Neth. J. Pl. Path. 74 (1968) 35–43

# Serological recognition of Streptomyces species causing scab on potato tubers

H. Vruggink and D. Z. Maat

Institute of Phytopathological Research (IPO), Wageningen

Accepted 10 December, 1967

**Abstract**

Antisera were prepared against extracts of two tyrosinase-positive *Streptomyces* spp., one of which caused a "deep" and the other a "russet" scab. Tyrosinase-positive *Streptomyces* isolates not reacting with either of these antisera proved to be non-pathogenic to potato tubers, with few exceptions only. Not all isolates reacting with one or both antisera, however, were pathogenic and so all the serological positive ones had to be tested for pathogenicity to potato tubers. To obtain this relative specificity the antisera had to be absorbed with an extract of a non-pathogenic tyrosinase-positive isolate.

## Introduction

Scab-producing *Streptomyces* species in soil are generally found among those colonies which produce a dark pigment when grown on a nutrient agar containing tyrosine. Tyrosinase-negative pathogens do occur; Corbaz (1964), for example, isolated a number of actinomycetes from scabby tubers of which 26 % were tyrosinase-negative and some of these proved to be weakly pathogenic. Menzies and Dade (1959), however, concluded that the number of tyrosinase-negative pathogens was negligible. As the tyrosinase reaction is no proof of pathogenicity, all isolates showing this reaction have to be tested on the living plant, which takes much time, labour and greenhouse space. Therefore a technique is needed which is quicker and yet reliable.
Bowman and Weinhold (1963) showed that extracts from *Streptomyces scabies* isolates contain an antigen lacking in most of the other *Streptomyces* species not pathogenic to potato tubers. Therefore the object of this research was to see if a serological test, following selection using the tyrosinase reaction, could give a quick diagnosis of the scab-producing isolates from among a number of tyrosinase-positive *Streptomyces* species. Two types of scab are recognized: (a) "normal", including deep and superficial lesions and (b) "russet" scab (Harrison and Eide, 1962). Antisera against both were prepared.

## Materials and methods

*Methods used in the isolation and culture of actinomycetes*
In the isolation of actinomycetes a soil dilution technique was used. From a thoroughly mixed bulk sample of soil a subsample of 100 g was taken and mixed with 1 l water in a mixer for 2 min. From this aliquots were taken and diluted with a mixture of water and phenol to reach a final concentration of 0.7% phenol, this to eliminate bacterial contamination (Lawrence, 1956). After 10 min a dilution series was made using sterile water. One ml from the final dilution (1:10.000 or 1:100.000) was added to 10 ml Conn's glycerol-asparagine agar with 0.05% l-tyrosine ("GAT"-medium) (Conn, 1921). After 5–7 days incubation at 28 °C, a number of tyrosinase-positive colonies, taken at random, were transferred to potato glucose agar slopes in test tubes. When growth was sufficient, the isolates were transferred to 40 ml potato-glucose + 0.7% peptone solution in 100 ml flasks and shake-cultured at about 20 °C for 10 days.

*Test for pathogenicity*
The pathogenicity test was that devised by R.E. Labruyère (unpublished) in which the wet mycelium of a shake culture was homogenized in small fragments by treating it with a high speed grinder, (Ultra Turrax) for 2–4 sec. After centrifuging at 5000 r.p.m. for 5 min the sediment was mixed with a small quantity of quartz sand. A polyethylene bag placed in an earthenware pot (12.5 cm diameter and 15 cm high) was filled with steam-sterilized potsoil (a potting compost, consisting of peat-dust, sand and farm yard manure) to half the height of the pot and on top of this an equal amount of soil was placed consisting of a mixture of sterile potsoil and quartz sand with mycelium. The empty part of the bag was rolled back so that about 10 cm protruded above the level of the pot to prevent splashing of inoculum from one pot to the other when watering. Pots were planted with sprouts of scab-free tubers. During growth of the potato plants the moisture content was kept at a low level (near wilting point) to obtain the best conditions for maximum attack. After 2–3 months the amount of scab was assessed on the newly-formed tubers.

*Preparation of antisera*
Two antisera were prepared, one against normal scab (S 79) and one against russet scab (S 13).
The mycelium was washed three times in distilled water by suspending, centrifuging at 5000 r.p.m. for 5 min and resuspending again. The sediment was ground with fine quartz sand and the homogenate centrifuged. The supernatant was used as extract for inoculation into rabbits. These were injected with 0.5 ml extract intravenously and at the same time with 2 ml extract emulgated in 2 ml of Freund's incomplete adjuvant subcutaneously on three occasions at weekly intervals. Two weeks after the last injection blood samples were taken and the titer assessed. These were rather low and varied from 32 to 128.

