Biology and Epidemiology of *Erwinia rhapontici*, Causal Agent of Pink Seed and Crown Rot of Plants

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ABSTRACT


*Erwinia rhapontici* is one of the few bacterial species that produces a distinct diffusible pink pigment on sucrose-peptone agar. It is an opportunistic plant pathogen that attacks a wide range of hosts causing pink seed and crown rot or soft rot. This review article summarizes current research progress on this unique bacterial pathogen, in the areas of disease occurrence, causal agent, host range, and control measures. The review also identifies the need for future research, particularly in the areas of epidemiology, ecology and control of the disease, as well as research to determine if *E. rhapontici* is a potential foodborne pathogen for humans and other animals.

Key words : disease control, *Erwinia rhapontici*, epidemiology, host specificity, pink seed, crown rot, proferrorosamine A, survival

INTRODUCTION

*Erwinia rhapontici* (Millard) Burkholder is an opportunistic bacterial plant pathogen (50). It has been reported to occur in North America, Europe, northeast Asia (Japan and Korea), and the Middle East (Israel) (4,10). The bacteria have been isolated from water (47), soil (19), seeds (31,33), and other plant tissues (38,50,56). *E. rhapontici* was first reported as a plant pathogen in 1924 and derives its name from the host plant, rhubarb (*Rheum rhaponticum* L.), on which it caused crown rot (38,42,43). The pathogen causes two kinds of plant diseases, pink seed and crown rot or soft rot, on more than 15 species of plants. The purpose of this article is to review current knowledge on biology and epidemiology of *E. rhapontici* and discuss the potential impact of this pathogen on various crops.

THE PATHOGEN

The genus *Erwinia* is subdivided into four "natural groups" including amylovora, carotovora, herbicola and atypical erwinias (15,16,17,18). *Erwinia rhapontici* belongs to the 'carotovora' group, which includes bacteria that produce pectolytic enzymes and cause soft rot in plants (16). However, Lelliott (36) reported that both *E. rhapontici* and *E. cyripedii* (Hori) Bergey *et al.* belong to the 'carotovora' group but they are incapable of causing degradation of pectate. The morphological and physiological differences between *E. rhapontici* and other *Erwinia* spp. are further discussed below.

Physiological characteristics of *E. rhapontici*

*E. rhapontici* is a facultative, anaerobic bacterial species that produces a diffusible pink pigment on sucrose-peptone agar (36,41,48). The bacterial cells have been isolated from water (47), soil (19), seeds (31,33), and other plant tissues (38,50,56). *E. rhapontici* was first reported as a plant pathogen in 1924 and derives its name from the host plant, rhubarb (*Rheum rhaponticum* L.), on which it caused crown rot (38,42,43). The pathogen causes two kinds of plant diseases, pink seed and crown rot or soft rot, on more than 15 species of plants. The purpose of this article is to review current knowledge on biology and epidemiology of *E. rhapontici* and discuss the potential impact of this pathogen on various crops.
auromycin, chloramphenicol, streptomycin, terramycin, tetracycline and dimethyl chlorotetracycline. 

E. rhapontici can be distinguished from E. cypripedii, E. rubrificans Wilson et al., and other non-pectolytic species of Erwina in the "carotovora group" by its failure to grow at 37°C, inability to produce gas from glucose, inhibition by KCN, production of acetoin, ability to reduce sucrose, and production of acid from raffinose, melezitose, dulcitol and lactose. Hao et al. isolated five strains (3 from tomatoes, 1 from banana and 1 from cucumber) of Gram-negative, oxidase-negative, facultatively anaerobic, fermentative, motile, rod-shaped bacterium which also produced a water-soluble pink pigment. However, they identified these strains as Erwina persicinus Hao et al. based on their negative reactions in tests for methyl red, N-acetylglucosamine and DL-tartrate assimilation; and no acid production from amygdalin, dulcitol, D-fucose, betagentiobiose, alpha-methyl-D-glucoside, glycerol, D-lyxose, melezitose, D-turanose, xyitol, and D-xylene; and a positive reaction for acetoin (Voges-Proskauer test)

Production of pink pigments

The most obvious characteristic of strains of E. rhapontici is the production of a diffusible pink pigment. The pigment is soluble in water and alcohol, but not in chloroform. This ability to produce pink pigment is shared by only four of the more than 100 species in the Enterobacteriaceae (Erwinia, Pseudomonas, E. rhapontici, E. rubrifaciens, E. persicinus, and Serratia marcescens) and is thus helpful in obtaining rapid tentative identification of the bacteria. In studies of various strains of E. rhapontici, most were capable of producing a water-soluble pink pigment on potato dextrose agar (PDA)

