

# Identification of *Erwinia carotovora* from Soft Rot Diseased Plants by Random Amplified Polymorphic DNA (RAPD) Analysis

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## ABSTRACT

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*Erwinia carotovora* and pectolytic pseudomonads are the most prevalent bacteria associated with soft rot of plants. A procedure based on random amplified polymorphic DNA (RAPD) analysis was developed to distinguish these bacteria rapidly and easily. Forty bacterial strains isolated from diseased plants in the province of Québec were used to screen RAPD primers. Two primers were chosen based on cost considerations and on their capacity to discriminate between the bacterial strains. The combination of the two primers was sufficient for adequate distinction of *Erwinia carotovora* from pectolytic, fluorescent *Pseudomonas* spp. Furthermore, *E. carotovora* subsps. *atroseptica* and *carotovora* could also be distinguished from each other. To simplify the identification work, a quantitative identification tool was elaborated, based on selected markers produced with the two primers. To verify the RAPD analysis accuracy, 49 additional soft rot bacteria from diseased plants were submitted to the RAPD procedure and all the identifications were in agreement with those produced by standard biochemical tests. In addition, the RAPD analysis correctly identified 25 bacteria from collections outside of Québec.

Bacterial soft rot is the primary cause of postharvest decay of perishable vegetables (1,2). In Québec, more than one third (243 cases) of the bacterial diseases referred to the diagnostic laboratory of the province between 1989 and 1994 were related to soft rot (M. Lacroix, unpublished). *Erwinia carotovora* and pectolytic pseudomonads such as *Pseudomonas fluorescens*, *P. marginalis*, and *P. viridiflava* are the most prevalent bacteria associated with soft rot (10,13).

*Erwinia carotovora* subspecies have different host ranges. *E. carotovora* subsp. *atroseptica* is primarily restricted to potato crops, whereas *E. carotovora* subsp. *carotovora*, also an important potato pathogen, is polyphagous. Thus, identification of these subspecies is important for potato growers in making adequate crop rotation choices when they face a field contamination problem. Although *E. carotovora* subsp. *atroseptica* and *E. carotovora* subsp. *carotovora* are associated with blackleg and soft rot of potato, respectively, strains from one subspecies may sometimes be responsible for the disease

usually associated with the other subspecies strains (8,15). In addition to being a serious pathogen of field plants, *E. carotovora* subsp. *carotovora* was identified as the responsible agent of stem rot and vascular wilt symptoms of hydroponically grown plants (16). Differentiation of *E. carotovora* from pectolytic pseudomonads is also of significance as the latter organisms are able to grow and macerate plant tissues at cool temperatures, and flourish in refrigeration devices that prolong the life of fresh products (13). These pectolytic pseudomonads also are better able to overwinter in cold climates (4).

Pectolytic soft rot bacteria are usually identified by biochemical tests. These tests are lengthy and costly. DNA analysis techniques are now widely used and could replace biochemical tests. Specific detections of *E. carotovora* and *E. carotovora* subsp. *atroseptica* were obtained with DNA probes (17,18) but are also limited by time and cost considerations. Recently, the random amplified polymorphic DNA (RAPD) technique was developed for the genetic analysis of DNA (19,20). The technique is based on the enzymatic amplification of nonselected DNA fragments, initiated by arbitrarily chosen DNA primers. With this technique, individual genomes can be grouped and classified by screening primers and selecting those that generate adequate levels of DNA polymorphism. The RAPD technique is simple, fast, and inexpensive, and hence could be an alternative to biochemical identification of soft rot bacteria. However, complex

patterns are often generated by RAPD primers and their analysis may appear difficult to users and discourage them from using the method. To be adopted, a RAPD procedure must focus on a limited number of significant markers and primers. Our objective was to develop such a procedure in order to distinguish pectolytic pseudomonads from *E. carotovora* and to discriminate between subspecies of the latter species.

## MATERIALS AND METHODS

**Bacterial isolation and identification by biochemical tests.** A piece of decayed plant tissue was soaked in saline solution (0.85% NaCl) for 30 min to allow the migration of bacteria into solution. One hundred microliters per plate of the resulting bacterial suspension was spread on MS (14) and King's B (11) media and incubated 48 h at 26°C. Isolated colonies were subcultured three times for purification and then transferred to nutrient glucose agar (NGA) for multiplication before performing biochemical and physiological tests. Fluorescence on King's B medium was the criterion used to discern *Pseudomonas* colonies (11). The following tests were used to identify *Pseudomonas* species: levan production, oxidase activity, arginine dihydrolase activity, tobacco hypersensitivity (12), and pectolytic activity (9). Orange colonies with irregular margins were selected on MS medium (14) and their identity as pectolytic *Erwinia* was ascertained by tests for oxidase activity, catalase activity, glucose degradation under anaerobic conditions, and pectolytic activity (5). The following tests were used to identify *Erwinia* species and subspecies: indole production, sucrose reduction, acid production from methylglucoside, and growth at 36°C (5).

