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HOLARCTIC ECOLOGY 5: 27–34. Copenhagen 1982

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Nº H. 800

Regeneration of Scots pine Pinus sylvestris at a natural tree-line in the Cairngorm Mountains, Scotland

G. R. Miller and R. P. Cummins

Miller, G. R. and Cummins, R. P. 1982. Regeneration of Scots pine *Pinus sylvestris* at a natural tree-line in the Cairngorm Mountains, Scotland. – Holarct. Ecol. 5: 27–34.

The age, density, distribution and reproductive capacity of Scots pine *Pinus sylvestris* L. were investigated along an altitudinal gradient through the only undisturbed tree-line remaining in the Cairngorm Mountains. Saplings at 300–410 m a.s.l. were unlikely to develop to reproductive maturity because of repeated browsing by red deer *Cervus elaphus* L. By contrast, pines were regenerating successfully in scrub at 531–590 m, where the population included individuals of all ages up to 300 yr. Above 590 m, there grew only saplings aged less than 30 yr and these declined in density with increasing altitude up to 730 m.
The climate at 531–590 m was not so severe as to prevent pines from growing to reproductive maturity. However, it may have been severe enough to restrict the activities of large herbivores and so the pine saplings there escaped the heavy browsing suffered by plants at lower altitude. Presumably zones of successfully regenerating pine scrub might occur more widely in the Cairngorms but for a shortage of seed-bearing trees.

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1. Introduction

The aims of this study were to investigate (a) the density and age structure of a forest of Scots pine *Pinus sylvestris* L. at its upper altitudinal limit, (b) the regenerative capacity of the trees and (c) the habitat and size of individual pine saplings.

The upper edge of pine forest rarely exceeds an altitude of 500 m a.s.l. in the Cairngorm Mountains, although the potential limit may be up to 650 m (Pears 1967). Almost all the present tree-lines show an abrupt change from tall pines to dwarf shrubs without the transitional sub-alpine scrub that occurs widely in Scandinavia (Poore and McVean 1957). This suggests that they are artificial and not determined climatically. Presumably the natural tree-lines of the Cairngorms have been destroyed by fire or by felling. Grazing or a shortage of viable seeds are possible causes of the failure of the trees to regenerate. One large area of apparently undisturbed tree-line remains at Creag Fhiaclach on the north-western edge of the Cairngorms massif. The site, first described by Watt and Jones (1948) and subsequently mentioned by several authors (e.g. Poore and McVean 1957, Pears 1967) may be unique in Britain.

In Scotland, many pinewoods at low altitude fail to regenerate because saplings are browsed by red deer *Cervus elaphus* L. and sheep (McVean 1963). However, recent research (Miller et al. 1983) has shown that saplings planted at 600 m or above are less liable to browsing than those on lower ground. The undisturbed tree-line at Creag Fhiaclach enabled us to determine whether pines can indeed produce viable seeds and regenerate successfully at their upper altitudinal limit.

2. Study area

Creag Fhiaclach is an exposed, west-facing spur rising to

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The role of the seed tuber in the contamination by *Erwinia carotovora* of potato crops in Scotland

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Accepted for publication 27 November 1973

Zusammenfassung, Résumé p. 197

Summary

Some mother tubers rotted as early as July and most were extensively rotted by the end of August. *Erwinia carotovora* var. *carotovora* and *E. carotovora* var. *atroseptica* were frequently isolated from them and from the soil in their vicinity. Daughter tubers became contaminated only after the rotting mother tubers had liberated bacteria into the soil. By the end of August and early September most of these were contaminated. Moreover, the absence of contamination in progeny tubers of pot and field grown plants without mother tubers in contrast to its presence where mother tubers were present further supports the view that the seed tuber is the main source of contamination by *E. carotovora* in potato crops in Scotland.

In the early stages of multiplication, virus tested clones raised from single tuber selections were more contaminated than those originating from virus tested stem cuttings. This observation is tentatively explained in terms of the presence or absence of mother tubers in the original propagative material and of the time of harvest.

Introduction

When examined after harvest, stocks of all seed grades and cultivars of potato in Scotland have been found to be extensively contaminated by *Erwinia carotovora* var. *carotovora* (Jones) Dye and by *E. carotovora* var. *atroseptica* (van Hall) Dye, the blackleg pathogen, irrespective of the level of blackleg in the crops (Pérombelon, 1972a). Both organisms were shown to survive in the lenticels and to a lesser extent on the tuber surface during the storage period (Pérombelon, 1973). Thus, a high proportion of seed tubers at planting time are contaminated and they probably form the main source of contamination for the growing crop since it is now generally accepted that the bacteria are no longer considered to be free living in soil (Graham 1958, 1962; Voronkovich, 1960; Lazar & Bucur, 1964; Logan, 1968; Pérombelon & Lowe, 1970).

This paper presents more direct evidence in support of this assertion and shows how and when tubers are contaminated in the field.

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Differentiation of virulent and avirulent cultures of Erwinia chrysanthemi (maize pathotype)

Differenzierung von virulenten und avirulenten Stämmen bei Erwinia chrysanthemi (Maispathotyp)

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Summary

Differences between virulent and avirulent cultures of the soft rot organism *Erwinia chrysanthemi* (maize pathotype) have been observed. These pertain to pectolytic activity, colony types on agar medium with 2,3,5-triphenyl tetrazolium bromide dye and motility. The virulent strain (MSR 12) produced larger and deeper wells/depressions when plated on sodium polypectate agar than the non-virulent one (MSR 1). Although both induced soft rot of potato slices that with the former was extensive and rapid. The cells of the former strain exhibited abundant motility as judged visually and also when grown on motility medium. Electron micrographs also showed presence of numerous peritrichous flagella on cells of this strain while they were absent on those of the non-virulent strain. Colonies of the virulent isolate on plating on triphenyl tetrazolium bromide agar medium showed large deep-red centres with small, narrow colourless peripheries; the reverse was the case with the avirulent strain (small red centres and wide colourless borders). In pathogenicity tests the virulent strain caused soft rot of the inoculated maize internode as well as adjacent ones in 3–4 days; with the MSR 1 - strain, however, there was no expression of any disease symptom (soft rot, pith maceration, water-soaked appearance, etc.).