Cultural media which induce production of pink pigment include the following: yeast-dextrose-calcium carbonate agar, nutrient broth yeast extract agar, nutrient glycerol and potato glucose agar, King's B medium, yeast extract-glucose-carbonate medium, potato-glucose medium and yeast extract-peptone medium. The characteristic of producing a diffusible pink pigment varies with medium and possibly conditions. Lelliott recommended a medium containing 2% sucrose, 0.5% peptone, 0.05% K$_2$HPO$_4$, 0.025% MgSO$_4$, 2% agar, at pH 7.2-7.4, for most consistent pigment production.

Temperature and nutrients are also important factors influencing the production of pigment in E. rhapontici. Luisetti and Rapilly found that pigment production was favoured by culturing E. rhapontici at 30°C and then placing at 23°C. Feistner et al. reported that temperature dependence was tested on solid media at 23°C, 30°C and 36°C, and pigment production decreased with increasing temperature and failed at 36°C. Luisetti and Rapilly also found that pigment production was favoured in a synthetic medium containing 1% glycerol or rhamnose. If one omits iron from the gluconate or mannitol medium, E. rhapontici neither produces the pink pigment nor the propigment. Under conditions of iron deficiency, the production of pigment (proferrorosamine A) by E. rhapontici and Pseudomonas roseus fluorescens Marchal (syn. Bacillus roseus fluorescens) can be stimulated by asparagine.

Composition of pink pigments of E. rhapontici

Feistner et al. reported that the pink pigment of E. rhapontici was proferrorosamine A which chelates iron, converting to ferrosoamine A. Proferrorosamine A (L-2-(2-pyridyl)-1-pyrroline-5-carboxylic acid) is a metabolite of P. roseus fluorescens. Pseudomonas GH (which was reclassified as a strain of E. rhapontici) revealed that at least some of the corresponding biosynthetic genes are located on the chromosome, suggesting that proferrorosamines have some useful or specific function. It has been proposed that proferrorosamine A in Pseudomonas GH may be essential for siderophore (iron (III)-chelator) production.
be determined, and this could be tested using specific mutants of *E. rhapontici* that are deficient in proferrorosamine synthesis (57).

*E. rhapontici* is also able to convert sucrose into isomaltulose (palatinose, 6-O-alpha-D-glucopyranosyl-D-fructose) and trehalulose (1-O-alpha-D-glucopyranosyl-D-fructose) by the activity of a sucrose isomerase (3). The role of sucrose isomerase production in pathogenicity of *E. rhapontici* is not clear at present. However, Boernke *et al.* (3) reported that these sucrose isomers cannot be metabolized by plant cells and most other microorganisms and therefore may be advantageous to the pathogen (5).

**HOST SPECIFICITY OF ERWINIA RHAPONTICI**

*E. rhapontici* is a bacterial species that does not degrade pectate. It causes rotting of onion bulbs or cucumber slices slowly and weakly (13,42,50), but does not cause rotting in carrots, potato tubers, lettuce, cauliflower, cabbage, turnip, parsnip and green stems of potato and tomato (42). *E. rhapontici* did not attack young petioles of celery but caused a localized rot, with pink pigment formation, in old petioles (42). On white onion, *E. rhapontici* also secreted a pink pigment which coloured the rotted tissues (42).

Roberts (48) reported that strains of *E. rhapontici* from natural crown rot of rhubarb caused pink discoloration of wheat grains. This suggests that host specificity may be lacking in *E. rhapontici*. Huang *et al.* (32) cross-inoculated seven strains of *E. rhapontici* from bean, pea and soil into pods of pea and bean and found that host specificity among these strains was lacking. All of the strains tested were pathogenic on pea and bean, causing pink or pinkish-brown lesions on pods and discoloration of seeds. Recent field experiments from another study showed that a strain of *E. rhapontici* from pea could infect kernels of durum wheat, resulting in pink wheat grains (Huang *et al.*, unpublished).

Additional studies on host specificity of *E. rhapontici* show that isolates of *E. rhapontici* from healthy or diseased plants can cause infection in healthy plants, resulting in typical symptoms of pink seed or soft rot. Volcani (61) demonstrated that neither of the isolated organisms produced any lesions on clover, and one of them was found to be pathogenic to citrus causing brown pink spot, and to tomato leaves causing brown spot. Sellwood and Lelliott (50) used the isolates of *E. rhapontici* from buds of apple and pear canker to inoculate the bulbs of hyacinth, and found that only one of the three isolates from apple could cause soft rot symptoms.

