Survival of *Pseudomonas pseudomallei* Strains at 5°C

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**Abstract**

The ability of 15 *Pseudomonas pseudomallei* strains of different origin to survive at 5°C was tested, in comparison with the type strains of *Pseudomonas aeruginosa* and *Pseudomonas cepacia*. Viable cells of each strain were suspended in tryptic soy broth (diluted 1:10) at a concentration of about 10⁶ CFU/ml. The suspensions were kept at 5°C, and the number of viable cells was determined by counting colonies every 10 days. Four strains of *P. pseudomallei*, including the type strain for the species, died within 90–100 days at 5°C. Ten other *P. pseudomallei* strains together with the type strain of *P. cepacia* survived 160 days or more, although viable counts of these strains decreased to 10¹⁴ CFU/ml. The Oklahoma strain of *P. pseudomallei* and the type strain of *P. aeruginosa* maintained 10⁵ CFU/ml after 170–190 days. It was concluded that resistance of *P. pseudomallei* to low temperature differs from strain to strain. From the results of our experiments and those reported in the literature of nation-wide soil contamination by *P. pseudomallei* in France, prejudice regarding the organism as a tropical inhabitant must be corrected.

**Introduction**

Since Whitmore¹) first named and described *Bacillus pseudomallei* in 1913 as the etiologic agent of a hitherto undescribed disease which was later named melioidosis by Stanton and Fletcher²), it was believed that this disease occurred in or was related to tropical areas, especially Southeast Asia. *B. pseudomallei* was transferred to the genus *Pseudomonas* and a new combination *Pseudomonas pseudomallei* was proposed by Haynes³). In order to determine the relationship between the geographical location of disease and environmental temperature, the survival of viable cells of *P. pseudomallei* strains incubated at 5°C was monitored for 170–190 days.

**Materials and Methods**

*Bacterial strains used*

The histories and designations of 15 *P. pseudomallei* strains and the type strain of *P. aeruginosa* and *P. cepacia* are listed in Table 1. The strain designation Ragaviah for EY 1979 was the name of a patient, a young Indian laborer, from whom the strain was isolated⁴). Strain EY 2005 was isolated from infected wound of an Oklahoma farmer, and an organism with the same attributes was obtained from the soil where the farmer was injured in farming accident. EY 2005 was originally reported as a strain of *P. pseudomallei*-like organism⁵). However, the strain was reidentified recently as a strain of *P. pseudomallei*⁶).
Table 1 Histories and designations of 17 strains of three *Pseudomonas* species

<table>
<thead>
<tr>
<th>Strain</th>
<th>Strain source</th>
<th>Type</th>
<th>Soil or Animal</th>
<th>Designation</th>
</tr>
</thead>
<tbody>
<tr>
<td>EY 3241</td>
<td>Blood, Japanese male</td>
<td>P. pseudomallei</td>
<td></td>
<td>2004</td>
</tr>
<tr>
<td>EY 3383</td>
<td>Surface soil, Ubon Ratchathani</td>
<td>P. pseudomallei</td>
<td></td>
<td>2005</td>
</tr>
<tr>
<td>EY 3384</td>
<td>Soil, 30 cm depth, Ubon Ratchathani</td>
<td>P. pseudomallei</td>
<td></td>
<td>2005</td>
</tr>
<tr>
<td>EY 3395</td>
<td>Surface soil, Ubon Ratchathani</td>
<td>P. pseudomallei</td>
<td></td>
<td>2005</td>
</tr>
<tr>
<td>EY 3448</td>
<td>Surface soil, Ubon Ratchathani</td>
<td>P. pseudomallei</td>
<td></td>
<td>2005</td>
</tr>
<tr>
<td>EY 3453</td>
<td>Human infection, Vietnam</td>
<td>P. pseudomallei</td>
<td></td>
<td>2005</td>
</tr>
<tr>
<td>EY 3455</td>
<td>Human infection, Vietnam</td>
<td>P. pseudomallei</td>
<td></td>
<td>2005</td>
</tr>
</tbody>
</table>

Strain EY 3241 was isolated from the blood of the first melioidosis patient in Japan. Strains EY 3383 and 3384 were isolated from the soil, in December 1989, at Ubon Ratchathani, Thailand. It was the exact place where a 13-year-old boy was injured by falling off a bicycle and developed melioidosis, 6 months before the soil sampling.

**Estimation of viable counts**

Each of the 17 strains was cultured in heart infusion (HI) broth (Difco) at 35°C for 20 h. A volume of 0.5 ml of each broth culture was added to 50 ml of tryptic soy broth (Difco) at 1/10 concentration, to make the number of viable bacterial about 10^6 CFU/ml. Immediately after preparing the bacterial suspension, the viable number was determined by inoculating the suspension on HI agar plates. Then 3-ml quantities of each suspension of the 17 strains were distributed into 20 sterile screw-capped test tubes and kept in a refrigerator at 5°C. Every 10 days, each one test tube was used to determine viable number, by avoiding any contamination due to repeated sampling.