**RAPD tests.** Bacterial DNA was extracted following a shortened version of the hexadecyltrimethylammonium bromide (CTAB) method (6). In this version, a loopful of bacteria was taken from colonies grown 2 h on NGA medium and placed in a 1.5-ml microcentrifuge tube, to which 600 µl of hot (60°C) CTAB solution (100 mM Tris-HCl, pH 8.0, 1.4 M NaCl, 20 mM EDTA, 2% CTAB and 0.2% 2-mercaptoethanol) was added. The suspension was vortexed, then incubated at 60°C for 30 min. The solution was extracted with 600 µl of chloroform/isoamyl alcohol

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(24:1). The phases were separated by centrifugation at 5,000 × g for 10 min and the aqueous phase was transferred to a new tube. The DNA was precipitated by the addition of 400 µl isopropanol at -20°C and then concentrated by centrifugation at 10,000 × g for 10 min. The pellet was washed with 1 ml of 76% ethanol in 10 mM ammonium acetate. After vacuum drying, the pellet was dissolved in 200 µl of water.

Conditions for the production of RAPD markers were modified from Williams et al. (20). Amplification reactions were carried out in volumes of 25 µl containing 10 mM Tris-HCl pH 8.8, 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.1% Triton X-100, 100 µM of each nucleotide dATP, dCTP, dGTP, and dTTP, 0.2 µM primer (10-base oligonucleotides, Operon Technologies, Alameda, CA), 20 ng of DNA, and 2 units of *Taq* DNA polymerase (Perkin-Elmer Cetus, Norwalk, CT). Amplifications were done in a thermal cycler (GeneAmp PCR System 9600, Perkin-Elmer Cetus) programmed for 45 cycles of 15 s at 92°C, 1 min at 38°C, and 2 min at 72°C, using the fastest transition to reach 92°C and a speed of 0.5°C s<sup>-1</sup> to achieve the subsequent temperatures. The amplification cycles were preceded by a denaturation step of 10 min at 92°C and followed by an elongation step of 10 min at 72°C. The amplified products were subjected to electrophoresis in 1.4% agarose gels and stained with ethidium bromide. DNA standards (1 Kb DNA Ladder, Gibco BRL) were included in each electrophoresis.

**RAPD identification procedure.** Useful RAPD primers were selected in a series of three steps. Three strains each of *E. carotovora* subsp. *atroseptica*, *E. carotovora* subsp. *carotovora*, *P. fluorescens* group IVb, *P. marginalis*, and arginine dihydrolase negative *Pseudomonas* (*P. syringae* and *P. viridiflava*) were initially used to screen roughly a set of 45 primers and identify by visual examination those that might produce genus-specific or *E. carotovora* subspecies-specific patterns. Secondly, primers with potential were used to generate RAPD patterns from a reference set of bacterial strains. The reference set was established using 40 strains isolated from specimens with soft rot symptoms received at the plant diagnostic laboratory of the Province of Québec between 1990 and 1993 (Table 1). The strains were chosen to ensure a representation of the bacteria in relation to their occurrence in Québec as estimated by the total soft rot bacteria identifications during this period.

In order to look for differences between *E. carotovora* and *Pseudomonas* spp., the strains from the reference set were split into two groups, one with the 17 strains of *E. carotovora* and the other with the 23 strains of *Pseudomonas* spp. In the same way, the *E. carotovora* group was further divided into two subgroups, *E. carotovora*

subsp. *atroseptica* (5 strains) and *E. carotovora* subsp. *carotovora* (12 strains). For each primer, all strains were amplified twice in separate experiments to ensure the reproducibility of the patterns. For every primer, patterns were examined for markers showing intergroup polymorphism and low intragroup variability. A marker was generally associated with the presence of a single band, but sometimes combinations of two or three bands were formed to constitute additional markers. Furthermore, for some markers, the brightness of the bands was also considered.