Key words: maize; *Erwinia chrysanthemi;* virulence; pectolytic activity; soft rot; culture characteristics; motility

Zusammenfassung

Zwischen virulenten und avirulenten Stämmen des Weichfäule-Erregers *Erwinia chrysanthemi* (Maispathotyp) wurden Unterschiede in der pektolytischen Aktivität, im Kolonie-Typ auf mit 2,3,5-Triphenyltetrazoliumbromid versetztem Agar und in der Bewegungsfähigkeit beobachtet. Der virulente Stamm (MSR 12) erzeugte auf Natriumpolypektat-Agar größere und tiefere Löcher als der nicht virulente Stamm MSR 1. Beide Stämme verursachten an Kartoffelscheiben eine Weichfäule, wobei sich jedoch die durch den virulenten Stamm hervorgerufene schneller und stärker entwickelte. Die Zellen

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EFFECT OF FERTILIZER APPLICATION ON THE INCIDENCE OF BACTERIAL STALK ROT OF MAIZE*

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ABSTRACT

In glass house tests, high dose of nitrogen (N) application without phosphorus (P) and potassium (K) resulted in severe incidence of bacterial stalk rot of maize (*Erwinia chrysanthemi* corn pathotype), whereas, high doses of P and K helped in decreasing the disease incidence. Under field tests also, high and very high doses of N (120 and 80 kg/ha) increased the disease significantly as compared to low dose (60 kg/ha). It is suggested to follow a reduced dose of N (60 kg/ha) combined with 60 kg P and 40 kg/ha K for minimising the disease incidence in the field.

कांचघर परीक्षणों में फास्फोरस (पी) और पोटाश (के) के बिना नाइट्रोजन की उच्च मात्रा देने पर मक्का में जीवाणिक वृंत विगलन (इर्विनिया क्रिसेन्थिमाइ मक्का रोग प्ररुप) का उग्र प्रकोप देखा गया, जबकि फास्फोरस तथा पोटाश की उच्च मात्रा रोग का प्रकोप घटाने में सहायक हुई। क्षेत्र परीक्षणों में भी नाइट्रोजन की उच्च से अति उच्च मात्राएं (80-120 कि.ग्रा. प्रति हैक्टर) इसकी निम्न मात्रा (60 कि. ग्रा. प्रति हैंक्टर) की अपेक्षा रोग का प्रकोप बढ़ाने में अधिक सहायक हुई। अतः खेत में रोग का प्रकोप घटने के लिए नाइट्रोजन को निम्न मात्रा (60 कि. ग्रा. प्रति हैक्टर) के साथ 60 कि. ग्रा फास्फोरस तथा 40 कि. ग्रा. पोटाश प्रति हैक्टर देने की सिफारिश की जाती है।

Bacterial stalk rot of maize incited by *winia chrysanthemi* pv. zeae (Sabet 1954) ctoria, Arboleda & Munoz (1975), a major sease in India, is prevalent in Himachal adesh, Punjab, Delhi, Uttar Pradesh, Bihar, West Bengal, Rajasthan, Madhya Pradesh and Andhra Pradesh (Payak, 1972). With the introduction of hybrid maize, the occurrence of this disease has become a unique feature in several parts of the country, particularly in the

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USE OF KLOROCIN FOR THE CONTROL OF BACTERIAL STALK ROT AND ITS ABSORPTION, TRANSLOCATION AND PERSISTENCE IN MAIZE TISSUE

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Received for publication March 4, 1980

ABSTRACT

Klorocin, streptocycline and Agrimycine were evaluated against *Erwinia chrysanthemi* corn pathotype under conditions of artificial inoculation, Klorocin (250 ppm a.i.) when applied 24 hr before inoculation provided significant control (48.26%) of stalk rot of maize. When the roots of maize plant were dipped in 1000 ppm Klorocin solution for 24 hrs., 80.25 ppm chlorine was absorbed and only 56.50 ppm was translocated to leaves. Persistence of chlorine was also noticed in basal stem portion.

अप्राकृतिक संक्रमण की स्थिति में इरविनिया क्राइसेंथीमी (मक्का प्रभेद) पर क्लोरोसीन, स्ट्रेप्टोसायक्लीन व एग्रीमायसीन के प्रभाव का परिक्षण किया गया। संक्रमण से 24 घंटे पूर्व क्लोरोसीन (250 भाग प्रति दस लाख) के प्रयोग ने मक्का में तनासड़न रोग को प्रभाव्य रूप से नियंत्रित किया। मक्का के पौधों की जड़ों को क्लोरोसीन के 1000 भाग प्रति दस लाख सांद्रता के घोल में 24 घंटे डुबोने पर केवल 80.25 भाग प्रति दस लाख भाग क्लोरीन का अवशोषन हुआ तथा 56.5 भाग प्रति दस लाख भाग क्लोरीन का स्थानान्तरण पत्तियों में हुआ। क्लोरीन का जमाव तने के नीचे वाले भाग में भी पाया गया।

Bacterial stalk rot of maize (Zea mays L. caused by *Erwinia chrysanthemi* corn pathotype was first reported from India by Hingorani et al. (1959). Since then it has been occurring more or less regularly causing heavy losses. Some studies have been made to control this disease through cultural practices (Sabet 1956a, 1957) and use of chemicals (Rangarajan and Chakravarti, 1970; Thind and Payak, 1972; Sangam Lal et al., 1970; Thompson, 1965 annd Randhawa, 1977).

