ジャガイモ疫病菌遊走子のうの間接発芽に及ぼす数種無機塩類および水素イオン濃度の影響

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Effect of Some Inorganic Salts and Hydrogen Ion Concentration on Indirect Germination of the Sporangia of *Phytophthora infestans*

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Abstract

When the sporangia of *Phytophthora infestans* washed twice in distilled water were tested for indirect germination at 14°C, they did not germinate well (9% in 3 hr) in distilled water, while in the tap water of Hokkaido Natl. Agric. Exp. Stn., they germinated well (78% in 3 hr). Since some inorganic ions in the tap water appeared effective, various concentrations (0.01-10 mM) of the salts, $CaCl_2$, $Ca(NO_3)_2$, $MgCl_2$, $MgSO_4$, KCl, K_2SO_4, NaCl and Na_2SO_4, were evaluated for the effectiveness on indirect germination. Sporangial suspensions were preincubated for 22 hr at 22°C for sporangial maturation and postincubated at 14°C for indirect germination. The most effective solution was 0.3 mM $CaCl_2$ (71% germination in 1 hr), followed by 0.3 mM $MgSO_4$ (65%). Potassium and sodium salts were not very effective (<23%). At the cation concentration of 10 mM all the salts except Na_2SO_4 inhibited indirect germination (0-5%). Hydrogen ion concentration markedly affected indirect germination, optimum at pH 8.0 (90% germination at 0.1 mM $CaCl_2$) but completely inhibitory at pH 4.5 (0%). Considering the results and the cations contained in the tap water, a synthetic salts solution favorable for indirect germination was devised: 0.2 mM $CaCl_2$, 0.05 mM $MgSO_4$, 0.05 mM KH_2PO_4, 0.5 mM $NaHCO_3$, 0.01 mM Fe-EDTA-Na. Using this solution the effect of sporangial concentration on indirect germination was also investigated. Sporangial suspensions below 2.5×10^4 sporangia/ml were favorable for indirect germination (≧97% in 2 hr) but those above 10^5 were not.

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Key words: *Phytophthora infestans*, sporangium, indirect germination, germination solution.

INTRODUCTION

To consider the inoculum potential of the potato late blight fungus *Phytophthora infestans*, we have to pay attention not only to the amount of sporangia but also to the proportion and rapidity of indirect germination, because the zoospores released by indirect germination are the main agents for the disease infection[7]. To research indirect germination, germination tests have usually been carried out in distilled water[1,4], or distilled water was used as control[2,3]. Since indirect germination in distilled water varied widely (0-100%) even at the optimum temperatures[1,5], physiological research has been disturbed greatly. On the contrary, in the tap water of Hokkaido Natl. Agric. Exp. Stn. almost all (>90%) sporangia germinated invariably within several hours at 14°C. Using this water indirect germination was investigated in detail by the author[8,9]. It was revealed that the rapidity of germination was markedly affected by sporangial age. The sporangia soon after formation required several hours (>6 hr) to germinate at 14°C, while the sporangia aged for several hours (>6 hr, depending on environmental conditions)

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acquired the ability of rapid germination (c. 90% in 1 hr at 14°C). The author called the physiological change of sporangia by aging as 'maturation'[8]. Also, it was revealed that sporangia had their own limit temperature of indirect germination[9]. By application of the knowledge, it was easy to prepare inoculum suspensions of synchronistically released zoospores. Thus, to research sporangial germination, it is essential to use a water favorable for indirect germination.

Therefore, the effect of some inorganic salts and pH on indirect germination was investigated to devise a synthetic salts solution as favorable for indirect germination as the tap water.

