Plant Archives Vol. 25, Supplement 1, 2025 pp. 450-461 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2025.v25.supplement-1.060

IDENTIFICATION AND PHENOTYPIC CHARACTERIZATION OF DIFFERENT ISOLATES OF *STREPTOMYCES SCABIES* [(THAXTER) WAKSMAN & HENRICI] FROM VARIOUS DISTRICT OF UTTAR PRADESH INDIA

Shivam Kuma[1], S. K. Biswas[1*], Ravi Kumar[1], Sucharita Mohapatra[2], Kishan Lal[3], Sandeep Kumar[1], Shenaz S. Ahmed[4], Girijesh Kumar Jaisval[1], Prabha Siddharth[1], Dipake Shital[1] and Saurabh Saini[1]

[1]Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, 208002 (Uttar Pradesh), India
[2]Department of Plant Pathology, Odisha University of Agriculture and Technology, 751003, Odisha, India
[3]Department of Plant Pathology, National P.G. College, Barhalganj, Gorakhpur, 273402 (U.P.), India
[4]Department of Plant Pathology, Assam Agriculture University, Assam, India
*Corresponding author E-mail: biswas.plpath@gmail.com
(Date of Receiving : 21-07-2024; Date of Acceptance : 30-09-2024)

ABSTRACT

Potato is the most significant vegetable crop in the world, belonging to the family Solanaceae, which designated as a 'Poor Men's Friend'. It is susceptible to several diseases caused by plant pathogenic fungi, bacteria, viruses, nematodes, and several non-pathogenic or physiological disorder. Among these diseases, the common scab of potato is an economically significant seed-soil borne disease primarily caused by *Streptomyces scabies* (Thaxter) Waksman & Henrici. The different purified isolates of *S. scabies* were identified on the basis of its colonies color of mature spores were sowing as light brown to dark brown in color. However, the most of the isolates of *S. scabies viz.,* AgS, KaS, FiS, HaS, KnS, BuS appearing as light brown in color whereas FaS, MeS and AlS, EtS isolates are showing as brown and dark brown in color, respectively. The slide of *S. scabies* isolates were observed under Phase contrast microscope at 100x after gram-staining, visible as filamentous, spore-forming in chains on spiral or flexuous hyphae and consists of slender, branched mycelium. Among all the isolates, the maximum number of bacterial isolates of *S. scabies viz.,* KaS, FiS, HaS, EtS, KnS, BuS, MeS, produces cylindrical spores in chain on specialized spiral hyphae except AgS, FaS and AlS isolates produces cylindrical spores in chain on flexuous hyphae. After Gram staining, the most of isolates of *S. scabies viz.,* FiS, HaS, EtS, BuS, also retain as bluish purple in colour, whereas KaS, KnS, MeS and AgS, FaS, AlS isolates are showing as light bluish purple and dark bluish purple in colour, respectively. The colonies color of mature spores of *S. scabies* isolates *viz.,* AgS, KaS, FiS, HaS, KnS, BuS, FaS, MeS, AlS, EtS, were grown on yeast malt extract agar (YMEA) media sowing as light brown to dark brown colouration around colonies being indicating the melanin pigment production after seven days. The pathogenicity of 10 isolates of *Streptomyces scabies* was tested by tuber slice method and found that the six isolates of *S. scabies viz.,* FaS, AlS, EtS, KnS, BuS, MeS were severely pathogenic and produced deep pits along with the dark lesions of tissues & two isolates of *S. scabies viz.,* AgS, KaS were medium in pathogenicity and formed brown to dark lesions of tissues with slight deep pits. Remaining two isolates of *S. scabies viz.,* FiS, HaS were produced light to brown lesion of tissues with slight pits and were kept under the mild pathogenic category.

Keyword **:** Isolates, *S. scabies,* Identification, Morphological Characterization, Pathogenicity test

Introduction

Potato is the most significant vegetable crop in the world, belonging to the family Solanaceae, which designated as a 'Poor Men's Friend'. The English word "Potato" comes from the Spanish word 'patata'. It was the first domesticated vegetable in the region of Southern Peru and North-western Bolivia of Andreas Mountain (South America) between 8,000 B.C. and 5,000 B.C. But the native of Potato is South America (Pushkarnath, 1976). Potato is a temperate crop, growing and yielding well in cool and humid climates or seasons, but it is also cultivated in tropical to sub-polar climatic regions, and represents a major food crop in many Asian countries, particularly in India (Motalebifard, 2017). Potato production ranked fourth in the world after wheat, rice, and maize (Miyu *et al.,* 2019) and is growing in about 150 countries of the world. The total harvested area of the world in 2022 is about 17,788,408 hectares with a production of 375 million tonnes. During the fiscal year 2023-24, potato produced across India accounted for over 59.72 million tonnes, which was increase of over 3.54 million tonnes from the previous fiscal year as 56.18 million tonnes (Source: Statista, 2024). In India, potato is extensively cultivated in northern state of Uttar Pradesh, which ranks first in potato production in India and contribute alone 32 per cent of the total production in India. In Uttar Pradesh, top 10 districts, which contributes the most of total potato production. Agra contributes alone 11% of total potato production, which ranks first in potato production in Uttar Pradesh followed by Kannauj, Firozabad, Hathras, Farrukhabad, Aligarh, and Etawah are comes in the Doab region. Kanpur Nagar which ranks 9th is also situated in Doab, while the other two, Barabanki (8th) and Budaun (10th) are in Avadh and Ruhelkhand, respectively. In Uttar Pradesh's potato belt extending from Agra in the western part of the state to Kanpur in the central part of the state data was reported at 259.63 quintal/ha in 2022. This record an increase from the previous number of 254.84 quintal/ha for 2021. Uttar Pradesh data is updated yearly, averaging 241.48 quintal/ha from Mar 2001 to 259.63 quintal/ha in 2022, with 22 observations. The data reached an all-time high of 259.63 quintal/ha in 2022 and a record low of 205.00 quintal/ha in 2009. The daily need of potato is increasing day by day resulting from increasing population pressure of the world. Therefore, there is a demand for more production from per capital available land.