The bacterium survives in the soil and the disease starts from the basal portion of the plant. The soil application of chemicals, therefore, has been found more

1343
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Damage of Unknown Origin in Flower Primordia of Apricot Trees

Schäden unbekannter Ursache in Blütenknospen von Aprikosenbäumen

T. Buban, Z. Klement, G. Bodnar and I. Turi

Introduction

In the last years a distinct dark brown discolouration in buds of apricots was observed during autumn. This leads to rapid decay of the flower primordia. The wilt of apricot flower primordia has not been reported in the literature. Because of the susceptibility of apricot trees to bacteria and fungi, it was attempted to identify the causes. There are, however, neither reviews (KLEMENT 1974, 1977) nor other papers published recently (DANCSNÉ-ROZSNYAY et al. 1972, CARTER and MOLLER 1977, MORVAN 1977, POPUSHOI 1977, ROZSNYAY 1977, ROZSNYAY and KLEMENT 1977, SAGASTA 1977) suggesting any indication in this respect.

Materials and Methods

Using isolated flower primordia, bacteriological investigations were carried out in autumn and winter of 1978/1979 and 1979/80. The symptoms can be recognized first at the end of September or beginning of October. On the other hand, the infection of apricots by phytopathogenic bacteria occurs after leaf fall, during the winter season (KLEMENT and DANCSNÉ-ROZSNYAY 1972, BÖTTCHER and KLEMENT 1972).

In the first year of investigations, flower primordia with typical symptoms were isolated from the buds under a dissecting microscope, using sterile appliances. 30 flower primordia were homogenized with sterile quarz sand in 2 ml of a physiological salt solution. The homogenate and intact flower primordia without homogenization, resp., were put into broth culture (bouillon). Following the incubation of 24 hours at 22, 37 or 44° C the bacteria were streaked out (and cultured at the same temperature) on solid plates of nutrient agar, brillantegreen agar, desoxycholate-citrate agar and agar plates containing 5 per cent of bovine blood, respectively.

According to the colony morphology, 6 different strains have been selected and it was tried to identify them with special respect to *Pseudomonas syringae* as employed by KLEMENT (1971a, b): incubation on the plates of the King B medium, estimating fluorescent pigment production and testing phytopathogenic character by means of hypersensitive reaction (HR) in leaves of *Pelargonium* and tobacco plants. The oxidase reaction, the potato soft rot test, and the arginine test were used. The biochemical tests (listed in Table 5) were done according to CROSSE and CARRETT (1963), LELLIOT et al. (1966), KLEMENT (1970), and VISNYOVSZKY et al. (1971). — All investigations were repeated at least three times.

One of the most significant tests of *Pseudomonas syringae* is the syringomycin production of the bacterium. All strains isolated from diseased tissues were investigated for syringomycin production.

Next year the investigations of flower primordia were repeated in aseptical conditions. To isolate bacteria from the necrotic tissues of primordia was unsuccessful in 1979/80. Only a few bacterial colonies appeared on nutrient agar plates which were not characteristic for pathogenic forms, however.

Results

The development of symptoms resulting in destruction of flower primordia are shown in figures 1 to 8. There is no discolouration or any alteration on the surface and inside the symptomless flower primordia (fig. 1 and 2). The distinct browning at the connecting point — i. e. at the basis — of the flower primordia (fig. 3'c') can be observed as early as September or October. Dissecting such flower primordia made obvious that this external discolouration indicates the same damage both within the tissues of the connecting point of flower primordia (fig. 4'c') and the bottom part of the pistil primordia (fig. 4'b').

Somewhat later dark brown necrotic spots appear on the surface of the sepal primordia (fig. 5's'). Behind the necrotized surface tissues there are early symptoms of damage of the anther primordia (fig. 6'a') and at the bottom part of the pistil primordia (fig. 6'b') as well as at the connecting point of the flower primordia (see 'c' in fig. 5 and 6).

The necrotized proportion of the surface is getting more and more extended (fig. 7). By the time the surface became damaged as shown in fig. 7, inside the flower primordia there is a dead pistil primordium and perhaps still several unharmed anther primordia (fig. 8).

Longitudinal sections (fig. 9 to 12) show the damage of the surface which is not only an external one (fig. 9). The deterioration in the bottom part of the pistil primordia proceeds gradually (fig. 10 and 'b' in fig. 11, 12). The peculiar staining in the tissues of anther and filament primordia (fig. 12'a' and 'f') belongs to the

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MULTIPLE DISEASE RESISTANCE IN MAIZE

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S.C. SHARMA and V. K. SAXENA[1]

SUMMARY

Thirty-seven indigenous collections of maize (*Zea mays* L.) were evaluated for resistance to maydis leaf blight (*Drechslera* state of *Cochliobolus heterostrophus* (DrechsL) Nisikado & Miyaki), Philippine downy mildew (*Peronosclerospora philippinensis* (Weston) Shaw) and bacterial stalk rot (*Erwinia chrysanthemi* pv. *zeae* (= *E. chrysanthemi* (Burkh.) McFadden & Dimock)) under artificial epiphytotic conditions. Multiple disease resistance was measured by calculating, for each collection, a mean score and a standard deviation considering the three diseases together. Six collections showed multiple disease resistance (low mean score) of which four possessed uniform multiple disease resistance (low mean score + low standard deviation). These six collections gave a good agronomic performance and a germplasm complex has been developed from them.

Breeding for disease resistance is more economical and safer than chemical and biological control measures. This involves an evaluation of germplasm to identify sources of resistance to various diseases, followed by their use in breeding programmes. In India, maize suffers from many diseases of which maydis leaf blight, Philippine downy mildew and bacterial stalk rot are among the most serious ones. The present study was conducted to define and identify sources with multiple resistance to these diseases.

