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FINAL REPORT

PROJECT NO. I-1418-88

**Application of Recombinant DNA and
Immunological Technologies for Development
of Diagnostic Tools for Phytopathogenic
*Streptomyces Spp.***

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Application of recombinant DNA and immunological technologies
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Streptomyces spp.

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ABSTRACT

Approximately 130 pathogenic Streptomyces strains have been collected in Israel from scab lesions on potato, soil of potato fields and shell warts of peanuts. Approximately 250 strains have been collected from potato producing regions in the U.S. Results indicate that pathogenic as well as nonpathogenic strains were isolated and that most of the Israeli pathogenic strains caused deep-pitted scab. Many of the latter appeared also to cause shell warts on peanuts. Most pathogenic strains from the U.S. have characteristics typical to S. scabies and the same pathogen may cause russet, slightly raised, slightly pitted and deeply pitted scab lesions. S. acidiscabies was also isolated from several locations.

Spore chain morphology of the Israeli strains varied from spiral which is typical to S. scabies to flexuous or reticulate whereas spore ornamentation varied from smooth to spiny or warty. High diversity in biochemical characteristics of these strains have been recorded. Initial studies of numerical taxonomy have been performed. Results indicate that the majority of the pathogenic strains are grouped within the cluster named S. violaceus. However, pathogenic strains which fell within the clusters of S. griseus and S. rochei were also characterized. Considering the fact that each cluster is comprised of at least several different species, the variability is indeed substantial. Our studies also indicate no apparent separation of strains causing common scab from those causing deep scab.

Various probes and restriction enzymes for RFLPs analysis have been tested. A rRNA operon from S. coelicolor and the restriction enzymes Sall, BamH1 and BstEII were finally selected for further analysis (ribotyping). Four groups with 100% similarity coefficient could be distinguished within 29 pathogenic strains that were digested with BamH1. However, strains that significantly differed from most of the other and displayed zero similarity coefficient with many of them could also be identified. Our preliminary and limited results suggest that no correlation exists between the sorting of strains according to RFLPs and the phenotypic classification according to cluster groups.

Plasmids could only be detected in about 30% of the pathogenic strains that were examined suggesting that they neither play a role in pathogenicity nor significantly contribute to the observed genetic diversity.

Polyclonal antibodies against S. scabies were prepared in chicken. Purified antibody preparation can be used to distinguish between S. scabies and S. acidiscabies in an ELISA test. Development of monoclonal antibodies for specificity to S. scabies has been started.

Results obtained in this report lead us to conclude that genes for pathogenicity which are responsible for scab formation are distributed among different Streptomyces spp. and that the latter phenomenon might be more common than previously realized.

D. OBJECTIVES OF THE ORIGINAL RESEARCH PROPOSAL

The original objectives of the present proposal were:

- a) Isolation of a large number of pathogenic and nonpathogenic strains of Streptomyces scabies and other Streptomyces spp.
- b) Characterization of their micromorphology and biochemical properties.
- c) Detection of plasmids and giant plasmids and investigating their relationship to pathogenicity.
- d) Analysis of restriction fragment length polymorphisms (RFLPs) in pathogenic and nonpathogenic strains and devising conditions for their distinction by RFLPs.
- e) Cloning an esterase encoding gene which might be associated with pathogenic strains and adapt its utilization for diagnosis.
- f) Producing monoclonal antibodies to pathogenic strains of S. scabies and S. acidiscabies for their detection by ELISA.

The foregoing objectives were designed for a duration of 3 years. However, a feasibility grant for only one year (1989-90) was supported by BARD. During this year we largely completed objectives a and b, made a substantial progress in objective d and initiated objective f. In addition, we made some preliminary tests on the remaining objectives (c and e). Results of these studies are presented in the following report. On the basis of our results, we have resubmitted a new BARD proposal.

E. BODY OF THE REPORT

1. Biochemical Characterization and Numerical Classification of Pathogenic and Non-Pathogenic Streptomyces Strains Isolated in Israel

Introduction

Scab diseases of potatoes (Solanum tuberosum), which are characterized by deep or superficial corky lesions, are incited by Streptomyces that historically have been referred to as "Streptomyces scabies". However, certain scab pathogens may belong to other species. Additional virulent isolates have been placed in S. griseus^(1,2), S. olivaceus⁽¹⁾, S. aureofaciens⁽³⁾, S. flaveolus⁽⁴⁾ and other species^(4,5).

During the early sixties, with the intensification of potato cultivation in Israel, scab became a limiting factor in potato production in the southern part of the country⁽⁷⁾. During the following years, shallow and deep-pitted scab were controlled in Israel mainly by improved irrigation techniques as described latter by Lapwood et al.⁽⁸⁾. The appearance of severe deep-pitted scab after 1981 was associated with Streptomyces strains which appeared different from S. scabies⁽⁹⁾. Moreover, these strains were not controlled by high soil moisture as was S. scabies in earlier period. We therefore postulated that the population of the pathogenic Streptomyces strains in Israel has been changed and apparently includes strains which deviate from the classical description of S. scabies. The latter species has recently been revived by Lambert and Loria⁽¹⁰⁾.

During recent years, a new disease in peanuts appeared in Israel which was designated as "Shell warts". The disease is characterized by the appearance of a dark circular raised areas on shells of developing pods which severely reduce the market value of the infected peanuts. Preliminary studies (Kritzman, unpublished results) demonstrated that this disease is caused by isolates of Streptomyces spp. which incite deep scab.

Failure of taxonomists in the past to find reliable tests for the identification of Streptomyces has resulted in the proliferation of "species". In 1964, a collaborative study, the International Streptomyces Project (ISP), was established to furnish reliable descriptions of authentic type strains of Streptomyces and related taxa, using a small number of traditional tests applied under standard conditions^(11,12). Over 450 species were redescribed, but

attempts to compare and delineate Streptomyces species were beyond the scope of the project. Despite the limitation of the ISP, especially its narrow data base, the results were used by several workers to construct identification schemes⁽¹³⁾. In the current edition of Bergey's Manual of Determinative Bacteriology⁽¹⁴⁾, the classification of the genus Streptomyces is based upon ISP data. Thus, 463 species are classified into 43 groups based on micromorphology and pigmentation; the groups are subdivided by carbon utilization patterns.

In contrast to the traditional approach to Streptomyces taxonomy in which the recognition of taxa is based on a few subjective chosen characters, in numerical taxonomy no single character is either indispensable or sufficient to entitle an organism to group membership. Instead, as many as possible characters are characterized per each strain and a statistical analysis is applied for calculating similarity coefficients among the different strains and accordingly, their classification.

A comprehensive numerical classification of Streptomyces and related genera has been proposed in 1983 by William et al.⁽¹⁵⁾ and has been widely accepted for classification of Streptomyces into groups (clusters) and subclusters. The aim of the following study was to isolate and characterize the micromorphology and biochemical characters of pathogenic and non-pathogenic strains of Streptomyces associated with potato scab in Israel and attempt to evaluate their diversity by using numerical taxonomy.

Materials and Methods

Isolation procedures

Two methods were employed for isolation of Streptomyces from scabby potato, shell warts of peanut, or soil. The first procedure was according to Lawrence⁽¹⁶⁾ with some modifications. The infected potato tuber was first rinsed with tap water following by washing with sterile water. Internal part of the symptom area (1-3g) was aseptically removed and homogenized with a pestle and mortar in 3-6 ml of sterile water. Fifty microliters of the latter suspension were thoroughly mixed with phenol: water (1:140, V/V) and allowed to stand for 10 min. Two hundred microliters of this suspension were then spread on the surface of plates containing glucose-asparagine agar⁽¹⁶⁾. The plates were incubated at 30 °C. In an alternative procedure, ground infected tissue was incubated overnight in the presence of sterile L-ascorbic acid (5ppm) in saline

prior to isolation on asparagine-glucose medium. Isolation from soil was performed by incubating a soil sample (1-3 gr) in 5 ml of 0.1% water agar containing 0.1% metham sodium overnight prior to isolation on yeast extract-starch medium containing nalidixic acid, 10 mg polymyxine B, 1 mg; penicillin G, 200 mg; and actidion, 150 mg per liter. Each strain was originated from a single colony and stored in 20% glycerol at -13°C ⁽¹⁷⁾. Cultures were also maintained on trypton soya broth (TSB) agar⁽¹⁷⁾.

Pathogenicity test

Each Streptomyces strain was grown in 200 ml of TSB in 1 liter Erlenmyer flask. The flasks were incubated for 3-4 days on a rotary shaker at 30°C . The cultures were used to inoculate sterile perlit crumbs #1 supplemented with liquid yeast-extract-starch medium with antibiotics as described above. After 4-days of incubation at room temperature the perlit crumbs were mixed with steam-sterilized sandy soil. Potato seed tubers or peanut seeds were planted in the latter mixture and grown in the greenhouse. Appearance of scab symptoms on potato tubers or shell warts on peanut pods were tested after 3 months.

Morphology and pigmentation

Spore chain morphology and spore ornamentation were examined by light and scanning electron microscopy of 2-weeks-old culture on TSB agar. Aerial spore mass color and reverse mycelium color were examined as described by Williams et al.⁽¹⁵⁾ on the following 4 different media: Salts starch, Oatmeal, glycerol asparagine and yeast malt^(12,17).

Biochemical tests

Utilization of sugars, organic acids and various nitrogen sources were carried out by conventional procedures as described elsewhere^(11,12,14). Additional tests included growth in the presence of various concentrations of NaCl, hydrolysis of p-nitrophenyl derivatives of sugars, amino-acids and additional compounds and acid formation from different carbon sources. Resistance to various antibiotics was also tested⁽¹⁵⁾.

Results

Strains of Streptomyces spp have been obtained from various types of lesions collected from potato scab (shallow or deep-pitted lesions) shell warts of peanuts and soils of potato fields in Israel. With the isolates collected in the previous year, we have now reached approximately 130 strains. Following isolation, we have initiated to characterize their pathogenicity, micromorphology and biochemical properties. A limited characterization of a randomly selected group of strains is illustrated in Table 1.

Results indicate that pathogenic as well as non-pathogenic strains were isolated and that most of the pathogenic strains caused deep-pitted-scab. Many of the strains that cause deep-pitted-scab in potato appeared also to cause shell warts on peanut.