MATERIALS AND METHODS

Test solutions. Distilled deionized water (abbr. distilled water), the tap water of Hokkaido Natl. Agric. Exp. Stn., and various concentrations (0.01-10 mM) of inorganic salt solutions were used for the germination tests. The tap water contained calcium (0.12 mM), magnesium (0.07 mM), potassium (0.04 mM) and sodium (0.59 mM) ions as main cations, and chloric and carbonic ions as main anions, and the pH was 7.7. Ferric, zinc, manganese and other ions were also contained as minor cations (personal communication from S. Yamazaki). The inorganic salt solutions tested were prepared from the special grade reagents: $CaCl_2$, $Ca(NO_3)_2$, $MgCl_2 \cdot 6H_2O$, $MgSO_4 \cdot 7H_2O$, KCl, K_2SO_4, NaCl, and Na_2SO_4. To test the effect of pH, test solutions were prepared by mixing the following solutions and checking the pH by a glass rod pH meter (TOA Inc.). For pH 4.5 and 5.0, 0.5 mM KH_2PO_4 solution and 0.01 N HCl were mixed. For pH 5.5-8.0, 0.5 mM KH_2PO_4 solution and 0.5 mM Na_2HPO_4 solution were mixed. For pH 9.0, 0.5 mM Na_2HPO_4 solution and 1 mM Na_2CO_3 solution were mixed.

Sporangial suspension. An isolate (H-1) of *P. infestans*, race 1 and A1 mating type, was used. Potato tuber slices (8-10 mm thick) of cv. Irish Cobbler were placed on the V-shaped glass rods on the moist newspaper sheet in a petri dish (18 cm dia.) and sprayed with a dilute sporangial suspension (c. 500 sporangia/ml). The petri dishes were kept in a temperature controlled room at about 18°C. Sporulation on the slices was few in 5 days but abundant in 6 and 7 days. Sporangial suspensions were prepared dipping several slices in c. 100 ml distilled water at 22-24°C. After removing mycelia by filtration through a gauze, the sporangia were collected by centrifugation (1,000 rpm, 1 min). For washing, the sporangia were suspended in 50 ml distilled water and again collected by centrifugation, and this was repeated two or three times. These sporangia were suspended in distilled water at the concentration of about 20×10^4 sporangia/ml. Then the suspension was added to test solutions. The final concentration of sporangia was about 1×10^4 sporangia/ml unless otherwise stated, and the suspension was dispensed in test tubes (15 mm diam.), 2 ml each.

Determination of the proportion of germination. The sporangial suspensions were incubated in an electric water bath at 14°C to test indirect germination either immediately or after preincubation for 12 or 22 hr at 22°C for sporangial maturation[8]. After desired periods of incubation at 14°C the suspension was added with a few drops of 10% formalin to stop further germination. The proportion of the sporangia which had already released zoospores and just been releasing them was determined by observing at least 100 sporangia under a microscope, and the mean percentage of three test tubes was used to indicate the proportion of germination in a suspension. Experiments were repeated at least two times.

RESULTS

Indirect germination in distilled water and the tap water

The effect of washing of sporangia in distilled water on indirect germination was examined first. Sporangia were collected from the 7-day-old tuber slice cultures, dipping two slices for a few seconds in 100 ml distilled water. A large part (c. 50%) of them were matured and had the ability to germinate rapidly. A small part of the suspension was dispended in test tubes without washing, and incubated for 3 hr at 14°C for indirect germination. The sporangia in the rest suspension were collected by centrifugation. After washing twice in distilled water the sporangia were resuspended in distilled water and also

incubated for 3 hr at 14°C. The experiment was repeated five times. In the case of nonwashing the proportion of indirect germination was high, 86.0% (s.d.=±1.5%), while in the case of washing it was low, 13.5% (s.d.=±2.1%). Thus, washing of sporangia in distilled water caused remarkable loss of the power of indirect germination. Without washing, some elements eluted from tuber slice cultures appeared to inhibit the loss of the power of indirect germination.

Then, recovery of the power of indirect germination was examined in the tap water. Sporangia were collected from the 6-day-old tuber slice cultures. After washing twice in distilled water, the sporangia were suspended in the tap water and distilled water and then incubated at 14°C. The proportion of indirect germination was determined after 1, 2, 3 and 24 hr of incubation. The results of triplicated experiments are shown in Fig. 1. In distilled water indirect germination was as slow as 9% in 3 hr and 28% in 24 hr, while in the tap water it was as rapid as 78% in 3 hr and 96% in 24 hr. Thus, some inorganic ions in the tap water appeared to be useful for the recovery of the power of indirect germination.