Thirty-seven indigenous collections from different parts of Punjab, India, were evaluated during 1979-1981 under artificial epiphytotic conditions conducive to maydis leaf blight, Philippine downy mildew and bacterial stalk rot. During the 3 years, the diseases developed satisfactorily except for bacterial stalk rot in 1980. Separate experiments were laid out for screening against the different diseases. A randomized complete block design with two replications was used in each experiment. Data were recorded on 20 plants in each plot.

The inoculation for Philippine downy mildew was performed by putting infected *kans* grass (*Saccharum spontaneum* L.) bits bearing mycelium and sporangia of the pathogen into the whorls of maize seedlings 2 days after emergence followed by a second inoculation 10 days later. In the case of maydis leaf blight, inoculation with conidia was performed twice, 30 and 40 days after emergence, following the method of Lim (1975). A single-colony culture of *E. chrysanthemi* pv. *zeae* was multiplied on potato-sucrose-peptone agar (200 g potato, 10 g sucrose, 5 g peptone, 20 g agar in 1 litre H_2O). Inoculation was performed by hypodermic syringe (Rangarajan and Chakrabarty, 1970) on 40-day old plants.

Incidence of maydis leaf blight was recorded on a 0–5 scale (Miller *et al.*, 1970). For Philippine downy mildew and bacterial stalk rot, percentages of infected plants were recorded. The percentage incidence was then transformed by arcsine before carrying out analysis.

To study multiple disease resistance the reaction was expressed on a uniform scale of 0–5. The percentage incidence was as follows:

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1345
N°M.807

A BACTERIAL STALK ROT OF IRRIGATED CORN IN NORTH CAROLINA

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Summary

A bacterial stalk rot has been observed following extensive overhead irrigation of corn at several locations in North Carolina. Leaf and stem tissue is rapidly invaded, becoming tan to brown and water soaked. Parenchymatous tissue is rotted quickly and affected portions of leaves are shredded. Diseased plants frequently topple over at the point of infection. A Gram-negative bacterium with peritrichous flagella was isolated from diseased plants and its pathogenicity to corn was demonstrated in greenhouse inoculations. The causal organism can also cause a pith decay of tobacco and soft rot of potato tubers, carrots, squash, cucumbers, cabbage and onions. Routine biochemical tests indicated that the bacterium can be considered a species of Erwinia, but that it differs from previously described species with respect to one or more biochemical reactions and pathogenicity to corn.

A severe stalk rot of field corn (Zea mays L.) was observed in breeding plots at the McCullers Test Farm of the North Carolina Agricultural Experiment Station in the summer of 1953 and again in the summers of 1954 and 1955. In all instances the appearance of the disease followed a period of extensive overhead irrigation. In 1954 specimens were also collected from a field of irrigated corn near Winston-Salem, North Carolina. The percentage of plants affected by this disease in a given field rarely exceeded 10. However, all diseased plants were either killed or so severely damaged that no ears were formed. There was no evidence of secondary spread from diseased to healthy plants once overhead irrigation was discontinued.

In isolations from material obtained at the McCullers Station and the farm in the vicinity of Winston-Salem, one type of bacterium was consistently isolated from the advanced margin of necrotic areas in the stalk. During the 1956 growing season additional isolates of the same bacterium were obtained from the corn breeding plots of the North Carolina Agricultural Experiment Station Test Farm at Clayton and also from a field of an inbred, N. C. 7, near Winterville, North Carolina.

Reports of bacterial stalk rot diseases of corn have been made by Ark (1), Boewe (2), Johnson et al. (3, 4), Lanza (5), Ludbrook (6), Prasad (7), Rosen (8, 9, 10), Sabet (11), Stanley (12), and Stanely and Orton (13). However, preliminary results obtained in several routine biochemical tests indicated that the bacterium isolated from diseased plants differed from other bacteria previously associated with stalk rot diseases of corn. This study was initiated to determine 1) whether the bacterium isolated from diseased corn plants was the primary pathogen, 2) whether this bacterium could also cause a typical soft rot of plants other than corn, and 3) whether it could be distinguished from the other stalk rot and soft rot bacteria previously described.

SYMPTOMATOLOGY

The most striking characteristic of the disease is the breaking over or toppling of plants above the fourth or fifth node (Fig. 1, A, B). This symptom differs from that described by Rosen (8, 9, 10) for the stalk rot disease caused by Erwinia dissolvens (Rosen) Burk. which usually attacks the stalk close to the ground line. Diseased leaf and stem tissues become water soaked and light tan to brown. The pith is disintegrated and often reduced to a slimy consistency; water soaked streaks extend short distances into the lamina of the leaf. Shredding of leaf tissue at the juncture with the stem is common. In advanced decay only the vascular bundles remain intact. In addition, diseased plants have a distinctly unpleasant offensive odor.

[1] Grateful appreciation is expressed to Dr. A. Husain for assistance in the laboratory studies.

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Chemical control of bacterial stalk rot *(Erwinia chrysanthemi* pv. *zeae)* and leaf stripe *(Pseudomonas rubrilineans)* of maize

Chemische Bekämpfung der bakteriellen Stengelfäule *(Erwinia chrysanthemi* pv. *zeae)* und der Blattstreifenkrankheit *(Pseudomonas rubilineans)*

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Summary

The evaluation of Streptocycline (100 μg/ml), Agrimycin-100 (100 μg/ml a.i.), stable bleaching powder (100 μg/ml chlorine), Blitox-50 W (2000 μg/ml), potassium permanganate (100 μg/ml) and combination of Streptocycline + Blitox-50 W in glasshouse and field experiments revealed that the spray and soil drench applications of Streptocycline alone and its combination with Blitox-50 W proved most effective for the control of maize stalk rot caused by *Erwinia chrysanthemi* pv. *zeae*. The application of these chemicals 24 h before inoculation yielded better results than 24 h after inoculation and soil drench application was better than spray application. The disease incidence decreased with the increase in concentration of chlorine from 100 to 1000 μg/ml. Keeping in view the survival of this bacterium in the soil in form of diseased plant debris and the suitability of stable bleaching powder (SBP) for soil drenching on account of its longer stability in soil, SBP has been recommended for the control of bacterial stalk rot of maize in comparison to Streptocycline and Agrimycin-100. Streptocycline alone and its combinations with glycerine or copper sulphate proved more effective for controlling leaf stripe of maize *(Pseudomonas rubrilineans)* than Agrimycin-100, SBP, potassium permanganate and Blitox-50 W.

