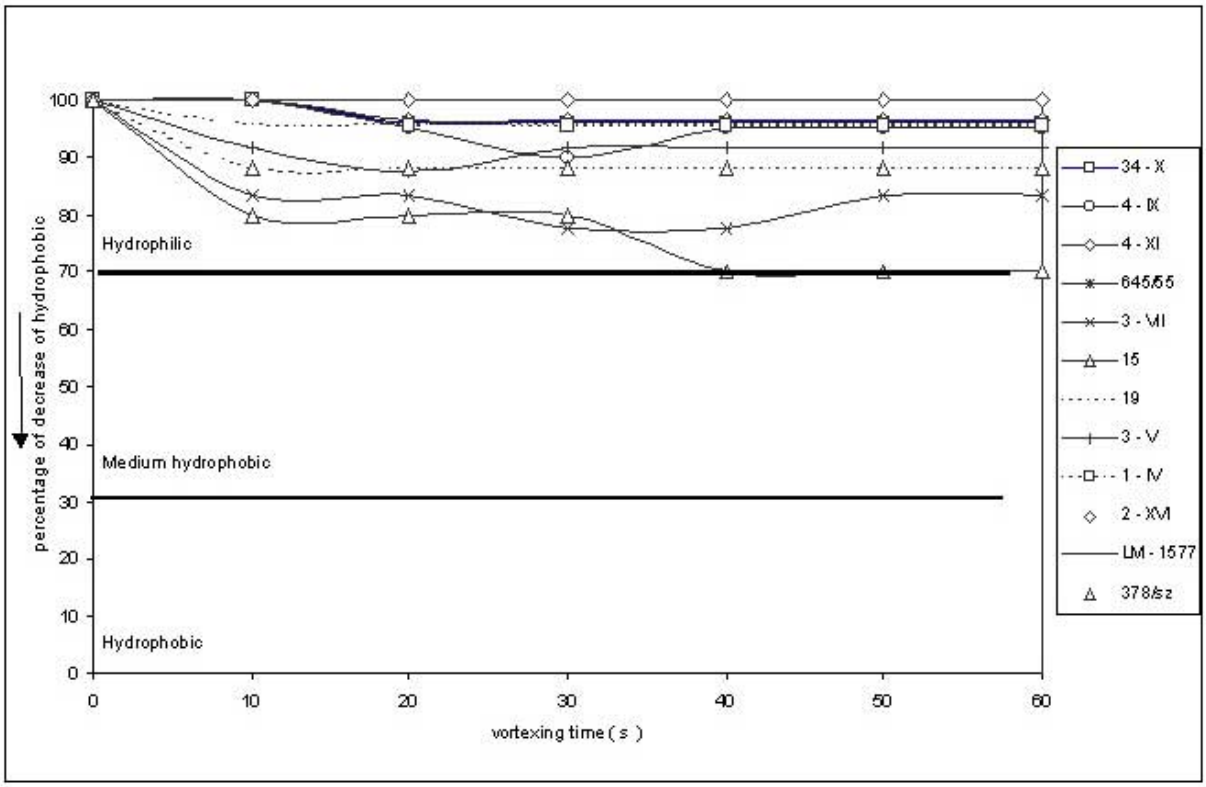


**Fig. 8. Hydrophobicity of *L. monocytogenes* strains incubated on BHI in vaccum contitions for 48h at 30°C**

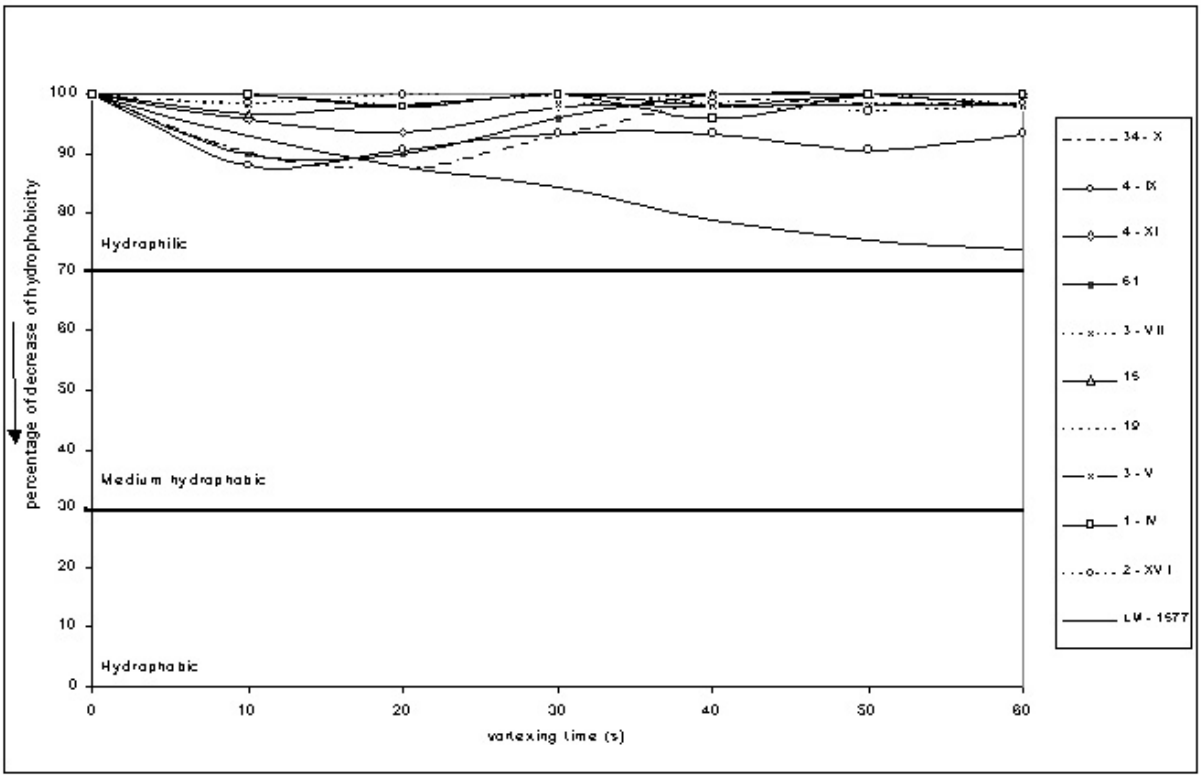

**Table 2. Comprehensive results of experiments on *Listeria* hydrophobic transformations**

| Strain | Culture conditions | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 30°C BHI | 20°C BHI | 5°C BHI | Agar 30°C | Lactic acid | Acetic acid | Hydrochloric acid | Vacuum 30°C | Vacuum 5°C | 8%NaCl BHI |
| **Lm.34-X 5/10**** | | | | + | + | + | + | | | + |
| **Lm.4-IX 4/10** | | + | | | + | + | + | | | |
| **L.m.4-XI 4/10** | | | + | + | + | | + | | | |
| **L.m.3-VII 4/10** | + | | | | + | | + | | | + |
| **L.m.3-V 5/10** | | | + | + | + | + | | | | + |
| **L.m.61 3/10** | | | + | | + | + | | | | |
| **L.m.15 4/10** | | | + | + | | + | | | | + |
| **L.m.19 0/10** | | | | | | | | | | |
| **L.m.1-IV 4/10** | | + | + | | | | + | | | + |
| **L.m.2-XVI 2/10** | | | | | + | | | | | + |
| **L.m.1577 7/10** | + | + | + | + | + | | + | | | + |
| | **2/11*** | **3/11** | **6/11** | **5/11** | **8/11** | **5/11** | **6/11** | **0/11** | **0/11** | **7/11** |

Research on the temperature influence conducted at 3 different incubation temperatures showed that 2 out of 11 tested strains revealed hydrophobic properties after incubation at 30°C. At 20°C - 3 strains and at 5°C - 6 strains were hydrophobic. It suggests that reducing incubation temperature causes transformation of strains into hydrophobic.

Cultures on low-nutrient medium like agar supplemented with meat extract affected *L. monocytogenes* transformation. In this case, 5 out of 11 strains became hydrophobic. Transformation was also clearly affected by adding acids to culture media. The strongest effect was caused by lactic acid (8 strains became hydrophobic). Hydrochloric acid produced a lower effect and acetic acid the lowest.

Addition of NaCl caused significant changes of 7 strains into hydrophobic. Strains from cultures incubated in vacuum conditions at 30°C and 5°C were all hydrophilic.