Effect of some inorganic salts on indirect germination

Since the tap water contained calcium, magnesium, potassium and sodium ions as main cations, the water solutions of various cation concentrations (0.01–10 mM) of the salts, $CaCl_2$, $Ca(NO_3)_2$, $MgCl_2$,

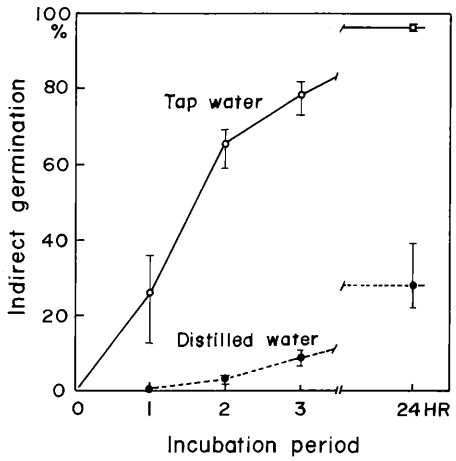

Fig. 1. Indirect germination of the sporangia of *Phytophthora infestans* in distilled water and the tap water of Hokkaido Natl. Agric. Exp. Stn. at 14°C, using the sporangia washed twice in distilled water. Points indicate the mean of triplicates and the bars the range.

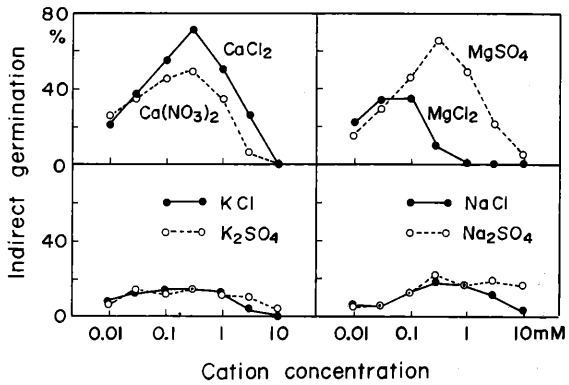

Fig. 2. Effect of some inorganic salts on indirect germination of the sporangia of *Phytophthora infestans*, using the sporangia washed three times in distilled water. Points indicate the mean of duplicates.

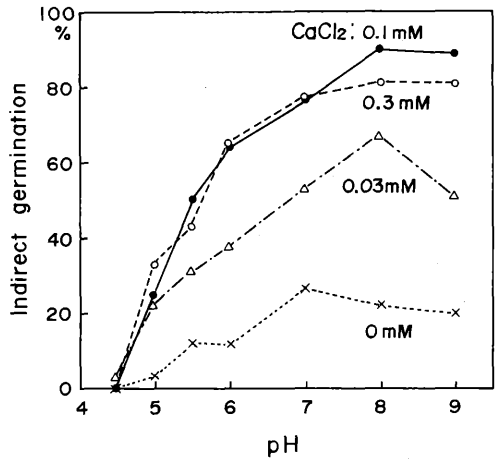

Fig. 3. Effect of pH and calcium chloride concentration on indirect germination of the sporangia of *Phytophthora infestans*, using the sporangia washed three times in distilled water. A representative result is shown.

$MgSO_4$, KCl, K_2SO_4, NaCl and Na_2SO_4, were tested on the effect on indirect germination. Sporangia were collected from the 6-day-old tuber slice cultures. After washing three times in distilled water, the sporangia were suspended in the salt solutions. The suspensions were preincubated for 22 hr at 22°C for sporangial maturation and then postincubated for 1 hr at 14°C for indirect germination. The results of duplicated experiments are shown in Fig. 2. In distilled water the proportion of indirect germination was as low as 8%. The proportion of indirect germination was highest (71%) in the solution of 0.3 mM $CaCl_2$, followed by 0.3 mM $MgSO_4$ (65%), 0.3 mM $Ca(NO_3)_2$ (49%) and 0.1 mM $MgCl_2$ (35%). It was noticeable that 1 mM $MgCl_2$ inhibited indirect germination almost completely. In the solutions of KCl, K_2SO_4, NaCl and Na_2SO_4 the proportion of indirect germination was relatively low, 22.4% at the highest (in 0.3 mM Na_2SO_4). At the cation concentration of 10 mM, all the salts except Na_2SO_4 inhibited indirect germination almost completely (0-4.6%).

