A Comparative Study of the Scab Pathogens of Potato, Sweet Potato and Sugar Beet in Bangladesh

A Dissertation Submitted to the University of Dhaka in Partial Fulfilment of the Requirements for the Degree of Master of Philosophy in Botany.

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Certificate

This is to certify that the research work entitled " A Comparative Study of the Scab Pathogens of Potato, Sweet Potato and Sugar Beet in Bangladesh" by Ashrafunnesa Flora, has been carried out under my supervision in the Laboratory of Microbiology, Department of Botany, University of Dhaka. It is further certified that the research work presented here is original and suitable for partial fulfilment of the Degree of Master of Philosophy in Botany.

08/10/2008

(Professor Mahbubar Rahman Khan)
Department of Botany
University of Dhaka.

Dedicated to

My family and teachers

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$	dollar
&	and
α	Alpha
β	Beta
cm	centimeter
Co.	Company
Conc.	Concentration
e.g.	Exempli gratia (for example)
et.al.	et alibi (with others)
etc.	Etcetra
Fig.	Figure
gm	Grams
h	Hour
i.e.	that is
lb	Pound
Ltd	Limited
mg	Milligram
min	Minute
ml	Milliliter
mm	Millimeter
μ	Micron
μm	Micrometer
NaCl	Sodium Chloride
nm	Nanometer
N_2	Nitrogen
No	Number
^oC	Degree Celsius
P^H	Negative logarithm of hydrogen ion concentration
%	percent
sol.	solution
sp.	species(singular)
Spp.	Species(plural)
Sec.	Second
UV	Ultra Violet
Vol.	Volume
W/V	Weight/Volume
V/V	Volume/Volume
viz.	Videli (namely)

ABSTRACT

The potato, beet and sweet potato samples with scab symptoms were collected from the local markets of Dhaka metropolitan city. Surface sterilized inocula were prepared from the diseased areas of the samples and these were used for the inolation of the associated organisms from a total of 35 isolates, 33 were studied in detail and their provisional identification was attempted. All the 33 isolates, were found to belong to the genus *Streptomyces*.

The isolates were grown on different ISP media and their growth responses were studied. Colonial characteristics, mass color, reverse color, diffusible pigment, growth response in ISP broth etc. were recorded. Production of melanin pigment, spore chain morphology, lecithinase production, pectinase activity and nitrate reduction activity etc. were also observed. The selected isolates were grown in different concentrations of NaCl, at different pH and at different temperature The organisms were also tested for their carbon source utilization.

All the studied organisms were studied in detail and (were) compared with the organisms described in the available literatures. In spite of the prevailing humid tropical conditions of Bangladesh, the isolated organisms showed no significant variation is their characters.

CHAPTER- 1

Introduction

Introduction

Potato is an annual, dicot, herbaceous plant under the family Solanaceae with potential perennial capacity because of reproduction through tuber. It is a plant with non-woody stem and compound leaf. The most common potato is the tetraploid (2n = 4x =48 chromosomes).

Potato (*Solanum tuberosum*) originated in the highlands of South America, where it has been consumed for more than 8000 years. The potato was introduced into Spain sometime before 1573, when it was first mentioned as a food source. Spanish explorers brought the plant to Europe in the late 16th century as a botanical curiosity. Its use in England was first reported in Herbals in 1596. From northern Europe it was returned to North America in 1719 and grown in the colonies. By the 19th century it had spread throughout the continent, providing cheap and abundant food for the workers of the Industrial Revolution (Hooker, 1983) (World Potato Atlas).

The United Nations have officially declared the year 2008 as the *International Year of the Potato* in order to "increase awareness of the importance of the potato as a food in developing nations" and calling the vegetable a "hidden treasure" (Wikipedia). The conference, held in Cusco in the potato's native land of Peru, brought together scientists and policy makers to help devise strategies to strengthen the role of what they are calling "the food of the future" in agriculture, the economy and food security, especially in the world's poorest countries, according to the UN Food and Agriculture Organization (FAO).Grown in more than 100 countries, the potato is already an integral part of the global food system, said FAO, which sponsored the conference along with the International Potato Centre (CIP). With a record 320 million tons produced in 2007, it is the world's number one non-grain food commodity. Consumption is expanding strongly in developing countries, which now account for more than half of the global harvest and where the potato's ease of cultivation and high energy content have made it a valuable cash crop for millions of farmers, according to the agency, June 10, 2008 was celebrated as National Potato Day in Peru and was supported by the International Potato Center (CIP)

(http:www.potatonews.com/pressreleases/press_detail). 'Bangladesh Potato campaign 2008' was held in China-Bangladesh Friendship Hall on 7-9th May 2008 and was organized jointly by Ministry of Agriculture, CSD Bangladesh and FAO to make awareness of increased potato consumption.

A B

Fig.- 1 : A) Logo of International year of the potato 2008, B) Potato campaign in China Bangladesh Friendship Hall, Bangladesh.

A single medium-sized potato contains about half the daily adult requirement of vitamin C. Other staples such as rice and wheat have none. Potato is very low in fat, with just 5 percent of the fat content of wheat, and one-fourth the calories of bread. Boiled, it has more protein than maize, and nearly twice the calcium (CIP).

Today, potato is the fourth most important food crop in the world, with annual production approaching 300 million tons (World Potato Atlas). The international potato center (Centro Internacional de la Papa-CIP) was established is 1895 in La Molina, Lima, Peru. As well as a food source, Potato is also used as theme of toy, art etc. to impress people.

Table 1: Nutritional value per 100 g Potato, raw, withpeel (3.5 oz) Energy 80 kcal 320 kJ

Carbohydrates	19 g
- Starch 15 g	
- Dietary fiber 2.2 g	
Fat	0.1 g
Protein	2 g
Water	75 g
Thiamin (Vit. B1) 0.08 mg	6%
Riboflavin (Vit. B2) 0.03 mg	2%
Niacin (Vit. B3) 1.1 mg	7%
Vitamin B6 0.25 mg	19%
Vitamin C 20 mg	33%
Calcium 12 mg	1%
Iron 1.8 mg	14%
Magnesium 23 mg	5%
Phosphorus 57 mg	8%
Potassium 421 mg	9%
Sodium 6 mg	0%

Percentages are relative to US recommendations for adults. (Source: Wikipedia)

More than one-third of the global potato output now comes from developing countries, up from just 11 percent in the early 1960s. Potato ranked first among tuber crops in Bangladesh. The Bangladesh Agricultural Research Institute (BARI) maintains a Tuber Crops Research Center (TCRC), established in 1986 in Gazipur.

CIP has played an important role in this shift, providing resource-poor farmers with a range of new technologies and potato breeding lines specifically designed for developing-country conditions.

