Isolation, identification, and characterization of Streptomyces species from common scab lesions

By

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Declaration

It is hereby declared that

1. The thesis submitted is my own original work while completing degree at BRAC University.
2. The thesis does not contain material previously published or written by a third party, exceptwhere this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any otherdegree or diploma at a university or other institution.
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Approval

The thesis titled “Isolation, identification, and characterization of Streptomyces species from common scablesions.” submitted by Fayruz Maysha (19176010) of spring, 2019 has been accepted as satisfactory in partial fulfillment of the requirementfor the degree of Master of Science in Biotechnology.

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Ethics Statement

No human or animal model was used in this study.

Abstract

Streptomyces scabiei is largely accepted as the causal organism of common scab on potato in Debiganj, Rangpur and other Streptomyces species associated with common scab are not often considered. This study, therefore, aims to determine the diversity and prevalence of Streptomycetes associated with a common scab on potatoes in Debiganj, Rangpur. Isolates from 11 of the 16 potato-producing regions in Debiganj, Rangpur were characterized morphologically,physiologically, and genetically. Most isolates resembled S. scabiei based on morphology and physiology. Most pathogenic isolates were S. acidiscabies or S. turgidiscabies, and no S. scabiei and S. stelliscabiei isolates were found. All three pathogenicity/ virulencepathogenicity/ virulencegenes (txtAB, nec1, tomA) were found in Debiganj, Rangpur isolates. Pathogenicity could not belinked to the presence of a single one or any combination of two of the three genes. These resultsrepresent the most comprehensive published survey of Streptomycetes isolated from common scab lesions on potatoes in Debiganj, Rangpur.

Keywords: Streptomyces; Potato; Common scab; Characterisation

Dedicated to my parents

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As I write the last paragraph, I have just realized that I have copied much of the words and phrases used here from the acknowledgement section of my undergraduate thesis! Seems like the "pro- procrastinator" in me has reached a new height! Maybe, life has changed much (or maybe it hasn't) for many individuals mentioned in my undergraduate and postgraduate thesis book. Some have even left us for the life hereafter. But wherever we are or whatever we do, I hope we all become good human beings at the end of the day. I might have lost the will to write new words rather than just copying some of them, but may we never lose the will to become a better human being at anyphase of our lives.

Fayruz Maysha

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Chapter 1: Introduction

1.1Background

The potato, *Solanum tuberosum* is the sixth most important as well as consumed commodity in agriculture worldwide. Potato holds place right after sugarcane, wheat, rice, maize and cereals. The origin of potato was in Peru of South America (Bradshaw and Ramsay). Later, it was exported to the rest of the world by shipment, transportation and war expeditions. In Bangladesh,the potato is cultivated over an area of 0.5 million hectares with the annual production of 8.6 million tons (Khalil et al.).

In the world, China is the largest potato producing country with the production of 99.5 million tons every year and it is shared by 25.02% by other countries such as Russia, India and United States (Bradshaw and Ramsay). Despite the food security significance and an amazing market value, potato is susceptible to mane ailments caused by viruses, bacteria, nematodes and fungi. Among all the diseases caused by bacteria, the most devastating is the common scab disease ofpotato.

Despite its food security significance and great market value, the potato crop is susceptible to many ailments caused by bacteria, viruses, nematodes, and fungi (Mehboob et al.). Among bacterial diseases, the common scab is the most devastating disease of the potato, which causes economic losses to the potato-growing countries of the world (Enciso-Rodriguez et al.). This disease is caused by *Streptomyces scabies*, which belongs to the phylum actinobacteria, which isone of the biggest taxonomic units among the 18 major lineages of bacteria and its divergence from other bacterial species is so ancient that it is currently not possible to identify their most

closely related group (Spooner et al.). Arguably, actinobacteria's best-studied genus isStreptomyces, which have complex developmental life cycles (Hampson).

Potato scab disease, which is caused by *Streptomyces scabies*, has been reported in many countries such as China, South Africa, Pakistan, Iran, Russia, India, United States and several other countries of the world. This disease includes many symptoms such as raised, deep pitted,sunken lesions, and scab-like surface on the tuber (Hampson).