Key words: maize; *Erwinia chrysanthemi* pv. *zeae; Pseudomonas rubrilineans;* stalk rot; leaf stripe; chemical control

Zusammenfassung

Untersuchungen über die Wirkung von Streptocyclin (100 μg/ml), Agrimycin-100 (100 μg/ml a. i.), "stable bleaching powder" (100 μg/ml Chlor), Blitox-50 W (2000 μg/ml) Kaliumpermanganat (100 μg/ml) und der Kombination von Streptocyclin mit Blitox-50 W in Gewächshaus- und Feldexperimenten ergaben, daß Spritzungen und Bodenapplikation von Streptocyclin allein oder in der erwähnten Kombination am besten die durch *Erwinia chrysanthemi* pv. *zeae* hervorgerufene bakterielle Stengelfäule des Maises bekämpften. Die Anwendung dieser Chemikalien 24 h vor der Inokulation führte zu besseren Ergebnissen als die 24 h nach Inokulation und Bodenapplikation erwies sich als günstiger als Spritzungen. Der Befall verminderte sich bei zunehmenden Chlorkonzentrationen (100 bis 1000 μg/ml). Unter Berücksichtigung des Überlebens dieses Bakteriums an befallenen Pflanzenresten im Boden und der Eignung des "stable bleaching powders" (SBP) zur Bodenbehandlung wegen der

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Evaluation of Selective Media for Isolation of Soft-Rot Bacteria from Soil and Plant Tissue

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Research supported by the College of Agricultural and Life Sciences, University of Wisconsin, Madison, Project No. 1621.

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ABSTRACT

A medium (CVP) containing $NaNO_3$, sodium polypectate, and crystal violet was compared with other selective media for the isolation of soft-rot bacteria from soil. Pectolytic colonies of *Erwinia* spp. could be distinguished from those of *Pseudomonas* spp. by the type of depressions formed in the pectate medium and colonial morphology. Recovery of *E. carotovora* and *E. atroseptica* from field soil on CVP range from 65 to 100%, depending upon soil type and the number of bacteria added to soil samples. Approximately 96% of the natural soil bacteria (ca. 6×10^7 cells/g dry wt soil) were eliminated on CVP. Addition of manganese sulfate (monohydrate) to CVP further reduced the soil bacterial populations without markedly lowering the percent recovery of soft-rot *Erwinia*. When CVP was used for detection of soft-rot bacteria in soil samples from cabbage, carrot, and potato fields, fluorescent pseudomonads (not soft-rot *Erwinia*) were the pectolytic gram-negative bacteria most frequently isolated.

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Additional key words: *Pseudomonas marginalis*, blackleg.

Selective media have been used with different degrees of success to isolate soft-rot bacteria from soil and decaying plant tissue. Direct soil plating techniques on agar media were considered by Leach (26) to be neither selective nor sensitive enough to detect small populations of soft-rot *Erwinia* that might survive the winter in soil in Minnesota; thus, he developed a potato (*Solanum tuberosum* L.) tuber-tissue assay method. Gram-negative soft-rot bacteria were isolated by a modification of Leach's method from a range of different types of soil in Scotland (22). Subsequently, Graham (11) found that the gram-negative soft-rot bacteria isolated by the tuber-tissue procedure from soil were not soft-rot *Erwinia* spp., but fluorescent pseudomonads. Other workers also have found that the use of plant tissue to determine numbers of soft-rot bacteria in soil was often neither specific nor accurate (24).

Many selective media containing pectin or sodium polypectate have been devised for detection of pectolytic soft-rot bacteria (2, 8, 18, 28, 35, 36, 38, 39, 44, 46, 49). Certain of these media, however, are unsuitable for plating large numbers of samples from soil because of inconvenient procedures, or lack of specificity. Media selective for soft-rot *Erwinia* that do not include pectic substrates have also been tested; examples are the crystal violet-bile agar medium of Patel (33, 34), the antibiotic-eosin-methylene blue medium of Segall (41), the modified Drigalski medium of Tsuyama and Sakamoto (48), and the salicin-sodium taurocholate-bromthymol blue medium of Noble and Graham (32).

More recently, Kado and Heskett (20) and Miller and Schroth (29, 30) have reported the development of media effective for the isolation of *Erwinia* spp., including the soft-rot group.

Among other techniques evaluated, an immunofluorescent staining procedure (23) for detection of *Erwinia aroideae* (Towns.) Holland in soil was found to be unsuitable for examining large numbers of soil samples containing diverse strains of the pathogen.

In initial studies to determine the populations of soft-rot *Erwinia* in potato field soils in Wisconsin at different seasons of the year, various media tested proved to be either time-consuming in preparation or ineffective for the detection of low populations of pectolytic bacteria from soil. The objective of this investigation was to develop an improved selective medium to be used in assaying soils and plant tissues for gram-negative aerobic soft-rot bacteria, in general, and, in particular, for the *Erwinia* that rot potato tubers. A preliminary report on this study has been presented (6).