(A) (B)

Fig 2: (A) Various potato dishes, (B) Potato Ceramic. Moche Culture. Larco Museum Collection (Source: Wikipedia)

Fig- 3 : Mr. Potato Head is a popular children's toy, first sold in 1952 by Hasbro, consisting of a plastic model of a potato which can be decorated with attachable plastic parts, such as a mustache, hat and nose, to make a face (Source: Wikipedia).

The dedication and effort of pioneer taxonomist Carlos Ochoa formed the foundation of the CIP-held collection of potato genetic resources. This Peruvian scholar, whose crusade to find wild species in the Andes began more than 40 years ago, has discovered 80 different species—about one-third of all the wild potatoes known to exist.

Russian geneticist Nikolai I. Vavilov and his colleagues were the first plant collectors and explorers to gather comprehensive potato samples from the Andean region. Vavilov conducted explorations in more than 50 countries during the 1920s and 1930s. He obtained numerous samples of potatoes and their wild relatives in expeditions to South America. Afterwards, Peruvians like Ochoa continued the arduous labor of exploration and collection of plant species.

CIP maintains the world's largest bank of potato germplasm, including some 1500 samples of about 100 wild species collected in eight Latin American countries and 3800 traditional Andean cultivated potatoes. The collection is maintained under the auspices of the FAO and is available to plant breeders worldwide free upon request.

The potato (*Solanum tuberosum* L.) was introduced to some coastal areas of southern Asia in the late sixteenth or early seventeenth century, most likely by mariners from Portugal and subsequently other European nations. The early historical record of the potato in the region is unclear since the term "potato" is derived from "batata," the Carib term for sweetpotato (*Ipomoea batatas*), which preceded the potato by eighty years in its introduction to Europe from its American site of origin (World Potato Atlas).

Warren Hastings, British colonial governor from 1772 to 1785, promoted the cultivation of potato widely throughout the region, beginning in areas around Calcutta in the Bengal region. By the late eighteenth to early nineteenth century, potatoes were sufficiently established in the hills and plains that varieties had acquired local names, such as: *Phulwa* (flowering in the plains), *Gola* (round potatoes), and *Satha* (maturing in sixty days) (World Potato Atlas).

Consistent with the trend for South Asia, the potato crop of Bangladesh has grown very rapidly over several decades. Average yields rose at a modest, but consistent rate through the 1990s, from about ten to eleven tons per hectare (T/HA), and have risen more sharply in recent years, reported at over fourteen T/HA in 2004. Given the cropping intensity of arable land in Bangladesh, future production gains will have to rely more on higher yield than on expanded area of cultivation. Trends over forty years are summarized below.

Table 2: Bangladesh: Potato Production Trends

	1961-63	2001-03	Comparison
Total Area Cultivated ('000 Hectares)	56	244	436%
Per Capita Area Cultivated (Square Meters)	10.3	17.0	165%
Average Yields (Tons per Hectare)	6.1	13.1	215%
Total Production ('000 Tons)	347	3,198	922%
Per Capita Production (Kilograms)	6.4	22.2	348%

- Data are three-year averages, comparison of 2001-03 to 1961-03.
- Population estimates for per capita data: 1961-63 = 54,341,000; 2001-03 = 143,808,000.
- Source: FAOSTAT.

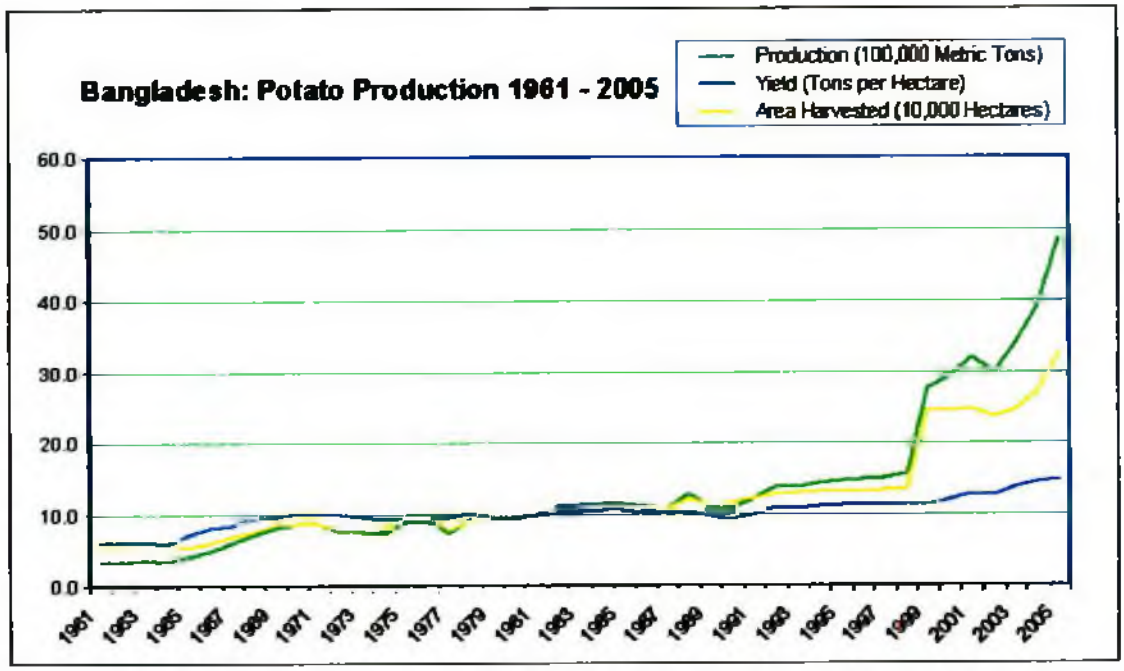


Fig- 4 : Potato Production of Bangladesh 1961-2005 (*Source:* World Potato Atlas.)

Also consistent with a general trend in South Asia, the potato in Bangladesh is not grown as a cheap starchy staple, but is a relatively expensive source of calories grown mostly for cash sale.

Potato provides a critically important element to the diets of many people in Bangladesh as a source of vitamin C and amino acids not provided by rice, and is a

source of cash income to farmers and laborers which complements other staple crops. Potato is now the third most important food item of Bangladesh by tonnage production (FAOSTAT).

Bangladesh maintains a delicate balance of food security. Although some degree of chronic malnutrition persists for many of Bangladesh's people, major famine has been diverted for the past few decades by significant growth in agricultural productivity, mostly of the main staple of rice, but also in recent years due to a shift to "market gardening" of high value crops, including the potato.

The potato crop is very widely grown across Bangladesh, with the greatest concentration of area (though generally not the highest yields) occurring in the northwestern region of the country, especially the districts of Rangpur and Bogra. An area of concentrated cultivation in the vicinity of the capital city, Dhaka, supplies the high demand for potato.