*The serological test*
Test extracts were prepared as for the antiserum preparation, except that mycelium was not washed and only a proportion of the mycelium was used; the remainder was used for testing the pathogenicity on the living plant.

Table 1. Relationship between pathogenicity and serological reaction of 27 isolates from tubers and soil

| *Type* | *Number of isolates tested** | *Serological reaction* |
|---|---|---|
| Non pathogenic | 7 | 7 negative |
| "Normal" scab | 16 | 16 positive |
| "Russet" scab | 4 | 2 positive (weak)<br>2 negative |

* Pathogenicity test done before serological test

*Tabel 1. Verband tussen pathogeniteit en serologische reactie van 27 isolaties uit knollen en uit grond*

To increase specificity, antisera were absorbed with extract of a non-pathogenic tyrosinase-positive isolate (No. 9). Procedure then followed the standard Ouchterlony agar gel diffusion test (Ball, 1961). The distance between the centre of the antigen- and antiserum holes was 5 mm and the diameter of the holes 3 mm.

## Experiments and results

*Experiments with "normal" scab antiserum S 79*

To compare pathogenicity with serological reaction a first experiment was carried out with 27 isolates originating from tubers as well as from soil, of which the pathogenicity was known before the test. These isolates were tested with the antiserum against S 79 absorbed with two parts extract of isolate No. 9. The results are shown in Table 1. It appears that the 7 non-pathogenic isolates reacted serologically negative, while from the 20 pathogenic isolates only 2 russet scab ones gave no positive reaction.

To obtain further information about the correlation between pathogenicity and serological reaction a second experiment was carried out with 85 tyrosinase-positive actinomycetes, isolated from soil. The pathogenicity of these isolates was tested on 'Bintje'. The results are given in Table 2. In both experiments the pathogenic isolates are reacting serologically positive. From the second experiment it is clear that non-pathogenic isolates also may react serologically positive. This made it necessary to check all serologically positive isolates in subsequent experiments for pathogenicity. To improve on specificity, it was tried to absorb the antiserum S 79 with extracts of these serologically positive, but non-pathogenic isolates, instead of using the extract

Table 2. Relation between pathogenicity and serological reaction of 85 isolates from soil

| *Serological reaction* | *Number of isolates** | *Pathogenicity test* | | |
|---|---|---|---|---|
| | | *non-pathogenic* | *"normal scab"* | *"russet" scab* |
| Positive | 61 | 23 | 36 | 2 |
| Negative | 24 | 21 | 0 | 3 |

* Pathogenicity test done after serological test

*Tabel 2. Verband tussen pathogeniteit en serologische reacties van 85 isolaties uit grond*

Table 3. Tests with soils from different regions

| *Source of soil sample* | *Number of isolates* | *Serological reaction* | | | | *Pathogenicity test** | | |
|---|---|---|---|---|---|---|---|---|
| | | *negative* | | *positive* | | *pathogenic* | | *non-pathogenic* |
| | | No. | (%) | No. | (%) | No. | (%) | |
| Oost-Flevoland | 119 | 28 | (24) | 91 | (76) | 29 | (32) | 62 |
| Stiens | 195 | 101 | (52) | 94 | (48) | 66 | (70) | 28 |
| Uithuizermeeden | 64 | 22 | (34) | 42 | (66) | 0 | ( 0) | 42 |
| Niehove | 24 | 16 | (67) | 8 | (33) | 8 | (100) | 0 |

* Serologically positive reacting isolates only

*Table 3. Proeven met grond van verschillende herkomst*

of No. 9. In doing so, however, all serological reactions became negative. As serologically negative isolates appeared to be non-pathogenic with the exception of a few russet scab isolates, a special antiserum against these isolates was prepared. This will be discussed in the next section. In the following experiments with isolates from soils in which no russet scab was known to occur, the serologically negative isolates were no longer tested on pathogenicity.