MATERIALS AND METHODS.—*Cultures.*—A strain of *Erwinia carotovora* (Jones) Holland (SR 53), originally isolated by L. R. Jones and listed by the American Type Culture Collection as type culture ATCC 495, was used throughout this work. Cultures SR 10, isolated in 1969 from rotting carrot (*Daucus carota* L.) tissue from Lake Mills, Wisconsin, and SR 8, isolated the same year from a potato plant with blackleg symptoms from Hancock, Wisconsin, were also used; these isolates had the essential characteristics of *E. carotovora* and *E. atroseptica* (van Hall) Jennison, respectively.

Test media.—The medium (CPG) used to grow bacterial inoculum contained (g/liter): glucose, 10; Bacto-peptone, 10; Bacto-casamino acids, 1; and Bacto-agar, 18 (21).

Initially, a pectate medium (PM) was prepared following the method of Beraha (2): (i) 1 N NaOH (4.5 ml), 10% $CaCl_2 \cdot H_2O$ (3 ml freshly prepared), 1.5% aqueous bromthymol blue (0.5 ml), Bacto-agar

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Physiological and pathological characteristics of *Erwinia chrysanthemi* isolates from potato tubers

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Nine isolates of *Erwinia chrysanthemi* from rotting potato tubers were compared with six type or reference strains of this species. Phenotypic properties of the potato isolates closely agreed with those of *Erw. chrysanthemi* pv. *zeae* and with the characteristics proposed for Dickey's infrasubspecific subdivision IV (1979) and Samson & Nassan-Agha's biovar 3 (1978), where *Zea mays* was among the most common host species. Pathogenicity tests on 20 ornamental and agricultural species showed only *Cyclamen* sp. and *Z. mays* to be susceptible. In Ouchterlony double diffusion tests, antisera to whole live cells of one potato strain reacted with four of the six pathovars of *Erw. chrysanthemi*. Tuber isolates did not produce blackleg symptoms in inoculated stems. The rationale of intensive pathogenicity testing is discussed.

Erwinia chrysanthemi Burkholder, McFadden and Dimock has long been recognized as a pathogen of ornamental plants and of some tropical and subtropical crops. It was first reported from potato stems by Tanii & Baba (1971) in Japan but as their isolates could possibly have been strains of *Erw. carotovora*, the first record may be that of Graham (1972) from potato blackleg in Brazil. The potato, usually considered a temperate vegetable, is now being actively researched in tropical and subtropical areas under the auspices of the International Potato Centre, and *Erw. chrysanthemi* has recently been isolated from potato roots, stems and tubers in Peru (de Lindo *et al.* 1978; French & Lindo 1979). In addition, it has been identified as causing severe losses of planted seed tubers in a semi-arid irrigation area in Australia (Cother 1980).

Two recent studies have suggested that strains of *Erw. chrysanthemi* isolated from a given host tend to belong to a distinctly identifiable biovar or specific subdivision. Dickey (1979) proposed five subspecific subdivisions based on 12 physiological properties and Samson & Nassan-Agha (1978) proposed three biovars based on six such properties. In either study, *Zea mays* was the only temperate host from which several strains were compared, the others being from ornamentals or tropical fruit. In the Southern Riverina region of New South Wales, traditional agriculture comprised dryland grazing and cereal production prior to the expansion of irrigation. Of the dominant irrigation crops, rice, maize, sorghum, onions, potatoes and tomatoes, only rice and maize are recognized hosts of this pathogen.

In view of the above relationships between host of origin and subdivision, the following studies were carried out to determine whether the potato isolates of *Erw. chrysanthemi* possessed any characteristics distinctly different from the type strains isolated from other hosts and whether these isolates belonged to any proposed subdivisions or biovars.

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Water Relations of *Erwinia chrysanthemi*: Intracellular and Extracellular Pectate Lyase Production

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(*Received 24 November 1982; revised 25 April 1983*)

When *Erwinia chrysanthemi* was grown in a sodium polypectate/yeast extract/salts medium adjusted with sorbitol to water activity (a_w) values of 0·990 and 0·980, extracellular pectate lyase (PL) production was repressed, whereas intracellular PL levels were not affected. Inorganic solutes (NaCl, LiCl, KCl, Na_2SO_4 and a NaCl/KCl/Na_2SO_4 mixture) at 0·990 a_w strongly stimulated the intracellular levels of PL (6- to 17-fold greater than the control), whereas extracellular levels were only slightly increased. Intra- and extracellular PL levels were not affected by LiCl, NaCl and KCl at 0·995 a_w. A NaCl/sorbitol mixture (0·990 a_w) completely inhibited extracellular but not intracellular PL production. Lowering of the a_w of cultures during mid-exponential growth phase with NaCl (0·990 a_w) resulted within 30 min in a 70-fold increase in intracellular PL activity. When sorbitol was used in similar experiments, extracellular PL production was inhibited to a greater extent than intracellular levels.

INTRODUCTION

Extracellular production of pectic acid lyase (PL), also known as polygalacturonic acid transeliminase (EC 4.2.2.2), by *Erwinia chrysanthemi* is profoundly influenced by the water activity (a_w) of the growth medium (Mildenhall *et al.*, 1981 *a*, *b*). PL production was increasingly repressed with lowered a_w when organic solutes (lactose, mannose, sorbitol and D-arabinose) were used as a_w adjusters whereas NaCl stimulated enzyme production at 0·990 a_w. A major problem encountered in a_w studies is to resolve whether the effects of a particular solute are due to its effect upon water removal or to the effects of the solute *per se* upon the organism. Sodium chloride is the standard inorganic solute used in a_w studies. Because the effect of NaCl upon extracellular PL production was different from that of the organic solutes we extended our studies to include LiCl, KCl and Na_2SO_4 as a_w adjusters. Sodium sulphate was included for comparison in order to assess whether chloride ions exerted a specific effect.