Potato provides balanced source of starch, vitamins and minerals to many communities in the global villages. In Bangladesh potato is the third largest crop after rice and wheat. It is used primarily as a vegetable and has potential as a staple food. Potato cultivation in the Bengal was promoted by a British Governor in 1770s and then it was a well-established garden vegetable. Annual consumption of potato has been growing rapidly, from around 7 kg per capita in 1990 to more than 25 kg in 2005. In Bangladesh, so far as many as 57 diseases in potato have been recorded (Khalil et al.). Among them late blight, stem rot /sclerotium rot, wilt, common scab, potato leaf roll and mosaic are the most important diseases. Common scab is widely distributed in Bangladesh which gives ugly appearance to wear potatoes. Though the disease does not cause appreciable reduction in yield, it can cause great loss due to reduction of market value of tuber. Moreover, infected seed tubers serve as the primary sources of inoculum for the next season. Theincidence, severity, etiology, epidemiology and control of common scab have been investigated extensively in many countries of the world and the disease has been the topic of various reports in Bangladesh during the end of the 18th century (Nasif et al.).

In Bangladesh potato common scab was initially a minor disease but now has become a major potato disease and incidence of the disease is increasing day by day. The information of potato

common scab is not available on the incidence of disease and its severity in different agro- ecological regions and the susceptibility of commercially cultivated potato in Bangladesh (International Journal of Science and Research (IJSR). Keeping all these in view, the present investigation was undertaken to study the regional variations on the disease incidence, severityand susceptibility of common scab disease of commercially cultivated potato in Bangladesh.

Common scab of potato is one of the most economically important worldwide diseases and it was named by Northern American growers in 1991 (Loria et al.). Common scab of potato is caused by several Streptomyces spp. (Loria et al.), but S. scabies is the predominant causal organism (Lambert & Loria). The symptoms of common potato scab are quite variable and are manifested on the upper surface of the potato tuber. Depending on many factors such as pathogen strain, cultivar susceptibility, environmental conditions, the symptoms of scab can appear as lesions of variable sizes and depth on tuber surfaces (Lorang et al.). The pathogen is disseminated by infected seed tubers or soil and easily can survive in the absence of host plants (Loria et al.; Wang & Lazarovits). Once established of the pathogen, it's really difficult to eliminate it from a field. However, there are several possible control methods for common scab,primarily chemical management, cultural controls such as irrigation (Lapwood), planting dates (Wilson), crop rotations, and cultivar selection (Hiltunen et al.). Although, potato common scabwas initially a minor disease in Bangladesh now has become a major one and its incidence and severity are increasing day by day. Therefore, the current study was designed to the integrated use of chemical (fungicides and fertilizers) and cultural (optimum planting time and crop duration) approaches for minimizing the common scab

disease of potato in Bangladesh.

Many species of Streptomyces such as S. scabies, S. ipomoeae, S. turgidiscabies, and S. acidiscabies because many symptoms on several hosts that include deep pitted and raised scab- like lesions on potato, beet, radish, and peanut crops. These crops are economically important, but they reduce these crops' market and consumption values. Potato scab disease is transmissiblefrom seed and soil sources (Moran and Crompton).

The disease develops when the tuber starts emerging in the first growth stages of tubers whenenlarges or direct penetration to the epidermis and enlarging the potato tuber (Clark et al.).

Programmed cell death occurs near the diseased areas of tubers. Then these spots/lesions consequently transfer into deep pitted shallow lesions due to the bacterization of nearby tuber areas, which are the initial symptom development of the disease. These lesions, which developon tubers, are circular when they are multiple; these merge to develop asymmetrical scabby lesions (Babcock et al.).

Some other factors can affect the production of the potato, such as the unavailability of seed, poor quality seed, and management problems (Bjor and Roer). All the above factors affect potatoyield, but among biotic factors, the factors that cause the most severe damage are diseases. The potato crop is susceptible to black scurf, powdery scab (*Spongospora subterranea*), wilt (*Verticillium albo-atrum*), but highly susceptible to common scab (*Streptomyces scabies* (Babcock et al.) which took place in the family Streptomycetaceae. *Streptomyces spp*. are the source for the production of numerous antibiotics; among the most important of these are streptomycin from (*S. griseus*), tetracycline (*S. rimosus*),

neomycin (*S. fradiae*), daptomycin (*S. oseosporus*), chloramphenicol (*S. venezuelae*), lincomycin (*S. lincolnesis*), fosfomycin (*S. radial*), oleandomycin and Pathogens 2020, 9, 760 3 of 26 boromycin (*S. antibioticus*), mycangimycin (*Streptomyces spp.* SPB74), tunicamycin (*S. orulosus*) and puromycin (*S. alboniger*) (Dees and Wanner).

