Survival of Mycoplasma, Acholeplasma and Spiroplasma at Different Temperatures

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Received for publication June 24, 1983

Summary: In order to determine the maintenance of viability of Mycoplasma pneumoniae, Mycoplasma salivarium, Acholeplasma laidlawii, Acholeplasma granularum, and Spiroplasma mirum, SMCA strain, outside the human and animal bodies and plant tissues, these mycoplasmal cells were kept in two kinds of solutions and at different temperatures, and their survival periods were measured. The results obtained indicate that, in general, except for M. salivarium, a solution containing a high concentration of protein, i.e. horse serum and albumin, is more suitable than a solution containing horse serum only for maintaining viability, and that a low temperature is better than a high temperature, as expected. In the results obtained here, the survival periods of M. pneumoniae, M. salivarium, A. laidlawii, A. granularum and S. mirum SMCA in phosphate buffered saline containing 1% horse serum and 0.1% bovine serum albumin V (PBS, HS, BSA) were approximately 80, 30, 230, 200 and 100 days, respectively.

Key words: mycoplasma — acholeplasma — spiroplasma — survival — temperatures

Introduction

Spiroplasmas that occur on the surface of flowers, presumably in nectar, were first isolated in culture from flowers of Liriodendron tulipifera L (Davis, 1978). Shortly thereafter, other isolates were recovered from flowers of Magnolia grandiflora L (Clark, 1978) and Circulifer haematocoe phala (McCoy et al. 1979). Very recently, Spiroplasma floricola, a new species, was isolated from the surface of flowers of the tulip tree, Liriodendron tulipifera L (Davis, 1978). Accordingly, it is now apparent that spiroplasmas are widespread in nature and that some strains associated with plants probably do not cause plant disease. It would be interesting, therefore, to know the survival period of spiroplasmas outside of the plants as well as that of other mycoplasmas and acholeplasmas.

The purpose of this paper is to report the survival periods of Mycoplasma, Acholeplasma and Spiroplasma kept at different temperatures, in order to contribute to studies on the significance of the isolation of Spiroplasma from plants and on epidemiological problems related to Mycoplasma and Acholeplasma.

Materials and Methods

Organisms tested

M. pneumoniae FH-Liu, M710 001084, M. salivarium PG 20, M712 001084, A.
laidlawii A PG8 M728 001084, A. granularum BTS-39 M719 001084, and S. mirum SMCA strain, ATCC 29335, were kindly supplied by Dr. M. F. Barile, Mycoplasma Branch, Bureau of Biologics, Food and Drug Administration, Bethesda, Md, U.S.A., and the strains have been maintained in our laboratory.

Maintenance solutions and culture medium

Phosphate buffered saline (pH 7.2) containing 1% horse serum (PBS, HS) and PBS, HS containing 0.1% bovine serum albumin V (PBS, HS, BSA) were used as maintenance solutions for observing the survival of mycoplasmas.

In the case of S. mirum R-8 (Chen, 1979) and SP-4 media were further used as maintenance solutions.

Experimental procedures

Broth cultures of mycoplasmal cells were first diluted 10- or 100-fold with PBS in order to eliminate the effect of horse serum contained in the culture medium and to make uniform the number of viable cells of each sample at “0” time, and then these diluted materials were added to the maintenance solutions at a proportion of 1:9. The tubes containing samples were then sealed with rubber stoppers and kept at 4°C, 30°C, and 37°C. Viability of each sample was measured after the desired incubation periods by the following methods.

For counting viable cells of the mycoplasmas, PPLO agar (Chanock, et al. 1962), was used and the number of viable cells was measured by the method of Smith (Smith, 1956) except in the case of S. mirum. For S. mirum, its mobility observed under a darkfield microscope was used as the indicator of viability.

Results

Survival period of M. pneumoniae at different temperatures

Cells of M. pneumoniae FH strain were suspended in PBS, HS as well as PBS, HS, BSA, and kept at 4°C, 30°C, and 37°C, then the survival periods were determined by the loss of colony-producing ability. The results obtained are illustrated in Fig. 1. It is obvious that cells of M. pneumoniae could survive much longer in PBS, HS, BSA solution than in PBS, HS solution. In the case of PBS, HS solution, there were no differences in survival periods of the cells incubated at 4°C, 30°C and 37°C, although the survival rate varied. In contrast, in the case of PBS, HS, BSA solution, there were marked differences between 4°C and 37°C as well as between 30°C and 37°C. As expected, cells of M. pneumoniae kept at 37°C lost their colony-forming ability more quickly than those at 4°C and 30°C. Unexpectedly, there was no difference between 4°C and 30°C.