**Results**

The survival periods of the 17 strains at 5°C are illustrated in Fig. 1. Four strains of *P. pseudomallei* including the type strain of the species (EY 2004) died within 70—110 days. Eleven *P. pseudomallei* strains
**Pseudomonas pseudomallei** at 5°C

Fig. 1 Survival periods of 15 *P. pseudomallei* strains, *P. aeruginosa* 274T, and *P. cepacia* 645T. The thick line at the top of each column indicates the final day the viable cells were detected. The dotted line indicates that the organism was viable but experiment was discontinued.

![Graph showing survival periods of 15 strains.](image)

Fig. 2 Decrease in the number of viable cells of 4 *P. pseudomallei* strains kept at 5°C.

![Graph showing decrease in viable cell counts.](image)

Together with *P. aeruginosa* EY 274T and *P. cepacia* EY 645T survived 150—190 days or more.

The decreases in viable counts of the four *P. pseudomallei* strains which died within 70—110 days are shown in Fig. 2. The number of viable cells of strains EY 1979 Regaviah and EY 2004T decreased rapidly and fell to 10^1 CFU/ml within 50 days.

Changes in the number of viable cells of 13 strains which survived five months or more are illustrated in Fig. 3. Among them, Oklahoma isolate EY 2005 and *P. aeruginosa* EY 274T remained at 10^6 CFU/ml, the starting concentration, for 120 days, and were still at 10^6 CFU/ml after 170 days. The Japanese isolate of *P. pseudomallei* from the blood of a patient, EY 3241, and *P. cepacia* EY 645T were also still viable on the 170th
day, although the viable cell number fell to $10^2$ and $10^1$ CFU/ml, respectively. For these four strains of three species, estimation of the viable cell number was discontinued after 170 days of incubation. The number of viable cells of four other P. pseudomallei strains, EY 1983, 3384, 3395, and 3448, was unanimously $10^2$ CFU/ml on the 190th day.

**Discussion**

From the results presented here, it is evident that there are differences in the resistance for low temperature (5°C) among the strains of P. pseudomallei. The most resistant strain was the Oklahoma isolate EY 2005, which equaled with P. aeruginosa EY 274. The geographical location of Oklahoma is 35°N, the same as Osaka and Nagoya. Two strains, EY 3383 and 3384, were isolated from the surface and 30-cm deep soils, respectively, at the same place in Ubon Ratchathani, Thailand. The surface isolate, EY 3383, died within 80 days at 5°C, whereas the 30-cm deep isolate, EY 3384, was still viable on the 190th day. The identity of these two strains should be clarified genetically.

An outbreak of animal melioidosis in France was first noted by the death of a female horse on 6 November 1975, and P. pseudomallei was isolated from 12 animals by 1986. These melioidosis animals were distributed from nearly 50°N to 44°N in France. Soil samples obtained at 32 geographical points, which covered almost the whole country of France, yielded $10^6$ CFU of P. pseudomallei per gram of soil. Although the source and route of transmission of P. pseudomallei was obscure, a panda sent from Mao-Tsetung to Pompidou in 1973 or infected horses imported from Iran, were thought to be the possible origin. The geographical location of France is almost the same as Sakhalin and Hokkaido. The French incidence clearly shows that P. pseudomallei strains are able to survive and spread in such a northern area far from the tropics.

From the results of our experiments and the unexpected outbreak of animal melioidosis, accompanied with elevated serum antibody titers against P. pseudomallei among the staff members of the zoo in Paris, and long-term contamination of the soil in France, our prejudice regarding the restricted habitat of P. pseudomallei in a tropical area should be corrected. Transmission of melioidosis from human to human has not been definitively confirmed. However, environmental P. pseudomallei contamination in France was thought to be caused by animal excreta which contained the organism. We have already experienced a
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meliodosis patient in Japan). Awareness by medical personnel of the importance of prompt diagnosis of melioidosis and appropriate control of such patients to avoid environmental contamination are required.

References

5°Cでの Pseudomonas pseudomallei の生存状況

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要旨

P. aeruginosa と P. cepacia の基準株を対照として、由来の異なる P. pseudomallei 15菌株の5°Cでの生存能を調べた。各被検菌株は約10⁶CFU/mlになるよう1/10濃度のtryptic soy brothに懸濁して5°Cに置き、10日毎に生菌数を測定した。P. pseudomallei の基準株を含む4菌株は70〜110日目に検出不能になった。その他の菌株及び P. cepacia の基準株は夫々160〜190日又はそれ以上生存したが、菌数は10⁵〜10⁴個まで低下した。これに対し P. pseudomallei のOklahoma株は P. aeruginosa の基準株と共に170日後にも10⁵個を維持していた。これらの実験結果から、5°Cでの P. pseudomallei の生存能は菌株によって著しく異なることが判明した。今回の実験結果と、フランス全国での動物メイオイドーシスの多発及び P. pseudomallei による土壤汚染の蔓延を考慮すると、この菌種の生存域が熱帯地域に限られていていう観念が既存の観念を修正しなければならない、日本での発病例は既に報告されており、この感染症に対する諸家の関心を高め、早期診断及び環境汚染防止のための患者管理を徹底する必要がある。