Concluding, factors which enable transformation of micro-organisms into hydrophobic are: acidification of the environment with lactic acid and a significant increase of NaCl concentration followed by a low-temperature incubation. Changes of hydrophilicity did not affect all strains to the same extent. Some of them, e.g. 1577, were particularly susceptible to hydrophobic transformation whereas others, like 2-XVI, remained hydrophilic. Analysing graphical representations of hydrophilic changes during a 60-second vortexing of suspension with hexadecane (Fig. 1) it is clearly seen that at the beginning some hydrophilic strains revealed hydrophobic properties, then after longer vortexing they became hydrophilic again. Interesting that, at the beginning some of them, like 3-VII, were stronger hydrophobic than the rest of strains to become the most hydrophilic at the end.

## DISCUSSION

It is well known that *L. monocytogenes* is frequently present in raw and processed food products. It is even suggested that it constitutes their natural microflora. A significant increase of listeriosis cases in highly industrialised countries, which has begun in 1985, focused public attention on this pathogen. As far the phenomenon of its increased incidence has not been explained. It has not been also explained why, although the number and frequency of *L. monocytogenes* presented in food exceed amounts of other food-borne pathogens, e.g. *Salmonella* sp. [8], the number of clinical listeriosis cases is incomparably lower.

First, it was explained by different serotypes characteristic for food products (serotype 1/2) and clinical cases (serotype 4a) [11]. However, epidemiological investigation proved that serotype 1/2 is also pathogenic [1]. It was also confirmed by analyses of virulence factors determining pathogenicity of *Listeria* strains isolated from food products. According to Brosh et al. [5] all *L. monocytogenes* strains pose serious health hazard not directly connected with their serotype or source of origin.

The other explanation was too low concentration of micro-organisms in particular lots of food not sufficient to cause infection. But many products were found which create the environment supporting growth of *L. monocytogenes* often to high concentrations. As already mentioned hydrophobicity of cell wall is a feature which promotes pathogenicity of bacteria and yeasts [10, 9]. It enables adhesion of micro-organisms to hydrophobic cell membranes of an intestinal epithelium. Clearly our results show that culture conditions may promote formation either hydrophobic or hydrophilic strains. Cultured in optimal thermal and nutrient conditions *Listeria* strains are hydrophilic. The growth in unfavourable conditions causes that part of them undergoes transformation into hydrophobic. It affects particular strains to a certain degree. Some strains, e.g. 19, remained hydrophilic, irrespective of culture conditions, whereas a strain 1577 in 7 out of 10 tests easily transformed into hydrophobic. Interestingly enough, it was the 4b-serotype strain isolated from a patient. Unfortunately, lack of clinical strains in the collection does not let state unequivocally whether clinical strains become hydrophobic easier than environmental or not. Our results may only suggest that the reason of low number of listeriosis cases in spite of frequent *Listeria* presence in food is a result of inbred or acquired (e.g. in vacuum culture conditions) hydrophilicity of strains. It was proved by *Listeria* strains cultured in vacuum conditions and incubated at low-temperature. Despite low-temperature which promotes hydrophobicity such strains all became hydrophilic.

It is well known that vacuum packaging has recently started to be very popular. Our previous research on vacuum-packed smoked salmon detected a high percentage of *L. monocytogenes*-positive samples [6]. Their ‘best before’ period lasts 14 days in cold storage conditions.

As *Listeria* strains may grow in vacuum conditions and at low temperatures [3], it is highly probable that during storage despite their multiplication, they become hydrophilic and less dangerous for consumers.

We also noticed that some strains vortexed for 10 s in a 10-minutes intervals were hydrophobic at the beginning then changed into hydrophilic. Such changes of strains properties indicate a presence of a chemical substance responsible for hydrophobicity loosely attached to a surface of the cell wall. It may belong to a group of biosurfactants [2], whose one hydrophilic end is firmly fixed with peptidoglycan of the cell wall and the other end is loose and hydrophobic. Intensive sixfold vortexing may cause detachment of biosurfactant what produces hydrophilicity of the strain. Santiago et al. [15] typed surface proteins which may be involved in *Listeria* adhesion to human Caco-2 erythrocytes. Their expression was influenced by temperature and a growth phase.

Presented results show that it is not possible to state unequivocally if *L. monocytogenes* strains are hydrophilic or hydrophobic. It depends on culture conditions and strain-distinctive features. Nevertheless, further analyses of hydrophobic transformations may lead to explanation of misapprehensions connected with pathogenicity of these micro-organisms. As complete elimination of *L. monocytogenes* without physical and chemical sterilisation is not possible and hydrophilic strains are less dangerous, food processing and storage should be modify to promote strain transformation from hydrophobic into hydrophilic.