This disease is caused by Streptomyces scabies that belongs to the phylum actinobacteria, whichis one of the biggest taxonomic units among the 18 major lineages of bacteria and its divergence from other bacterial species is so ancient that it is currently not possible to identify their most closely related group. Arguably, actinobacteria's best-studied genus is Streptomyces, which havecomplex developmental life cycles. Potato scab disease, which is caused by Streptomyces scabies, has been reported in many countries such as China, South Africa, Bangladesh, Pakistan,Iran, Russia, India, United States and several other countries of the world. This disease includes many symptoms such as raised, deep pitted, sunken lesions and scab like surface on the tuber.

Many economical and medicinal values, such as two-thirds of antibiotics, are developed from Actinomycetes worldwide and about 80% of antibiotics are developed from Streptomyces spp.

Chapter 2: Methods and Materials

2.1 Source of potato tubers

Potato tubers showing symptoms of common scab were obtained from potato growers, farmer advisers and the potato industry. The tubers sampled originated from 11 fields in Debiganj, Rangpur after the growing seasons in 2018 and 2019.

2.2 Bacterial isolates

Symptoms on the scabby potato tubers were recorded before the tubers were surface-disinfected in 70% ethanol for 20 s and then rinsed several times in sterile distilled water (SDW). Thereafter, from each tuber, a small piece of potato tissue was cut from under the surface of a single lesion, at the border between healthy and infected tissue, and then homogenized in 200 lL SDW and incubated for 30 min at room temperature. A 100 lL aliquot of the homogenate was plated out on water agar and incubated at 28LC in the dark. Single isolation was performed from each of a total of 957 independent tubers. From each plate, up to three single colonies, phenotypically characteristic of Streptomyces, were transferred to yeast malt extract agar (YME) (Loria) after approximately 6 days. Subsequent transfer to fresh medium was done to obtain pure cultures. Streptomyces isolates were grown on YME agar plates at 28LC and stored on YME agar plugs at –80LC.

2.3 Isolation of genomic DNA

Streptomyces isolates were grown on YME at 28LC for 6–8 days and thereafter cells were scraped from the plate into a mortar and ground with a pestle in liquid nitrogen. DNA was extracted using the DNeasy Plant Mini Kit (QIAGEN) according to the manufacturer's instructions. Lysis buffer and RNase were added and the samples were incubated at 65LC for 1

h. DNA was eluted in 40 lL AE buffer. The quality and amount of DNA were determined by agarose gel electrophoresis.

2.4 Identification of putative pathogenic Streptomyces by PCR

Polymerase chain reaction using primers txtAB 1 and txtAB 2 (Table 1) was performed as previously described (Wanner) to detect the txtAB operon encoding thaxtomin synthetase. The PCR mix (15 lL) contained primers at 0Æ5 lM, 0Æ2 mM dNTPs, 0Æ02 U lL) Phusion High- Fidelity DNA polymerase (Finnzymes) 5 · Phusion HF buffer, 3% dimethyl sulphoxide (DMSO) and 2Æ5 lL template DNA. The PCR cycle included initial DNA denaturation at 94LC for 3 min followed by 30 cycles of denaturation at 94LC for 30 s, primer annealing at 46LC for 30 s, extension at 72LC for 35 s, and final extension at 72LC for 7 min. The experiment was repeated once.