Potato varieties in Bangladesh, as across the territory of colonial India, have been derived from two subspecies, *Solanum tuberosum* subspecies *tuberosum* and subspecies *andigena* (Shekhawat *et. al.* 1992). As of independence from UK (i.e. of India and Pakistan in 1947), most varieties were of recent European import, and few were suitable to the short-day conditions of the Indo-Gangetic Plains.

Potatoes in Bangladesh are sometimes designated as *deshi*, a term often treated as synonymous with "local varieties" or "indigenous," generally referring to varieties introduced prior to independence from UK in 1947 which are favored within a particular region. Most are relatively low yielding and have somewhat longer vegetative cycles than more recently introduced varieties, but are retained by farmers for other factors, such as storage properties and cooking and culinary qualities (World Potato Atlas).

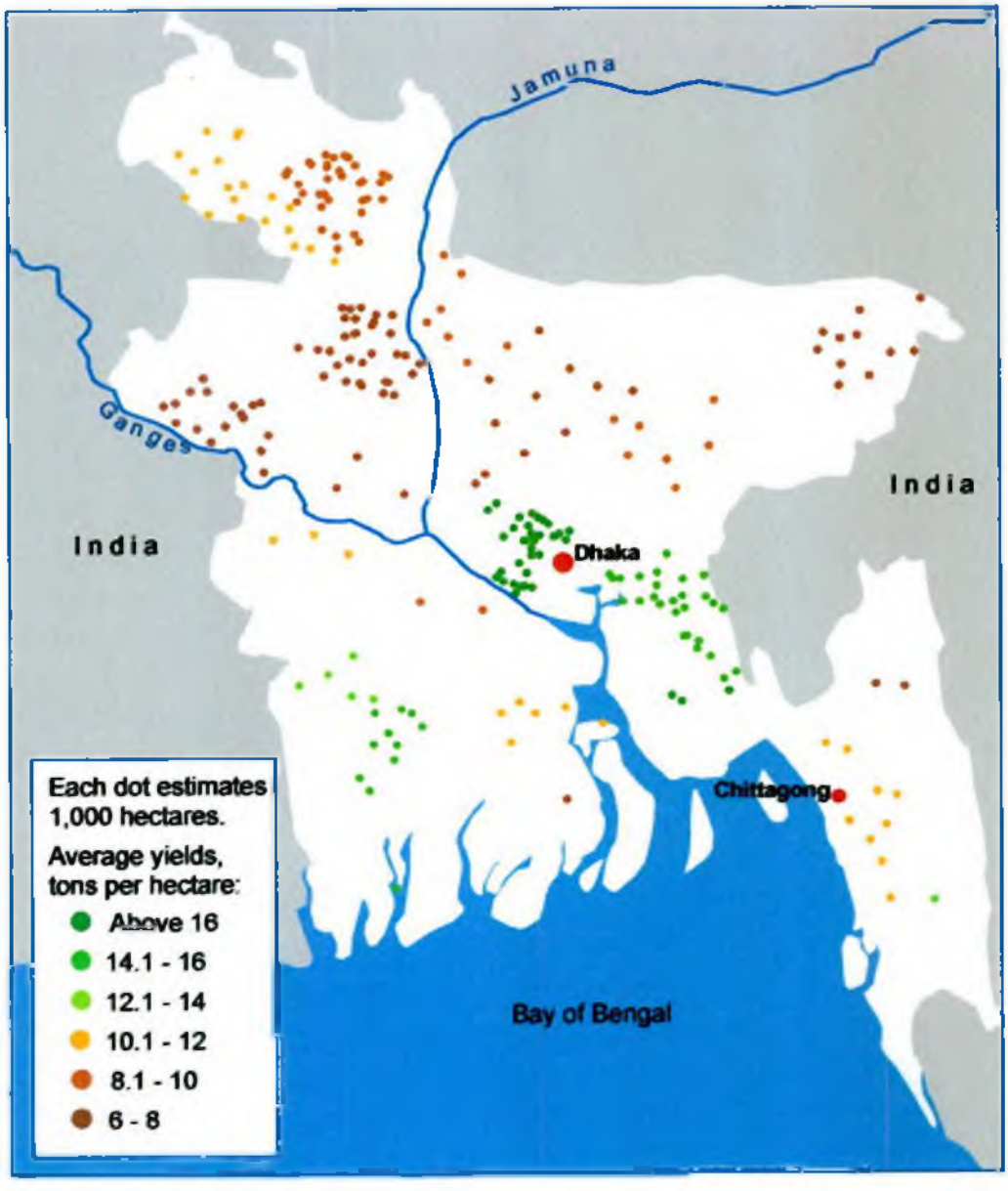


Fig- 5 : Potato Production areas and Average Yield (Source: World Potato Atlas)

From the list of "local varieties" which have been blessed with a local name, it seems that a reddish tuber color is a popular quality. Local names include (Banglapedia: Potato):

- *Sheel Bilatee*: mostly cultivated in Rangpur, with oblong reddish tubers;
- *Lal Sheel* , also known as *Lal Madda* and *Bograi*: grown primarily in Bogra with rounder and reddish tubers;
- *Lal Pakri* : widely grown in Dinajpur, Bogra, and Sirajganj districts with round, also reddish tubers;
- *Du Hajari*: mostly cultivated in the Chittagong area, with round and more pale tubers.

Beginning in the 1970s, several dozen potentially higher yielding varieties (HYVs) have been introduced to Bangladesh. Most have proven unsuitable to local conditions, but several which have been retained have proven capable of much higher average yields. The Bangladesh Agricultural Research Institute (BARI) now maintains a facility in Panchagar District where HYVs are evaluated and seed tubers are produced. Among the more popular HYVs are (Banglapedia: Potato; Khurana and Garg 2003, p. 176):

- Cardinal, perhaps the most popular HYV, also with reddish tubers;
- Diament, reported as fairly resistant to disease;
- Kufri Shindhury, introduced from India, also reportedly resistant to many diseases and pests;
- Other more recently introduced exotic varieties: Alisa, Alpha, Archa, Baraka, Chamak, Cleopatra, Dheera, Granola, Hira, Kronia, Maurin, Mondial, Morena, Multa, Origo, and Patronis.

As of data available for 2000-2001, HYVs are consistently outyielding "local varieties," though the difference is locally variable, greatest where overall yields and production are highest. For example, in the Dhaka district (in the vicinity of the capital), the average yields of HYVs and local varieties are 17.3 and 8.7 tons per hectare, respectively (World Potato Atlas, Source: Bangladesh Bureau of Statistics) .