Revival of the name Streptomyces scabies has been recently proposed by Lambert and Loria⁽¹⁰⁾ for the predominant species causing common scab of potato. The common scab strains were identified by them on the basis of approximately 40 phenotypic criteria some of which are presented in Table 1. Results obtained in our study indicate significant variations among the strains tested in spite of the fact that only a limited number of characters have been used for their differentiation. Thus, spore chain morphology varied from spiral, which is typical to S. scabies⁽¹⁰⁾ to flexuous or reticulate, whereas spore ornamentation varied from smooth to spiny or warty among pathogenic strains (Table 1, Fig. 1). Variation in the pattern of sugar utilization could also be detected (Table 1) and was also observed in other strains tested (not shown).

The limited number of criteria for strain characterization described in Table 1 was further extended by utilizing 130 different characters which are included in Table 2. The 77 pathogenic strains that were examined by the 130 characters exhibited amplification of the variation expressed in Table 1 (results not shown) and further emphasized that the population of the Israeli pathogenic strains is phenotypically diverse and deviates from the typical S. scabies. In order to express the genetic variation within this population by conventional nomenclature we have decided to employ the numerical classification proposed by Williams et al.⁽¹⁵⁾. To achieve this goal we have initiated a close cooperation with Dr. P. Kampfer of the Technische Universitat, Berlin. Dr. Kampfer has at his disposal an automatic analyzer which can determine simultaneously a large number of characters for each of several strains.

Furthermore, the obtained data are immediately analyzed and compared with the computer-stored data base of the numerical taxonomy according to Williams et al.⁽¹⁵⁾. The 329 tests which have been used per each Streptomyces strain are summarized in Table 2.

The numerical classification of Israeli as well as strains from the U.S.A. is currently being continued. Initial results are given in Table 3 and summarized in Table 4. From the 17 strains which are presented the majority are grouped within a cluster named S. violaceus⁽¹⁵⁾. The strains of this group were isolated from either infected potatoes or infected peanuts or soil and include 2 strains obtained from our cooperating scientist, Dr. R. Loria. Although most of the strains of these groups caused deep-pitted scab on potato, 2 strains producing shallow scab and 2 non-pathogenic strains were also recorded. Three strains were clustered in a subcluster named S. griseus 2 of which caused deep scab and 1 was non-pathogenic. Again, these strains were isolated from potato, peanut or soil. One strain isolated from peanut and causing deep scab, fell within a cluster named S. rochei whereas another strain causing shallow scab could not be assigned to any group and forms a single member cluster. As indicated earlier, the numerical classification of the Israeli strains has only started. We intend to analyze many more of the Israeli strains and also to include strains from the USA in order to obtain a comprehensive view on the clustering of pathogenic Streptomyces strains.

Discussion

Analysis of the pathogenic Streptomyces strains clearly indicate a diversity in micromorphology and biochemical characteristics. In contrast to U.S.A.⁽¹⁰⁾ and common knowledge, which indicates that S. scabies predominates among the Streptomyces spp. causing potato scab, many of the isolated strains in Israel differ from the recently redescribed S. scabies⁽¹⁰⁾. Since earlier reports⁽¹⁷⁾ and records in Israel suggested that S. scabies was in past years the main scab causing species, it appears that genetic changes in the composition of the Israeli pathogenic Streptomyces population occurred during the last decade. The latter conclusion is corroborated by the appearance of shell warts in peanut in Israel. The latter is a new disease caused by Streptomyces strains responsible for deep-pitted scab production in potato.

The majority of the strains so far examined are grouped within S. violaceus cluster (Table 4). This cluster contained 118 strains and is defined at a similarity level of 81.7% (Ssm) and is mainly composed of strains from William's groups A₁₈ (S. cyanus) and A₁₉ (S. diasticus) beside many strains designated as S. chartreusis and S. viridochromogenes (Kampfer, personal communication). Three reference strains of S. scabies were also found within that cluster: S. scabies DSM 40997, DSM 40998, and DSM 41000. Interestingly, the 2 S. scabies strains obtained from R. Loria (Table 4) are also grouped within this cluster.

Additional 3 strains are clustered in a subcluster named S. griseus. This is composed mainly of strains found in subcluster AIB of Williams et al.⁽¹⁵⁾, named S. albidus. The cluster S. rochei which contained one pathogenic strain, is defined at a 81.7% level (Ssm) and contained beside the majority of strains from Williams' group A12 (S. rochei), 6 strains of group A21 (S. grieoruber) and the majority of S. violaceoruber.

Results obtained suggest that there is no apparent separation of strains causing common (shallow) scab from those causing deep scab as strains from both groups are found within different clusters. Furthermore, even from the limited data so far obtained it can be concluded that scab producing strains are highly diverse and may belong even to different clusters. Considering the fact that each cluster is composed of at least several different species defined according to the ISP nomenclature^(11,12,15) the variability is indeed substantial. The foregoing findings lead us to the hypothesis that genes for pathogenicity, which are responsible for scab formation, are distributed in different Streptomyces spp. The latter species may sometimes be taxonomically far apart since they can belong even to different clusters. However, they should have one property in common, namely intimate association with potatoes during tuber development.

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TABLE 1

Characterization of selected Streptomyces strains from Israel

Isolate	GSH	PBS1	P2	2	11	12	21	23	27	31	43	44	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP
													111	112	113	114	115	211	212	214	215	
Origin ¹	G	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	VI	
Pathogenicity ²																						
Potato	CS	*	*	*	DP	CS	DP	DP	DP	DP	DP	DP	CS	CS	NP	NP	NP	NP	NP	NP	NP	
Peanuts	NP	*	*	SW	SW	NP	SW	SW	SW	SW	SW	SW	NP	NP	NP	NP	NP	NP	NP	NP	NP	
Spore mass color ³ on																						
Salts starch medium	W	-	-	-	-	-	W	-	-	-	-	-	-	-	-	-	W	-	-	-	-	
Oatmeal medium	GY	W	*	W	W	W	-	W	W	W	W	W	GY	W	W	W	-	W	-	-	W	
Glycerol asparagine medium	W	W	W	-	-	W	W	W	-	W	W	W	GY	GY	W	W	-	W	-	GY	W	
Yeast malt medium	GY	W	GY	W	GY	W	W	GY	GY	GY	GY	GY	GY	GY	GY	GY	-	GY	GY	GY	W	
Reverse mycelium color ³ on																						
Salts starch medium	Y	BR	Y	BR	Y	BR	Y	Y	BR	R	BR	W	R	R	W	R	R	BR	BR	-	Y	
Oatmeal medium	BR	W	*	Y	BR	BR	BR	BR	Y	R	BR	W	R	R	GY	R	R	GY	BR	W	Y	
Glycerol asparagine medium	BR	BR	BR	BR	BR	BR	BR	BR	Y	R	BR	GY	R	R	BR	R	R	Y	BR	BR	GY	
Yeast malt medium	GY	W	GY	W	GY	W	W	GY	GY	GY	GY	GY	GY	GY	GY	GY	-	GY	GY	GY	W	
Spore chain morphology ⁴	SP	F	RT	SP	RT	SP	R	SP	SP	SP	SP	SP	SP	R	R	VE	SP	RT	SP	SP	SP	
Spore ornamentation ⁵	SM	SM	SY	SM	SY	SM	SM	SM	SM	WA	SM	SM	SM	SM	SM	HA	SY	SM	SM	SY	SM	
Diffusable pigment ⁶	BR	BR	*	Y	*	BR	BR	BR	BRY	BRR	BR	BR	BRR	BRR	BRY	BRR	BRR	BR	BR	BR	*	
Carbon source usage ⁷																						
L-arabinose	++	++	+	++	+	++	+	++	++	++	+	++	++	+	++	+	+	+	++	++	+	
D-fructose	++	++	+	-	+	+	++	+	++	++	++	++	++	++	+	++	++	+	++	+	++	
D-glucose	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	-	++	++	++	++	
D-mannitol	+	++	+	++	+	++	++	++	++	-	++	++	++	++	+	-	+	++	+	±	++	
raffinose	-	±	±	±	±	-	-	-	++	+	±	±	++	++	+	+	++	++	+	++	+	
rhamnose	++	++	++	++	++	+	+	+	++	++	++	++	+	++	++	++	++	+	++	++	++	
sucrose	++	+	+	-	-	-	++	±	++	+	+	++	++	++	+	++	+	++	+	+	+	
D-xvlose	++	++	+	++	+	+	+	+	++	+	+	++	++	++	+	++	++	++	+	++	++	
meso-inositol	-	-	+	++	+	-	±	-	++	++	++	++	+	++	+	+	++	++	++	+	++	

- * - not detected
¹ VI- Dr. Kritzman, Volcani Center Israel; G - Prof. Schrempf, Germany
² CS- common scab; DP- deep pitted scab; SW- shell's warts; NP- non pathogenic.
³ GY- grey; W- white; Y- yellow; BR-brown; BRR-brown red; BRY- BROWN YELLOW; - no sporulation within 3 weeks.
⁴ F - flexuous; RT- reticulate; SP- spirale.
⁵ SM- smooth; SY- spiny HA- hairy; WA- warty.
⁶ - - negative utilization; ± - utilization doubtful; + positive utilization; ++ - strongly positive utilization

TABLE 2 Biochemical tests used for numerical taxonomy

Utilization of:

AGA	N-ACETYL-D-GALACTOSAMINE	IBU	ISOBUTYRATE
AGL	N-ACETYL-D-GLUCOSAMINE	IVA	ISOVALERATE
AMY	AMYGDALIN	PRO	PROPIONATE
DAR	D-ARABINOSE	SBS	SORBATE
LAR	L-ARABINOSE	ACT	cis-ACONITATE
ARS	ARABONIC ACID	ACO	trans-ACONITATE
ARB	p-ARBUTIN	ADI	ADIPATE
CEL	D-CELLOBIOSE	AZE	AZELATE
DEX	DEXTRANE	CTR	CITRACONATE
FRU	D-FRUCTOSE	CIT	CITRATE
DFU	D-FUCOSE	FUM	FUMARATE
LFU	L-FUCOSE	GLT	GLUTARATE
GAL	D-GALACTOSE	GCT	GLYCERATE
GAK	alpha-D-GALACTURONAT	GOP	GLYCEROPHOSPHATE
GSÄ	D-GLUCARATE	GOC	GLYCOLATE
GLK	GLUCONATE	GYX	GLYOXYLATE
GLA	D-GLUCOSAMINE	3HB	DL-3-HYDROXYBUTYRATE
GLÄ	D-GLUCOSAMINIC ACID	4HB	4-HYDROXYBUTYRATE
GLU	D-GLUCOSE	HIB	DL-2-HYDROXYISOBUTYRATE
GUÄ	D-GLUCURONATE	HIC	L-2-HYDROXYISOCAPRATE
GCV	GLYCOGEN	HIV	DL-2-HYDROXYISOVALERATE
GYV	GLYCYRRHIZINATE	2HV	2-HYDROXYVALERATE
INU	INULIN	ICT	DL-ISOCITRATE
LAC	LACTOSE	ITA	ITACONATE
LCU	LACTULOSE	LTT	D-LACTATE
DLY	D-LYXOSE	LCT	DL-LACTATE
LLY	L-LYXOSE	LTC	L-LACTATE
MAL	D-MALTOSE	LAE	LAEVULINATE
MAN	D-MANNOSE	MAA	DL-MALATE
MLN	L-MANNOSE	MAT	L-MALATE
MHP	D-MANNOHEPTULOSE	MEA	MALEATE
MEZ	alpha-D-MELEZITOSE	MAO	MALONATE
MEL	alpha-D-MELIBIOSE	MES	MESACONATE
MMA	alpha-METHYL-D-MANNOSIDE	MEO	MESOXALATE
MGL	METHYL-alpha-D-GLUCOPYRANOSIDE	OXA	OXALACETATE
MXL	BETA-METHYL-D-XYLOSIDE	OGT	2-OXOGLUTARATE
MUC	MUCATE	OIC	2-OXOISOCAPRATE
UGL	5-OXOGLUCONATE	PIM	PIMELATE
RAF	alpha-D-RAFFINOSE	PYR	PYRUVATE
RHA	L-RHAMNOSE	SUB	SUBERATE
RIB	D-RIBOSE	SUC	SUCCINATE
SAC	D-SUCROSE	DTA	D-TARTRATE
SAL	SALICIN	MTA	m-TARTRATE
SOR	L-SORBOSE	LTA	L-TARTRATE
STÄ	STARCH	TRT	TARTRONATE
TAG	D-TAGATOSE	1BU	1-BUTANOL
TRE	D-TREHALOSE	2BU	2-BUTANOL
TUR	D-TURANOSE	ETH	ETHANOL
DXV	D-XYLOSE	HEX	1-HEXANOL
LXY	L-XYLOSE	OCT	1,8-OCTANDIOL
ADO	ADONITOL	AAC	ACETAMIDOCAPRATE
DAT	D-ARABITOL	AGT	ACETYL-L-GLUTAMATE
LAT	L-ARABITOL	AGM	ACETYL-L-GLUTAMINE
DUL	DULCITOL	AGY	ACETYL-GLYCINE
ERY	m-ERYTHRITOL	AGM	ACETYL-DL-METHIONINE
GLY	GLYCEROL	AGP	ACETYL-L-PROLINE
INO	i-INOSITOL	DAL	D-ALANINE
MLT	MALTITOL	LAL	L-ALANINE
MNT	D-MANNITOL	BAL	BETA-ALANINE
TRB	TRIBUTYRINE	2AA	DL-2-AMINOADIPATE
XYT	D-XYLITOL	2AB	DL-2-AMINOBYTYRATE
ACE	ACETATE	3AB	DL-3-AMINOBYTYRATE
BUT	BUTYRATE	2AI	DL-2-AMINOISOBUTYRATE
		4AB	4-AMINOBYTYRATE
		AMX	AMINOXYACETATE

ADG D-ARGININE
 ARG L-ARGININE
 ADN D-ASPARAGINE
 ASN L-ASPARAGINE
 ASP L-ASPARTATE
 APT DL-ASPARTATE
 BET BETAINE
 CAS CASEIN
 CYI L-CYSTEINATE
 CTU L-CITRULLINE
 DIB DL-2,4-DIAMINO BUTYRATE
 DAP DIAMINOPIMELATE
 DIP 2,3-DIAMINOPROPIONATE
 DMG DIMETHYLGLYCINE
 GLÄ D-GLUTAMATE
 GLM L-GLUTAMATE
 GLN L-GLUTAMINE
 GCI L-GLYCINE
 GCA GLYCINANHYDRID
 DHI D-HISTIDINE
 HIS L-HISTIDINE
 HOM L-HOMOSERINE
 HLL DL-delta-HYDROXYLYSINE
 HPL L-HYDROXYPROLINE
 DIS DL-ISOLEUCINE
 ISL L-ISOLEUCINE
 DLE D-LEUCINE
 LEU L-LEUCINE
 DLY D-LYSINE
 LYS L-LYSINE
 DME D-METHIONINE
 MET L-METHIONINE
 MSU DL-METHIONINESULFONE
 NOR L-NORLEUCINE
 DNO D-NORVALINE
 DNV L-NORVALINE
 ORN L-ORNITHINE
 DPH D-PHENYLALANINE
 PHE L-PHENYLALANINE
 PHG PHENYLGLYCINE
 DPR D-PROLINE
 PRO L-PROLINE
 SSI SARCOSINE
 DSE D-SERINE
 SER L-SERINE
 THR L-THREONINE
 DTR D-TRYPTOPHANE
 LTR L-TRYPTOPHANE
 VAL L-VALINE
 BUY BUTYLAMINE
 CAD CADAVERINE
 CTN CARNITINE
 CRI CREATINE
 CTI CREATININE
 EOA ETHANOLAMINE
 ELA ETHYLAMINE
 URE UREA
 HEY HEXYLAMINE
 PUT PUTRESCINE
 SPD SPERMIDINE
 SPM SPERMINE
 TAU TAURINE
 TRG TRIGONELLINE
 TRP TRYPTAMINE
 TYM TYRAMINE
 AAD ACETAMIDE
 ALL ALLANTOINE

CYO CYTOSINE
 GCD GLYCINAMIDE
 HPP HIPPURATE
 I3A INDOL-3-ACETATE
 LLA L-LEUCINAMIDE
 OAS OXAMID ACID
 SAY SALICYLAMIDE
 THI THIAMINE
 URC URACIL
 URI UREIDOSUCCINATE
 LVA L-VALINAMIDE
 2AZ ANTHRANILATE
 3AZ 3-AMINO BENZOATE
 4AZ 4-AMINO BENZOATE
 BEN BENZOATE
 F2C FURAN-2-CARBONIC ACID
 3HY 3-HYDROXYBENZOATE
 4HY 4-HYDROXYBENZOATE
 2AC 2-HYDROXYPHENYLACETATE
 3AC 3-HYDROXYPHENYLACETATE
 4AC 4-HYDROXYPHENYLACETATE
 IPH ISOPHTHALATE
 DMA D-MANDELATE
 LMA L-MANDELATE
 PXY PHENOXYACETATE
 PAC PHENYLACETATE
 PLA PHENYLLACTATE
 PPR PHENYLPROPIONATE
 PPY PHENYLPYRUVATE
 PHT PHTHALATE
 PCA PROTOCATECHUATE
 QUI QUINATE
 SAI SALICYLATE
 TRO TROPIC ACID
 VAN VANILLATE

Growth in presence of:

NA1 NACL 1% + GLUCOSE
 NA2 NACL 2% + GLUCOSE
 NA3 NACL 5% + GLUCOSE
 NA4 NACL 7% + GLUCOSE
 NA5 NACL 10%+ GLUCOSE
 NZ1 NAZ 0.005% + GLUCOSE
 NZ2 NAZ 0.01% + GLUCOSE
 NZ3 NAZ 0.02% + GLUCOSE

Hydrolysis of:

CAK pNP-N-ACETYL-beta-D-GALAKT
 CAG pNP-N-ACETYL-alpha-D-GLUCO
 CBG pNP-N-ACETYL-beta-D-GLUCOS
 CAP pNP-alpha-L-ARABINOPYRANOS
 CAC pNP-beta-D-CELLOBIOSIDE
 CAF pNP-alpha-L-FUCOPYRANOSIDE
 CBF pNP-beta-L-FUCOPYRANOSIDE
 CGB pNP-beta-D-GALACTOPYRANOSID
 CGU pNP-beta-D-GALACURONIDE
 CAU pNP-alpha-D-GLUCOPYRANOSID
 CBU pNP-beta-D-GLUCOPYRANOSIDE
 CGL pNP-beta-D-GLUCURONIDE
 CLA pNP-beta-D-LACTOSIDE
 CMN pNP-alpha-D-MANNOPYRANOSID
 CML pNP-alpha-D-MALTOSIDE
 CBL pNP-beta-D-MALTOSIDE
 CEM pNP-beta-D-MELIBIOSE

CRH pNP- α -L-RHAMNOPYRANOSIDE
CBX pNP- β -D-XYLOSIDE
CSU pNP-SULPHATE
CP3 pNP-PHOSPHATE pH7 CHA-Salt
CP4 pNP-PHOSPHATE pH5.4 Ph-Eth-Sa.
CP5 pNP-PHOSPHATE pH8.2 Ph-Eth-Sa.
CBP BIS-pNP-PHOSPHATE
CPP pNP-PHENYL-PHOSPHONATE
CPC pNP-PHOSPHORYL CHOLINE
CDH 2-DEOXYTHYMIDINE-5'-pNP-PHOSP.
CAN ACETYL-L-ALANINE-pNA
CEU ACETYL-L-LEUCINE-pNA
CAA D-ALANINE-pNA
CAL L-ALANINE-pNA
CAG L-ARGININE-pNA
CBA BENZOYL-DL-ARGININE-pNA
CGU GLUTAMATE-1-pNA
CGM γ -L-GLUTAMATE-pNA
CGB L-GLUTAMATE- γ -3-CARB.-pNA
CCY GLYCINE-pNA
CLY L-LYSINE-pNA
CHE L-PHENYLALANINE-pNA
CPR L-PROLINE-pNA
CSP SUCCINYL-L-PHENYLALANINE-pNA
CVA L-VALINE-pNA