Markers that were present at high frequency in one group and at low frequency in the other possessed a capacity to discriminate between the groups. Based on this assumption, markers present in more than 50% of the strains in one group and less than 50% in the other group were selected as potentially useful. Numerical values related to the capacity for a marker to discriminate between groups, used as indices of discrimination, were calculated by doing the absolute difference between the percentages of presence in each group. In the last selection step, primers were selected by the examination of the indices of

discrimination of their markers. The marker selection process applied to groups was also used for the *E. carotovora* subgroups. To minimize costs, the smallest combination of primers, capable of simultaneous and reliable identification of both groups and subgroups, was searched for.

Since a reference set represents only a fraction of the possible strains, when additional strains are analyzed it is likely that strains with atypical patterns of apparently contradictory RAPD markers will be found. So identification is not always obvious and reliable when based simply on visual examination of markers. A quantitative identification tool to help decision making and reduce uncertainty was then constructed with the useful markers from the selected primers. Values of 1 to 5 were attributed to arbitrarily chosen ranges of indices of discrimination, the value of 5 corresponding to the highest indices. Different ranges were assayed until the quantitative tool could correctly identify the strains from the reference set. To identify a strain, the presence of every marker was recorded and the following scoring scheme was applied: when a marker was present, its value was assigned to the

**Table 1.** Soft rot bacterial strains used as a reference set for the development of a random amplified polymorphic DNA (RAPD) identification procedure

| Identification                                      | Strain number                                 | Host                 | Year of isolation   |      |
|-----------------------------------------------------|-----------------------------------------------|----------------------|---------------------|------|
| <i>Erwinia carotovora</i> subsp. <i>atroseptica</i> | 062                                           | Potato               | 1990                |      |
|                                                     | 157                                           | Potato               | 1990                |      |
|                                                     | 690                                           | Potato               | 1993                |      |
|                                                     | 707                                           | Potato               | 1993                |      |
|                                                     | 749                                           | Potato               | 1993                |      |
|                                                     | <i>E. carotovora</i> subsp. <i>carotovora</i> | 131                  | Potato              | 1990 |
|                                                     |                                               | 138                  | Potato              | 1990 |
|                                                     |                                               | 153                  | Potato              | 1990 |
|                                                     |                                               | 164                  | Christmas cactus    | 1991 |
|                                                     |                                               | 172                  | Lily                | 1991 |
| 182                                                 |                                               | <i>Euphorbia</i> sp. | 1991                |      |
| 227                                                 |                                               | Cucumber             | 1991                |      |
| 245                                                 |                                               | Potato               | 1991                |      |
| 253                                                 |                                               | Potato               | 1991                |      |
| 375                                                 |                                               | Lettuce              | 1991                |      |
| <i>Pseudomonas fluorescens</i> group IVb            | 382                                           | Potato               | 1991                |      |
|                                                     | 413                                           | Potato               | 1991                |      |
|                                                     | 110                                           | Fennel               | 1990                |      |
|                                                     | 120                                           | Cabbage              | 1990                |      |
|                                                     | 129                                           | Potato               | 1990                |      |
|                                                     | 142                                           | Chinese cabbage      | 1990                |      |
|                                                     | 296                                           | Lettuce              | 1991                |      |
|                                                     | 322                                           | Tomato               | 1991                |      |
|                                                     | 326                                           | Tomato               | 1991                |      |
|                                                     | 328                                           | Tomato               | 1991                |      |
|                                                     | 362                                           | Onion                | 1991                |      |
|                                                     | 403                                           | Potato               | 1991                |      |
|                                                     | 408                                           | Cauliflower          | 1991                |      |
|                                                     | <i>P. marginalis</i>                          | 094                  | Pepper              | 1990 |
|                                                     |                                               | 101                  | Celery              | 1990 |
|                                                     |                                               | 192                  | Onion               | 1991 |
|                                                     |                                               | 215                  | <i>Aconitum</i> sp. | 1991 |
| 221                                                 |                                               | <i>Aconitum</i> sp.  | 1991                |      |
| 292                                                 |                                               | Lettuce              | 1991                |      |
| 357                                                 |                                               | Tomato               | 1991                |      |
| <i>P. syringae</i>                                  | 468                                           | Carrot               | 1992                |      |
|                                                     | 097                                           | Pepper               | 1990                |      |
|                                                     | 135                                           | Cauliflower          | 1990                |      |
| <i>P. viridiflava</i>                               | 183                                           | Eggplant             | 1991                |      |
|                                                     | 659                                           | Pumpkin              | 1992                |      |