In the experiment of Bowman and Weinhold (1963) with about 60 isolates, nearly 97% reacted serologically negative. In our experiment with 85 isolates this percentage was only 28% (24/85 × 100), while in an experiment with another soil 85% of the isolates were found to react serologically negative. To see if there was any relation between the origin of the soil and the percentage of serologically negative isolates, an experiment was carried out with isolates from four different soils, with a different scab history each, coming from Oost-Flevoland, Stiens, Uithuizermeeden and Niehove. The sample of Oost-Flevoland was a mixture from two adjacent fields of which one yielded very scabby tubers while the other gave nearly clean ones. The soil from Stiens was a well known "scab" soil. In Uithuizermeeden a field trial was carried out in which scabbiness proved to be on a very low level. From the soil from Niehove the scab history is not known. The results are given in Table 3. The soil samples from Oost-Flevoland and Uithuizermeeden show significantly less serologically negative isolates (24% and 34%) than the soil samples from Stiens and Niehove (52% and 67%), confirming that the percentage of serologically negative isolates varies from soil to soil.

The percentages of pathogens among the serologically positive isolates also differed greatly; ranging from 0 to 100%. Often qualitative differences were also observed in the isolates of different soil samples. Predominant representatives of a certain group of actinomycetes in one soil sample frequently were missing in another. Evidently percentages of serologically negative isolates as well as percentages of pathogens among the positive ones are depending on the compostition of the actinomycete-flora of the soil in question.

*Experiments with "normal" – (S 79) and "russet" scab (S 13) antisera*

In The Netherlands "normal" scab is much more important than "russet" scab which is rather rare and attacks only a few potato varieties. De Bruyn (1939) found that russet scab isolates only attacked the varieties 'Bintje' and 'Industrie'. This was con-

Fig. 1. Type of scab caused by a russet scab isolate on 'Bintje' (left) and 'Eigenheimer' (right)

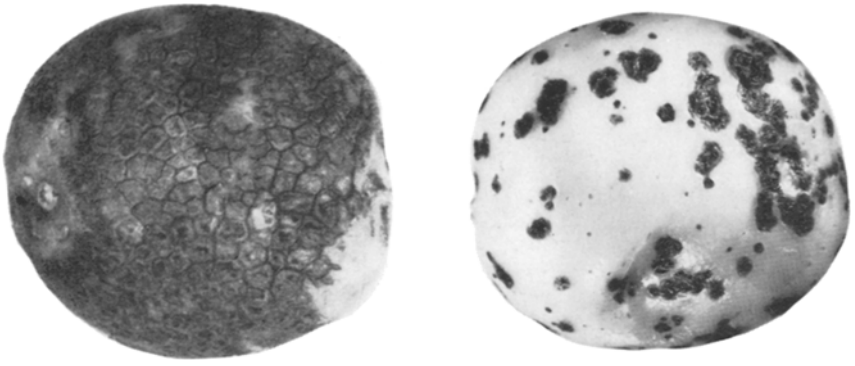

*Fig. 1. Aantasting door een bepaalde netschurftisolatie op 'Bintje' (links) en 'Eigenheimer' (rechts)*

firmed by Mygind (1965) for 'Bintje'. We found that the variety 'Climax' was also susceptible. Two russet scab isolates caused not only russet scab on 'Bintje' but also normal scab on 'Eigenheimer' (Fig. 1). Because of existing differences between russet scab isolates and normal scab isolates in infection of the tuber and the root system, it was of interest to know more about serological relationship between these two types of pathogens. As is shown in Table 1 and 2, not all russet scab isolates react with the antiserum S 79. Therefore an antiserum against the russet scab isolate S 13 was prepared and several russet scab isolates were tested with both antisera. The results of this experiment are given in Table 4. From this table it can be seen that the antiserum S 13 reacted with all isolates from soil, whereas antiserum S 79 reacted only with 3 out of 7. However, with the isolates from tubers both antisera S 13 and S 79 missed 4 out of 5.

Unpublished data from previous work showed, that the number of pathogens among tyrosinase-positive isolates originating from the glycerol-asparagine-tyrosine (GAT) medium was higher than on the tyrosine-caseinate-nitrate (TNC) medium of Menzies and Dade (1959).To see if the percentage of serologically positive isolates as well as the number of pathogens was affected by the medium on which the isolates were collected, the antisera S 79 and S 13 were used with isolates of a soil in which russet scab was the predominant. The results are given in Table 5. With both antisera S 79

Table 4. Differences between two antisera, using "russet" scab isolates

| *Isolate source* | *Number of isolated tested* | *Serological reaction with antiserum* | | | | *Pathogenicity on* | |
|---|---|---|---|---|---|---|---|
| | | *S 13* | | *S 79* | | *Bintje* | *Eigenheimer* |
| | | (+) | (–) | (+) | (–) | | |
| Soil | 7 | 7 | 0 | 3 | 4 | 7 | 1* |
| Tuber | 5 | 1 | 4** | 1 | 4 | 5 | 1* |