Some strains of *E. rhapontici* are nonpathogenic to the plant species from which the bacteria were originally isolated. Vantomme *et al.* (60) isolated three strains of *E. rhapontici* from chicory seeds, but they were non-pathogenic to chicory. Although *E. rhapontici* did not induce galling on *Gypsophila paniculata* L., it was consistently recovered from the browning and rotting galled tissues induced by *E. herbicola* in inoculations on cuttings (13). The role of nonpathogenic strains of *E. rhapontici* in relation to their hosts needs further clarification.

**DISEASE SYMPTOMS**

*E. rhapontici* can cause two types of disease, soft rot or crown rot and pink seed. The record of occurrence, hosts and type of disease of *E. rhapontici* is listed in Table 1.

**Crown rot, soft rot, bulb rot and blossom rot**

*E. rhapontici* causes root rot of vegetables in south Korea (34), soft rot of onion (44,45), and wasabi (*Eutrema wasabi* Maxim.) (23) in Japan, bulb rot of garlic in south Korea (39), yellow-brown necroses on inner skins and upper third part of the bulb of hyacinth (*Hyacinthus orientalis* L.) in Czechoslovakia (35) and in England (50), soft rot disease of tomato in Ukraine (51), brown spot of tomato (61) and brown pink spot of citrus in Israel (61), blossom rot of carrion (*Hippeastrum* sp.) and knight’s-star (*Dianthus* sp.) in Lithuania (55), as well as crown rot of rhubarb (*Rheum rhaponticum*) in Canada (38) and England (42,43), on cyclamen (*Cyclamen persicum* Mill.) in Italy (4), and on sugar beet (*Beta vulgaris* L.) in Iran (27). *E. rhapontici* is also a major pathogen for shoot and stem rot of mulberry in Korea (50) and bacterial rot of gerbera in Russia (28).

**Pink seed**

*E. rhapontici* causes formation of pink seeds on numerous crops such as small grain cereals in Canada (5,38), USA (21,41,48), France (40), Belgium (14), and Yugoslavia (1); field peas (*Pisum sativum* L.) in Canada (33) and Montana, USA (49), and common bean (*Phaseolus vulgaris*) (31), lentil and chickpea in Canada (Huang *et al.*, unpublished data). Howe and Simmonds (28) later reported that *Serratia rubefaciens* (Zimmerman) Bergey *et al.* (= *E. rhapontici*) caused bacterial pink blotch of wheat. All isolates produced a distinct pink coloration in potato dextrose medium (28). The infected grains are pink and have an internal cavity below the hilum. Pink and shriveled seeds of wheat do not germinate well and show growth retardation at the seedling stage (40).

*E. rhapontici* was reported as a pathogen of the weed *Amaranthus hybridus* L. (22). Also, it was isolated from the surface of medicinal plants such as roman chamomile (*Chamaemelum nobile* L.), lemon balm (*Melissa officinalis* L.) and peppermint (*Mentha piperita* L.) in France (56).

**EPIDEMIOLOGY**

The reported distribution of *E. rhapontici* includes North America, Europe, the Middle East (Israel) (4), and northeast Asia (Japan and Korea) (8,45). The bacterium can be isolated from water (47), soil (19), and plant surfaces (38,50,56). *E. rhapontici* is an opportunistic bacterial plant pathogen (50) and infects its hosts through wounds (41,42,45,48). Volcani (61) reported that no infection was obtained when drops of bacterial suspension of *E. rhapontici* were applied to the surface of fruit without injury by pricking. Similarly, pink grain was reproduced with *E. rhapontici* when developing grains of
wheat plants were inoculated, but only after wounding (48). Huang et al. (39) demonstrated that pink pea seeds are induced only by pod injection with the bacterial suspension, and not by other inoculation methods. The findings of these studies confirm that *E. rhapontici* is a wound pathogen.