Z40 DULCITOL
Z41 m-ERYTHRITOL
Z42 GLYCEROL
Z43 i-INOSITOL
Z44 MALTITOL
Z45 D-MANNITOL
Z46 D-XYLITOL
Z47 TRIBUTYRIN

Acid formation from:

Z01 N-ACETYL-D-GLUCOSAMINE
Z02 D-ARABINOSE
Z03 L-ARABINOSE
Z04 ARABONIC ACID
Z05 D-CELLOBIOSE
Z06 DEXTRANE
Z07 D-FRUCTOSE
Z08 D-GALACTOSE
Z09 α -D-GALACTURONATE
Z10 GLUCONATE
Z11 D-GLUCOSAMINE
Z12 D-GLUCOSE
Z13 D-GLUCURONATE
Z14 GLYCOGEN
Z15 INULIN
Z16 LACTOSE
Z17 LACTOBIONIC ACID
Z18 LACTULOSE
Z19 L-LYXOSE
Z20 D-MALTOSE
Z21 D-MANNOSE
Z22 α -D-MELEZITOSE
Z23 α -D-MELIBIOSE
Z24 α -METHYL-D-MANNOSIDE
Z25 METHYL- α -D-GLUCOPYRANOSIDE
Z26 β -METHYL-D-XYLOSIDE
Z27 MUCATE
Z28 α -D-RAFFINOSE
Z29 L-RHAMNOSE
Z30 D-RIBOSE
Z31 D-SUCROSE
Z32 SALICIN
Z33 L-SORBOSE
Z34 STARCH
Z35 D-TAGATOSE
Z36 D-TREHALOSE
Z37 D-TURANOSE
Z38 D-XYLOSE
Z39 L-XYLOSE

TABLE 3: Biochemical characterization of Streptomyces strains.
The description of tests and their abbreviations are described in Table 2.
In order to save space only, the data for the clusters of S. rochei and
S. violaceus are presented. The rest will be provided upon request.

1 : S.rochei like (2)

1000	AGA	AGL	AMY	DAR	LAR	ARS	ARB	CEL	DEX	FRU	DFU	LFU	GAL	GAK	GSÄ	GLK	GLA
DAA2108	+	+	-	-	+	-	+	+	-	+	-	+	+	-	+	+	+
DAA2109	-	+	+	-	+	-	+	+	-	+	-	-	+	+	-	+	+
1000	GLÄ	GLU	GUÄ	GCY	GYG	INU	LAC	LCU	DLY	LLY	MAL	MAN	MLN	MHP	MEZ	MEL	MMA
DAA2108	-	+	+	+	-	+	+	+	+	+	+	+	-	-	-	-	+
DAA2109	+	+	+	+	-	+	+	+	+	-	+	+	-	-	-	+	+
1000	MGL	MXL	MUC	UGL	RAF	RHA	RIB	SAC	SAL	SOR	STÄ	TAG	TRE	TUR	DXY	LXY	ADO
DAA2108	-	-	+	+	-	+	+	-	+	-	+	-	+	+	+	-	-
DAA2109	+	-	-	-	-	+	+	+	+	-	+	-	+	+	+	-	-
1000	DAT	LAT	DUL	ERY	GLY	INO	MLT	MNT	TRB	XYT	ACE	BUT	CAP	HEP	IBU	IVA	PRO
DAA2108	+	-	-	-	+	+	-	+	-	-	+	+	+	+	+	+	+
DAA2109	+	-	-	-	+	+	+	+	-	+	+	-	+	-	-	-	+
1000	SBS	ACT	ACO	ADI	AZE	CTR	CIT	FUM	GLT	GCT	GOP	GOC	GYX	3HB	4HB	HIB	HIC
DAA2108	+	+	-	-	+	-	+	+	-	-	-	-	+	+	-	-	-
DAA2109	-	-	-	-	-	-	+	+	-	+	-	-	+	+	-	-	-
1000	HIV	2HV	ICT	ITA	LTT	LCT	LTC	LAE	MAA	MAT	MEA	MAO	MES	MEQ	OXA	OGT	OIC
DAA2108	-	+	+	-	-	-	+	+	-	+	-	+	-	+	+	+	+
DAA2109	-	+	+	-	-	+	-	+	+	+	-	+	-	-	+	+	+
1000	PIM	PYR	SUB	SUC	DTA	MTA	LTA	TRT	1BU	2BU	ETH	HEX	OCT	AAC	AGT	AGM	AGY
DAA2108	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-
DAA2109	-	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-
1000	AGM	AGP	DAL	LAL	BAL	2AA	2AB	3AB	2AI	4AB	AMX	ADG	ARG	ADN	ASN	ASP	APT
DAA2108	-	-	-	+	+	-	+	+	-	+	-	-	+	-	+	+	+
DAA2109	-	-	-	+	+	-	-	-	-	+	-	-	+	-	+	+	-
1000	BET	CAS	CYI	CTU	DIB	DAP	DIP	DMG	GLÄ	GLM	GLN	GCI	GCA	DHI	HIS	HOM	HLL
DAA2108	-	+	+	+	-	-	-	-	+	+	+	+	-	-	+	-	-
DAA2109	-	+	-	+	-	-	-	-	+	+	+	+	-	-	+	-	-
1000	HPL	DIS	ISL	DLE	LEU	DLY	LYS	DME	MET	MSU	NOR	DNO	DNV	ORN	DPH	PHE	PHG
DAA2108	+	+	+	-	+	-	+	-	-	-	-	-	-	+	-	+	-
DAA2109	-	-	+	-	+	-	-	-	-	-	-	-	-	+	-	+	-
1000	DPR	PRO	SSI	DSE	SER	THR	DTR	LTR	VAL	BUY	CAD	CTN	CRI	CTI	EOA	ELA	URE

DAA2109	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
- 16 -																	
1000	THI	URC	URI	LVA	2AZ	3AZ	4AZ	BEN	F2C	3HY	4HY	2AC	3AC	4AC	IPH	DMA	LMA
DAA2108	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
DAA2109	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
1000	PXY	PAC	PLA	PPR	PPY	PHT	PCA	QUI	SAI	TRO	VAN	NA1	NA2	NA3	NA4	NA5	NZ1
DAA2108	-	+	-	-	+	-	-	-	-	-	-	+	+	+	+	+	+
DAA2109	-	+	+	-	+	-	-	-	-	-	-	+	+	+	+	+	+
1000	NZ2	NZ3	CAK	CAG	CBG	CAP	CAC	CAF	CBF	CGB	CGU	CAU	CBU	CGL	CLA	CMN	CML
DAA2108	+	+	+	-	+	+	-	-	-	+	-	+	+	-	+	+	+
DAA2109	+	+	+	-	+	+	+	-	-	+	-	+	+	-	+	+	+
1000	CBL	CEM	CRH	CBX	CSU	CP3	CP4	CP5	CBP	CPP	CPC	CDH	CAN	CEU	CAA	CAL	CAG
DAA2108	+	-	+	+	-	+	+	+	+	+	+	+	-	-	+	+	+
DAA2109	+	+	-	+	-	+	+	+	+	+	+	+	-	-	+	+	+
1000	CBA	CGU	CGM	CGB	CCY	CLY	CHE	CPR	CSP	CVA	Z01	Z02	Z03	Z04	Z05	Z06	Z07
DAA2108	-	+	+	+	+	-	-	+	-	-	+	-	-	-	-	-	+
DAA2109	-	+	+	+	+	+	-	+	+	-	+	-	-	-	+	-	+
1000	Z08	Z09	Z10	Z11	Z12	Z13	Z14	Z15	Z16	Z17	Z18	Z19	Z20	Z21	Z22	Z23	Z24
DAA2108	+	-	-	-	+	-	-	-	+	+	-	-	-	+	-	-	-
DAA2109	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
1000	Z25	Z26	Z27	Z28	Z29	Z30	Z31	Z32	Z33	Z34	Z35	Z36	Z37	Z38	Z39	Z40	Z41
DAA2108	-	-	-	-	+	+	-	-	-	+	-	-	-	-	-	-	-
DAA2109	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-
1000	Z42	Z43	Z44	Z45	Z46	Z47											
DAA2108	+	-	-	-	-	-											
1000	Z42	Z43	Z44	Z45	Z46	Z47											
DAA2109	+	+	-	-	-	-											

2 : S.violaceus like (13)

1000	AGA	AGL	AMY	DAR	LAR	ARS	ARB	CEL	DEX	FRU	DFU	LFU	GAL	GAK	GSÄ	GLK	GLA
DAA2104	+	+	+	-	+	-	+	+	-	+	-	-	+	+	-	+	+
DAA2106	+	+	-	+	+	-	-	+	-	+	-	+	+	-	+	+	+
DAA2110	+	+	+	-	+	-	+	+	+	+	-	+	+	+	-	+	+
DAA2111	+	+	+	-	+	-	+	+	+	+	-	+	+	+	-	+	+
DAA2112	+	+	-	+	+	-	+	+	-	+	-	+	+	+	-	+	+
DAA2113	+	+	+	-	+	-	-	+	-	+	-	-	+	-	-	+	+
DAA2114	-	+	+	-	+	-	+	+	-	+	-	+	+	-	-	+	+
DAA2115	-	+	+	-	+	-	+	+	+	+	-	+	+	-	-	+	+
DAA2116	-	+	+	-	+	-	+	+	+	+	-	-	+	-	-	+	+
DAA2117	+	+	+	+	+	-	+	+	-	+	-	+	+	-	-	+	+
DAA2119	+	+	-	-	+	-	+	+	+	+	-	-	+	-	-	+	+
DAA2120	+	+	-	+	+	+	-	+	-	+	-	+	+	+	-	+	+
DAA2121	+	+	+	-	+	-	+	+	+	+	-	+	+	+	-	+	+

DAA2112	-	+	+	+	-	+	-	+	-	+	+	+	+	+	-	+	-
DAA2113	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	-
DAA2114	+	+	+	+	-	+	-	+	-	+	+	+	+	+	-	-	+
DAA2115	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	-	-
DAA2116	-	+	+	+	-	+	+	+	-	+	+	+	+	+	-	-	-
DAA2117	-	+	-	+	-	+	+	+	-	+	+	+	+	+	-	-	-
DAA2119	-	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	+
DAA2120	+	+	+	+	-	+	+	+	-	-	+	+	+	+	-	-	+
DAA2121	+	+	+	+	-	+	+	+	+	-	+	+	+	+	-	-	+