Bangladesh

Potato Production by Area and Average Yields of Long-Established and Recently Introduced Varieties

Fig- 6 : Bangladesh: Distribution of Long-Established and Recently Introduced Potato Varieties (Source: World Potato Atlas)

Note: This map displays the area of potato cultivation in Bangladesh, distinguishing older established varieties and more recently introduced high-yielding varieties, or HYV's. (Please see the text of the "Varieties and Seed Systems" section for more information about varieties.) Data are provided by the Government of Bangladesh, Bureau of Statistics, reported for 2000-2001.

The base of each rectangle represents the area of cultivation within each district of HYV's (in **red**) and longer-established varieties (in **green**), ranging from as high as 31,000 hectares of HYV cultivation in Dhaka District, to as little as 1,000 hectares in several districts. (Data have been slightly rounded and generalized for mapping display.)

The height of each rectangle represents average yields in tons per hectare (T/ HA), ranging from 4 T/HA of older varieties in Khagrachari District to 23 T/HA of HYV's in Noakhali District.

Map design and production by Kelly Theisen, using ESRI ArcGIS 9/ ArcMAP at CIP Research Informatics Unit, Lima, Peru. June 2006.

Distribution of varieties by land under potato cultivation has been estimated as 33 percent "local," 65 percent HYV, and 2 percent "Indian" (Banglapedia). Since the border with India has been open and porous since the independence of Bangladesh, it is likely that some varieties developed by the Central Potato Research Institute (CPRI) of India since its founding in 1949 have made their way to Bangladesh, though perhaps currently known by alternate names.

Since climatic conditions in Bangladesh allow only for a winter crop, tubers retained for seed must be stored for several months from one season's harvest to the next planting. Storage of seed tubers can be accomplished via the limited cold storage facilities of Bangladesh, or possibly via locations in surrounding countries which permit cultivation during other seasons.

The potato seeds are produced in Bangladesh mainly by the formal system and the informal system. The formal system refers to seed tubers produced and distributed by state-sponsored institutions, with some involvement of the private sector. Seed from the formal sector has generally been subject to an inspection and control process intended to assure that the seed is of the variety claimed, with low incidence of disease or pest infestation, and otherwise viable. Such seed is often referred to as "certified seed," having been produced from "pre-foundation" and "foundation" seed, although the precise definitions of these terms are locally variable.

The Tuber Crops Research Center affiliated with the Bangladesh Agricultural Research Institute (BARI) uses imported tuber seed to produce from 500 to 700 tons of pre-foundation seed annually, which it supplies to the Bangladesh Agriculture Development Corporation (BADC), which in turn produces and supplies certified seed. BADC is able to produce about 6,000 tons of certified seed each year, approximately 7.3 percent of the required tuber seed of HYVs grown in Bangladesh, or about 4.8 percent of the nation's total requirement of tuber seed (World Potato Atlas). To the extent that subsequent generations of certified seed are sown, beyond just one cropping season, the quantitative impact of certified seed can be considerably greater than these percentage figures would suggest (World Potato Atlas).

Another alternative that has been explored in Bangladesh to increase the availability of high quality disease-free seed tubers without depending on imports is multiplication via true potato seed (TPS). TPS research was initiated in Bangladesh in 1980-81 at the Tuber Crops Research Center. CIP has contributed several families of potato cultivars to this effort, but one challenge remains finding TPS families which meet farmers' preferences at an affordable price (World Potato Atlas).

A significant challenge to potato cultivation across the Indo-Gangetic Plains has always been that, unlike temperate regions where the potato harvest is followed by a cold winter, the harvest in February and March occurs when both temperatures and humidity are increasing. As of 1998, only about 200 cold storage facilities were in operation across Bangladesh. As of 1996, roughly 675,000 tons of potato were in cold storage, or about 40 percent of the total production that year (Banglapedia: Potato: FAOSTAT). Development of cold storage has not kept pace with an increase of crop production of over twice the 1996 volume (World Potato Atlas).

Commercial production of most potatoes is primarily through vegetative propagation by means of lateral buds formed on the tuber, a modified stem. Trough such vegetative propagation many disease affects each of the types listed above.

Much of potato pathology has been done with S. tuberosum in northern Europe and North America. Increased importance of the crop in the tropics and subtropics has occasioned unprecedented work during the past half century on tropical diseases and evaluation of wild and cultivated genotypes as sources of disease resistance.

Potato scab was ranked as the fourth most important disease of this crop in North America (Loria et al. 1997) and a common disease that occur through out the potato growing regions of the world including Bangladesh. Potato can be considered as a good substitute of rice especially in these days of rice shortage.

Disease severity is often assed on the proportion of tuber surface affected (Richardson & Heeg, 1954, Large & Honey,1955). A spot sampling method can be

used (Lowings & Ridgman,1959) on a few tubers and the damage done within the affected area assed (Mckee, 1958).

S. scabies (Thaxter) Waksman & Henrici is considered the main causal organism although Corbaz (1964) claimed that *S. griseus* Waksman & Henrici, *S. aureofasciens* Duggar and *S. flaveolus* (Waksman) Waksman & Henrici can also cause the disease. Russet scab may be caused by a species close to *S. tenuis or S. marginatus* (Millard & Burr) Waksman (Harrison,1962) and Bonde & McIntyre (1968) described acid tolerant pathogenic *S.* spp. not identical with *S. scabies*.(Lapwood,1973). Recent studies reveal that scab diseases of potato and other root crops, except sweet potato, are characterized by corky lesions on potato tubers and expanded tap roots and cause economically significant losses in yield (Loria *et al*,1997). These diseases are known to be caused by *Streptomyces scabiei* (Lambert& Loria, 1989a; Truper& Clari,1997), *Streptomyces acidiscabies (*Lambert & Loria, 1989b), *Streptomyces turgidiscabies* (Miyajima *et al*.,1998) and other species (Faucher *et al*.,1995; Goyer *et al*.,1996; Bouchek-Mechiche *et al*.,2000). *Streptomyces scabiei* is the predominant causal agent and most closely related to the Diastatochromogenes group encompassing *Streptomyces diastatochromogenes, Streptomyces bottrropensis* and *Streptomyces neyagawaensis (*Takeuchi *et al*., 196). *Streptomyces acidiscabies* and other pathogenic strains fall into a group with similarities to the *Streptomyces albidoflavus* group of (Williums *et al*., 1983; Song *et al*., 2004).

Common scab of potato occurs throughout the world. It is most prevalent in neutral or slightly alkaline and light sandy soils, especially during relatively dry years. The same pathogen also affects garden beets, sugar beets, radish and other crops. The disease, by its usually superficial blemishes on tubers and roots, reduces the value rather than the yield of crop. But severe root infection may reduce yields. Deep scabs result in increasing the waste in peeling (Agrios, 1997).