In a field study, Huang et al. (32) reported that mechanical injury of pea plants at the pod development stage resulted in a higher rate of infection of seeds by *E. rhapontici* compared to the non-injured treatments, regardless of whether the plants originated from healthy or pink seeds. Injury of host plants by pests also increases the opportunity for infection by *E. rhapontici*. Metcalfe (42) reported that infection of healthy plants from the soil usually occurs when eelworms (*Anguillulina dipsaci* Kuhn) were present. In the absence of the eelworm, rotting ensues only if the bacterium is introduced into the tissue through a penetrating wound. Luisetti and Rapilly (40) noted that the rather severe outbreak of pink grain of wheat in France in 1966 coincided with an infestation of gall midges (Cecidomyidae). *E. rhapontici* was also isolated from the bark beetle *Anisandrus dispar* F. (6), suggesting that the beetle may play a role in transmission of the disease, possibly by creating injury sites as a result of feeding on host plants.

In addition to the presence of wounds on the host plant, the other condition conducive to infection by *E. rhapontici* is a prolonged period of high humidity (5,28,41,48). The conditions of injury and high humidity may explain the casual observation that outbreaks of disease caused by *E. rhapontici* often follow rainy weather or hailstorms.

**SURVIVAL OF ERWINIA RHAPONTICI**

Information on survival of *E. rhapontici* is meager. Huang and Erickson (29) reported that isolation of the tissues from plants originating from infected seeds in greenhouse experiments showed that *E. rhapontici* was most readily isolated from tap roots and basal stems (97% and 99%, respectively). A 2-year field study demonstrated that *E. rhapontici* survived Canadian prairie winters on infected seeds and stems of pea (*Pisum sativum* L.), regardless of burial depth at 0 or 6 cm (30). The study suggests that *E. rhapontici* in infected pea seeds or stems can be an important source of inoculum for pink seed of dry peas and other legume crops under Canadian prairie conditions (30).

*E. rhapontici* can overwinter on infected tissues of crops in western Canada (30). It can also survive on the weed *Amaranthus hybridus* L. (22). In addition, strains of *E. rhapontici* are not host-specific (32) and the pathogen has a wide range of hosts including dry pea (33), dry bean (31), common wheat (*Triticum aestivum* L.) (48) and durum wheat (*T. durum* Desf.) (41) (Huang et al., unpublished). These findings suggest that cultural practices such as the popular pulse-wheat rotations in North America may not be effective for control of this disease.

**ECONOMIC IMPACT**

*E. rhapontici* can affect seed yield and seed quality. Field studies of pink seed of dry pea revealed that *E. rhapontici* reduced the seed size, seedling emergence and pre-elongation seedling height (29). The 100-kernel weight for pink seeds was 14% lower than for healthy seeds. The seedling height was reduced by 46% compared to plants from healthy seeds (29). Other studies on wheat in the USA revealed that pink seed in durum wheat can result in rejection of this crop for pasta because of the resulting pink semolina (49). Studies in the UK indicated that pink grain of wheat has not reached economic proportions, but confusion might arise because of the superficial likeness of pink grain associated with *E. rhapontici* to the pink color of some organo-mercury seed dressings (48). Many crops such as dry pea, dry bean and wheat are used as food for humans and animals, and it remains unknown whether *E. rhapontici* is a foodborne pathogen for them.

**CONTROL**

Practical methods such as seed treatment, biocontrol, and control of vectors can be used to reduce the potential damage on plants due to diseases caused by *E. rhapontici*.

**Seed treatment**

Since *E. rhapontici* is seedborne, the use of disease free seed is a pre-requisite for controlling seedborne inoculum. Effective chemical-based seed treatments for control of *E. rhapontici* remain unavailable, but further investigation in this field may be of merit since Sidorovich (54) reported that phytobacteriomycin and dithane M-45 possess the highest antibacterial activity against this pathogen.

**Biological control**

*E. rhapontici* has been reported as a good potential biocontrol agent, due to its characteristics of good survival and easy colonization on plant roots (2,22,39). Liang et al. (39) reported that strains of *E. rhapontici* were antagonistic to *Pythium* sp. 'group G', a sterile form of *Pythium ultimum*, causing damping-off of safflower. Seed treatment with *E. rhapontici* was effective in controlling damping-off of safflower (39) and sugar beet (2) caused by *Pythium* sp. "group G". However, it is impractical to use *E. rhapontici* as a biocontrol agent for plant diseases (2,39) because it is a plant pathogen with no host specificity. In addition, *E. rhapontici* has been proposed as a potential biological control agent for control of the weed *Amaranthus hybridus* L. (22). This method may also be impractical, as *E. rhapontici* is not only a pathogen of the weed *A. hybridus*, but also a pathogen of numerous crops.