Materials and Methods

Experiments site

The present investigation, laboratory experiments on common scab (*Streptomyces scabies*) of potato were carried out during the period 2022-23 in the Plant Bacteriology Lab, Department of Plant Pathology, Chandra Shekhar Azad University of Agriculture & Technology, Kanpur, 208002 (Uttar Pradesh). The details of materials used and the methodology adopted in the present investigation are briefly described below:-

Collection of scab infected tuber samples

Potato tubers with typical scab symptoms were collected from 10 district of Uttar Pradesh *viz.,* Gadima village of Achhnera block (Agra), Paraspur village of Jalalabad block (Kannauj), Bilaspur village of Jasrana block (Firozabad), Gukhrauli village of Sadabad block (Hathras), Punpalpur village of Mohammadabad block (Farrukhabad), Ahamadpura village of Atrauli block (Aligarh), Bhootha village of Basrehar block (Etawah), Student Instructional Farm, CSA Uni. of Agri. & Tech. (Kanpur Nagar), Dahmu village of Ujhani block (Budaun) and ICAR-CPRI RS-Modipuram (Meeruth). Infected tubers with deep scab lesion were taken from the field and washed in sterilized water. The infected tuber with deep scab lesion were microscopically examined to confirm the presence of the bacteria. After confirming for the presence of bacterial, the sample was placed in between two folds of sterilized blotter paper and preserved at 4-6^{0}C in refrigerator for isolation and purification of pathogen. The purified pathogen was later used for laboratory, field and pot experiments.

Isolation, purification and maintenance of *Streptomyces scabies* from disease tubers

Small tuber pieces containing a scab lesion were cut out with sterilized scalpel blade into small bits in such way that each bit contains infected portions along with some healthy parts and surface disinfected for one minute in 1 % sodium hypochlorite solution. After several rinses in sterile distilled water, the tissue was ground in a sterilized pestle mortar in 3-5 ml of sterile water depending upon the tissue size. Serial dilutions were spread-plated on to Yeast Malt Agar media (Peptone 5g, Yeast extract 3g, Malt extract 3g, Dextrose 10g, Agar 20g and distilled water to make the final volume 1 lit, pH 6.2). The plates were incubated at 28 $\pm$1^{0}C for 10 days. Individual characteristics of bacterial colonies started appearing at 1-2 days after isolation. The individual colonies were picked by inoculation needle and serially transferred to fresh media plates until a pure culture was obtained. For

general use, cultures were maintained on YMA plates at 20°C. Strains were also stored for long term as a spore suspension at -20°C in a 30% glycerol stocks are viable for several years even after multiple freeze thaw cycles. Cultures were grown on YMA for one week. Sterile storage medium (5 ml) consisting of 1/3 strength YME with 30% glycerol was transferred to each plate and the surface scraped to loosen colonies. The resulting spore suspension was transferred to a storage vial.

Pathogenicity test of different isolates of *Streptomyces scabies* collected from various districts of Uttar Pradesh

Many techniques have been proposed for assessing pathogenicity of *Streptomyces* strains causing common scab of potato *viz.,* Tuber slice assay and Pot assay of pathogenicity. These techniques involve artificial inoculation of the pathogen and creation of disease epiphytotics.

Tuber slice assay of pathogenicity

Pathogenicity of 10 *Streptomyces* isolates was tested by tuber slice method and the severity was determined based on the lesion and pit formation on tuber slices. Based on the symptoms developed, the isolates were grouped into three different categories *viz.,* severe, medium, and mild-pathogenic. Out of ten, four isolates were severely pathogenic and produced deep pits along with the dark lesions of tissues. Four isolates were medium in pathogenicity and formed brown to dark lesions of tissues with slight pits. Two isolates produced light to brown lesion of tissues with no or slight pits and were kept under the mild pathogenic category. Pathogenic *Streptomyces scabies* synthesize the phytotoxin thaxtomin A, which elicits scab symptoms and is the primary pathogenicity determinant in common scab-causing species.