## CONCLUSIONS

1. Strains of *L. monocytogenes* cultured in optimal conditions present hydrophilic properties.
2. Deteriorating of culture condition (reducing temperature and pH, using low-nutrient media) causes transformation of strains from hydrophilic into hydrophobic.
3. A kind of acid used to acidify a medium produces various effects on changes of hydrophobic properties of *L. monocytogenes*.
4. All strains cultured in vacuum conditions are hydrophilic.
5. Susceptibility to hydrophobic transformation is a strain-distinctive feature; some strains remain hydrophilic, other may easily change into hydrophobic.

## REFERENCES


1. Aarts H.J.M., Hakemulder L.E., Van Hoef A.M.A., 1999, Genomic typing of *Listeria monocytogenes* strains by automated laser fluorescence analysis of amplified fragment length polymorphism fingerprint patterns. Int. J. Food Microbiol. 49: 95-102.
2. Arino S., Marchal R., Vandecasteele J.P., 1998, Involvement of a rhamnolipid-producing strain of *Pseudomonas Aeruginosa* in the degradation of polycyclic aromatic hydrocarbons by a bacterial community. J. Appl. Microbiol. 84: 769-776.
3. Bayles D. O., Annous B. A., Wilkinson B. J., 1996, Cold stress proteins induced in *Listeria monocytogenes* in response to temperature downshock and growth at low temperatures. Appl. Env. Microbiol. 62: 1116-1119.
4. Beachey E.H., 1981, J. Infect. Dis. 143: 325.
5. Brosch R., Catimel B., Milon G., Buchrieser C., Vindel E., Rocourt J., 1993, Virulence heterogeneity of *Listeria monocytogenes* strains from various sources (food, human, animal) in immunocompetent mice and its association with typing characteristics. J. Food Prot. 56: 296-301.
6. Dąbrowski W., Iwański R., Bednarska M., Mędrala D., 2001, The effect of culture condictions of hydrophobicity of strains of *Listeria monocytogenes* isolated from food. EJPAU.

7. Dickson J.S., Koohmaraie M., 1989, Cell surface charge characteristics and their relationship to bacterial attachment to meat surfaces. Appl. Env. Microbiol. Apr. 55, 4: 832-836.
8. Heinitz M.L., Johnson J.M., 1998, The incidence of *Listeria* spp., *Salmonella* spp. and *Clostridium botulinum* in smoked fish and shellfish. J. Food Prot. 61: 318-323.
9. Hoštacka A., Šipošova E., 1998, *Klebsiella pneumoniae* clinical isolates: susceptibility to some antibiotics and surface hydrophobicity. Microbios 95: 101-107.
10. Jankowski S., Sarowska J., Zarczyńska H., Cisowska A., 1997: Hydrophobic properties of *Pseudomonas aeruginosa* strains. Med. Dośw.. Mikrobiol. 49: 187-190.[in Polish]
11. Kwiatek K., 1993, Appeareance of *L. monocytogenes* in meat and meat products, Życie Wet. 12: 304-306, [in Polish].
12. Ławrynowicz I., Tylewska S., Hryniewicz W., 1985, Physicochemical properties of cell surfaces of *Streptococcus agalactiae*. Med. Dośw. Mikrobiol. 37: 131. [in Polish]
13. Maisnier-Patin S., Richard J., 1996, Cell wall changes in nisin-resistant variants of *Listeria innocua* grown in the presence of high nisin concentrations. FEMS Microbiol. Lett., 140(1): 29-35.
14. Miller A. L., Smith J. L., Somkuti G. A., 1990, Foodborne Listeriosis. **ELSEVIER**, 5: 25.
15. Santiago N.I., Zipf A., Bhunia A.K., 1999, Influence of temperature and growth phase on expression of 104-kilodalton *Listeria* adhesion protein in *Listeria monocytogenes*. Appl. Environ. Microbiol. 65: 2765-2769.
16. Takeuchi K., Matute C.M., Hassan A.N., Frank J.F., 2000, Comparison of attachment of *Escherichia coli* O157:H7, *Listeria monocytogenes, Salmonella typhimurium*, and *Pseudomonas fluorescens* to lettuce leaves. J. Food Prot. 63: 1433-1437.
17. Van der Mei H.C., De Vries J., Busscher H.J., 1993, Hydrophobic and electrostatic cell surface properties of thermophilic sairy *Streptococci*. Appl. Env. Microbiol. 59, 12: 4305-4312.

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