Survival period of M. salivarium at different temperatures

The results obtained indicate, as shown in Fig. 2, a quite different pattern of the loss of colony-forming ability of M. salivarium from that of M. pneumoniae; namely, there was no difference in survival period of M. salivarium between PBS, HS solution and PBS, HS, BSA solution. As far as the temperature is concerned, as expected, the lower the temperature the longer was the survival period.

Survival periods of A. laidlawii and A. granularum at different temperatures

The results obtained are shown in Figs. 3 and 4. The same tendency was generally noted in both acholeplasmas tested, that is, PBS, HS, BSA solution was more suitable than PBS, HS solution for maintenance of viable cells of A. laidlawii A and A. granularum in the cold. (4°C). However, in the case of A. laidlawii, no difference between the inactivation rates of the cells at 30°C and 37°C in PBS, HS solution and in PBS, HS, BSA solution was observed.
In contrast, in the case of A. granularum, a similar inactivation curve in the two solutions at 37 °C was found, but viable cells in PBS, HS solution were more quickly inactivated at 4 °C than at 30 °C.

Finally, the above results are summarized in Table 1. In general, viable cells of Mycoplasma and Acholeplasma could be maintained in the cold longer than at 30 °C and 37 °C as expected, except for A. granularum in PBS, HS solution.

**Inactivation of SMCA at different temperatures**

Cells of SMCA propagated in SP-4 medium were suspended in PBS, PBS, HS, BSA solution and R-8 and SP-4 medium, and inactivation of the cells was estimated by the loss of mobility observed under a dark-field microscope. The results obtained are shown in Table 2. No complete or slight inactivation of cells in any of the solutions tested was observed either at 30 °C or 37 °C and 4 °C after 115 days' incubation.
Fig. 3. Survival period of *A. laidlawii* A in different solutions at different temperatures.

Fig. 4. Survival period of *A. granularum* in different solutions and at different temperatures.
Survival of Mycoplasma and Acholeplasma

<table>
<thead>
<tr>
<th>Maintenance solution</th>
<th>Incubation temp. (°C)</th>
<th>Mycoplasma species tested</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. pneumoniae</td>
<td>M. salivarium</td>
</tr>
<tr>
<td>PBS • HS</td>
<td>37</td>
<td>28*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>28</td>
</tr>
<tr>
<td>PBS • HS • BSA</td>
<td>37</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>78</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>82</td>
</tr>
</tbody>
</table>

PBS • HS = PBS + 1% horse serum
PBS • HS • BSA = PBS + 1% horse serum + 0.1% bovine serum albumin V (Armour Co.)

* : Survival period (days)

TABLE 2

Inactivation of SMCA during incubation

<table>
<thead>
<tr>
<th>Maintenance solution</th>
<th>Incubation temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>37</td>
</tr>
<tr>
<td>PBS</td>
<td>++</td>
</tr>
<tr>
<td>PBS • HS • BSA</td>
<td>++</td>
</tr>
<tr>
<td>R • 8</td>
<td>++</td>
</tr>
<tr>
<td>S • 4</td>
<td>NT</td>
</tr>
</tbody>
</table>

Estimated after 115 days of incubation.
NT: Not tested.

*: The symbols indicate as follows: + ; One mobile cell, ++ ; less than one hundred cells, +++ ; more than one hundred cells per one field under a dark-field microscope.

Discussion

It is essential to study the viability of mycoplasmas, acholeplasmas, and spiroplasmas outside the human and animal bodies, and plant tissues, respectively, from the epidemiological viewpoint, because it is not yet clear how M. pneumoniae is transmitted from person to person in an epidemic of mycoplasmal pneumonia, and the mode of transmission of plant diseases caused by mycoplasmas also is not yet determined. As stated at the beginning of this paper, it might be considered that spiroplasmas are widely spread among plants which are apparently healthy.

The results obtained here indicate that the viability of M. salivarium, M. pneumoniae, A. laidlawii, A. granularum in a solution containing protein in the cold was maintained for approximately one month, more than 80 days, more than 230 days, and 200 days, respectively, and that S. mirum could survive for more than 100 days in the four kinds of solutions tested.

From the results, it can be stated in general that a solution containing a high concentration of protein is better than one containing a low concentration of protein, and that the lower temperature is more suitable than the higher temperature for maintaining the viability of mycoplasmas, as expected. However, in the case of M. salivarium, unexpectedly there was no difference between the two solutions PBS. HS and PBS. HS. BSA for maintaining its viability. This conflicting property of M. salivarium in comparison with the other mycoplasmas should be studied in the future. In addition, the fact that the viability of A. granularum was lost more quickly in the cold than at 30 °C should be confirmed in a repeated experiment.

Although some unsolved problems re-
main the data presented here might add very significant information to our knowledge of the general properties of mycoplasmas and should contribute to the epidemiological studies of mycoplasmal infections.

References