group with the high frequency of presence in the reference set; conversely, a marker being absent in a strain gave its value to the group with the low frequency. The values obtained for each group were added and identification of the strain was inferred from the group with the highest score. Similar calculations were made for the *E. carotovora* subgroups when applicable. For example, a hypothetical marker, found at very high frequency for the *E. carotovora* strains of the reference set and at very low frequency for the *Pseudomonas* spp. strains, has a high index of discrimination and received a value of 5. In the identification process of a strain, if this marker is seen, its value of 5 is given to the *E. carotovora* group and the *Pseudomonas* spp. group receives nothing. The same operation is repeated with the other markers. If the total of the values attributed to the *E. carotovora* group is higher than the total of the *Pseudomonas* spp. group, then the strain is identified as *E. carotovora*.

**Verification of the RAPD procedure.** In 1993 and 1994, 29 and 20 additional

bacterial strains (Table 2), respectively, were obtained from plants with soft rot symptoms representing all soft rot specimens received at the diagnostic laboratory of Québec between September and December of both years. At least three strains were isolated from each sample and RAPD tested. These strains were used to verify the accuracy of the procedure in correctly identifying unknown soft rot bacteria from Québec. Strains were identified in parallel by biochemical tests described above and by the RAPD procedure. In addition, 25 strains originating from outside Québec and identified by the senders were also obtained (Table 2), amplified twice in separate RAPD experiments, and used to check the possible generalization of the procedure.

## RESULTS AND DISCUSSION

**RAPD identification procedure.** In the first screening step, we selected primers that generated group-specific patterns. Nine of the 45 initial primers (OPF02, OPF12, OPG05, OPG07, OPG09, OPG10,

OPG17, OPH03, and OPH05) were retained for further examination. The other primers were rejected for one of the following reasons: high intragroup variations (polymorphic patterns within groups), no intergroup polymorphism (all patterns look alike), or no amplification at all. Further analysis of the 40 strains from the reference set with the nine selected primers revealed 22 markers with polymorphism between groups and low variability within the groups (Table 3). These markers were generated by only six of the primers (OPF12, OPG09, OPG10, OPG17, OPH03, and OPH05). Although the initial selection seemed promising, primers OPF02, OPG05, and OPG07 gave no potentially useful markers in this more complete step.

As we wished to restrict the number of primers to simplify the analysis and reduce costs, we first examined the markers with an index of discrimination of 100% (Table 3). Only primer OPG17 had a marker with such an index value to differentiate the *E. carotovora* group from the *Pseudomonas*

**Table 2.** Identification and numerical analysis of bacterial strains used in the verification procedure

| Origin | Strain number <sup>a</sup> | Host     | Results of quantitative identification tool <sup>b</sup> |                         |                              |                 | Identification |                  |
|--------|----------------------------|----------|----------------------------------------------------------|-------------------------|------------------------------|-----------------|----------------|------------------|
|        |                            |          | Genera level                                             |                         | Subspecies level             |                 | RAPD procedure | Standard tests   |
|        |                            |          | <i>E. carotovora</i>                                     | <i>Pseudomonas</i> spp. | <i>E. c. a.</i> <sup>c</sup> | <i>E. c. c.</i> |                |                  |
| Québec | 93-823                     | Leek     | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-824                     | Cucumber | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-826                     | Tomato   | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. virid.</i> |
| Québec | 93-828                     | Potato   | 13                                                       | 2                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-830                     | Potato   | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-832                     | Potato   | 15                                                       | 0                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-842                     | Potato   | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-845                     | Potato   | 15                                                       | 0                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-850                     | Turnip   | 15                                                       | 0                       | 9                            | 14              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-854                     | Potato   | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-856                     | Lettuce  | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. virid.</i> |
| Québec | 93-859                     | Potato   | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-860                     | Cabbage  | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. fluor.</i> |
| Québec | 93-866                     | Potato   | 3                                                        | 12                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. margi.</i> |
| Québec | 93-868                     | Turnip   | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. fluor.</i> |
| Québec | 93-874                     | Potato   | 10                                                       | 5                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-877                     | Potato   | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-878                     | Leek     | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-882                     | Potato   | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-884                     | Lettuce  | 3                                                        | 12                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. margi.</i> |
| Québec | 93-886                     | Bokchoy  | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. margi.</i> |
| Québec | 93-888                     | Lettuce  | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. virid.</i> |
| Québec | 93-892                     | Potato   | 15                                                       | 0                       | 9                            | 14              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-896                     | Potato   | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. fluor.</i> |
| Québec | 93-905                     | Potato   | 13                                                       | 2                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-909                     | Potato   | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. margi.</i> |
| Québec | 93-924                     | Potato   | 13                                                       | 2                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-1923                    | Potato   | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 93-1938                    | Potato   | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. fluor.</i> |
| Québec | 94-693                     | Potato   | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 94-748                     | Potato   | 13                                                       | 2                       | 23                           | 0               | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 94-755                     | Potato   | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 94-763                     | Potato   | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec | 94-848                     | Potato   | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |

(continued on next page)

<sup>a</sup> First two numbers indicate the year of isolation for Québec strains.

<sup>b</sup> Presence of every marker recorded for each strain. When a marker was present, a value, related to its index of discrimination, was given to the group with the high frequency of presence in the reference set. The value was given to the group with the low frequency when the marker was absent. Same process was repeated for *E. carotovora* subsp. subgroups when applicable.

<sup>c</sup> Abbreviations: *E.c.a.* = *Erwinia carotovora* subsp. *atroseptica*; *E.c.c.* = *E. carotovora* subsp. *carotovora*; *Pseud.* = *Pseudomonas* spp.; *P. fluor.* = *P. fluorescens*; *P. margi.* = *P. marginalis*; *P. virid.* = *P. viridiflava*; NA = not applicable.

spp. group, and was thus selected. In addition, this primer had two markers with an index of discrimination of 100% to differentiate the *E. carotovora* subgroups. In order to alleviate potential problems of identification caused by an unusual OPG17 RAPD amplification, such as chance occurrence or absence of one selected marker, it was decided to use more markers through the addition of a second primer. Another primer added strength to the analysis while keeping the test technically and economically attractive. Four other primers (OPF12, OPG09, OPH03, and OPH05) had a marker with an index of discrimination of 100% for the *E. carotovora* subgroups. However, primer OPH05 was chosen because it produced a second marker with a high index of discrimination (92%) for these subgroups. Furthermore when these two markers of OPH05 were combined, it was possible to obtain an additional marker (OPH05z; Table 4) with an index of discrimination of 100% to differentiate *E. carotovora* and *Pseudomonas* spp. groups. Therefore, the patterns obtained with primers OPG17 and OPH05

(Fig. 1) were retained for the development of the quantitative identification tool.

Strains from the reference set were used to assign a value to the markers to be used in the quantitative identification tool. Arbitrary values were assayed and when values of 1 to 5 were attributed to indices of discrimination of 60 to 69, 70 to 79, 80 to 89, 90 to 99, and 100 respectively, exact correspondence with the previous biochemical identifications was obtained. Descriptions and values of the useful markers were then fixed (Table 4) and used unmodified in the remaining analyses. Thus, the total number of points attributed by the tool was 15 at the genus level and 23 at the *E. carotovora* subspecies level.

The *E. carotovora* subsp. *atroseptica* strains from the reference set revealed very similar patterns; for eight out of the nine tested primers, patterns looked alike. In contrast, only three primers gave similar patterns among *E. carotovora* subsp. *carotovora* strains or among the *Pseudomonas* spp. strains. This probably reflects greater genetic homogeneity of *E. carotovora* subsp. *atroseptica*. It could be attrib-

uted to the host specialization of *E. carotovora* subsp. *atroseptica* compared with the polyphagous habits of *E. carotovora* subsp. *carotovora*. In Québec, *E. carotovora* subsp. *atroseptica* is almost exclusively found on potato. Host-specialized populations of the pathogenic fungus *Colletotrichum orbiculare* are also characterized by limited genetic diversity among strains from a single host, as determined by diverse DNA analysis techniques including RAPD (3). As an indication of an ongoing process of host specialization, isolates of the fungus *Discula umbrinella* were also clustered by RAPD into groups corresponding to their host of origin (7).

In addition to the *Erwinia-Pseudomonas* and the *E. carotovora* subspecies distinctions, attempts were made to discriminate *Pseudomonas* species (J.-G. Parent and D. Pagé, unpublished). With the best quantitative tool we could develop, 20 out of 23 strains of the reference set were correctly identified at the species level. It was impossible to identify all the strains, even with additional primers.