Table 1 Primers used for detection of genes from Streptomyces isolates

Primer name		**Primer sequence (5¢–3¢)**	**Target**	**Reference**
txtAB 1	txtAB 1	CCACCAGGACCTGCTCTTC	txtAB operon	1
txtAB 2	txtAB 2	TCGAGTGGACCTCACAGATG	txtAB operon	
Nf	Nf	ATGAGCGCGAACGGAAGCCCC GGA	nec1 gene	2
Nr	Nr	GCAGGTCGTCACGAAGGATCG	nec1 gene	
Tom3	Tom3	GAGGCGTTGGTGGAGTTCTA	tomA gene	1
Tom4	Tom4	TTGGGGTTGTACTCCTCGTC	tomA gene	
ITS-L	ITS-L	GTCAAGTCATCATGCCCCTT	16S intergenic region	3
ITS-R	ITS-R	AAACTTGGCCACAGATGCTC	16S intergenic region	

pA	pA	AGAGTTTGATCCTGGCTCAG	Universal Streptomyces (16S rRNA gene)	4
pH¢	pH¢	AAGGAGGTGATCCAGCCGCA	Universal Streptomyces (16S rRNA	

			gene)	
ScabI	ScabI	CAACACTCTCGGGCATCCGA	S. scabies (16S rRNA gene)	5
ScabII	ScabII	TTCGACAGCTCCCTCCTTAC	S. scabies (16S rRNA gene)	
TurgI	TurgI	CCTCGCATGGGGGTGGGTTA	S. turgidiscabies (16S rRNA gene)	5
TurgII	TurgII	CGACAGCTCCCTCCCCGTAA	S. turgidiscabies (16S rRNA gene)	
BOXA1R	BOXA1R	CTACGGCAAGGCGACGCTGACG	Repetitive DNA sequences	6

[b]References: 1 (Wanner); 2 (Bukhalid et al.); 3 (Song et al.; Flores-Gonzalez et al.); 4(Edwards et al.); 5 (Kreuze et al; Lehtonen et al.); 6 (Clark et al.).

2.5 Species identification by PCR

The primers developed for the 16S rRNA gene sequences by Lehtonen et al. were used to detect DNA of S. scabies and S. turgidiscabies by PCR as described by the authors. However, S. scabies and S. europaeiscabiei cannot be distinguished by investigating the 16S rRNA gene sequences because those sequences are almost identical. Therefore, the intergenic transcribed spacer (ITS) region of the 16S operon was amplified using the primer pair ITS-R/ITS-L (Table 1) and the amplicons were subjected to digestion with the restriction enzyme Hpy99I (New England Biolabs), as previously described (Song et al.; Flores-Gonzalez et al.). The S. scabies type isolate ATCC49173 (Hpy99I+, i.e. amplicon-digestible with Hpy99I) and the three isolates ME01-11h (S. scabies; Hpy99I+), ID02-12 (S. europaeiscabiei; Hpy99I–) and PE07-1C (Hpy99I–) were used as controls (courtesy of Dr L. Wanner, USDA, Beltsville, MD, USA).

2.6 PAI marker genes

The genes nec1 and tomA were detected using the primer pairs Nf/Nr and Tom3/Tom4, respectively (Table 1), as previously described (Bukhalid et al.; Wanner). The PCR cycle was thesame as above, except that the annealing temperatures were 57 and 54LC for nec1 and tomA, respectively.

2.7 Pathogenicity assay on radish

A selection of 46 putative pathogenic Streptomyces isolates (txtAB-positive) was subsequently tested for pathogenicity on radish seedlings as previously described (Flores- Gonzalez et al.). In short, radish cv. Cherry Belle seeds were disinfected in 0Æ5% sodium hypochlorite solution for 1 min and then rinsed several times in SDW. Thereafter, the seeds were placed on an 8-day old culture of a Streptomyces isolate growing on Difco Oatmeal Agar (OMA) and incubated at room temperature for 8 days. Growth of seeds on OMA plates

with four txtAB-negative Streptomyces isolates and OMA plates without bacteria were used as controls. The appearance of the seedlings after growth with the bacteria was recorded. A bacterial isolate was considered pathogenic if the seedlings showed abnormal growth and hypertrophy, or if the seeds did not even germinate. The experiment was performed twice in duplicate.

2.8 Pathogenicity assay on the potato cultivar

Twenty-one txtAB-positive and three txtAB-negative Streptomyces isolates were tested for pathogenicity on potato (Table 3). Disease free minitubers of highly susceptible to CS, were used in the experiment.