Several patterns of PL synthesis and excretion have been reported in *Erwinia*, *Yersinia* and *Klebsiella* (Chatterjee *et al.*, 1979). In their strain of *E. chrysanthemi*, PL activity was located mainly extracellularly whereas in *Erwinia carotovora* the enzyme was found in the cytoplasm, periplasm and extracellular fluid. In *Klebsiella pneumoniae* PL was confined to the cytoplasm. The effects of a_w, reported previously, upon extracellular PL levels in *E. chrysanthemi* suggested that this system may help elucidate the mechanism of PL regulation in this organism.

We investigated (i) the effect of a_w (sorbitol, NaCl, Na_2SO_4, KCl and LiCl) upon intracellular and extracellular PL production and (ii) the effect of changing the a_w (sorbitol; NaCl) of the medium in the mid-exponential phase of growth, upon PL synthesis and excretion in *E. chrysanthemi*. The results show that intracellular PL levels are generally elevated by inorganic solutes at 0·990 a_w, particularly by the sodium salts and LiCl whereas sorbitol failed to exert this effect. Extracellular PL production is severely repressed by sorbitol.

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[42] Selection of Clones from Libraries: Overview

By ALAN R. KIMMEL

There are several procedures for selecting cloned sequences of interest from recombinant DNA libraries. Individual recombinants within libraries can be screened for homology with a nucleic acid sequence, for expression of an antigen (antibody recognition), or for expression of a phenotype. In addition, populations can be screened as mixtures of recombinants. Selection using only a single approach is rarely proof that a clone has been identified correctly, because even the most stringent criterion for screening may select false positives. Therefore, a combination of selection schemes is often needed and further characterization is usually essential for corroboration. Such analyses must rely on information which supplements that originally used for screening [54] (numbers in brackets refer to chapters in this volume).

Nucleic Acids Probes

Screening with nucleic acid probes is dependent upon molecular hybridization, the annealing of single-stranded nucleic acid molecules to form duplex structures stabilized by sequence-specific hydrogen bonds [43]. Only nucleic acids of related sequence organization will base pair or hybridize with each other. The stability of hybrids is a function of the relatedness of the two nucleic acid sequences. Theoretical considerations and practical applications for screening with nucleic acid probes are critical for discriminating sequence-specific hybridization from nonspecific interactions. Empirical procedures are described which relate the effect of probe length, probe base composition, reaction temperature, reaction time, organic solvent concentration, cation concentration, probe concentration, base-pair mismatch, and differences in reactivity of RNA and DNA on the ability to form stable hybrids [43].

Before screening, recombinant libraries must be organized to permit the isolation of one or a few positive colonies or plaques from the $>10^4$ clones probed. Libraries are distributed on master agar plates; clones are transferred to filter matrices and oriented to permit alignment of master plates with the pattern of recombinants replicated on filters [44]. Often duplicate filters are made. DNA from these clones is then immobilized *in situ* and hybridized with denatured or single-stranded radiolabeled nucleic acid probes [45]. Under appropriate conditions, only clones containing DNA sequences that share homology with the probe will hybridize. Posi-

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Soft rot *Erwinia* bacteria in surface and underground waters in southern Scotland and in Colorado, United States

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MCCARTER, N.J., FRANC, G.D., HARRISON, M.D., MICHAUD, J.E., QUINN, C.E., SELLS, I.A. & GRAHAM, D.C. 1984. Soft rot *Erwinia* bacteria in surface and underground waters in southern Scotland and in Colorado, United States. *Journal of Applied Bacteriology* **57**, 95–105.

An anaerobic liquid enrichment method followed by plating on a selective medium revealed that the soft rot coliform bacterium *Erwinia carotovora* subsp. *carotovora* was generally present in water from drains, ditches, streams, rivers and lakes (including reservoirs) in southern Scotland and in Colorado, United States, in mountainous, upland and arable areas through the year. Many sites were remote from susceptible or diseased crops. *Erwinia carotovora* subsp. *atraseptica* was isolated much less frequently and no *Erwinia* bacteria were isolated from underground waters. *Erwinia* bacteria were also found in rain-water in Scotland, in winter snow from mountain passes in Colorado, and in sea water from the west and east coasts of Scotland and from the coasts of Oregon, California, Texas, Louisiana and Florida. The significance of the occurrence of these bacteria in water is discussed in relation to the control of blackleg and soft rot diseases of potato by production of *Erwinia*-free stocks.

A study was made of the occurrence of *Erwinia* spp. in association with weed roots in Scotland and in Colorado (McCarter-Zorner *et al.* 1982a) as part of a general investigation into sources of soft rot erwinias in the environment, from which the organisms might spread and contaminate erwinia-free potato stocks produced from stem cuttings or by micropropagation. In late spring 1980, random testing in Scotland showed that the roots of aquatic weeds and water in ditches very often yielded these organisms. As a result, an investigation was made into their presence in water of drains, ditches, streams, rivers, lakes (including reservoirs) and underground sources over the period July 1980 until July 1982 in southern Scotland. Although generally considered a rainy country, many parts of the Scottish potato growing areas have a moisture deficit in the growing season and irrigation is becoming more widely practised. Much of the irrigation water is obtained from dammed ditches, streams and rivers in arable areas, which therefore might be sources of contamination. Many streams and rivers rise in mountainous or upland areas where arable crops are not grown, and water from these sites was also examined.