1000 |MGL|MXL|MUC|OGL|RAF|RHA|RIB|SAC|SAL|SOR|STÄ|TAG|TRE|TUR|DXY|LXY|ADO

DAA2104	+	-	+	-	-	+	+	-	+	-	+	-	+	-	+	-	+
DAA2106	-	-	+	+	+	+	+	+	+	-	+	-	+	-	+	-	+
DAA2110	-	-	-	-	-	+	+	-	+	-	+	-	+	+	+	-	+
DAA2111	+	-	-	-	-	+	+	-	+	-	+	-	+	+	+	-	-
DAA2112	-	-	+	+	+	+	+	+	+	-	+	-	+	-	+	-	-
DAA2113	-	-	-	-	-	+	+	-	+	-	+	-	+	-	+	-	-
DAA2114	-	-	-	+	+	+	+	+	+	-	+	-	+	-	+	-	-
DAA2115	+	-	-	+	+	+	+	+	+	-	+	-	+	+	+	-	-
DAA2116	+	-	+	+	+	+	+	+	+	-	+	-	+	+	+	-	+
DAA2117	-	-	-	-	-	+	+	-	+	-	+	-	+	-	+	-	-
DAA2119	+	+	+	-	-	+	+	+	+	-	+	-	+	+	+	-	-
DAA2120	-	-	-	-	+	+	+	+	-	-	+	-	+	-	+	-	-
DAA2121	+	-	-	-	-	+	+	-	+	-	+	-	+	+	+	-	-

1000 |DAT|LAT|DUL|ERY|GLY|INO|MLT|MNT|TRB|XYT|ACE|BUT|CAP|HEP|IBU|IVA|PRO

DAA2104	+	-	-	-	+	-	-	+	-	-	-	+	+	-	+	+	-
DAA2106	+	-	-	-	+	+	-	+	-	-	+	+	+	-	+	+	-
DAA2110	+	-	-	-	+	+	+	+	-	-	+	+	+	-	+	+	+
DAA2111	+	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+
DAA2112	+	-	-	-	+	+	-	+	-	-	+	+	-	-	+	+	+
DAA2113	+	-	-	-	+	+	-	+	-	-	+	+	+	-	+	+	+
DAA2114	+	-	-	-	+	+	-	-	+	-	+	-	+	-	+	+	+
DAA2115	+	-	-	-	+	+	+	+	-	-	+	+	-	-	+	+	+
DAA2116	-	-	-	-	+	+	+	-	-	-	+	+	-	-	+	+	+
DAA2117	+	-	-	-	+	+	-	+	-	-	+	+	+	-	+	+	+
DAA2119	+	-	-	-	+	+	+	+	-	-	+	+	+	+	+	+	+
DAA2120	-	-	-	-	+	+	-	-	-	-	+	+	+	-	+	+	+
DAA2121	+	-	-	-	+	+	+	+	-	-	+	+	+	-	+	+	+

1000 |SBS|ACT|ACO|ADI|AZE|CTR|CIT|FUM|GLT|GCT|GOP|GOC|GYX|3HB|4HB|HIB|HIC

DAA2104	-	+	-	-	+	-	+	+	-	+	-	-	+	+	-	-	-
DAA2106	-	+	-	-	-	-	+	+	-	+	-	-	-	+	-	-	-
DAA2110	+	+	-	+	+	-	+	+	+	+	-	-	+	+	-	-	-
DAA2111	+	+	-	+	+	-	+	+	-	-	-	-	+	+	-	-	-
DAA2112	-	-	-	+	+	-	+	+	-	+	+	-	+	+	-	-	-
DAA2113	+	-	-	+	-	-	+	+	-	+	-	-	+	+	-	-	-
DAA2114	-	+	-	+	-	-	+	+	-	+	-	-	+	+	-	-	-
DAA2115	-	-	-	+	+	+	+	+	-	-	-	-	+	+	-	-	-
DAA2116	-	-	-	-	-	-	+	+	-	+	-	-	+	+	-	-	-
DAA2117	+	-	-	+	+	-	+	+	-	+	-	-	+	+	-	-	-
DAA2119	+	+	-	-	-	-	+	+	-	-	-	-	+	+	-	-	-
DAA2120	-	-	-	+	+	-	+	+	-	+	-	-	-	+	-	-	-
DAA2121	+	+	-	+	+	-	+	+	-	+	-	-	+	+	-	-	+

1000 |HIV|2HV|ICT|ITA|LTT|LCT|LTC|LAE|MAA|MAT|MEA|MAO|MES|MEO|OXA|OGT|OIC

DAA2104	-	-	-	-	-	+	-	+	-	+	-	-	-	+	+	+	+
DAA2106	-	+	-	-	-	-	-	+	+	+	-	+	-	-	+	+	-
DAA2110	-	+	+	-	-	+	-	+	+	+	-	+	+	+	+	+	+
DAA2111	-	+	+	-	-	+	-	+	-	+	-	-	-	-	+	+	+
DAA2112	-	+	-	-	-	+	+	+	-	+	+	-	+	-	+	+	+
DAA2113	-	+	-	-	-	+	-	+	-	+	-	+	-	-	+	+	+

1000 | PIM | PYR | SUB | SUC | DTA | MTA | LTA | TRT | 1BU | 2BU | ETH | HEX | OCT | AAC | AGT | AGM | AGY

DAA2104	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DAA2106	-	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-	-
DAA2110	+	+	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-
DAA2111	-	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	+
DAA2112	-	+	+	+	+	-	-	-	-	-	-	-	-	+	-	+	-
DAA2113	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-
DAA2114	+	+	-	+	+	-	-	-	-	-	-	-	+	+	-	+	-
DAA2115	+	+	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-
DAA2116	+	+	+	+	+	-	-	-	-	-	-	-	+	+	-	-	-
DAA2117	-	+	+	+	-	-	-	-	-	-	-	-	+	-	-	-	-
DAA2119	-	+	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-
DAA2120	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	-
DAA2121	+	+	+	+	-	-	-	-	-	-	-	-	+	+	-	-	+

1000 | AGM | AGP | DAL | LAL | BAL | 2AA | 2AB | 3AB | 2AI | 4AB | AMX | ADG | ARG | ADN | ASN | ASP | APT

DAA2104	-	-	-	+	+	-	-	-	-	+	-	-	+	-	+	-	-
DAA2106	-	-	+	+	+	-	-	-	-	+	-	-	+	-	+	+	+
DAA2110	-	-	-	+	+	-	-	+	-	+	-	-	+	-	+	+	+
DAA2111	+	-	-	+	+	-	-	-	-	+	-	-	+	-	+	+	-
DAA2112	-	-	+	+	+	-	-	+	-	+	-	-	+	-	+	+	+
DAA2113	-	-	-	+	+	-	-	+	-	+	-	-	+	+	+	+	+
DAA2114	-	-	+	+	+	-	-	-	-	+	-	-	+	-	+	+	-
DAA2115	-	-	-	+	+	-	-	-	-	+	-	-	+	-	+	+	+
DAA2116	-	-	-	+	+	-	-	-	-	+	-	-	+	-	+	+	+
DAA2117	+	-	-	+	+	-	-	+	-	+	-	-	+	-	+	+	+
DAA2119	-	-	-	+	+	-	-	-	-	+	-	-	+	-	+	+	-
DAA2120	-	-	+	+	+	+	+	-	-	-	-	-	+	-	+	+	-
DAA2121	-	-	-	+	+	+	+	+	-	+	-	-	+	-	+	+	+

1000 | BET | CAS | CYI | CTU | DIB | DAP | DIP | DMG | GLA | GLM | GLN | GCI | GCA | DHI | HIS | HOM | HLL

DAA2104	-	+	-	-	-	-	-	-	-	+	+	+	-	-	+	-	-
DAA2106	+	+	-	+	-	-	-	-	+	+	+	+	-	-	+	-	-
DAA2110	-	+	-	+	-	-	-	-	-	+	+	-	-	-	+	-	-
DAA2111	-	+	-	+	-	-	-	-	-	+	+	-	-	-	+	-	-
DAA2112	-	+	-	+	-	-	-	-	-	+	+	+	-	-	+	-	-
DAA2113	-	+	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-
DAA2114	+	+	-	-	-	-	-	-	-	+	+	+	+	-	+	-	-
DAA2115	-	+	-	+	-	-	-	-	+	+	+	+	-	-	+	-	-
DAA2116	-	+	-	+	-	-	-	-	+	+	+	+	-	-	+	-	-
DAA2117	-	+	-	-	-	-	-	-	+	+	+	+	-	-	+	-	-
DAA2119	-	+	-	-	-	-	-	-	+	+	+	-	-	-	+	-	-
DAA2120	-	+	+	+	-	-	-	-	-	+	+	+	-	-	+	-	-
DAA2121	-	+	+	+	-	-	-	-	+	+	+	+	-	-	+	-	-

1000 | HPL | DIS | ISL | DLE | LEU | DLY | LYS | DME | MET | MSU | NOR | DNO | DNV | ORN | DPH | PHE | PHG

DAA2104	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	+	-
DAA2106	+	+	+	-	-	-	+	-	-	-	-	-	-	+	+	+	-
DAA2110	+	+	+	-	-	-	+	-	+	-	-	-	-	+	-	+	-
DAA2111	+	+	+	-	+	-	+	-	+	-	-	-	-	+	-	+	-
DAA2112	+	+	+	-	+	-	+	-	-	-	-	-	-	+	-	+	-
DAA2113	-	+	+	-	+	-	-	-	-	-	-	-	-	+	-	+	-
DAA2114	+	+	+	-	+	-	+	-	-	-	-	-	-	+	+	+	-
DAA2115	+	+	+	-	+	-	-	-	-	-	-	-	-	+	-	+	-
DAA2116	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	+	-
DAA2117	-	-	+	-	+	-	+	-	+	-	+	-	+	-	-	+	-
DAA2119	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-	+	-
DAA2120	+	-	+	-	+	-	+	-	-	-	+	-	-	+	-	+	-
DAA2121	+	+	+	-	+	-	-	-	-	+	+	-	+	+	-	+	-