Streptomyces isolates stored at 80LC were grown on YME agar for 2 weeks. P-Soil (mixture of peat and clay; Tjerbo Torvfabrikk) and agraperlite (Pull Rhenen) were autoclaved three times and mixed 50:50 (v / v) in 5-L pots. Two plates of each Streptomyces isolate were mixed thoroughly in the upper layer of the soil/perlite mixture before planting one potato tuber in each pot. Pots containing soil mixed with YME from uninoculated plates and pots containing soil without any treatment were used as controls. The plants were grown indoors from July to Augustat 18LC under natural light conditions. An automated drip watering system provided water containing fertilizer separately to each pot. Each treatment was run in three replicates and the tubers were harvested after 9 weeks. The lesion types and the surface area covered with symptoms were recorded for all tubers from each pot.

Chapter 3: Results

3.1 Symptoms of common scab on tubers sampled in the survey

The tubers sampled in this survey exhibited diverse symptoms, ranging from superficial to deep- pitted lesions. A few tubers had raised and warty lesions. Furthermore, scab severity varied from the occurrence of a few discrete lesions to deep-pitted, coalescent lesions that covered nearly the entire surface of a tuber.

3.2 Putative pathogenic Streptomyces isolates

Streptomycetes were isolated from potato tubers displaying symptoms of CS. After extracting DNA from pure cultures, the primer pair txtAB 1 / txtAB 2 was used to distinguish putative pathogenic isolates and probable non-pathogenic isolates. The 223 txtAB-positive isolates originated from 190 independent tubers and 11 different fields (Table 2). The various isolates came from different potato cultivars and 40% of them were obtained.

3.3 Species identification by PCR and distribution of the species

All of the putative pathogenic isolates were positive by PCR using the universal primers for the 16S rRNA gene of Streptomyces spp. Species determination was conducted using the primers developed by Lehtonen et al. (2004) for the 16S rRNA gene sequences. All the isolates were firsttested with the ScabI / ScabII-primer pair (Table 1). Of the 223 isolates, 152 (69%) produced amplicons with the ScabI / ScabII primer pair: none of those 152 isolates could be restricted with Hpy99I and thus they were all assigned to *S. europaeiscabiei* (Table 2).

All 223 of the putative pathogenic isolates were also tested with the primer pair TurgI / TurgII, because *S. turgidiscabies* is found. Of a total of 223 isolates, DNA of 71 of the isolates could be amplified using the primer pair TurgI / TurgII and hence these were assigned to *S.turgidiscabies*

(Table 2). Distribution of the species was the same both years; 69% of the isolates were assignedto *S. europaeiscabiei* and 31% to *S.turgidiscabies.*

The current results showed no pattern of geographical distribution of *S. europaeiscabiei* and *S.turgidiscabies*. Both species could occur in the same field and even in the same lesion.

Streptomyces europaeiscabiei was found in all in the survey.

ANOVA was performed to detect correlations between the following: Streptomyces species and geographical regions; PAI genotypes and geographical regions; species and percentage of the tuber surface covered with CS; and PAI genotypes and percentage of the tuber surface covered with CS. According to the results, there was no correlation between Streptomyces species and PAI genotype or the other factors. Furthermore, no specific pattern was observed in the geographical distribution of the species or the PAI genotypes (Table 2).

Streptomyces europaeiscabiei and *S. turgidiscabies* could be isolated from tubers with symptoms ranging from only a few superficial lesions to almost complete coverage with deep pitted lesions. No correlation was found between the percentage of tuber surface covered with CS and either Streptomyces species or PAI genotypes.