Effect of pH and calcium ion concentration on indirect germination

Sporangia were collected from the 6-day-old tuber slice cultures. After washing three times in distilled water, the sporangia were suspended in the various solutions of 0-0.3 mM $CaCl_2$ at the pHs of 4.5-9.0. After preincubation for 22 hr at 22°C for sporangial maturation, the suspensions were postincubated for 1 hr at 14°C for indirect germination. The experiment was repeated two times and a representative result is shown in Fig. 3. The highest proportion of indirect germination (90%) was found in the solution of 0.1 mM $CaCl_2$ at pH 8.0, and the pHs around 8.0 appeared optimum for indirect germination. Wihtout calcium ion the proportion of indirect germination was low (22%) even at the optimum pH of 8.0. At pH 4.5 indirect germination was inhibited almost completely (0-3%).

Considering the results and the cation concentrations in the tap water, a synthetic salts solution of the following formula was devised: 0.2 mM $CaCl_2$, 0.05 mM $MgSO_4$, 0.05 mM KH_2PO_4, 0.5 mM $NaHCO_3$ and 0.01 mM Fe-EDTA-Na. Ferric ion was added only for expecting some physiological stability for the later germination of zoospores. For the ordinary use, solutions of 200 mM $CaCl_2$, 50 mM $MgSO_4$, 50 mM KH_2PO_4, 500 mM $NaHCO_3$ and 10 mM Fe-EDTA-Na were prepared separately and stocked in a refrigerator, and when used, each 1 ml of the solutions was added to 995 ml distilled water. To confirm the usefulness of the synthetic solution, following experiments were made.

Effect of sporangial concentration on indirect germination

Sporangia were collected from the 5-day-old cultures which were mostly immature and delicate under the unfavorable water conditions. After washing twice in the synthetic salts solution, the sporangia were suspended in the solution at different concentrations ($2.5-40\times10^4$ sporangia/ml). One half of the suspensions were immediately incubated for 12 hr at 14°C for indirect germination, while the other half

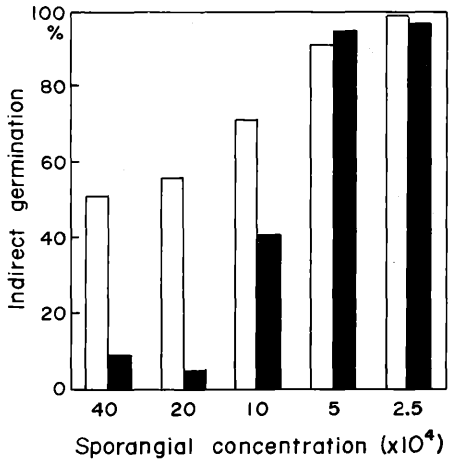

Fig. 4. Effect of the concentration of sporangia of *Phytophthora infestans* on indirect germination. After washing twice in the synthetic salts solution (see text) the sporangia were suspended in the solution. One half of the suspensions were incubated immediately for 12 hr at 14°C (□) for indirect germination. The other half of them were preincubated for 12 hr at 22°C for sporangial maturation and then postincubated for 2 hr at 14°C (■) for indirect germination. Histograms indicate the mean of duplicates.

of the suspensions were preincubated for 12 hr at 22°C for sporangial maturation and then postincubated for 2 hr at 14°C for indirect germination. The results of duplicated experiments are shown in Fig. 4. The lowest sporangial concentration (2.5×10^4 sporangia/ml) was most favorable for indirect germination (≧ 97%) in both cases. The proportion of germination decreased with increasing sporangial concentration. The decrease of germination was more remarkable in the suspensions preincubated at 22°C than in those immediately incubate at 14°C. At the concentrations above 20×10^4 sporangia/ml, only less than 10% sporangia germinated in the preincubated suspensions, while more than 50% sporangia germinated when they were incubated immediately for 12 hr at 14°C. The above tendency on germination in relation to sporangial concentration was similar to that found in the tap water. Thus, the synthetic salts solution was useful enough to prepare inoculum suspensions of synchronistically released zoospores.

DISCUSSION

The present results clearly showed that washing of sporangia in distilled water caused loss of the power of indirect germination, and the power was recovered by suspending them in a water solution containing appropriate concentrations of inorganic salts. Low concentration (0.1-0.3 mM) of calcium ion and the hydrogen ion concentrations around pH 8 appeared the optimum condition for indirect germination. Based on the results a synthetic salts solution favorable for indirect germination was devised.