The losses of saleable tubers caused by common scab, although not usually as severe as that by other potato diseases, may be considerable, especially if the tubers are to be washed and pre-packed. Moderately affected tubers are rendered unsightly and those

that are severely scabbed may be unmarketable. The consumer also suffers loss as deeper peeling is usually necessary.

Common scab is most prevalent in neutral or slightly alkaline and light sandy soils, especially during relatively dry years. The same pathogen also affects garden beets, sugar beets, radish and other crops. The disease, by its usually superficial blemishes on tubers and roots, reduces the value rather than the yield of crop. But severe root infection may reduce yields and deep scabs increase the waste in peeling (Agrios, 1997).

According to Agriculture Extension Department (AED), Bangladesh data, about 32 lac metric ton (MT) of potato have been produced in over 1 lac 78 thousand hectares in eight northern districts against last year's production of 14 lac 60 thousand MT from a little over 1 lac hectares. The production this year is 220 percent more than that of the previous year. This year's total yield is worth about *taka* 34 thousand crore (The Daily Star, March 18, 2008, pp 14). Fulfilling the local requirement potato is now making its way to hotels where exhibitions are being held on different innovative recipes of potatoes like sweet potato pie, potato *halwa*, sweet potato tart, potato cake and potato ice cream. Recently (May 7-9, 2008) a potato campaign was held in Dhaka, Bangladesh to make awareness and to encourage increased potato intake. Potato export from Bangladesh is expected to be 2 thousand MT this year mainly due to a bumper production, lower price and lack of storage facilities. All potatoes can not be exported. Each exportable potato must have a weight of at least 100 gram and should be spotless and fresh (The Daily Star, March 27, 2008). Like potato, beet and sweet potato also have large prospect to be treated as economically important crops.

The symptoms of common scab of potato are observed mostly on tubers. At first they consist of small brownish and slightly raised spots but later they may enlarge, coalesce and become very corky. Frequently the lesions extend below the tuber surface and when the corky tissue is removed, 3 to 4 mm deep pits are present in the tuber. Sometimes the lesions appear as small russeted areas and are so numerous that they almost cover the tuber surface or they may appear as slight protuberances with depressed centers covered with a small amount of corky tissue (Agrios, 1997).

Potato scab lesions may some times be confused with powdery scab, a disease caused by an entirely different pathogen, the fungi, *Spongospora subterranea* (Guerrero, 1996, Bulman et al. 1998). Common scab lesions lack the torn periderm around the lesion and the powdery masses of spores which are characteristic features of powdery scab (Dutt, 1979). Symptoms of scab disease are quite variable and distinctions have been made between russet (superficial corky tissue), erumpent (a raised corky area) and pitted (a shallow to deep hole) scab. All of these can be caused by the same pathogen, *Streptomyces scabies* (Loria,1991). According to Loria (Loria,1997), the type of lesion probably is determined by host resistance, aggressiveness of the pathogen strain, time of infections and environmental conditions. The term common scab generally refers to the response of the disease to soil pH. Common scab is suppressed at soil pH levels of 5.2 or lower. Acid scab occurs in soils below pH 5.2 and caused by *Streptomyces acidiscabies* and the symptom is identical to that of *Streptomyces scabies* (Loria, 1991)

Scab disease has also been reported to occur on other tuber crops e.g. beet, turnip, rutabaga, radish, carrot etc. (Michael et al. 1996). *Streptomyces* ipomoea cause scab in sweet potato (Gottlieb, 1973) which is also called soil rot and canker (Loria et al. 1997). *Streptomyces scabies* cause pod wart in peanut, root tumor of cucurbits and the symptom is not identical to scab of potato (Loria et al. 1997). Again the scab symptom similar to that of potato can be produced by completely different organisms e.g. citrus (*Elsinoe australis,*) (Chung & Timmer, 2005,), cucurbit (*Cladosponum cucumerinum)* (Zitter, 1986), wheat (*Fusarium graminearum*) (O'Donnel et al, 2000).The term common scab generally refers to response of the disease to soil pH. Common scab is wide spread and is caused by *Streptomyces scabies*. Acid scab has been reported below pH 5.2 and caused by *Streptomyces acidiscabies* (Loria et al. 1997).

Common scab of potatoes was first recorded in the U.K. in 1825 and is known to cause raised or depressed lesions on the surface of the potato tubers. The disease has been given various names such as scab, American scab, deep scab, corky scab, gale and schorf. Several other diseases of potato have been called scab to denote ulceration of the surface of the tubers (Dutt, 1979) . Six different types of scab lesions have been described by Millard and Burr (1926).

In 1890, Thaxter identified the causal agent of common scab and named it *Oospora scabies* (Thaxter, 1891). The type culture was not maintained, and the species was renamed *Actinomyces scabies* by Gussow (Gussow, 1914) and then *Streptomyces scabies* by Waksman and Henrici (Waksman and Henrici,1948). Later, confusion over the use of this nomenclature resulted in the species being considered *incertae sedis* (type strain not exant, many taxomically different strains available). The species name, *S. scabies*, has since been revived by Lambert and Loria. (Bramwell *et al.*1988).

Streptomyces scabies is a hardy saprophyte that can survive indefinitely either in its vegetative myceloid form or as spores in most soils except the most acidic ones. The vegetative form consists of slender branched mycelium with few or no cross walls. The spores are cylindrical or ellipsoid and are produced on specialized spiral hyphae that develop cross walls from the tip toward their base, and as the cross walls constrict, spores are pinched off at the tip and eventually break away from the hypha. The spores germinate by means of one or two germ tubes, which develop into the myceloid form (Agrios,1997).

The pathogen is spread through soil water, wind blown soil, and by infected potato seed tubers. It penetrates tissues through lenticels,wounds, stomata and in young tubers directly as they are more prone to infection .after penetration, the pathogen apparently grows between or through a few layers of cells, the cells die and the pathogen then lives off them as a saprophyte. In the meantime, however, the pathogen apparently secretes a substance that stimulates the living cells surrounding the lesion to divide rapidly and to produce several layers of cork cells that isolate the pathogen and several plant cells. As the cells that are cut off by the cork layer die, the pathogen subsists on them. Usually, several such groups of cork cell layers are produced, and as they are pushed outward and sloughed off, the pathogen grows and multiplies in the additional dead cells, and thereby large scab lesions develop (Agrios, 1997).