3.4 PAI marker genes

All the putative pathogenic isolates and about 10 of the non-pathogenic isolates were tested for presence of the genes nec1 and tomA, which are characteristic of the Streptomyces PAI. Amongst the isolates that were txtAB-positive, four different PAI genotypes were detected; nec1+/tomA+, nec1)tomA+, nec1+/tomA) and nec1)/tomA) (Table 2). The nec1 gene was missing in 60% of all the Streptomyces isolates and 37% of

the isolates lacked tomA. The nec1) ⁄ tomA+ PAI genotype predominated in *S. turgidiscabies*, whereas nec1) ⁄ tomA) and nec1+ ⁄ tomA+ were detected most frequently in the *S. europaeiscabiei* isolates. The combination nec1+⁄tomA+ was found in 41% of the *S. europaeiscabiei* isolates and 26% of the *S. turgidiscabies* isolates and nec1)⁄ tomA+ was observed in up to 63% of the S. turgid-iscabies isolates. The combination nec1+ ⁄ tomA) was detected in 2% of the S. europaeiscabiei isolates, but not in *S. turgidiscabies*. The combination nec1) ⁄ tomA) was found in almost half of the *S. europaeiscabiei* isolates (Table 2). None of the non-pathogenic isolates tested harboured the nec1 and tomA genes. Isolates with different combinations of the PAI marker genes nec1 and tomA were derived from the same field.

3.5 Pathogenicity assay on radish

Forty-six of the putative pathogenic Streptomyces isolates were tested for pathogenicity on radish seedlings. The ability of the bacterial isolates to inhibit radish seed germination and early seedling growth was consistent with the presence of the txtAB operon, the pathogenicity determinant (Table 3). The radish seeds that were grown with the txtAB-positive isolates showedhypertrophy or did not even germinate. By comparison, the seeds grown with txtAB-negative isolates displayed normal germination and growth, similar to the seedlings grown on OMA plateswithout bacteria (Fig. 1).

3.6 Pathogenicity test on potato

All 21 txtAB-positive isolates tested for pathogenicity on potato induced symptoms characteristic of CS on the tubers (Table 3; Fig. 2). All of the tubers harvested from pots inoculated with txtAB-positive isolates showed symptoms ranging from discrete superficial lesions to coalescing deep-pitted lesions. The scab lesions varied in appearance and severity and this symptom variation was observed between and within

species. Lesion severity was reproducible for each isolate tested in three independent replicates. All the tubers inoculated with txtAB-negative isolates were completely free of symptoms (Fig. 2). The mean surface area covered by symptoms was 8Æ5% (range 2Æ3–17Æ7%) in tubers infected with *S. europaeiscabiei*, but was 24Æ8% (5Æ0–46Æ7%) in tubers infected with *S. turgidiscabies.*

Table 2 Putative pathogenic Streptomyces isolates characterized in this study

	S. europaeiscabiei					***S. turgidiscabies***				
	PAI genotype					**`PAI genotype**				
Fields	**Isolates**	**TxN1 To**	**Tx**	**TxTo**	**TxN1**	**Isolates**	**TxN1 To**	**Tx**	**TxTo**	**TxN1**
Debiganj 1	4	4	0	0	0	2	0	0	2	0
Debiganj 2	5	3	2	0	0	2	0	0	2	0
Debiganj 3	4	4	0	0	0	2	1	0	1	0
Debiganj 4	4	2	1	1	0	0	0	0	0	0
Debiganj 5	27	6	17	4	0	15	3	2	10	0
Debiganj 6	3	0	2	1	0	3	0	1	2	0
Debiganj 7	34	5	25	2	2	3	0	1	2	0
Debiganj 8	14	3	9	0	2	4	1	0	3	0
Debiganj 9	1	0	0	1	0	3	3	0	0	0
Debiganj 10	4	0	4	0	0	0	0	0	0	0
Debiganj 11	1	1	0	0	0	0	0	0	0	0

Debiganj 12	11	3	4	3	1	10	5	3	2	0

Debiganj 13	1	0	1	0	0	4	2	0	2	0
Debiganj 14	12	11	1	0	0	4	0	1	3	0
Debiganj 15	3	1	1	1	0	4	0	0	4	0
Debiganj 16	24	14	2	8	0	15	6	0	9	0
Total	**152**	**57**	**69**	**21**	**5**	**71**	**21**	**8**	**42**	**0**

[a]PAI genotype: Tx = presence of txtAB operon; N1 = presence of nec1 gene; To = presence oftomA gene.