* "Normal" and not "russet" lesions on Eigenheimer
** Isolates from the same tuber

*Tabel 4. Verschillen tussen antisera ten opzichte van "netschurft"-isolaties*

Table 5. Comparison of two media used for isolation of Streptomycetes and of the specificity of the antisera S 13 and S 79

| *Isolation medium* | *Number of isolates* | *Antiserum* | *Serological reaction* | | | *Pathogenicity test** | | *Type of scab relation on 'Bintje'* | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | *negative* | *positive* | | *pathogenic* | *non-pathogenic* | *"russet"* | *"normal"* | |
| | | | | *No.* | (%) | | | | *superficial* | *deep* |
| GAT | 114 | S 79 | 73 | 41 | (36) | 31 | 10 | 21 | 6 | 4 |
| | | S 13 | 32 | 82 | (72) | 45 | 37 | 34 | 7 | 4 |
| TCN | 119 | S 79 | 103 | 16 | (13) | 10 | 6 | 2 | 8 | 0 |
| | | S 13 | 57 | 62 | (52) | 24 | 38 | 13 | 11 | 0 |

* Serologically positive reacting isolates only

*Table 5. Vergelijking van twee media gebruikt voor het isoleren van Streptomyceten en van de specificiteit van de antisera S 13 en S 79*

and S 13 higher numbers of serologically positive reacting isolates were found on the GAT medium in comparison to the TCN medium, also resulting in a higher number of pathogens, confirming the findings mentioned above.
From this table and from Table 4 it can be seen that when both scab types are present in a given soil the use of antisera S 13 is necessary because of the detection of a considerable number of russet scab pathogens, which are not found when using antiserum S 79. When only normal scab is present the use of antiserum S 13 is also preferable, while it gives slightly higher numbers of pathogens of this scab type too, although specificity is less, as can be seen in Table 5.

**Discussion**

The main object of this work has been to find a quick and yet reliable method of identifying *Streptomyces* species, causing scab on potatoes without the need of the laborious pathogenicity test on the living plant. If such a method could be developed it would be possible to predict the danger of a severe scab attack before potatoes were planted, which is of great importance to seed potato growers, who often rent land without knowing its scab history. A quick assessment of the number of pathogens in field and laboratory experiments during the experiment can also be of great value. This object has not been attained because the serological method gives no indication of the proportion of pathogens among tyrosinase-positive isolates. However, as most serologically negative isolates are non-pathogens, these need not be tested for pathogenicity, which in itself saves much labour and greenhouse space.
Bowman and Weinhold (1963) stated that: "... serological specificity does not appear to be directly related to pathogenicity". From our experiments, it appears that much depends on the choice of the starting material. For example, with the preparation of the antiserum against S 79, an antiserum was prepared against another pathogenic isolate. Even when absorbed with four parts of No. 9, this antiserum still reacted with more non-pathogens than the antiserum against S 79 and therefore was excluded in further experiments. Absorption of the antiserum S 79 with the extracts of other serologically negative non-pathogenic isolates gave the same or worse results as after absorption with No. 9. As mentioned before, also differences in specificity between the antiserum S 79 and the antiserum S 13 do exist. Therefore the possibility of finding a better combination of pathogen for antiserum production and non-pathogen for absorbing still remains possible.
Pathogenicity does not appear to be directly related to one species of *Streptomyces* only as shown by Corbaz (1964). We have found that among isolates which cause russet scab there are a number which differ biochemically and culturally from *Streptomyces scabies*. For example, on glycerol-asparagine agar the substrate growth is light grey-brown, while normal scab isolates are mostly crême-yellow; on milk after peptonisation, the milk turns colorless while normal scab isolates turn the milk brown colored; and also the tyrosinase reaction is less strong than with normal scab isolates. These were the four isolates which did not react with the russet scab antiserum as is shown in Table 4. Considerable differences also exist among different russet scab isolates, making it unlikely that they all belong to one species.
In Table 4 the isolates causing superficial scab which do react with antiserum S 13 but do not with the antiserum S 79 are different from the normal scab producing isola-