Streptomycetes may be characterized and identified on the basis of phenotypic and genotypic variation. In an extensive numerical taxonomic study, Williams et al. (1983) assigned *S. scabies* (ISP 5078) to *S. atrolovaceus* (cluster 3). A subsequent study characterized *S. scabies* sp. nov., nom. rev. (ATCC 49173; Lambert and Loria

1989) which had not been identified as closely related to any of the major clusters in the previous study. More extensive phenotypic studies (Kamper et al. 1991; Doering-Saad et al. 1992) revealed significant diversity among scab causing strains and assigned them to several clusters. Molecular techniques have also been applied to the classification of scab strains. Studies using DNA homology (Healy and Lambert 1991), Southern blotting (Doering-Saad et al. 1992), rep_PCR (Sadowsky et al.1996) and sequence analysis (Takeuchi et al. 1996) have suggested genetic diversity of scab causing strains The study examines the diversity of Streptomycetes causing potato scab using phenotypic identification, 16S rRNA sequence, and pathogenicity measurements. Pathogenicity did not correlate well with taxonomic identification, nor with phylogenetic position. The results suggest that neither phenotypic nor 16s characteristics are diagnostic for scab-causing strains. Additionally phenotypic characterization were not fully consistent with phylogenetic relationships. This incongruency highlights the difficulties of taxonomy within the streptomycetes and affirms the need for better integration of genetic and phenotypic characters in the systematic of this group (Bramwell *et al* 1998).

The disease scab and its pathogen has become a much studied topic throughout the world. Research works have been accomplished on this topic in different countries like Japan, China, Israel, Finland, U.S.A., U.K., Canada, Iran, Korea, France.

In Israel eighty *Streptomyces* isolates, including 35 potato scab inducing strains and 12 reference strains of *Streptomyces scabies*, were physiologically characterized by a total of 329 miniaturized tests. Overall similarities were determined by numerical taxonomy. The majority of the pathogenic isolates and reference strains were assigned to *S. violaceus* (57%) and *S. griseus* (22%). A DNA probe derived from the rRNA operon of *S. coelicolor* IMET 40271 was used to detect restriction fragment length polymorphism (RFLPs) among 40 pathogenic and nonpathogenic *Streptomyces* isolates. No significant correlation between numerical classification and RFLP grouping of *Streptomyces* strains could be revealed. The results obtained suggest that RFLP data are of minor importance in classification of *Streptomyces* species and that genes for pathogenecity determinants are spread among different *Streptomyces* species by mobilizable elements (Doering et al., 1992).

Streptomyces strains isolated from potato scab and potato russet scab lesions in Japan were characterized by morphological and physiological analyses, DNA homology, and restriction fragment length polymorphism (RFLPs). in Japan, potato scab is caused by at least four genetically distinct *Streptomyces* species, including S. scabies and *S. acidiscabies* (Sawada et al., 1995). The complete 16S rRNA sequences of 12 strains of *Streptomyces* spp. causing potato scab pathogens, were determined and phylogenetic analysis was done. The results confirmed the lack of close relationship among *Streptomyces* spp. that cause potato scab. The findings suggest that potato scab is caused by phylogenetically diverse *Streptomyces* spp and that the pathogenesities of these organisms develop independently. (Takeuchi., 1996).

In 1998 *Streptomyces turgidiscabies* was first reported in Eastern Hokkido, Japan. Symptoms included distinctly erumpent lesions. In culture, it is distinct from other scab causing *Streptomyces* spp. The levels of DNA similarity within *Streptomyces turgidiscabies* strains were high, but similarities between strains of this species and strains of *Streptomyces scabies, Streptomyces acidiscabies, S. caviscabies, S.griseus, S. setonii and S. tendae* were low (Miyajima et al, 1998). The existence of the causal agent of potato scab in high acid soils having a pH as low as 4.4 was confirmed in Japan (Tashiro et al., 1999) and was identified as *Streptomyces acidiscabies* (Tashiro, 2003). Identification of 132 potato scab samples collected in Hokkido, Japan, in 1994 showed the presence of *Streptomyces scabies, S. achromogenes, Streptomyces turgidiscabies and Streptomyces acidiscabies* (Tanaka, 2003).

In Korea, potato common scab disease was first reported to be associated with diseased tubers (Kim & Lee,1996) and subsequently, strains identified as *Streptomyces scabies, Streptomyces turgidiscabies and Streptomyces acidiscabies* were isolated from diseased tubers (Kim et al., 1998). Symptoms of potato scab in tubers collected from Jeju Island (raised corky lesions) were distinct from those typically found elsewhere in Korea; isolates derived from these lesions showed distinctly different morphological features. Studying their fatty acid profiles, 16S rRNA gene sequences, the pathogenicity- linked genes nec-1 and production of phytotoxin three novel plant pathogenic *Streptomyces* species were identified. They

are *Streptomyces luridiscabiei sp. nov, Streptomyces puniciscabiei sp. nov. and Streptomyces niveiscabiei sp. nov.* (Park et al.,2003). Phylogenetic analysis of *Streptomyces* spp. isolated from potato scab lesions in Korea on the basis of 16S rRNA gene and 16S-23S rDNA internally transcribed spacer sequences was done. (Song et al., 2004).

Phenotypic, genotypic and pathogenic variation among *Streptomyces* implicated in common scab disease was studied in UK. The study examines the diversity of *Streptomyces* causing potato scab using phenotypic identification,16S rRNA sequences and pathogenicity measurements. Pathogenicity did not correlate well with taxonomic identification, nor with phylogenetic relationships. It is concluded that this shows the problems in Streptomycetes taxonomy, and suggested the need for better integration of genetic and phenotypic characters in the systematics of this group (Bramwell et al.1998).

Identification of potato scab inducing and suppressive species of *Streptomyces* was done in USA (Lorang et al, 1995). Of 17 strains of *Streptomyces* (16 from potato tubers and one from garden beet), 13 were determined to be *Streptomyces scabies,* two were *Streptomyces diastatochromogenes,* one was *Streptomyces albogriseolus,* and one could not be identified. Only the 13 *Streptomyces scabies* strains and the one unidentified russet scab strain were pathogenic on leaf-bud tubers. Three strains, two *Streptomyces diastatochromogenes* and one *S. albogriseolus,* obtained from a potato scab plot that had become suppressive, produced antibiotic-like compounds against highly pathogenic strains of *Streptomyces scabies.* Dr. Rosemary Loria of Cornell University NY seems the most enthusiast worker on the topic *Streptomyces* and the potato scab disease. She is responsible for potato disease management in New York State and conducts research on soilborn pathogens of potato, including molecular analysis of pathogenecity in the genus *Streptomyces* (Loria,1997) and identification of the plant cell target for thaxtomin, the phytotoxin produced by *Streptomyces* spp. responsible for scab disease development in the susceptible plant (Loria, 2008). Most of other works are concerned to controlling the disease (Ryan et al.,2004; Liu et al.,1995; Ryan & Kinkel,1997; Becker et al.,1997).