Germination tests of the sporangia of *P. infestans* have usually been carried out in distilled water[1,4], or distilled water was used as a control[2,3]. The proportion of indirect germination was sometimes high (80-100%)[1,4] but sometimes low (0-60%)[2,4]. The low proportions of germination were found when sporangia were somewhat washed in distilled water[2,3], while the high proportions of germination were found when sporangia were not washed in distilled water[1,4]. In such a condition sporangia of various *Phytophthora* species are known to be able to germinate at high proportions[5]. Hence distilled water has been considered by many researchers to be favorable for indirect germination[5]. But it is not true as shown in the present results. Probably some inorganic ions eluted from the cultures provided good conditions for indirect germination.

Some organic compounds have also been tested to increase the proportion of indirect germination using the sporangia which showed low proportion of germination[2,4]. Crosier reported that sucrose was not effective[4]. Clark and Page once reported that hypoxanthin was somewhat effective[2], but later Clark

et al. obtained negative results using some purines and pyrimidines including hypoxanthine[3]. To increase the proportion of indirect germination organic compounds are not necessary but inorganic ions are essential as shown in the present results.

Gisi *et al.* previously reported the concentrations of inorganic salts inhibitory to indirect germination using the sporangia of *P. cactorum*[6]. Sporangia were suspended in distilled water, and without washing the suspensions were immediately used for the germination tests. In such a condition sporangia could germinate well. Inorganic salt solutions of 4 mM $CaCl_2$, 1.7 mM $MgCl_2$, and 12.5 mM KCl were completely inhibitory to indirect germination. The inhibitory concentrations of the salts were similar to the present results using the sporangia of *P. infestans*.

The effect of sporangial concentration on indirect germination was also investigated using the synthetic salts solution. Low concentrations below 2.5×10^4 sporangia/ml were favorable for indirect germination but high concentrations above 10^5 sporangia/ml were not. The synthetic solution was useful enough to prepare inoculum suspensions of synchronistically released zoospores, similarly to the tap water[8,9]. Also, the solution was useful to research sporangial germination not only for the isolate here used but also for all the isolates of *P. infestans* ever used in our laboratory.

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和 文 摘 要

佐藤章夫：ジャガイモ疫病菌遊走子のうの間接発芽に及ぼす数種無機塩類および水素イオン濃度の影響

蒸留水で2回洗浄したジャガイモ疫病菌の胞子（遊走子のう）を用い，直ちに14℃で発芽試験を行うと，再蒸留水に懸濁した胞子の間接発芽は強く抑制された（3時間で9％の発芽）が，北海道農業試験場の水道水に懸濁した胞子は速やかにかつ高率に発芽した（3時間で78％）。この水道水に含まれる無機イオンが間接発芽に好適と考えられたので，8種の無機塩（$CaCl_2$, $Ca(NO_3)_2$, $MgCl_2$, $MgSO_4$, KCl, K_2SO_4, NaCl および Na_2SO_4）の種々の濃度の溶液の間接発芽に及ぼす影響を調べた。これらの溶液に胞子を懸濁して22℃に22時間置いた後，14℃に冷却して発芽させたところ，発芽にもっとも好適な溶液は0.3 mMの$CaCl_2$で（1時間で71％の発芽），0.3 mM $MgSO_4$がこれに次いだ（65％）。ナトリウムおよびカリウム塩の効果は小さかった（<23％）。Na_2SO_4以外の供試塩類は陽イオン10 mMの濃度で発芽を強く抑制した（0～5％）。水素イオン濃度も間接発芽に影響し，$CaCl_2$濃度が0.1 mMの時，最適pHの8.0では1時間で90％の発芽が見られたが，pH 4.5では発芽は完全に抑制された。以上の結果および水道水の含まれる陽イオンの

種類と濃度を踏まえ次の処方の塩類溶液を調製したところ，間接発芽に極めて好適であった：0.2 mM $CaCl_2$, 0.05 mM $MgSO_4$, 0.05 mM KH_2PO_4, 0.5 mM $NaHCO_3$, 0.01 mM Fe-EDTA-Na。この溶液を用いて間接発芽に及ぼす胞子濃度の影響を調べたところ 2.5×10^4 個/ml 以下の濃度ではほとんどすべての胞子が発芽したが(2 時間で 97 %)，10^5 個/ml 以上では発芽が顕著に抑制された。