Table 2. (continued from preceding page)

| Origin           | Strain number <sup>a</sup> | Host             | Results of quantitative identification tool <sup>b</sup> |                         |                              |                 | Identification |                  |
|------------------|----------------------------|------------------|----------------------------------------------------------|-------------------------|------------------------------|-----------------|----------------|------------------|
|                  |                            |                  | Genera level                                             |                         | Subspecies level             |                 | RAPD procedure | Standard tests   |
|                  |                            |                  | <i>E. carotovora</i>                                     | <i>Pseudomonas</i> spp. | <i>E. c. a.</i> <sup>c</sup> | <i>E. c. c.</i> |                |                  |
| Québec           | 94-890                     | Potato           | 15                                                       | 0                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-915                     | Tomato           | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-937                     | Potato           | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1025                    | Potato           | 15                                                       | 0                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1026                    | Potato           | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1027                    | Potato           | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1028                    | Potato           | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1030                    | Potato           | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1032                    | Tomato           | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1034                    | Potato           | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1036                    | Chinese cabbage  | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1038                    | Potato           | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1048                    | Potato           | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1050                    | Potato           | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Québec           | 94-1054                    | Potato           | 13                                                       | 2                       | 23                           | 0               | <i>E.c.a.</i>  | <i>E.c.a.</i>    |
| British Columbia | 006                        | Potato           | 8                                                        | 7                       | 23                           | 0               | <i>E.c.a.</i>  | <i>E.c.a.</i>    |
| Wisconsin        | 031                        | Potato           | 8                                                        | 7                       | 23                           | 0               | <i>E.c.a.</i>  | <i>E.c.a.</i>    |
| Nova Scotia      | 051                        | Broccoli         | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. margi.</i> |
| Nova Scotia      | 052                        | Broccoli         | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. margi.</i> |
| Nova Scotia      | 053                        | Broccoli         | 5                                                        | 10                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. margi.</i> |
| Nova Scotia      | 054                        | Brussels sprouts | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. margi.</i> |
| Nova Scotia      | 056                        | Cabbage          | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. fluor.</i> |
| Nova Scotia      | 057                        | Broccoli         | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. fluor.</i> |
| Nova Scotia      | 058                        | Brussels sprouts | 3                                                        | 12                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. virid.</i> |
| Nova Scotia      | 059                        | Brussels sprouts | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. virid.</i> |
| Holland          | 065                        | Potato           | 10                                                       | 5                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Holland          | 071                        | Potato           | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| France           | 074                        | Potato           | 8                                                        | 7                       | 5                            | 18              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| France           | 081                        | <i>Musa</i> sp.  | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. margi.</i> |
| France           | 082                        | Celeriac         | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. fluor.</i> |
| France           | 083                        | Cauliflower      | 0                                                        | 15                      | NA                           | NA              | <i>Pseud.</i>  | <i>P. fluor.</i> |
| British Columbia | 092                        | Potato           | 8                                                        | 7                       | 9                            | 14              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Wisconsin        | 190                        | Potato           | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| British Columbia | 196                        | Potato           | 8                                                        | 7                       | 23                           | 0               | <i>E.c.a.</i>  | <i>E.c.a.</i>    |
| British Columbia | 198                        | Potato           | 8                                                        | 7                       | 23                           | 0               | <i>E.c.a.</i>  | <i>E.c.a.</i>    |
| United States    | ATCC 15713                 | potato           | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| New Zealand      | ATCC 495                   | Carrot           | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| Iowa             | ATCC 25206                 | Carrot           | 15                                                       | 0                       | 4                            | 19              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| United States    | ATCC 25270                 | Potato           | 15                                                       | 0                       | 0                            | 23              | <i>E.c.c.</i>  | <i>E.c.c.</i>    |
| United States    | ATCC 33260                 | Potato           | 8                                                        | 7                       | 23                           | 0               | <i>E.c.a.</i>  | <i>E.c.a.</i>    |