Using biocontrol agents to control diseases caused by *E. rhapontici* has shown some promise. Vanneste and Paulin (59) found that *E. rhapontici* was sensitive to one of 11 lytic phages tested. The potential of using bacterial phages for
control of *E. rhapontici* warrants further investigation. Kim *et al.* (34) reported using *Pseudomonas* sp. as an effective biocontrol agent of vegetable root rot caused by *E. rhapontici*. Another study found that a considerable number of *Erwinia* strains were sensitive to different strains of *Bacillus subtilis* (52). The mechanism of action of antagonistic substances of *B. subtilis* against *Erwinia* spp. was to damage the K⁺ ions' transport of sensitive phytopathogenic bacteria via cell walls (52). In preliminary studies conducted by Huang *et al.* (unpublished data), injection of *Bacillus cereus* isolate LRC 805 into young pea pods after inoculation with *E. rhapontici* reduced the percentage of pink seeds. Research on biocontrol of *E. rhapontici* by bacterial agents is still in its infancy and thus, further studies are required.

**Control of vectors**

Control of insect pests associated with plant disease by chemical or other means may be helpful in controlling the spread of *E. rhapontici*. Metcalfe (42) mentioned that it is possible that any control of eelworm may give some degree of control of crown rot disease caused by *E. rhapontici*. Similar strategies may be useful on other plant hosts, especially where significant insect-pathogen interactions can be documented.

**Other control measures**

To date, no information on host plant resistance to *E. rhapontici* has been reported, but the fact that it is an opportunistic pathogen suggests that control by resistance is possible. Although *E. rhapontici* overwinters in the debris of diseased tissues in the prairie region of western Canada (30), a carefully selected crop rotation using non-host crops might be helpful in preventing buildup of the pathogen. However, the host range of *E. rhapontici* needs to be fully documented first.

**CONCLUSION**

Although the information on pink seed and crown rot diseases caused by *E. rhapontici* is meager, this review of the published literature may stimulate more interest in this intriguing and unique plant pathogen. So far, most of the studies have focused on occurrence of the disease as well as taxonomy and etiology of the pathogen. There are signs of interest in the areas of economic impact of the disease, host range and host specificity of the pathogen, ecological and epidemiological factors affecting the disease, and control strategies for the pathogen. Continuing efforts in these areas are necessary to gain a better understanding of this pathogen.

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**Figures 1-4.** Pink seed of pea, bean and wheat caused by *Erwinia rhapontici*. Each figure shows diseased seeds (left) and healthy seeds (right). Note the diffusion of pink pigment from infected seeds of common bean cv. US1140 into the potato dextrose agar medium after incubation at room temperature (20 ±2°C) for 2 days (Fig. 1, left). Note also the distinct pink color developed on infected seeds of dry pea cv. Delta (Fig. 2, left), dry bean cv. US1140 (Fig. 3, left) and durum wheat cv. Kyle (Fig. 4, left) collected from commercial fields.
of research could lead to the development of effective methods for the control of pink seed and crown rot diseases caused by *E. rhapontici*. Since most of the host crops of *E. rhapontici* are used for human food and/or animal feed, it is also of paramount importance to conduct studies on effects of diseased seeds and/or other diseased tissues on the health of humans and other animals.

### LITERATURE CITED


**摘 要**

黃鴻鵠1,3, 謝廷芳2, R. S. Erickson1. 2003. 引起粉紅與冠腐病的病原細菌（Erwinia rhapontici）之生物學及流行病學的研究進展。植病會刊12:69-76. (1. 加拿大農業及農業食品部Lethbridge 研究中心；2. 台灣台中縣霧峰鄉行政院農業委員會農業試驗所；3. 聯絡作者：電子郵件huangh@agr.gc.ca；傳真：+0021-403-382-3156)

Erwinia rhapontici 爲少數幾種能於含糖培養基（如馬鈴薯葡萄糖洋菜培養基）上產生擴散性粉紅色色素的細菌之一。此一機會性病原細菌的寄主範圍廣泛，可造成粉紅色種子及植物冠腐或軟腐等病徵。本文旨在針對此一特殊病原細菌的研究現況及進展情形，包括病害發生、病原鑑定、寄主範圍，以及防治方法等作一綜合性評述。同時提出今後可能加強研究的方向，如流行病學、生態學、病害防治，以及罹病種子或病組織對人畜的毒害等加以討論。

關鍵詞：Erwinia rhapontici，粉紅色種子，冠腐，寄主專一性，proferrorosamine A，流行病學，存活