From August 1980 until December 1981, similar studies were made in central Colorado, United States. Colorado has a typical continental climate with long periods of dry warm weather during the potato growing season. Irrigation is therefore essential; water is taken from streams and rivers and often from aquifers, and applied to crops with centre pivot overhead sprinklers or by surface irrigation methods. These waters and some others from arable, upland and mountainous areas were examined

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Potato Res. 23 (1979) 75-84

Bacterial seed tuber decay in irrigated sandy soils of New South Wales

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Zusammenfassung, Résumé p. 82

Additional key-words: Erwinia chrysanthemi, E. carotovora, soil inoculum

Summary

Breakdown of newly sown seed tubers in semi-arid irrigated sands was shown to be caused by *Erwinia carotovora* var. *carotovora* and *E. chrysanthemi*. The soil-borne bacterial population appeared to be more important than seed tuber population in initiating rots in soil. Pectolytic activity of tuber-borne bacteria in an anaerobic environment increased with increasing temperature; there was comparatively little rotting below 25 °C. Average percentage weight loss of tubers was approximately seven times greater when they were dip- or puncture-inoculated with *E. chrysanthemi* than with *E. carotovora*. Control measures should perhaps be based on protection of the newly planted seed tuber rather than on development of clean seed sources. This is the first report of *E. chrysanthemi* caused soft-rotting of potato tubers.

Introduction

The potato industry of south-western New South Wales, Australia is located in the Murrumbidgee, Coleambally and Berriquin Irrigation Areas. It is a region of high solar input where 2 crops per year can be produced since frost damage does not usually occur outside the period of late May to late August. Cropping is confined to unconsolidated aeolian sands consisting of 5 % clay and 95 % sand, generally of one particle grouping. The uniform dunes have no surface horizons but often overlie cemented layers which can interfere with free drainage at the dune edge. The area is climatically semi-arid and the annual evapo-transpiration is about five times the annual rainfall. Prior to cropping the land is growing native pasture and *Callitris* pine. It is usual for two crops to be grown each year on the same field and if a break occurs in cropping, the land is left fallow under weeds or sown to oats to reduce wind erosion.

The plant densities of the autumn crop, sown in January/February, range from 40 to 80 % with an average of 70 %. Approximately 85 % of the gaps are caused by bacterial breakdown of whole seed tuber shortly after sowing, the remainder being due to missing seed; occasionally entire fields must be resown.

This paper describes the identification of bacteria involved in seed tuber decay, the source of infection and effect of temperature on tuber breakdown. The term seed tuber in this paper refers to whole uncut tubers weighing 45–75 g.

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[4] Large- and Small-Scale Phenol Extractions

By DONALD M. WALLACE

Of basic importance to molecular cloning is the ability to extract and purify nucleic acids from a variety of sources. Probably the most common method is phenol extraction. With appropriate modification of the conditions, the technique can be applied to such diverse operations as the isolation of RNA or DNA from complex mixtures of biological origin, e.g., cell extracts, or to the retrieval of DNA from relatively simple mixtures such as those encountered during molecular genetic manipulations *in vitro*. These operations require either large-scale (usually tens to hundreds of milliliters) or small-scale (microliters) phenol extractions, respectively.

A brief review of the literature shows that a plethora of phenol extraction methods have evolved, with little or no explanation of how or why they came about. Rather than describing them, the aim here is to discuss the basic factors which influence the procedure so that rational choices of methodology can be made. In addition, two detailed methods are presented: a large-scale phenol extraction method for RNA from complex mixtures, and a small-scale technique for DNA recovery from simple mixtures. Readers are referred to the relevant chapters of this volume for more detail on DNA and RNA isolation methods (see this volume [13,15,18,20–22] and elsewhere in this series[1]).

The fundamental aim of a phenol extraction is the deproteinization of an aqueous solution containing the desired nucleic acids. In simple terms the phenol reagent is mixed with the sample under conditions which favor the dissociation of proteins from nucleic acids. Centrifugation of the mixture yields two phases: a lower organic phenol phase carrying the protein (much of which actually segregates to the white flocculent interphase) and the less dense aqueous phase containing the intact nucleic acids. Ultimately, recognition of the optimal conditions for a particular extraction procedure is dependent on the nature of the nucleic acids to be isolated and on how they will be subsequently used.

Quality of Phenol

Many commercial suppliers provide liquified phenol which can be used directly, without further purification; it is clear and colorless. For

[1] This series, Vol. 12, Parts A and B.

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[13] Practical Aspects of Preparing Phage and Plasmid DNA: Growth, Maintenance, and Storage of Bacteria and Bacteriophage

By HARVEY MILLER

The practical aspects of cloning can be divided into four major topics: (I) handling, culturing, and storing bacteria; (II) preparation of competent cells for transformation; (III) growth of phage and the preparation of phage DNA; and (IV) isolation and purification of plasmid DNA. The majority of this chapter is devoted to providing detailed instructions for each of these broad categories. Brief explanations of theory are included where necessary. There are also three tables that contain basic information about bacterial strains used in cloning (Table I), buffers, media, and agar (Table II), and antibiotics (Table III).

Handling, Culturing, and Storing of Bacteria

Successful recombinant DNA techniques require the proper handling of a substantial number of bacterial strains. Although all are derivatives of *Escherichia coli* K12 strains, the strains have different characteristics making them suitable for a particular purpose. For example, many strains lack host restriction or modification systems which allow propagation of unmodified DNA. Some carry mutations of the *lac* operon which allows detection of inserts into phage and plasmids of the pUC and M13mp type. Strains containing nonsense suppressors permit the growth of specialized phage cloning vectors. Table I lists many common bacterial strains, their genotypes, and uses.

It is important when working with these bacteria that the cultures be kept pure, that the phenotypes be verified prior to use, and that they be stored properly. It is assumed that sterile glassware and media are available. A thorough discussion of basic microbiological techniques can be found in *Experiments in Molecular Genetics*.[1]

Pure Cultures. Pure cultures can be obtained by propagating bacterial cultures from single, isolated colonies on agar plates. The simplest method of obtaining isolated colonies is by dilution streaking with an inoculating loop. A small inoculum of bacterial culture is picked up with a

[1] J. H. Miller, "Experiments in Molecular Genetics." Cold Spring Harbor Lab., Cold Spring Harbor, New York, 1972.