Pot assay of pathogenicity in wire house

Pot assay of pathogenicity was carried out according to the method of Labruyere (1971). To confirm the pathogenicity test of all the 10 isolates of *Streptomyces scabies* were performed on healthy disease-free seed tubers of potato, were sown two tuber per pot, fill with sterile soil under wire house conditions (25 to 30°C). Later, each plot soil was inoculated with the spore suspension of respective isolates of *S. scabies.* The standard spore suspension was utilized for artificial inoculation on potato tubers in the wire house condition to observe the development of scab lesion. The spore suspension was prepared by transferring the 5–6 plugs of *S. scabies* pathogen on 500 ml of Yeast Malt Broth media without adding agar and incubated at an orbital rotator at 120 RPM at 28^0C for 72 h to obtain a concentration of 1×10^6 CFU ml^{-1}. After this, spore suspension was mixed immediately on the sterilized loamy soil. The susceptible potato varieties Kufri Jyoti were sown on infested soil with three replications for each treatment. Each pot carried the same inoculum (10^6 CFU mL^{-1}) and kept in a wire house with 25–30°C temperature and 75% relative humidity (RH). Symptoms were assessed after two months of sowing. Pathogenicity of identified isolates using inoculum (spore suspension) was performed to fulfill Koch's postulates. All the 10 isolates showed visible symptoms ranging from severe corky deep pitted scab to mild russet with no symptoms on above plant parts. The un-inoculated plants produced healthy tubers with no scab symptoms.

Results and Discussion

Isolation, purification and morphological characterization of different isolates of *Streptomyces scabies* collected from 10 districts of Uttar Pradesh

Potato tubers with typical scab symptoms were collected from Gadima village of Achhnera block (Agra), Paraspur village of Jalalabad block (Kannauj), Bilaspur village of Jasrana block (Firozabad), Gukhrauli village of Sadabad block (Hathras), Punpalpur village of Mohammadabad block (Farrukhabad), Ahamadpura village of Atrauli block (Aligarh), Bhootha village of Basrehar block (Etawah), Student Instructional Farm, CSA Uni. of Agri. & Tech. (Kanpur Nagar), Dahmu village of Ujhani block (Budaun) and ICAR-CPRI RS-Modipuram (Meeruth). Infected tubers with deep scab lesion were taken from the field and washed in sterilized water. The deep scab lesion were cut out with sterilized scalpel blade into small bits in such way that each bit contains infected portions along with some healthy parts and surface disinfected for one minute in 1 % sodium hypochlorite solution. After several rinses in sterile distilled water, the tissue was grind in a sterilized pestle mortar in 3-5 ml of sterile water and spread on sterilized Yeast Malt Agar media containing plates. The plates were incubated at 28 $\pm 1^0$C and the bacterial colonies started appearing at 1-2 days after isolation. The small bit of colonies were picked by inoculation needle and serially transferred to fresh media plates until a pure culture was obtained. The purified pathogen was identified on the basis of its morphological and cultural characters and pathogenic behavior towards the host and also by Gram staining method was given by Christian Gram (1884).

Table 1: Collection, isolation and purification of different isolates of *Streptomyces scabies* collected from various district of Uttar Pradesh (India)

S. No.	Village name	Block name	District	Isolate name	Isolation Source	Pathogen
1.	Gadima	Achhnera	Agra	AgS	Tuber	*S. scabies*
2.	Paraspur	Jalalabad	Kannauj	KaS	Tuber	*S. scabies*
3.	Bilaspur	Jasrana	Firozabad	FiS	Tuber	*S. scabies*
4.	Gukhrauli	Sadabad	Hathras	HaS	Tuber	*S. scabies*
5.	Punpalpur	Mohammadabad	Farrukhabad	FaS	Tuber	*S. scabies*
6.	Ahamadpura	Atrauli	Aligarh	AlS	Tuber	*S. scabies*
7.	Bhootha	Basrehar	Etawah	EtS	Tuber	*S. scabies*
8.	CSA Uni. of Agr. &Tech.	Kanpur	Kanpur Nagar	KnS	Tuber	*S. scabies*
9.	Dahmu	Ujhani	Budaun	BuS	Tuber	*S. scabies*
10.	ICAR-CPRI RS-Modipuram	Modipuram	Meeruth	MeS	Tuber	*S. scabies*

Cultural characteristics and pathogenicity test of different isolates of *Streptomyces scabies* collected from various district of Uttar Pradesh (India)

Methods for characterization of different isolates generally followed that of Lambert and Loria (1989a). A detailed description of methods is as follows:

Colony color of different isolates of *Streptomyces scabies*

The data illustrated in table-2 & plate-1, the colonies color of mature spores were grown on yeast malt extract agar (YMEA) media sowing as light brown to dark brown in color. However, the most of the isolates of *S. scabies viz.,* AgS, KaS, FiS, HaS, KnS, BuS appearing as light brown in color whereas FaS, MeS and AlS, EtS isolates are showing as brown and dark brown in color, respectively. Goyer and Beaulieu (1997) reported that the *S. scabies* showed brown to tan colonies with grey spore born in spiral chains. Spore were cylindrical measuring approximately 1.0 x 1.1 µm with smooth surface. Lindholm *et al.,* (1997) stated that *S. scabies* on YME (yeast malt extract) agar was characterized by formation of grey to brown colonies, grey spores, spiral sporophores and melanin pigmentation. Fischer *et al.,* (2003) characterized the *S. scabies* in colony colours ranging from gray to brown and from white to cream with and without production of pigment. The colonies formed were flexuous or spiral spore chain with variable size and producing or not aerial mycelium in spiral colonies.