DAA2113	-	+	-	-	+	+	-	19	+	-	-	-	-	-	+	-	-
DAA2114	-	+	-	-	+	+	-	+	+	-	+	-	-	-	+	-	-
DAA2115	-	+	-	-	+	+	-	+	+	-	+	-	-	-	+	-	-
DAA2116	-	+	-	-	+	+	-	+	+	-	+	-	-	-	+	-	-
DAA2117	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-
DAA2119	-	+	-	-	+	+	-	-	+	-	-	-	-	-	-	-	-
DAA2120	-	+	-	-	+	+	-	+	+	-	+	-	-	-	+	-	-
DAA2121	-	+	-	-	+	+	-	+	+	-	+	-	-	-	+	-	-

1000 |HEY|PUT|SPD|SPM|TAU|TRG|TRP|TYM|AAD|ALL|CYO|GCD|HPP|I3A|LLA|OAS|SAY

DAA2104	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
DAA2106	-	+	+	-	-	-	-	+	-	+	-	+	-	-	+	-	-
DAA2110	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
DAA2111	-	+	+	-	-	-	-	-	-	-	-	-	-	+	+	-	-
DAA2112	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
DAA2113	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
DAA2114	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-	-	-
DAA2115	-	+	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-
DAA2116	-	+	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-
DAA2117	-	+	+	-	-	-	-	-	-	-	-	-	+	-	+	-	-
DAA2119	-	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
DAA2120	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-
DAA2121	-	+	+	-	-	-	-	-	+	-	-	+	-	-	+	-	-

1000 |THI|URC|URI|LVA|2AZ|3AZ|4AZ|BEN|F2C|3HY|4HY|2AC|3AC|4AC|IPH|DMA|LMA

DAA2104	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
DAA2106	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-
DAA2110	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
DAA2111	-	-	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-
DAA2112	-	-	-	+	+	-	-	-	-	-	-	+	-	-	-	-	-
DAA2113	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-
DAA2114	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
DAA2115	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-
DAA2116	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-
DAA2117	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	-
DAA2119	-	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-	-
DAA2120	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-
DAA2121	-	-	-	+	+	-	-	-	-	-	+	-	-	-	-	-	-

1000 |PXY|PAC|PLA|PPR|PPY|PHT|PCA|QUI|SAI|TRO|VAN|NA1|NA2|NA3|NA4|NA5|NZ1

DAA2104	-	-	+	-	+	-	-	-	-	-	-	+	+	+	+	+	+
DAA2106	-	+	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+
DAA2110	-	+	+	-	+	-	-	+	-	-	+	+	+	+	+	+	+
DAA2111	-	+	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+
DAA2112	-	-	+	+	+	-	+	+	-	-	-	+	+	+	+	+	+
DAA2113	-	+	+	-	+	-	-	-	-	-	-	+	+	+	+	+	+
DAA2114	-	-	+	+	+	-	+	+	-	-	+	+	+	+	+	+	+
DAA2115	-	-	+	-	+	-	+	+	-	-	+	+	+	+	+	+	+
DAA2116	-	-	+	-	+	-	+	+	-	-	-	+	+	+	+	+	+
DAA2117	-	-	+	-	+	-	-	+	-	-	-	+	+	+	+	+	+
DAA2119	-	+	+	-	+	-	-	-	-	-	-	+	+	+	+	+	+
DAA2120	-	-	+	-	+	-	-	-	-	-	-	+	+	+	+	+	+
DAA2121	-	-	+	-	+	-	-	-	+	-	-	+	+	+	+	+	+

1000 |NZ2|NZ3|CAK|CAG|CBG|CAP|CAC|CAF|CBF|CGB|CGU|CAU|CBU|CGL|CLA|CMN|CML

DAA2104	+	+	+	-	+	+	+	+	-	-	-	+	+	-	+	+	+
DAA2106	+	+	+	-	+	+	+	+	-	+	-	+	+	-	+	+	+
DAA2110	+	+	+	-	+	+	+	-	-	+	-	+	+	-	+	+	+
DAA2111	+	+	+	-	+	+	+	-	+	+	-	+	+	-	+	+	+

Table 4: Assignment of Streptomyces strains associated with potato scab to cluster groups^a

Strain	Source	Pathogenicity on potatoes	Cluster group
2	potato	DPS	<u>S. violaceus</u>
4	potato	DPS	<u>S. violaceus</u>
11	peanut	DPS	<u>S. violaceus</u>
26	soil	CS	<u>S. violaceus</u>
27	soil	DPS	<u>S. violaceus</u>
31	potato	DPS	<u>S. violaceus</u>
NP 111	soil	CS	<u>S. violaceus</u>
NP 113	soil	NP	<u>S. violaceus</u>
B 30	soil	DPS	<u>S. violaceus</u>
P2	potato	DPS	<u>S. violaceus</u>
84.01.43 ^c	U.S.A.	DPS	<u>S. violaceus</u>
84.01.70 ^c	U.S.A.	NP	<u>S. violaceus</u>
43	peanut	DPS	<u>S. rochei</u>
20	peanut	DPS	<u>S. griseus</u>
NP 215	soil	NP	<u>S. griseus</u>
PBS1	potato	DPS	<u>S. griseus</u>
12	potato	CS	single member

^a Clustering of strains was performed according to Williams et al.⁽¹⁵⁾.

^b CS - common scab; DPS - deep pitted scab; NP - non-pathogenic.

^c Obtained from R. Loria, Cornell University.

Fig. 1 Comparative micromorphology of different pathogenic Streptomyces strains causing deep-pitted scab of potato as expressed by scanning elctro-micrograph. A) Strain PBS1 - flexous, smooth spores. B) Strain B 30- spiral, warty spores. C) Strain 26 - spiral, smooth spores.

2. Restriction Fragment Length Polymorphisms of Pathogenic and Non-Pathogenic Streptomyces strains Associated with Potato Scab

Introduction

During recent years a new class of genetic polymorphisms, restriction fragment length polymorphisms (RFLPs) has been uncovered by the use of restriction endonucleases which cleave DNA molecules at specific sites and cloned DNA probes which detect specific homologous DNA fragments^(1,2). By using specific unique DNA probes, a DNA fragment that occurs as rarely as one in a million can be detected⁽¹⁾. Any unique DNA sequences can serve as a probe in RFLP detection as long as it hybridizes with some part of one of the DNA fragments formed by the restriction process. Any changes that alter the sequences that are recognized by restriction enzymes (e.g. mutations, deletions, transpositions etc.) may abolish or create sites for restriction enzymes and contribute to the RFLPs. RFLPs have been widely used to identify DNA fingerprints specific for plants⁽³⁾, bacteria⁽⁴⁾ and fungi⁽⁵⁾.

rRNA operons comprising 16S, 23S, 5S and some tRNA are present in several copies in the bacterial chromosomes with the number and location of such operon copies varying widely in different microorganisms. RFLPs of microorganisms using rRNA operon as a probe (i.e. ribotyping) give rise to banding patterns specific for each organism. Such differentiation between strains is achieved without relying on expression of a phenotype. The number of bands detected varies depending upon the restriction enzyme used and the nature or probe used. The objective of the following study was to evaluate the genetic diversity between phytopathogenic Streptomyces strains and investigate whether specific banding patterns can be recognized for pathogenic versus non-pathogenic strains. An optimal procedure for the following study has only recently been obtained and consequently only initial results could be presented.

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Materials and Methods

Streptomyces strains and growth conditions

All strains used in this study were from the collection maintained at the Department of Plant Pathology and Botany at the Volcani Center and Tel-Aviv University respectively, as described in the previous section. Ordinarily, strains were grown at 30 °C on TSB medium (see previous section) on a rotary shaker for 2-3 days prior to DNA extraction.

DNA probes and restriction enzymes

For probe selection, a lambda library in EMBL-4 replacement vector has been constructed with DNA of Streptomyces strain 44 using conventional protocols⁽⁶⁾. Strain 44 causes deep-pitted scab on potato and shell warts on peanuts. DNA inserts from this library were screened for their use as probes for RFLPs. In addition, we have tested DNA sequences of S. lividans (library in Charon vector) which were kindly provided by Professor Schrepf of Osnabruck University, Germany, and a 16S rRNA of E. coli (pKK 3535)). However, all the above tested probes were not satisfactory since the number of obtained bands following DNA digestion and southern hybridization was too low for RFLP analysis.

The probe of choice was finally selected as a rRNA operon cloned from S. coelicolor. The latter was provided to us by the laboratory of Professor Schrepf where the principle investigator spent 3 months during last year.

Various restriction enzymes (e.g. KpnI, SmaI, PstI, SalI, BamHI, BstEII) were tested for digestion of genomic DNA following hybridization with the rRNA as a probe⁽⁹⁾. Results in Fig. 1 for example illustrate a Southern blot of DNA extracted from strains 44, 84.01.80 and 84.01.43 and digested with restriction enzymes BstEII, KpnI, and PstI, each of which was followed by BamHI digestion. BstEII gave an optimal pattern for RFLP analysis as compared to the other tested enzymes. BstEII, BamHI and SalI were selected as the best enzymes for further work on ribotyping of Streptomyces strains. Genomics DNA was obtained by a procedure described by Hopwood et al.⁽¹⁷⁾ with slight modifications. This procedure employs a lytic mix (Kirby mix) which protects the DNA against nucleolytic activity.

Similarity coefficients

Similarity coefficients for all pairwise combinations of RFLP groups were calculated by means of the formula.

$$F = (N_{xy} + N_{yx}) / (N_x + N_y)$$

in which N_x and N_y represent the number of major restriction fragments describing organisms X and Y respectively, N_{xy} is the number of major restriction fragments in strain X common with any of the restriction fragments in strain Y and N_{yx} is the number of major restriction fragments in strain Y common with any of the restriction fragments in strain X⁽⁸⁾.

Results and Discussion

RFLPs of pathogenic and nonpathogenic strains of Streptomyces from various cluster groups are currently being investigated. Results presented in the report are limited only to few strains whose DNA was digested with BamHI or Sall prior to ribotyping. Therefore, any conclusions on the classification of the pathogenic Streptomyces strains on the basis of their RFLPs should be delayed until this study is completed.