Taxonomic studies on *Streptomycetes* causing potato scab was done in Canada (Goyer et al, 1996). The symptoms of potato scab caused by *Streptomyces scabies, Streptomyces acidiscabies and, Streptomyces sp. are given. The diversity of S. scabies* and the morphological and physiological traits such as protein and fatty acid content of the species are discussed. DNA-DNA hybridization stydies which classify *Streptomyces scabies into* 3 genetic groups are included. The ability to produce phytotoxins called thaxtomins a physiological property shared by most pathogenic strains of Streptomyces,is discussed (Goyer et al, 1996). Most of the other works were concerned with the phytotoxin Thaxtomin (Doumbou et al, 1998 and King & Lawrence, 1996) and control of scab (Lazarovits et al, 1999 and conn et al,1998).

Actinomycetes isolated from scab lesions of potato tubers in Finland were characterized using physiological tests and production of necrosis on potato mini tubers. The majority of the pathogenic strains were similar to the *Streptomyces scabies* that is characterized by grey to brown colonies, grey spores, spiralsporophores, melanin production,and utilization of the International Streptomyces Project (ISP) sugars. Two groups of strains differed from *Streptomyces scabies* and a highly virulent strain differed from all other strains for its phenotypic traits (Lindholm et al, 1997). The sequences of the 16S rRNA genes from various *Streptomyces* strains pathogenic to potato were compared. These included 10 pathogenic *Streptomyces* strains isolated from potato scab lesions in Finland. Three Finnish strains were identified as *Streptomyces turgidiscsbies*, a species previously described only in Japan. Six Finnish strains were identified as *Streptomyces scabies*. In pathogenecity test, *Streptomyces scabies cause* characteristic symptoms of common scab , *Streptomyces turgidiscabies* caused mainly pitted scab and *Streptomyces auriofacience* caused netted scab (Kreuze et al, 1999). In a work a PCR based diagnostic method was developed for direct detection from tuber lesions of pathogenic Streptomyces causing common scab of potato in Western Europe. The method was evaluated on 84 naturally infected potato samples, in the Netherlands, the UK, France, Germany, and Spain. Pathogenic Streptomyces in tuber lesions were detected by PCR in 70 samples and were assigned to S. europaeiscabiei, which emerged as the main cause of potato common scab in Western Europe (Flores-Gonzalez et al 2008).

The potato growing zone of Bangladesh includes the districts: Dinajpur, Thakurgao, Rangpur, Lalmonirhat, Kurigram, Nilphamari, that produce maximum potato of the country have soil pH is 5.5-6. As a result insignificant scab disease is reported. In some other districts like Jessor, some area of Munsiganj, Daudkandi, Chandina, the soil pH is 6.5-7.5. So in water trace condition, during bulking period, this disease is reported (Dr. Rezaul Karim, Deputy Director, quality control, potato division, BADC, personal communication). In Bangladesh most of the work done so far concerned to this disease is related to control. A study was done in Tuber Crop Research center (TCRC) Bangladesh, to study the prevalence of the disease in imported seed potatoes (Anonymus, 1990). Next an experiment was done with ten different chemicals to evaluate their efficacy in controlling the disease (Anonymus, 1991). To explore the use of cultural and chemical practices in controlling the disease was also done (Ali et al, 1993). Use of chemicals and management of soil moisture have positive impact against the disease (Dey et al, 2006).

Aims & Objectives:

So far in Bangladesh no comprehensive work has been done with these organisms particularly their physiological and ecological specializations have not been worked out. After a careful analysis of all these facts and figures, the proposed work was planned and designed to address the following:

Objectives:

- Isolation of organisms from collected samples.
- characterization the isolated organisms for possible identification.
- Study the enzyme profile of the selected organisms.
- find out the carbon utilization pattern of the selected isolates.
- to find out their morphological, cultural, physiological & ecological specializations.
- to understand their nature of adaptation to our crops and environmental conditions.

CHAPTER- 2

Materials & Methods

Materials & Methods

Sampling site:

The samples potato, beet and sweet potato were collected from the local markets i.e.: Palashi bazaar, Dhaka new market, Mirpur-10 bazaar, Rupnagar bazaar, and Karwan bazaar. Priority was given to collect different varieties. Various potato samples were collected e.g. round potato *(Solanum tuberosum var. tuberosum)* (white), Deshi alu *(Solanum tuberosum var. indigena)*, lal alu, jum alu etc. Two varieties of sweet potato *(Ipomea batatas)* and a single variety of beet *(Beta vulgaris)* which were available in the market were also collected.

Sample collection:

For the purpose of sampling, some clean, dry polythene bags were taken to the sampling site. Sufficient amount of samples were collected observing the symptoms of scab disease over the tubers in naked eye. The tubers contain spots that are usually tan to brown in color and are rough in texture. The roughness may be erumpent or superficial in nature. Samples were tagged with labels, which includes the sample no, place, date and sample name. The samples were brought to the laboratory as soon as possible and preserved in the refrigerator.

Table-3. Samples and sampling sites

Sample no.	Date of Collection	Sample	Collected form
1	02.01.07	Round potato	Palashi Bazar
2	03.02.07	Round potato	Rupnagar Bazar
3	24.02.07	Round potato	Mirpur-10 Bazar
4	06.03.07	Round potato	Rupnagar Bazar
5	18.03.07	Jam alu & Deshi alu	Rupnagar Bazar
6	08.04.07	Lal alu , Jam alu, Deshi alu & Beet	New Market
7	17.04.07	Beet	New Market
8	17.04.07	Sweet Potato	Palashi Bazar
9	07.06.07	Lal alu , Jam alu & Deshi alu	Rupnagar Bazar
10	08.06.07	Beet	Karwan Bazar
11	12.08.07	Sweet Potato	New Market
12	12.08.08	Sweet Potato	Mirpur-10 Bazar

(A)

(B)

(C)

Fig- 7 : Samples collected A) Potato, B) Beet & C) Sweet Potato.

Isolation procedure:

For a detailed study, an attempt was made to isolate and cultivate those organisms. For the purpose the following technique was used:

Tubers with scab symptoms were cut into pieces, surface sterilized with 1.5% NaOCl for 1 min. and rinsed with sterile water. The whole tissue piece was placed on Nystatin, Polymixin, Penicillin, Cycloheximid added Yeast Malt Extract (NPPC YME) Agar plates. Plates were incubated for 10-14 days at 30°C. The method was a modified form of R. Loria and J.R. Davis(1988)

Culture media:

Oatmeal Agar medium (Shirling & Gottlieb,1966), Glucose asparagine Agar medium (Waksman 1967) and Bennett's Agar medium (Iwasa et al. 1970) were primarily used to isolate the organisms and Bennett's Agar medium was proved preferable to the organisms. In this investigation NPPC YME Agar was used (R. Loria and J.R. Davis, 1988) for isolating the pathogen.