**Table 3.** Occurrence and index of discrimination (ID) of potentially useful random amplified polymorphic DNA (RAPD) markers as determined after analysis of bacterial strains from the reference set

| Marker              | Genus level                                   |                                             |                     | <i>Erwinia carotovora</i> subspecies level    |                                              |                     |
|---------------------|-----------------------------------------------|---------------------------------------------|---------------------|-----------------------------------------------|----------------------------------------------|---------------------|
|                     | In <i>Erwinia carotovora</i> (%) <sup>a</sup> | In <i>Pseudomonas</i> spp. (%) <sup>a</sup> | ID (%) <sup>b</sup> | In subsp. <i>atroseptica</i> (%) <sup>a</sup> | In subsp. <i>carotovora</i> (%) <sup>a</sup> | ID (%) <sup>b</sup> |
| OPF12v <sup>c</sup> | 29                                            | 0                                           | 29                  | 100                                           | 0                                            | 100                 |
| OPF12w              | 47                                            | 30                                          | 17                  | 100                                           | 25                                           | 75                  |
| OPF12 x             | 29                                            | 91                                          | 62                  | 40                                            | 25                                           | 15                  |
| OPF12y              | 59                                            | 83                                          | 24                  | 40                                            | 67                                           | 27                  |
| OPG09h              | 47                                            | 65                                          | 18                  | 100                                           | 25                                           | 75                  |
| OPG09i              | 29                                            | 0                                           | 29                  | 100                                           | 0                                            | 100                 |
| OPG09j              | 59                                            | 13                                          | 46                  | 0                                             | 83                                           | 83                  |
| OPG10k              | 65                                            | 13                                          | 52                  | 100                                           | 50                                           | 50                  |
| OPG10l              | 41                                            | 4                                           | 37                  | 100                                           | 17                                           | 83                  |
| OPG10n              | 12                                            | 87                                          | 75                  | 0                                             | 17                                           | 17                  |
| OPG10p              | 35                                            | 83                                          | 48                  | 0                                             | 50                                           | 50                  |
| OPG10q              | 24                                            | 4                                           | 20                  | 80                                            | 0                                            | 80                  |
| OPG17a              | 71                                            | 0                                           | 71                  | 0                                             | 100                                          | 100                 |
| OPG17b              | 100                                           | 13                                          | 87                  | 100                                           | 100                                          | 0                   |
| OPG17c              | 100                                           | 0                                           | 100                 | 100                                           | 100                                          | 0                   |
| OPG17d              | 29                                            | 13                                          | 16                  | 100                                           | 0                                            | 100                 |
| OPG17e              | 35                                            | 4                                           | 31                  | 100                                           | 8                                            | 92                  |
| OPH03a              | 47                                            | 0                                           | 47                  | 100                                           | 25                                           | 75                  |
| OPH03b              | 47                                            | 22                                          | 25                  | 100                                           | 25                                           | 75                  |
| OPH03c              | 29                                            | 4                                           | 25                  | 100                                           | 0                                            | 100                 |
| OPH05f              | 71                                            | 0                                           | 71                  | 0                                             | 100                                          | 100                 |
| OPH05g              | 35                                            | 0                                           | 35                  | 100                                           | 8                                            | 92                  |

<sup>a</sup> Occurrence of markers in groups or subgroups formed with the bacterial strains from the reference set.

<sup>b</sup> Indices of discrimination defined as difference between the occurrence percentages of the two bacterial groups or subgroups.

<sup>c</sup> Markers identified by name of primer followed by a code letter. Description of markers is in Table 4 for primers used in the RAPD procedure.

**Table 4.** Descriptions and values of the markers used by the quantitative identification tool

| Marker | Description                                                | Values <sup>a</sup> |                  |
|--------|------------------------------------------------------------|---------------------|------------------|
|        |                                                            | Genus level         | Subspecies level |
| OPG17a | OPG17 <sub>1800</sub> as clearly the brightest band        | 2                   | 5                |
| OPG17b | Presence of OPG17 <sub>1800</sub>                          | 3                   | NA <sup>b</sup>  |
| OPG17c | OPG17 <sub>1800</sub> as one of the four brightest bands   | 5                   | NA               |
| OPG17d | Presence of OPG17 <sub>1435</sub>                          | NA                  | 5                |
| OPG17e | Presence of OPG17 <sub>1100</sub>                          | NA                  | 4                |
| OPH05f | Presence of OPH05 <sub>2700</sub> or OPH05 <sub>2800</sub> | NA                  | 5                |
| OPH05g | Presence of OPH05 <sub>2400</sub>                          | NA                  | 4                |
| OPH05z | Presence of OPH05f or OPH05g                               | 5                   | NA               |

<sup>a</sup> Values were arbitrarily attributed to markers to allow the correct identification of all the strains from the reference set by the quantitative identification tool.

<sup>b</sup> Not applicable.