Gram staining of different isolates of *Streptomyces scabies*

The data presented in table-2 & plate-1, showed that the isolates of gram-positive bacteria *viz., Streptomyces scabies*, observed light bluish purple to dark bluish purple colour under microscope at 100x using Phase contrast microscope after gram-staining (Christian Gram, 1884). The most of isolates *S. scabies viz.,* FiS, HaS, EtS, BuS, retain as bluish purple in colour, whereas KaS, KnS, MeS and AgS, FaS, AlS isolates are showing as light bluish purple and dark bluish purple in colour, respectively. Waksman and Henrici (1943) reported that *S. scabies* produced coenocytic hyphae measuring 0.5 to 2.0 µm in diameter and gave gram positive reaction.

Colony character of different isolates of *Streptomyces scabies*

The data mentioned in table-2 & plate-1, showed that the slide of *S. scabies* isolates observed under microscope at 100x using Phase contrast microscope after gram-staining are visible and reveal shape-size of bacteria. The isolates of *S. scabies* is a filamentous, spore-forming in chains on spiral or flexuous hyphae and consists of slender, branched mycelium. Among all the isolates, the maximum number of bacterial isolates of *S. scabies viz.,* KaS, FiS, HaS, EtS, KnS, BuS, MeS, produces cylindrical spores in chain on specialized spiral hyphae except AgS, FaS and AlS isolates produces cylindrical spores in chain on flexuous hyphae. Waksman (1919) reported that *S. scabies* produced wavy or straight, branched hyphae, conidia more or less cylindrical measuring 0.8 to 1.0 x 1.2 to 1.5 µm. Millard and Burr (1926) reported that conidiophore of *S. scabies* was spirally coiled and barrel shaped conidia were measured 0.8 x 1.7 µm in size.

Pigmentation of different isolates of *Streptomyces scabies*

The data illustrated in table-2 & plate-1, represented that the colonies color of mature spores of *S. scabies* isolates *viz.,* AgS, KaS, FiS, HaS, KnS, BuS, FaS, MeS, AlS, EtS, were grown on yeast malt extract agar (YMEA) media sowing as light brown to dark brown colouration around colonies being indicating the melanin pigment production after seven days. *Streptomyces scabies* produced melanin pigment when grown on medium containing tyrosine was found as a

diagnostic tool for identification of *S. scabies* causing common scab of potato *(*Taylor and Decker, 1947; Hollis, 1953; Vaisey *et al.,* 1954). Corbaz (1964) studied that aerial mycelium of *S. scabies* was grey, sporophores forming spirals and spores were smoot. He also observed melanin pigment on tyrosine agar medium. *S. scabies* was characterized by smooth gray spores, born in spiral chain and produced melanin pigment (Loria and Davis, 1988; Lambert and Loria, 1989; Faucher *et al.,* 1992).

Pathogenicity test

Tuber slice assay of pathogenicity

The presented in table-2 & plate -2, the pathogenicity of 10 isolates of *Streptomyces scabies* was tested by tuber slice method and the severity was determined based on the lesion and pit formation on tuber slices. Based on the symptoms developed, the isolates were grouped into three different categories *viz.,* severe, medium, and mild-pathogenic. Out of ten, six isolates of *S. scabies viz.,* FaS, AlS, EtS, KnS, BuS, MeS were severely pathogenic and produced deep pits along with the dark lesions of tissues & two isolates of *S. scabies viz.,* AgS, KaS were medium in pathogenicity and formed brown to dark lesions of tissues with slight deep pits. Remaining two isolates of *S. scabies viz.,* FiS, HaS were produced light to brown lesion of tissues with slight pits and were kept under the mild pathogenic category. Pathogenic *Streptomyces scabies* synthesize in large amount of phytotoxin i.e. thaxtomin A, which elicits scab symptoms and is the primary pathogenicity determinant in common scab-causing species. Padilla-reynaud *et al.,* (2015), suberin, a major constituent of the potato periderm, is known to promote the production of thaxtomins, the key virulence factors of the common scab-causing agent *Streptomyces scabiei* in pathogenicity test. The production of 13 out of the 14 cellulases produced by *S. scabiei* in cellulose-containing medium was stimulated by the presence of suberin.

Pathogenicity test of different isolates of *Streptomyces scabies* collected from various district of Uttar Pradesh (India) under pot culture

The pathogenicity test of different isolates of *S. scabies* were performed on healthy disease-free seed tubers of potato planted in pots of sterile soil. Later this soil was inoculated with the culture of the pathogen. The concentration of spore suspension (10^6 CFU/ml) was utilized for artificial inoculation on potato tubers in the wire house condition to see the development of scab lesions. The concentration was prepared by transferring the 5–6 plugs of actinomycetes bacterial pathogen on 500 ml of Yeast Malt Broth media without adding agar and incubated at an orbital rotator at 120 RPM at 28ºC for 72 h to obtain a concentration of 1×10^6 CFU ml^{-1}. After this, suspension was mixed immediately on the sterilized loamy soil. The susceptible potato varieties 'Kufri Jyoti' were sown on loamy soil with three replications for each treatment. Each pot carried the same inoculum (10^6 CFU mL^{-1}) and kept in a wire house with 25–30ºC temperature and 75% RH. Symptoms were assessed after 1 month of inoculation. Pathogenicity of identified isolates using inoculum was performed to fulfill Koch's postulates (plate- 3).