tes, because of the production of a red pigment on various culture media. These isolates are rather weak pathogenic.
We did give evidence that the medium from which isolates are obtained can be selective. Table 5 shows, that the number of tyrosinase-positive and serologically positive isolates from the GAT medium is higher than from the TNC medium. When the tyrosinase-positive isolates originating from the TNC medium were transferred to GAT slants, it appeared that a number of these positive isolates were tyrosinase-negative on the GAT medium. These very isolates that were missing among the tyrosinase positive ones on the GAT medium all proved to be serologically negative. If results concerning the number of pathogenic *Streptomyces* are to be compared, it is important that the same medium is always used when isolating them.
Recently we have found that the culture filtrate in which the isolates are growing might be used in the serology test rather than mycelium extract, provided cultures are shaken for at least 14 days at about 20 °C. The isolates, which gave a serologically positive reaction with the culture filtrate, were also positive with the mycelium extract, but from the negative ones some reacted positive with the mycelium extract, so that from these ones always the mycelium extract had to be prepared and tested subsequently. Part of the preparation of mycelium extract can now be omitted, which speeds up the testing process.

**Samenvatting**

*Het serologisch herkennen van Streptomyces-soorten die schurft op aardappelknollen veroorzaken*

Onderzocht is of de serologie een snelle en toch betrouwbare methode kan opleveren voor het herkennen van *Streptomyces*-soorten, die schurft geven op aardappel. Deze soorten vormen overwegend een donker pigment op een tyrosine-bevattend voedingsmedium. Tot nog toe was het noodzakelijk om al deze tyrosinase-positieve isolaties op pathogeniteit te toetsen op de levende plant, hetgeen veel werk, tijd en kasruimte in beslag nam. Tegen twee van deze tyrosinase-positieve isolaties zijn antisera bereid. Deze isolaties, S 79 en S 13, veroorzaken op 'Bintje' respectievelijk normale schurft en netschurft. Ter verkrijging van een specifieke reactie was het nodig de antisera te verzadigen met een extract van een niet-pathogene isolatie.
In de eerste proeven (Tabel 1 en 2) is gebleken, dat vrijwel alle isolaties, die niet met het antiserum tegen S 79 reageren, niet pathogeen zijn. Uitzonderingen hierop zijn enkele netschurftisolaties, die echter wel met het antiserum tegen S 13 reageren (Tabel 4). Onder deze netschurftisolaties bevonden zich twee die niet alleen de vatbare rassen 'Bintje', 'Industrie' en 'Climax' aantastten, doch ook het als niet vatbaar aangemerkte ras 'Eigenheimer', waarop zich een normale schurftaantasting ontwikkelde (Fig 1). De isolaties die met één of beide antisera wel reageren, zijn echter niet alle pathogeen en moeten dientengevolge nog steeds op hun pathogeniteit getoetst worden op de levende plant.
Het percentage serologisch negatieve isolaties kan, evenals het percentage pathogenen onder de serologisch positieve, sterk variëren (Tabel 3). Dit is waarschijnlijk mede afhankelijk van de samenstelling van de actinomyceten-flora van de desbetreffende grond.

Gebleken is verder, dat het medium waarvan men isoleert, een zekere selectiviteit bezit (Tabel 5).

**Acknowledgments**

The authors are much indebted to Dr. D. H. Lapwood, Rothamsted Experimental Station, Harpenden, England and Ir. R. E. Labruyère, IPO, Wageningen, for criticism and advice in preparing the manuscript.

**References**

Ball, E. M., 1961. Serological tests for the identification of plant viruses. Published by the American Phytopathological Society, Ithaca, N.Y.

Bowman, T. and Weinhold, A. R., 1963. Serological relationships of the potato scab organism and other species of Streptomyces. Nature, Lond. 200:599.

Bruyn, H. L. G. de, 1939. Onderzoekingen over enkele Actinomyceten, welke aardappelschurft verwekken. Tijdschr. Plziekt. 45:133–156.

Conn, H. J., 1921. The use of various culture media in characterizing actinomycetes. Tech. Bull. N.Y. agric. Exp. Stn 83.

Corbaz, R., 1964. Étude des streptomycètes provoquant la gale commune de la pomme de terre. Phytopath. Z. 51:351–360.

Harrison, M. D. and Eide, C. J., 1962. The cause of potato russet scab in Minnesota. Pl. Dis. Reptr 46:128–129.

Lawrence, C. H., 1956. A method of isolating Actinomycetes from scabby potato tissue and soil with minimal contamination. Can. J. Bot. 34:44–47.

Menzies, J. D. and Dade, E., 1959. A selective indicator medium for isolating Streptomyces scabies from potato tubers in soil. Phytopathology 49:457.

Mygind, H., 1965. Kartoffel-Netskurv. Tidsskr. PlAvl. 69:47–66.