Chapter 4: Discussion

The present study would appear to be the most comprehensive survey of CS-causing Streptomyces species conducted in Debiganj, Rangpur thus far. The aim was to isolate and characterize plant-pathogenic Streptomyces species from CS lesions on potatoes grown in Debiganj, Rangpur. *Streptomyces europaeiscabiei* was found to be the most abundant species (69%) isolated from CS lesions in Debiganj, Rangpur, while 31% of the isolates obtained in the study were *S. turgidiscabies*. Surprisingly, *S. scabies* was not found in this study, nor were otherstreptomycetes that are pathogenic on potatoes, such *as S. acidiscabies* and *S. stelliscabiei*.

Streptomyces europaeiscabiei can be mistaken for *S. scabies* if restriction analysis of the ribosomal DNA spacer region is not performed, and thus the real global distribution of *S. scabies*might differ from the picture presented in the literature.

Considering the findings of the present study in Debiganj, Rangpur, there was no pattern in the geographical distribution of *S. europaeiscabiei* and *S. turgidiscabies*. The lack of a pattern in thegeographical distribution of *S. europaeiscabiei* and *S. turgidiscabies* or the PAI genotypes may have been caused by the use of certified seed potatoes and the subsequent dispersal of infected seed tubers throughout the Debiganj, Rangpur. This is probably because Bangladeshi seed certification standards allow 5% (by weight) surface blemishes which can suffice to spread strains around the country. Another possible explanation is that both species may be natural inhabitants of soil in Debiganj.

Streptomyces europaeiscabiei was the most abundant species isolated from CS-lesions in Debiganj, and it is also found to be the prevalent species in Western Europe (Flores-Gonzalez et al.). The present study found that *S. turgidiscabies* is widespread in Debiganj, although it is less abundant than *S. europaeiscabiei*. Streptomyces turgidiscabies , whereas

it appears that this species is absent or less common in other parts of the world, such as western Europe and North America (Bouchek-Mechiche et al. ; Flores-Gonzalez et al.; Wanner).

However, in the cited studies, 23 of all the isolates from Western Europe came from France and 84 isolates originated from several different countries in northern Europe. In Wanner's extensivecollection of 1074 txtAB-positive isolates from North America, only two isolates could be assigned to *S. turgidiscabies* (Wanner). Besides the 71 isolates of *S. turgidiscabies* described in Debiganj, the largest collections of this species were gathered in Japan and Finland, with 22 and 38 isolates, respectively (Miyajima et al.; Kreuze et al.; Lehtonen et al.). The prevalence of *S. turgidiscabies* in Debiganj may be partly explained by the climatic conditions. Hiltunen et al. suggested that *S. turgidiscabies* competes with *S. scabies* for an ecological niche and is a potentially major cause of CS in Debiganj, Rangpur.

In the present study, *S. turgidiscabies* was detected in samples from 16 different cultivars, whichconfirms that this species can infect a broad range of potato cultivars grown in Debiganj, Rangpur. Many fields harbored both *S. europaeiscabiei* and *S. turgidiscabies*, and these two species could also be found in the same lesion. This is consistent with previous studies of CS showing that a single field could be infested with multiple pathogenic Streptomyces species, andeven a single lesion could contain more than one species (Lehtonen et al.; Wanner).

The Streptomyces isolates showed variation in PAI marker genes and the dominant PAI genotypes differed between *S. europaeiscabiei* and *S. turgidiscabies*. Although nec1 and tomAare both present in *S. scabies*, they are not located in the same chromosomal regions as the thaxtomin biosynthesis gene cluster and the separate presence or absence of these regions suggests that they are independently transferable (Aittamaa et al.). Aittamaa et al. concluded from one of their studies that pathogenicity- and virulence-

related gene clusters in *S. turgidiscabies* have multiple origins and that a PAI consists of a mosaic of regions that may undergo independent evolution.