Table 2 : Cultural characteristics and pathogenicity test of different isolates of *Streptomyces scabies* collected from various district of Uttar Pradesh (India)

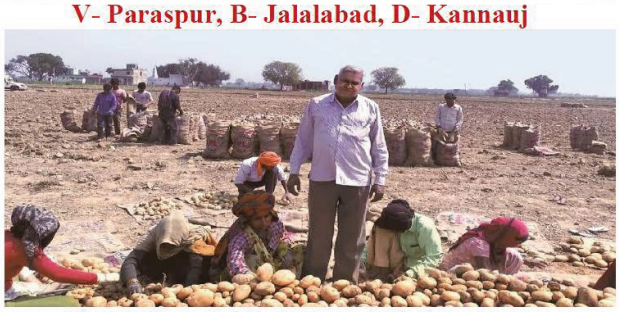
S. No.	Isolate name	Colony color	Gram Staining	Colony character	Pigmentation	Tuber slice assay
1.	**AgS**	Light brown	Dark bluish purple	Spore borne in chain on flexuous hyphae	Melanin pigment	Medium pathogenic
2.	**KaS**	Light brown	Light bluish purple	Spore borne in chain on spiral hyphae	Melanin pigment	Medium pathogenic
3.	**FiS**	Light brown	Bluish purple	Spore borne in chain on spiral hyphae	Melanin pigment	Mild pathogenic
4.	**HaS**	Light brown	Bluish purple	Spore borne in chain on spiral hyphae	Melanin pigment	Mild pathogenic
5.	**FaS**	Brown	Dark bluish purple	Spore borne in chain on flexuous hyphae	Melanin pigment	Severe pathogenic
6.	**AlS**	Dark brown	Dark Bluish purple	Spore borne in chain on flexuous hyphae	Melanin pigment	Severe pathogenic
7.	**EtS**	Dark brown	Bluish purple	Spore borne in chain on spiral hyphae	Melanin pigment	Severe pathogenic
8.	**KnS**	Light brown	Light bluish purple	Spore borne in chain on spiral hyphae	Melanin pigment	Severe pathogenic
9.	**BuS**	Light brown	Bluish purple	Spore borne in chain on spiral hyphae	Melanin pigment	Severe pathogenic
10.	**MeS**	Brown	Light bluish purple	Spore borne in chain on spiral hyphae	Melanin pigment	Severe pathogenic

Plate No.1 Collection of disease samples from different district of Uttar Pradesh (India)