Results in Fig. 2 and Table 1 illustrate the ribotyping blot and the calculated similarity coefficients respectively obtained when DNA preparations of 29 strains were digested with BamHI. Results in Fig. 3 and Table 2 illustrate the ribotyping blot of DNA from 9 strains and their calculated similarity coefficients respectively obtained following the digestion of DNA with Sall. The similarity coefficients among the strains restricted with BamHI ranged from 0 to 100% (Table 1). The following four groups with 100% similarity could be distinguished even within this limited number of pathogenic strains: group A includes strains 3, 11, P2 and 19 with 91% similarity, group B includes strain 12, 21 and 23 with 89% similarity, group C includes strains 20, 26, 42 and PBS-1, and group D includes strains 27, 29 and 31. It is noteworthy that groups B or C, for example, contained strains which incited shallow scab (e.g. strains 12 and 29) whereas the rest of the strains caused deep-pitted scab. The latter observation suggests that the 2 types of symptoms are not caused by genetically distinct strains.

In contrast to the groups which exhibited identical RFLPs, some pathogenic strains significantly differed from most of the other and displayed zero similarity coefficients with many of them. For example, strain 70 exhibit zero

similarity coefficient with 13 strains and its highest similarity coefficient with other strains did not exceed 26% (Table 1).

Our preliminary results may indicate that no correlation exists between the sorting of strains according to RFLPs and the phenotypic classification according to cluster groups. Thus strain 20 and PBSI which belong to the subcluster S. griseus are identical with strain 26 which was classified within the cluster S. violaceus. In addition, strain 44, for example, which belongs to S. violaceus cluster exhibited 73% similarity with strain 43 of the S. rochei cluster as compared to 36% similarity with strain NPIII which belongs to its own cluster. Another point of interest is that the strains 84.01.51, 85.01.29, 86.01.30, 84.01.70 and 84.01.43 which were isolated in the U.S.A. exhibited variable similarity with Israeli strains and among themselves (Table 1). Their highest similarity coefficients (above 80%) were recorded with Israeli strains rather than within their own groups.

The number of strains used for ribotyping following SalI digestion (Table 2) was too limited for drawing any conclusions. It might be of interest to find out whether RFLPs-based classification of Streptomyces strains with different restriction enzymes coincide with each other.

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Fig. 1: Ribotyping of total DNA from 3 Streptomyces strains following digestion with BstEII/BamHI(A); KpnI/BamHI(B) and PstI/BamHI(C). Strains tested are 44 (1), 840170(2) and 840143(3).

Fig. 2 Ribotyping of total DNA from 29 Streptomyces strains following digestion with Bam H1.

The following strains were analyzed:

a- 2	g- 20	n- 31	t- NP111	Z- 84.01.151
b- 3	h- 21	o- 33	u- NP112	aa- 85.01.29
c- 4	i- 23	p- 41	v- B30	ab- 86.01.30
d- 11	k- 26	q- 42	w- P2	ac- 84.01.70
e- 12	l- 27	r- 43	x- PBS 1	ad- 84.01.43
f- 19	m- 29	s- 44	y- 70	ae- λ Hind III

All strains tested were pathogenic with the exception of z, aa, ab, and ac.

Fig. 3 Ribotyping of total DNA from 9 Streptomyces strains following digestion with Sall.

The following strains were analyzed:

a- NP Act 9	p- 27
b- 42	g- 44
c- 33	h- NP 212
d- 3	i- NP 115
e- 12	

All the strains tested were pathogenic with the exception of a, h, and i.

Table 1: Similarity Coefficients of the Combined Data from Fig 2
Expressed as Described in Materials and Methods.

	2	4	11	26	27	31	44	RP111	B30	P2	20	FBS1	43	12	3	19	21	23	29	33	41	42	RP112	8	8401151	850129	860130	840170	840143
2	0																												
11	0.55	0.36																											
26	0.55	0.36	0.9																										
27	0.36	0.36	0.17																										
31	0.36	0.36	0.17	0.4																									
44	0.5	0	0.73	0.44	1																								
RP111	0.73	0.73	0.5	0.2	0.18	0.18	0.86																						
B30	0.53	0.66	0.63	0.29	0.75	0.75	0.53	0.63																					
P2	0.55	0.36	1	0.8	0.18	0.18	0.73	0.53	0.63																				
20	0.44	0	0.8	1	0.4	0.4	0.44	0.2	0.29	0.83																			
FBS1	0.44	0	0.67	0.3	0.33	0.33	0.73	0.33	0.75	0.67	0.7																		
43	0.73	0.46	0.43	0.33	0.43	0.43	0.15	0.43	0.56	0.36	0.33	0.33	0.7																
12	0.73	0.36	1	0.6	0.16	0.16	0.73	0.5	0.63	1	0.6	0.6	0.67	0.5															
3	0.3	0.6	0.91	0.22	0.37	0.37	0.4	0.55	0.67	0.91	0.22	0.22	0.67	0.46	0.91														
19	0.46	0.43	0.33	0.43	0.43	0.43	0.15	0.43	0.56	0.36	0.33	0.33	0.28	0.89	0.59	0.44	0.89												
21	0.38	0.46	0.39	0.6	0.47	0.47	0.38	0.47	0.57	0.47	0.6	0.6	0.53	0.89	0.59	0.44	0.89	0.5											
23	0.38	0.46	0.39	0.6	0.47	0.47	0.38	0.47	0.57	0.47	0.6	0.6	0.53	0.89	0.59	0.44	0.89	0.5	0.41										
29	0.73	0.36	0.95	0.4	1	1	0.36	0.5	0.63	0.66	0.6	0.6	0.53	0.89	0.59	0.44	0.89	0.5	0.41	0.55									
33	0.4	0.4	0.95	0.6	0.5	0.5	0.36	0.5	0.63	0.66	0.6	0.6	0.53	0.89	0.59	0.44	0.89	0.5	0.41	0.55	0.55								
41	0.4	0.4	0.95	0.6	0.5	0.5	0.36	0.5	0.63	0.66	0.6	0.6	0.53	0.89	0.59	0.44	0.89	0.5	0.41	0.55	0.55	0.55							
42	0.55	0.4	0.6	1	0.55	0.55	0.33	0.4	0.55	0.67	0.55	0.55	0.5	0.5	0.5	0.54	0.54	0.54	0.5	0.5	0.55	0.55	0.55						
RP112	0.42	0.33	0.33	0.64	0.77	0.77	0.5	0.4	0.28	0.6	0.55	0.55	0.5	0.5	0.5	0.54	0.54	0.54	0.5	0.5	0.55	0.55	0.55	0					
8	0	0	0.12	0.13	0	0	0.18	0.2	0.28	0.1	0.2	0.2	0.2	0.2	0.2	0.22	0.22	0.22	0.2	0.2	0.33	0.33	0	0					
8401151	0.36	0.55	0.58	0.2	0.42	0.42	0.68	0.83	0.83	0.58	0.2	0.2	0.58	0.43	0.59	0.44	0.54	0.54	0.5	0.5	0.55	0.55	0	0	0				
850129	0.45	0.55	0.5	0.2	0.33	0.33	0.55	0.33	0.33	0.5	0.4	0.4	0.75	0.43	0.5	0.54	0.54	0.54	0.5	0.5	0.55	0.55	0	0	0	0.18			
860130	0.44	0.44	0.6	0	0.5	0.5	0.66	0.6	0.6	0.6	0	0	0.6	0.42	0.5	0.54	0.54	0.54	0.5	0.5	0.55	0.55	0	0	0	0.31	0.18		
840170	0.8	0.4	0.72	0.56	0.55	0.55	0.4	0.55	0.55	0.55	0.56	0.56	0.56	0.54	0.72	0.5	0.54	0.54	0.5	0.5	0.55	0.55	0	0	0	0.45	0.4		
840143	0.72	0.18	0.5	0.5	0.66	0.66	0.36	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.55	0.55	0	0	0	0.45	0.44	0.4	0.91

Table 2: Similarity Coefficients of the Combined Data from Fig 3
Expressed as Described in Materials and Methods.

Sal Blot	27	44	12	NPA9	42	33	3	NP212	NP115
27									
44	0.2								
12	0.18	0.36							
NPA9	0.3	0.4	0.36						
42	0.36	0.55	0.17	0.55					
33	0.38	0.3	0.14	0.15	0.29				
3	0.36	0.55	0.5	0.36	0.33	0.43			
NP212	0.64	0.18	0.42	0.18	0.5	0.14	0.66		
NP115	0.4	0.2	0.55	0.6	0.55	0	0.18	0.36	

3. Preliminary studies on objectives C and E

Detection of plasmids (objective C)

Thirteen pathogenic and 12 nonpathogenic Streptomyces strains were examined for presence of plasmids as described by Hopewood et al. (reference 16 in section 1). Results given in Table 1 indicate that plasmids could be detected only in 4 pathogenic strains. The latter finding refutes any linkage between plasmids and pathogenicity. Furthermore, it appears that plasmid-located genes may not contribute significantly to the genetic diversity observed in pathogenic strains.

Cloning of an esterase encoding gene which might be associated with pathogenic strains (Objective E).

The objective was based on a report by McQueen and Schottel (J. of Bacteriol 1987, 169: 1967-71) which claimed that a zinc induced esterase that might be associated with suberin is present only in pathogenic strains. We have started experiments aiming at testing whether this enzyme is indeed present exclusively in pathogenic strains. Their claim was based on a very few examined strains. Using their methodologies we could confirm the enzyme in one but not in another pathogenic strain. We were also informed that the authors themselves do not claim anymore that such specificity indeed exists. Therefore, we cancelled any further work in this direction.

Table 1: Detection of plasmids in various pathogenic and nonpathogenic Streptomyces strains.

Pathogenic strains	Presence of plasmids	Nonpathogenic strains	Presence of plasmid plasmids
3	-	NP 211	-
4	-	83.01.39	-
11	-	84.01.70	-
20	+	87.01.60	-
26	+	NP Act 5	-
31	-		-
41	-	NP Act 6	-
42	+	NP Act 11	-
44	-	NP Act 12	-
B30	-	NP Act 13	-
PBS1	+	NP Act 14	-
70	-	NP Act 18	-
83.01.03	-	NP Act 22	-

4. Research Activities on the Project performed at Cornell University

The report is organized according to the objectives of the original proposal (see section D). During the past year we have actually completed objectives a and b and made a significant progress on objective f. Though not part of the original proposal, a rapid reliable pathogenicity test would greatly facilitate research on the mode of pathogenicity. Therefore, we have concentrated on developing such tests and it is recorded under "other activities".