Table 3 Streptomyces isolates selected for pathogenicity tests on potato and radish

			Potato	Radish
	Isolate	Lesion type[a]	Percentage of tuber surface area covered with symptoms[b]	Pathogenic on radish seedlings
S. europaeiscabiei	08-05-02-1	3	10	+
	08-05-04-1	nt	nt	+
	08-06-03-4	nt	nt	+
	08-08-01-1	nt	nt	+
	08-08-02-1	nt	nt	+
	08-12-01-1	3	10	+
	08-15-01-1	nt	nt	+
	08-20-	nt	nt	+

	01-1			
	08-30-4b-1	3	5	+
	08-74-04-1	3	13	+
	08-88-5-1	3	5	+
	09-63-2-1	3	18	+
	09-185-2-1	3	13	+
	09-192-3-1	2	2Æ5	+
	09-196-4-2	3	6	+
	09-204-2-1	3	4	+
	09-210-2-1	3	4	+

S. turgidiscabies	1B	2	13	+
	14	nt	nt	+
	08-02-05-1a	1	18	+
	08-04-02-1a	nt	nt	+
	08-04-04-1	nt	nt	+
	08-13-01-1	3	5	+
	08-18-02-2	nt	nt	+
	08-35-01-1	1	23	+
	08-45-02-3	1	23	+
	08-54-05-1	1	11	+
	09-22-1-3	1	47	+
	09-176-3-3	1	37	+

	09-213-1-1	1	30	+
	10-129-3-1	1	40	+
Control	08-06-03-1b	0	0	)
	08-23-01-1	0	0	)
	YME[c]	0	0	)
	YME	0	0	)
	No treatment	0	0	)
	No treatment	0	0	)
	No treatment	0	0	)

[a]Lesion type: 0 = none; 1 = deep pitted lesions; 2 = superficial lesions; 3 = both deep pittedlesions and superficial lesions; nt = not tested on potato. [b]Mean percentage of tuber surfacecovered with symptoms, results from three independent replicates per isolate. [c]Yeast malt extract agar.

The results of the present study demonstrate that there is genetic variation within species andthus they do not spread simply through clonal expansion. This confirms the novel findings concerning the genetic variability within *S. europaeiscabiei* that were obtained in a previousinvestigation (Dees et al.).

Figure 1 Scab on Potato

The pathogenicity tests on potatoes revealed that, compared with *S. europaeiscabiei*, *S. turgidiscabies* induced more severe damage of the skin in general and caused symptoms over a larger proportion of the surface of tubers. The results of pathogenicity tests on

potatoes also showed that species had a greater effect than PAI genotype on the proportion of the tuber surfacedisplaying symptoms. No correlation was found between the surface area of the tuber covered with symptoms and the Streptomyces species isolated from it. Indeed, both *S. europaeiscabiei*

and *S. turgidiscabies* could be isolated from tubers showing a wide range of symptoms ranging from the presence of a few discrete lesions to coalescent lesions covering nearly the entire surface. The development of symptoms depends on a number of factors including potato cultivar,environmental conditions, and the pathogenicity and virulence of the bacterial species (Bouchek-Mechiche et al.; Wanner).

Much knowledge has been gained in recent years on various aspects of CS, including pathogenicspecies and their distribution, detection methods, mechanisms of pathogenicity, and interactions between the bacteria and the plant. Nonetheless, there are still no reliable methods for controllingCS. Disease-resistant potato cultivars would be the best and most desirable control method, but no commercially available cultivar has yet been shown to be completely resistant. In as much as CS leads to diminished market value and impaired appearance of infected tubers, this disease continues to be an important quality problem in the production of potatoes worldwide. Most of the work done with the aim of controlling CS is based on *S. scabies*, but it is possible that the other pathogenic species may respond differently to agricultural practices than *S. scabies*.
Therefore, when developing methods to manage CS, it is important, to begin with, a survey ofthe pathogenic species that are present in the country of interest.

In summary, a total of 223 putative pathogenic Streptomyces isolates were obtained from CS lesions on potato tubers originating from Debiganj, Rangpur. PCR, using species-specific primers, and restriction analysis of parts of the ITS region, identified the isolates as *S. europaeiscabiei* or *S. turgidiscabies*. The distribution of PAI genotypes amongst the isolates washigh.

It would be valuable to include the collection of plant-pathogenic streptomycetes in populationgenetic studies of isolates obtained from CS lesions. Furthermore, it might be possible to applythe new knowledge about the Debiganj Streptomyces population to develop strategies for managing CS and national breeding programs aimed at acquiring CS-tolerant varieties the most abundant biological entity on earth (or beyond, who knows!): viruses.

Chapter 5: References

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