V- Gadima, B- Achhnera, D- Agra

V- Paraspur, B- Jalalabad, D- Kannauj

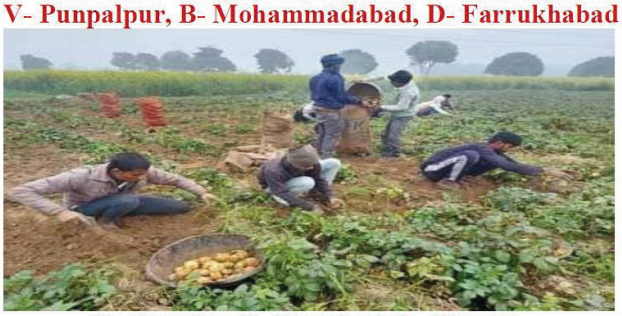
V- Bilaspur, B- Jasrana, D- Firozabad



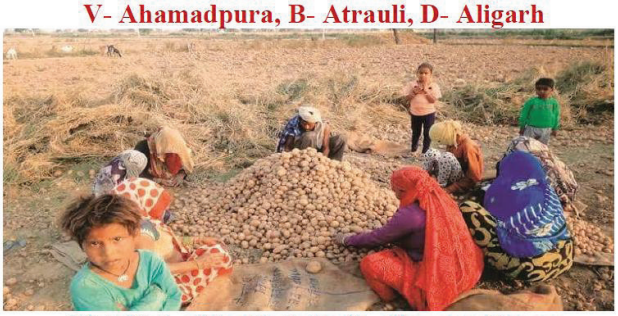
V- Gukhrauli, B- Sadabad, D- Hathras

V- Punpalpur, B- Mohammadabad, D- Farrukhabad

V- Ahamadpura, B- Atrauli, D- Aligarh



V- Bhootha, B- Basrehar, D- Etawah



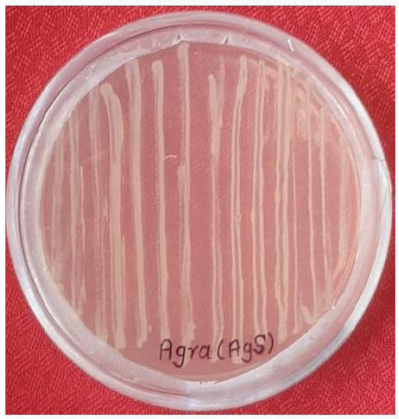
CSA Uni. of Agri. & Tech., Kanpur Nagar

V- Dahmu, B- Ujhani, D- Budaun

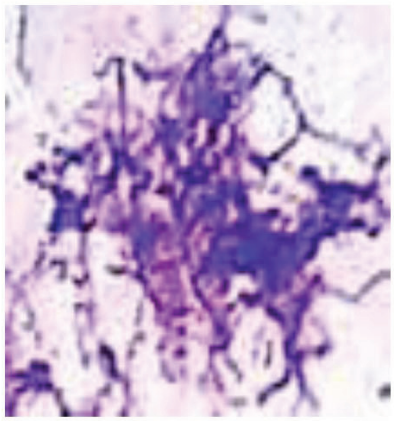
ICAR-CPRI Regional Station, Modipuram, Meeruth

Plate No. 2 Isolation, purification and staining of different isolates of *Streptomyces scabies* collected from various district of Uttar Pradesh (India)