Objective A

Approximately 250 Streptomyces spp. have been collected from potato producing regions in the United States. Isolates were obtained from scab lesions on potato tubers or from colleagues. A very diverse group of Streptomyces strains has been accumulated which is representative of potato scab causing species and saprophytic Streptomyces spp. associated with potato tubers in the United States. Streptomyces spp. were obtained from New York, Maine, Virginia, West Virginia, Florida, Ohio, Idaho, New Jersey, Pennsylvania, Wisconsin, Massachusetts, Alaska, Minnesota, North Carolina, California, Louisiana, Washington and Michigan. Several strains were also obtained from Canada. Strains were obtained from a large number of potato cultivars, including Katahdin, Hudson, Sunrise, FL 657, Shepody, Kennebec, Green Mountain, Bintje, Red Pontiac, Chippewa, Superior, Hampton, Atlantic, Russet Burbank, Norland, Ontario, Monona, Viking, Denali and a number of potato breeding lines. Approximately 50% of the strains in the collection are pathogenic on potato tubers in greenhouse assays.

Objective B

The biochemical and micromorphological properties of the pathogenic strains, and many of the nonpathogenic strains, have been determined. Criteria and methods for evaluating strains were those specified by the International Streptomyces Project. Data on selected United States strains are presented in Table 1. Most pathogenic strains have characteristics typical of S. scabies: gray ariel mass color, spores in spiral chains, yellow to brown substrate,

melanoid pigment production and a complete carbon utilization pattern. Strain 87-01-22 is typical of this group. S. scabies was found to be the primary scab pathogen in the U.S., causing disease in all potato production regions. This pathogen was isolated from russet, slightly raised, slightly pitted and deeply pitted scab lesions. Therefore claims that these lesion types are caused by different Streptomyces spp. in the United States do not appear to have merit. The acid scab pathogen, S. acidiscabies, was also isolated from several locations, primarily in the Northeastern U.S. These strains, typified by 88-01-11, have pale gray to yellow/orange mass color, yellow to brown substrate, spores in flexuous chains, do not produce melanoid pigments or utilize raffinose. This pathogen is not as widely distributed as S. scabies and is found rarely. Therefore S. scabies and to a lesser extent S. acidiscabies, appear to be responsible for potato scab in the United States.

Objective F

Good progress has been made in the purification of polyclonal antibodies to S. Scabies and S. acidiscabies. These antibodies were previously produced in chickens. Egg yolks were collected, grouped according to day after immunization, frozen and subsequently processed. A crude protein fraction containing polyclonal antibodies was extracted from the egg yolks. Antibody titers in this crude preparation were determined using an indirect enzyme linked immunosorbent assay (ELISA). Useful antibody titers were obtained for both pathogens. Highest titers occurred 7 - 8 weeks after immunization. These crude antibody preparations were further purified using ammonium sulfate precipitation and DEAE-cellulose chromatography. The purified antibody preparation was much more active and specific than the crude preparation. Antibodies produced to S. scabies can be used to distinguish between S. scabies and S. acidiscabies in an ELISA test. These antibodies were used to develop an ELISA protocol for screening monoclonal antibodies for specificity to S. scabies. Antigen preparation and antigen, antibody and reagent concentrations have been optimized. A screening technique is now in place for use in monoclonal evaluations. Five BALB C mice have been immunized with S. scabies and serum has been collected and evaluated for antibody titer. Based on these assessments, two mice will be chosen for use in the hybridoma production, which will be carried out in December 1990.

Other Activities

We have begun work on an in vitro pathogenicity test for Streptomyces spp. on potato. Though not part of our original proposal, a rapid, reliable pathogenicity test would greatly facilitate research on the molecular basis of pathogenicity in S. scabies. Scab lesions have been produced on microtubers in vitro using the scab susceptible variety Chippewa, and a pathogenic strain of s. scabies. This work was done with whole plantlets in a liquid medium. Though successful, this technique is not particularly rapid or efficient in its use of plant material. However, tubers can be produced on nodal cuttings from potato plantlets on a solid medium through manipulation of temperature and photoperiod. This technique, described by Kwiatkowski, has been used successfully in Loria's laboratory with Chippewa. Large numbers of nodal cuttings can be produced rapidly and efficiently. We have now optimized the conditions for infection of Chippewa with a pathogenic isolate of S. scabies. The optimum age for infection of microtubers is two weeks. The receptivity of the tubers to infection by S. scabies is increased by immersion in ethanol for 30 seconds. Tubers are allowed to air dry before inoculating them with a spore suspension. Tubers are incubated in the dark at 25C for 24 - 48 hours before evaluating symptoms.

F. DESCRIPTION OF COOPERATION

A very close cooperation was established between Dr. Loria of Cornell University and Drs. Barash, Manulis and Kritzman of Tel-Aviv University and the Volcani Center. The cooperation was expressed by frequent consultations and exchange of strains for RFLPs analysis. Dr. Barash visited Dr. Loria at Cornell University last June for reviewing the progress made so far and discussing ideas for the continuation of the project.

G. EVALUATION OF THE RESEARCH ACHIEVEMENTS

The characterization of Streptomyces strains associated with potato scab and peanut shell warts in Israel by their micromorphology and biochemical properties (objectives a & b) clearly indicate the presence of phenotypically diverse population. The observation that pathogenic strains were distributed even among different cluster groups further emphasize the latter point. In contrast to Israel, most pathogenic strains in the U.S. have characteristics typical to S. scabies. S. scabies was found to be the primary scab pathogen in all potato regions. The acid scab pathogen, S. acidiscabies, is not as widely distributed as S. scabies. At present it is too early to speculate about the difference in diversity between the scab-inciting-Streptomyces populations in Israel and the U.S.

The limited RFLPs analysis (objective d) obtained with the Israeli strains clearly support the phenotypic diversity discussed earlier. However, it is noteworthy that no correlation could so far be established between the genomic diversity expressed by RFLPs and diversity based on biochemical characteristics (see section 2 this report). Results also indicate that classification of pathogenic Streptomyces strains into groups on the basis of RFLPs similarity is feasible. Examination of a large number of strains is required in order to determine whether certain patterns of RFLPs might be characteristic to pathogenic strains and consequently be used for diagnosis. It would be worthwhile to extend the RFLPs analysis into the U.S. strains which mostly consist of S. scabies. It is noteworthy that in contrast to the high genetic diversity observed by RFLPs, many of the pathogenic strains examined did not contain plasmids. Thus, the genetic variation of the strains resided primarily in the chromosome (or giant plasmids) and seem not to be expressed by plasmids. Furthermore, plasmids do not appear to be associated with pathogenicity.

How can the observed immense genetic diversity in the Israeli scab producing strains be explained? It is well known that Streptomyces spp. are quite widespread as soil inhabitants. We hypothesize that the genes required for pathogenicity are distributed among diverse streptomyces strains which have one property in common, namely, the capacity to be intimately associated with potato tubers (or peanut pods) during their development. It might be assumed that these genes are responsible for the biosynthesis and release of biologically active metabolites (e.g. toxins, hormones or hormone inhibitors) which trigger the scab symptoms. The possible involvement of toxin in common scab production has recently been reported⁽¹⁾. We further hypothesize that the pathogenicity genes may be transferred and exchanged among genetically diverse Streptomyces strains through conjugation following by genetic recombination. The phenomenon of conjugation among Streptomyces strains even with gram negative bacteria has recently been reported⁽²⁾ and might be more frequent than being realized.

If the above-mentioned supposition is correct than the best, if not the only way, to identify pathogenic Streptomyces strains is by means of DNA probes which can specifically hybridize with pathogenicity genes. Therefore, our major efforts should be directed towards identification and isolation of pathogenicity genes. Transposon mutagenesis is not yet available for experimental use in Streptomyces for identification of pathogenicity genes. Therefore, to pursue this goal we intend to use the following 2 approaches: The first involves the subtractive hybridization of the DNA of a pathogenic strain by DNA from genetically similar nonpathogenic strains. Subtractive hybridization has been successfully used to isolate strain-specific DNA sequences in bacteria⁽³⁾. For the latter procedure we intend to select pathogenic and nonpathogenic strains which exhibit high similarity in their RFLPs and fatty acids analysis. The significance of the latter procedure in expressing the diversity of the population is discussed in our resubmitted proposal to BARD.

The second approach involves the biochemical identification of pathogenicity determinants and subsequent identification of their genes. On the basis of symptoms development we hypothesize that toxins or hormone-like metabolites released by the pathogen are potential candidates to fulfill such a role. A crucial procedure for the determination of pathogenicity factors is a reasonable pathogenicity test. In view of the extreme incubation period encountered in the current pathogenicity assay (about 3 months), we intend to

continue our efforts in developing a short and reliable pathogenicity assay (see "other activities" in the U.S. report). With the availability of the latter assay, we shall be able to isolate biologically active compounds produced by the pathogen which may play a role in scab production.

Since the population of Streptomyces strains in the U.S. is relatively homogenous and predominantly S. scabies, immunological identification, particularly monoclonal antibodies, might be practical for diagnosis. Therefore, efforts in the U.S. are now in progress (objective f) to develop poly- and monoclonal antibodies. It would be of interest to use the various antibodies also with the Israeli strains.

Based on our preliminary results, we intend to continue our research by pursuing the following objectives:

- a) Complete evaluation of the genetic diversity of pathogenic and non-pathogenic strains by RFLPs and fatty acids analysis and determine if these procedures are capable of distinguishing between pathogens and non-pathogens.
- b) Develop DNA probes specific for pathogenic strains by using subtractive hybridization strategies.
- c) Produce monoclonal antibodies to pathogenic strains of S. scabies and S. acidiscabies for their detection by ELISA.
- d) Develop a rapid pathogenicity test.
- e) Attempt to identify biochemical determinants of pathogenicity (e.g. toxins, plant growth regulators).

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