Although not taken into account during the identification by RAPD, checking the general aspect of shared patterns before making a final decision is also important, especially for pseudomonads that are characterized by the absence of markers. Hence the misidentification of unrelated pectolytic bacteria as pseudomonads would be avoided, for although these bacteria are rare, they are sometimes responsible for soft rot (10,13). Similar patterns were shared by pseudomonads when primer OPG17 was used (Fig. 1), therefore this primer was used to prevent this problem.

**Verification of the RAPD procedure.** Forty-nine additional strains of Québec's soft rot bacteria were tested by biochemical and RAPD tests. Identifications from RAPD tests were obtained by using the quantitative identification tool. Thirty-six

*E. carotovora* subsp. *carotovora*, two *E. carotovora* subsp. *atroseptica*, and 11 *Pseudomonas* spp. were identified by both methods (Table 2). These results confirmed the efficiency of our RAPD procedure for correctly identifying the Québec soft rot bacteria.

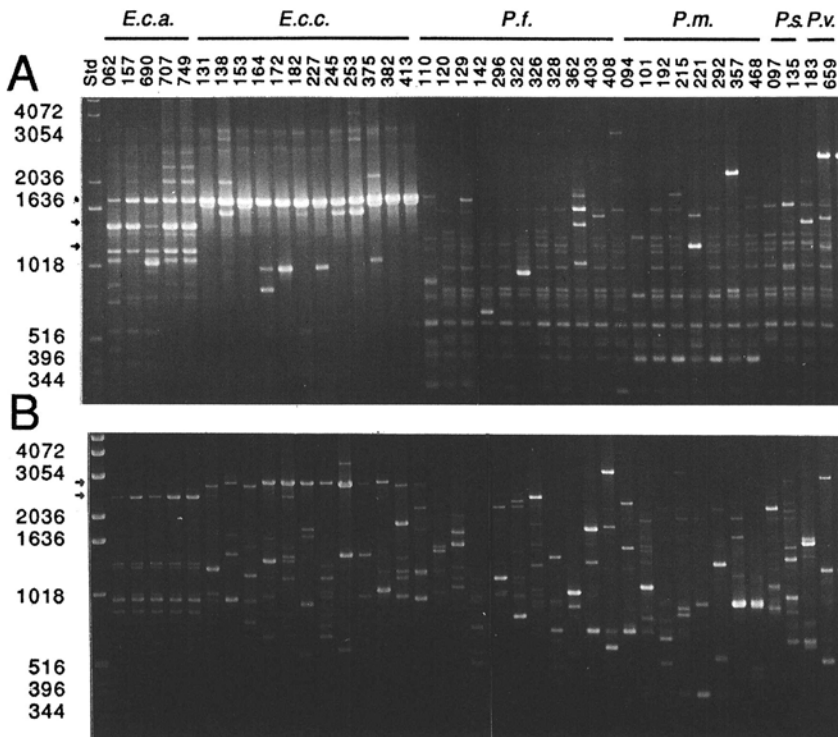
Trials with 25 strains from outside of Québec were also done using the RAPD procedure. After calculations with the quantitative tool, results of the RAPD procedure corresponded to the previous identifications made by the original senders of the strains (Table 2). Although tested with only a limited number of strains from outside of Québec, our RAPD procedure, it appears, could be used to identify soft rot bacteria from sources worldwide.

Our aim was to develop a simple and inexpensive RAPD procedure for the identification, by a diagnostic laboratory, of the

major soft rot bacteria. With RAPD, the identification period is reduced to about 24 h and the cost is only a few dollars per sample. Our method was developed from an original set of 45 primers. With the availability of thousands of RAPD primers, many other primer combinations likely exist. However, we obtained a quick and reliable method to attain our objective with the addition of a quantitative identification tool. Our procedure is now routinely used in our diagnostic laboratory.

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**Fig. 1.** Photographs of electrophoresis gels containing random amplified polymorphic DNA fragments of soft rot bacteria from a reference set. Tested bacteria were *Erwinia carotovora* subsp. *atro-septica* (*E.c.a.*), *E. carotovora* subsp. *carotovora* (*E.c.c.*), *Pseudomonas fluorescens* (*P.f.*), *P. marginalis* (*P.m.*), *P. syringae* (*P.s.*), and *P. viridiflava* (*P.v.*). Strains are identified by their number. Arrows indicate the position of markers used in the identification procedure. Numbers at left are sizes, in base pairs, of standard DNAs (Std). Primers used were (A) OPG17 and (B) OPH05.

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