Sample of Agra District



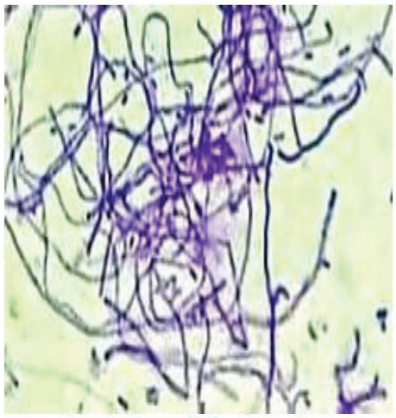
AgS isolate



Staining of AgS isolate

Sample of Kannauj District

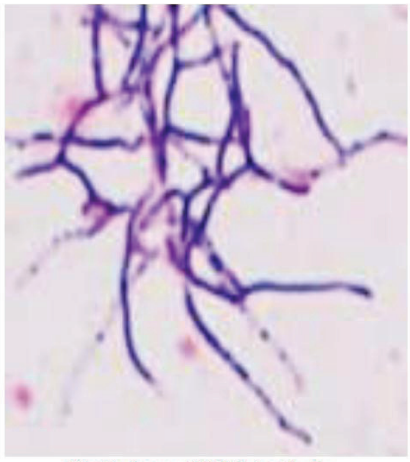
KaS isolate



Staining of KaS isolate

Sample of Firozabad District

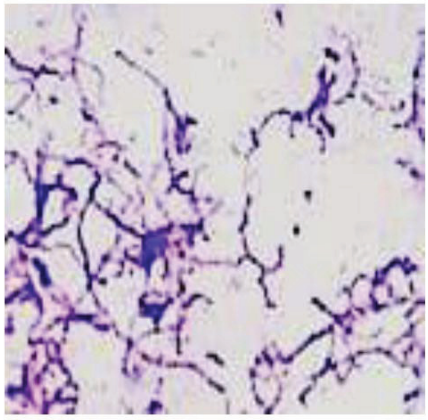
FiS isolate



Staining of FiS isolate

Sample of Hathras District

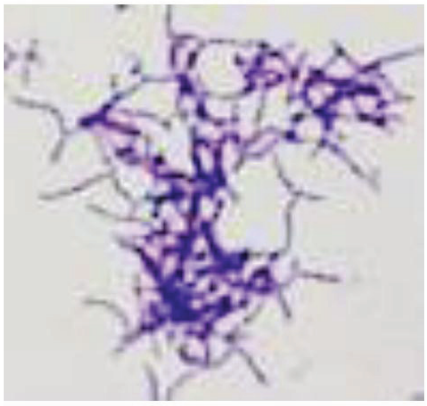
HaS isolate



Staining of HaS isolate

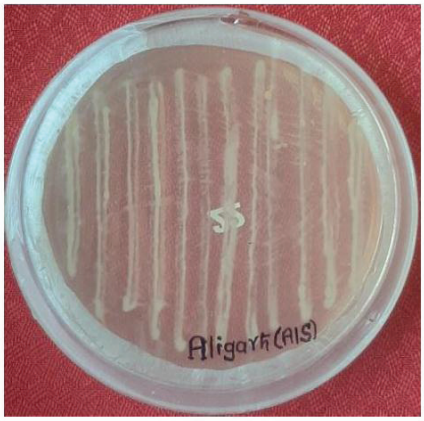
Sample of Farrukhabad District

FaS Isolate

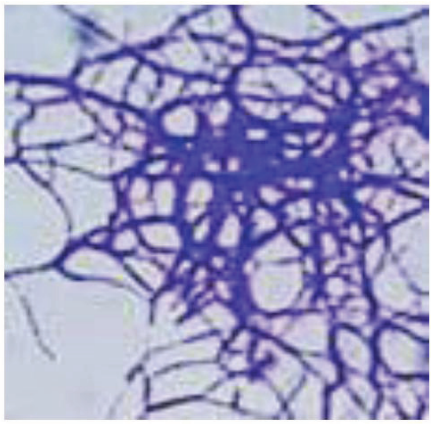


Staining of FaS isolate

Sample of Aligarh District



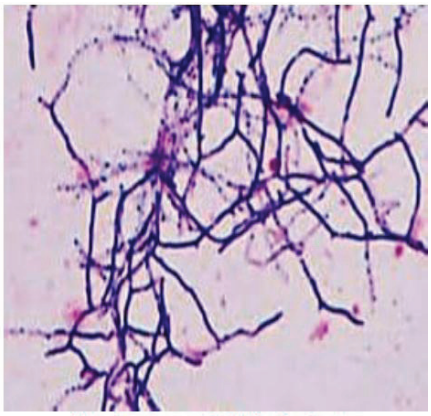
AlS isolate



Staining of AlS isolates

Sample of Etawah District

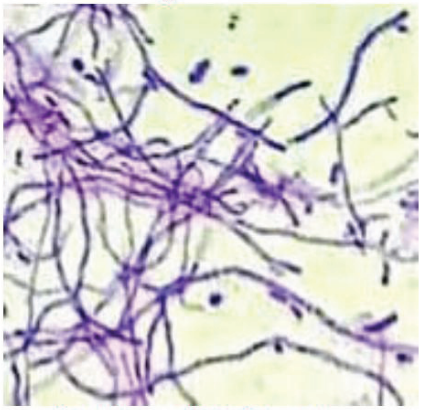
EtS isolate



Staining of EtS isolate

Sample of Kanpur Nagar

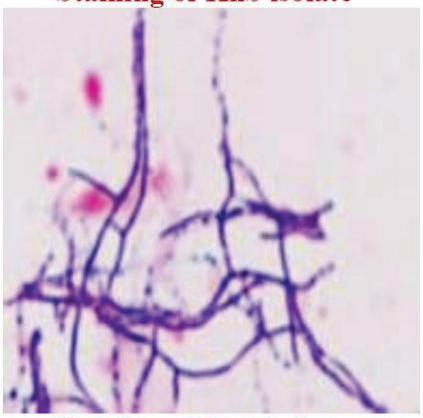
KnS isolate



Staining of KnS isolate

Sample of Budaun District

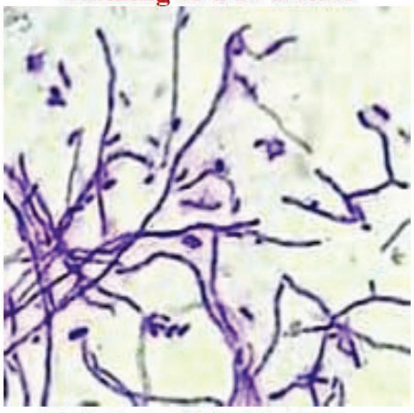
BuS isolate



Staining of BuS isolate

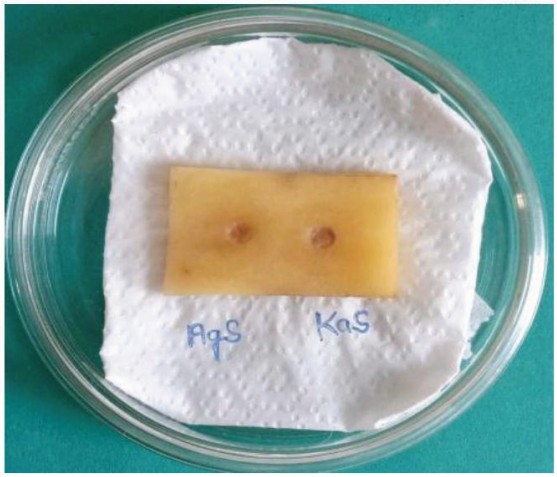
Sample of Meerut District

MeS isolate

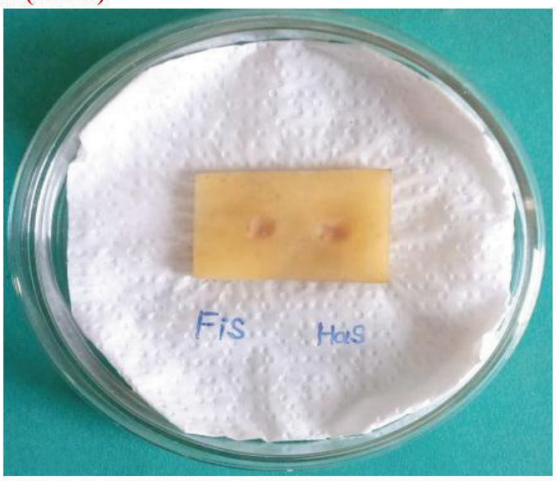


Staining of MeS isolate

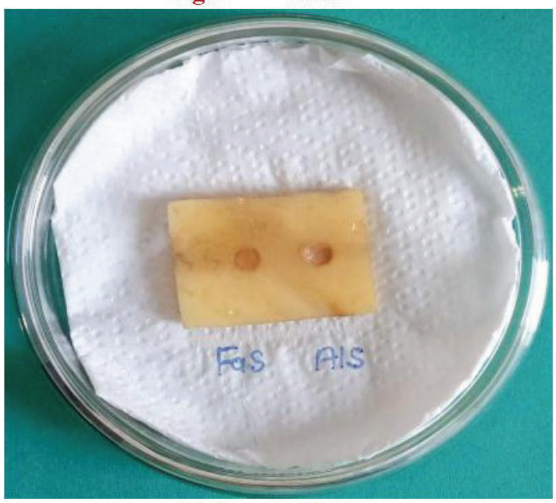
Plate No. 3 Tuber slice assay of pathogenicity of different isolates of *S. scabies* collected from various district of Uttar Pradesh (India)



