SURVIVAL STUDY OF LISTERIA MONOCYTOGENES IN SEAWATER

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Abstract

We studied the behaviour of *Listeria monocytogenes* in sterile seawater. Recuperation of this bacterium on Tryptone Soya Agar shows that it can survive in sterile seawater for a long period of time in atypical colonies. Characters permitting the differentiation of *Listeria monocytogenes* and *Listeria innocua* are then modified.

Key words: Listeria monocytogenes, survival, seawater, adaptation.

Introduction

Until the late 1970s, it was generally assumed that *Listeria monocytogenes* does not survive in seawater (1). This view has been challenged by the finding that this bacterium can form a dormant life stage. *L. monocytogenes* is distributed widely in many natural environments (2). The success of *L. monocytogenes* as a pathogen seems to be related to its ability to adapt to its environment (3). The purpose of this work was to determined the morphological and metabolic modifications of *L.monocytogenes* growing in seawater.

Material and methods

Bacteria strain and cultural media

This study was carried out with *Listeria monocytogenes* strain isolated at the INSERM unity 452. Bacteria are recupered on Tryptone Soya Broth supplemented with yeast extract (0.6 %) (TSB) at 37°C for 24 h.

Growth agar was Tryptone Soya Agar supplemented with yeast extract (0.6 %) (TSA), or prepared with seawater (TSAM).

Starvation

Cells were grown for 24 h at 37°C and then washed three times in normal saline solution after centrifugation. The final pellet was suspended in 200 ml of filtered seawater.

Survival and recuperation tests

Culturability was assayed by spread plate counts. Serially diluted samples (0.1 ml) in sterilized seawater were spread in triplicate on plate media. After 24 to 48 h of incubation at 37°C, Colony-Forming Units (CFU) at appropriate dilutions were counted.

Biochemical profile of cells

The phenotypic profile was determined by Api *listeria* galleries.

Statistical analysis

Each point on the curves for enumeration of culturable cells presented in this study represents an average of 3 petri dishes. Statistical analysis was made by the Anova test carried out with the help of Program Stat View™ 512+.

Results

Quantitative variation analysis of *L. monocytogenes* incubated in microcosm seawater, showed a decrease by 99 % of CFU/ml after 24h of incubation in the seawater on TSA and TSAEM. The culturable forms continue to decrease until five weeks.

After one month in seawater microcosm, *L. monocytogenes* exhibited cultural modifications. Yellow pigmented, orange pigmented colonies appeared. Atypical colonies were cocci-rod forms with positive Gram, positive catalase and negative oxydase.

After one month of incubation in seawater, biochemical characters modifications are noted in the atypical colonies after their isolation from seawater microcosm (Table 1).

Table 1. L. monocytogenes biochemical characters before and after incubation in the seawater.

Characters / Colonies	DIM	ESC	_MAN	DARL	XYL	RHA	MDG	RIB	G1P	TAG
L.monocytogenes	-	+	+	+	-	+	+	-	-	-
Yellow colonies	(+)	+	+	+	-	(-)	+	-	-	-
Orange colonies	(+)	+	(-)	+	-	+	+	-	-	-

DIM: Differentiation L. innocua / L. monocytogenes; **ESC**: Esculine; ?**MAN**: ?-Mannosidase; **DARL**: D-Arbitol; **XYL**: D-Xylose; **MDG**: ?-Methyl-D-Glucoside; **RIB**: Ribose; **G1P**: Glucose-1-Phosphate; **TAG**: D-Tagatose; (): modified characters of atypical colonies compared with the typical colonies.

The enzyme that allows the differentiation between *L. innocua* and *L. monocytogenes* (DIM on Api *Listeria*) changed. The yellow colonies lost their rhamnose enzyme and orange colonies lost their mannosidase activity.

Discussion and conclusion

In this work we showed that *L. monocytogenes* survives in the seawater for a long period under cultivable forms. Subsequently, the cells may be evolve towards viable but non cultivable forms as recently demonstrated by Besnard et *al.* (4).

After one month of *L. monocytogenes* survival in seawater, we have observed yellow and orange pigmented colonies. It was shown that 60 % of marine bacteria are pigmented (5). This pigmentation varied from yellow to red (6).

We showed that rods develop towards coccoid forms. This result agrees with morphological modifications described also to occur in *L. monocytogenes* subjected to high hydrostatic pressure (7). A morphological changes of cells and/or colonies can be explain by biochemical modifications of the envelopes of bacteria (8).

The results of Api *Listeria* tests show that the modifications in the phenotypic profile of the stressed colonies involved essentially the key characters of *L. monocytogenes* determination. The instability of enzymes cause problems in the characterisation of species isolated from the environment.

In an oligotrophic aquatic environment and at low organic matter concentrations, *L. monocytogenes* can adapt to environmental stress. This adaptation involves, biochemical and morphological modifications generally used in taxonomy.

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