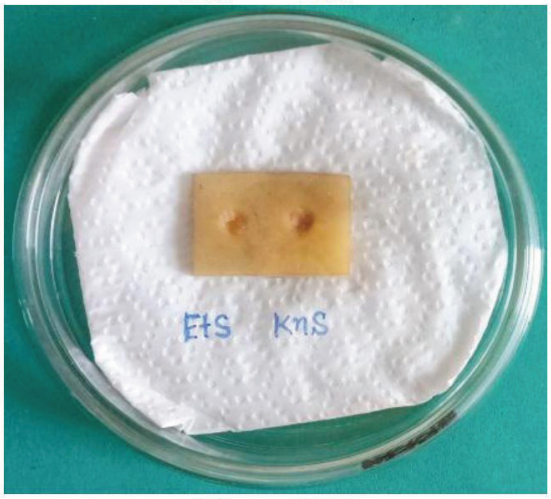
AgS KaS



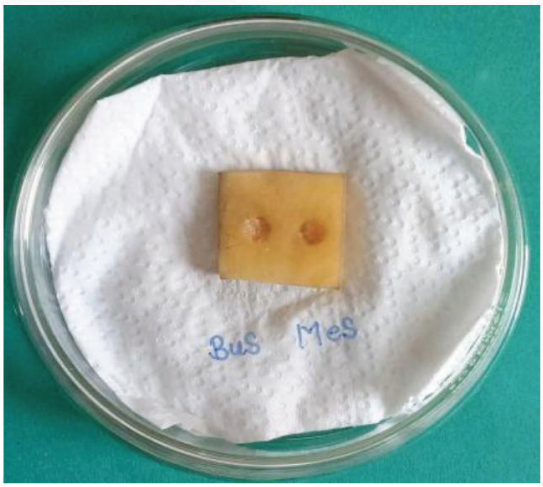
FiS HaS



FaS AlS



EtS KnS



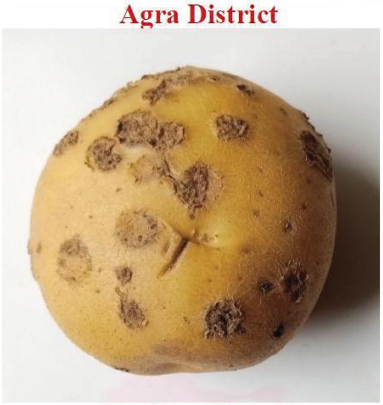
BuS MeS

Plate No. 4 Disease developed after Pathogenecity test of different isolates of *S. scabies* collected from various district of Uttar Pradesh (India)

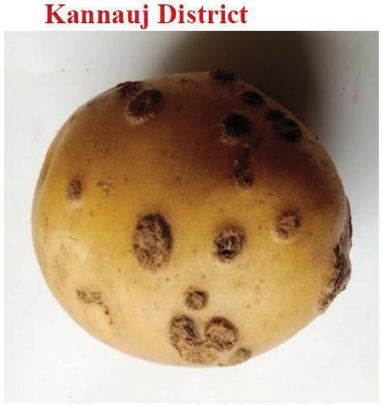
Agra District

Kannauj District

Firozabad District



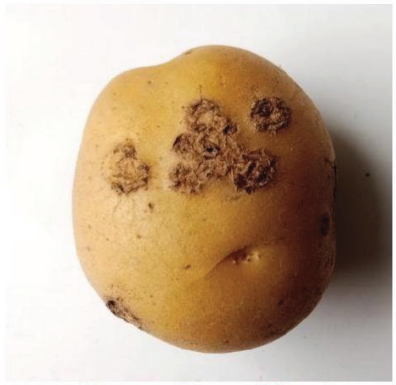
Hathras District



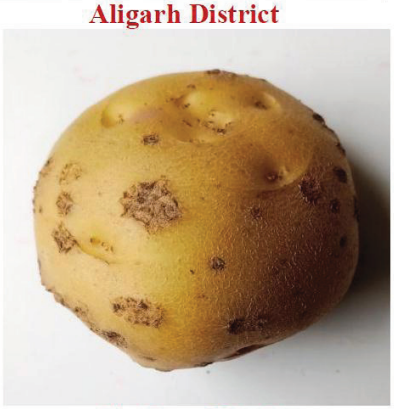
Farrukhabad District

Aligarh District

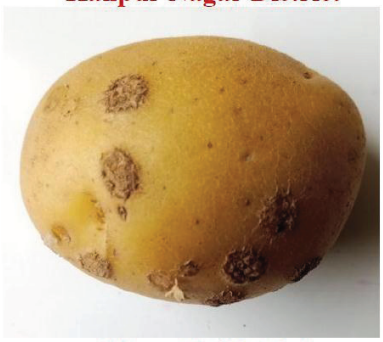
Etawah District



Kanpur Nagar District



Budaun District



Meeruth District

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