Preparation of NPPC YME Agar:

Preparation of antibiotic stock sol. (NPPC):

At first the antibiotic stock sol. was prepared by adding Nystatin, Polymixin B Sulfate, Sodium Penicillin G and Cycloheximide to a bottle of Distilled water(100 ml) which was autoclaved and cooled. This stock sol. was stored up 4 weeks at 5^0C (Davies & Williams, 1970). This antibiotic stock sol. was incorporated in the YME medium at a concentration of 10ml/l.

Preparation of YME:

All the ingredients of Yeast Malt Extract (YME) agar medium was taken in a conical flask containing distilled water and mixed thoroughly. The pH of the YME medium was adjusted to 7.3 with NaOH before adding agar. The agar was dissolved in water

by boiling and then autoclaved for 15 min. at 15 lb pressure. When the medium was cooled down to about 45 to 50°C, the antibiotic was added (Pridham et al, 1956-57).

Preparation of agar plates:

Glass Petri dishes were sterilized by heat sterilizer at 160°C for 2 and a ½ hour before pouring melted agar medium. Then 15ml YME agar medium was poured aseptically into each Petri dish, covered with their respective lids and left undisturbed until they solidify. After the medium had been solidified, the plates were kept in an incubator at 37°C for 24 h. before using them to drive away excess moisture and to find out whether the plates were contaminated.

Inoculation and incubation:

The infected potato tubers were taken and cut into small pieces containing disease symptom. These were surface sterilized with Clorox and repeatedly washed in sterile water, then placed onto NPPC YME agar and incubated for 10-14 days at 30°C before the colonies were transferred to YME agar (modified R. Loria & J.R. Davis, 1988).

Isolation of colonies:

The plates with white or grey colonies were selected for isolation. Selected colonies were marked and their colonial characteristics were recorded. These colonies were transferred to fresh agar plates and agar slants containing corresponding medium.

Purification of the isolates:

The cultures of *Streptomyces* that was contaminated with bacteria and/or fungi was purified by streak plate technique (Williams and Cross, 1965). A small amount of the *Streptomyces* cultures was streaked on YME (modified R. Loria & J.R.Davis, 1988). The medium was supplemented with antibiotic stock sol., that was used previously. This streaking procedure was continued until a single type of discrete colonies of *Streptomyces* was found on agar plate. The colony was transferred onto agar slants of the same medium.

Maintenance and preservation of the isolates:

The purified *Streptomyces* isolates were maintained throughout the study on YME agar slant. The culture tubes were kept in a refrigerator at 4°C. These isolates were transferred to fresh medium periodically to keep them viable.

Taxonomic study for identification:

For this purpose an array of microscopic and cultural tests were performed that served for the identification of the isolates.

Morphological studies and determination of growth characters for identification of the selected isolates:

Colonial morphology:

The growth of *Streptomyces* colonies on plating medium was morphologically studied as their form, elevation, margin, surface, pigmentation, opacity and the rate of growth.

Broth culture:

ISP-1, ISP-2 and ISP-8 media were inoculated with selected strains. The characteristic growth in the broth media was noted. Characters like amount, surface growth, subsurface growth, sedimentation in the broth were observed.

Mycelia and spores :

Characters of mycelia and spores were studied microscopically and photomicrographs were taken with a Nikon Optiphot (FX35 WA Japan) microscope fitted with photomicrography attachment.

Special technique used for microscopic studies:

The following methods were successfully used for the morphological studies of selected isolates.

Coverslip culture in solid medium (Kawato and Shinobu, 1959):

The cover slip culture technique (Kawato and Shinobu, 1959) was used to find the development of the germinating spore as well as spore pattern. Pre sterilized glass cover slips were stabbed at an angle of 45° into solidified medium in a plate, so that half of the cover slip remained protruded from the medium. An inoculum from a slope culture was then streaked with a fine inoculating needle along the line where upper surface of the cover slip met the agar. The plates were incubated at 37°C for 7 days. Each plate was inoculated with a single isolate. During incubation, the organisms grew both on the medium and uppersurface of the cover slip. The cover slips were withdrawn from the medium gently along with a portion of growth mass, mounted on slides, with lactophenol cotton blue and pressed with blotting paper to avoid excess fluid. Then the slides were observed under microscope .

Cover slip culture in liquid medium (Luedemann 1969):

In this method, a clean, dry cover slip was put into a MacCurtny bottle. ISP-2 broth was then dispensed into the bottle in such a way that 1/3rd of the cover slip remained drowned in the broth and the rest 2/3rd above the broth. Several bottles were prepared in this manner and autoclaved. Each bottle was inoculated with a single isolate and incubated at 37°C for 7 days. During this incubation period organisms grew on the cover slips, producing spores and showed their actual growth patterns. The cover slips were then withdrawn gently, mounted on a slide with lactophenol cotton blue and observed under microscope.

Biochemical Studies of the Isolates:

Bergey's Manual of Systematic Bacteriology is considered as the most authentic manual for microbiologists all over the world. Following Bergey's Manual (Bergey's Manual of Systematic Bacteriology, Sneath et al. 1986, vol. 4, 9th ed.) the physiological and biochemical tests of the isolated organisms were carried out. Along with Bergey's Manual several other manuals such as Manual of Microbiological Methods (SAB 1957), Microbiological Methods (Collins and Lyne 1984) and Understanding Microbes (Claus 1995) were also consulted.

Determination of growth pattern on different ISP media:

Some special media (ISP media) formulated by the collaboration in the "International *Streptomyces* Project" (ISP) for characterization of type and neotype strains of the genus "*Streptomyces*" were used to study the growth response and chromogenesis of the selected isolates. For this purpose different ISP media, viz.ISP-2, ISP-3, ISP-4, ISP-5, ISP-6, ISP-7 and ISP-9 were inoculated and incubated at 37°C for 7 days. The growth pattern, sporulation, aerial mass color, reverse color, diffusible pigment of the isolates in different ISP media (Shirling & Gottlieb,1966) were recorded.

The media were:

ISP medium 1: Tryptone-yeast extract broth
ISP medium 2: Yeast extract-malt extract agar
ISP medium 3: Oatmeal agar
ISP medium 4: Inorganic salts-starch agar
ISP medium 5: glycerol-asparagine agar
ISP medium 6: Peptone-yeast extract iron agar
ISP medium 7: Tyrosin agar
ISP medium 8: Bacto nitrate broth
ISP medium 9: Mineral salt agar

Determination of diffusible pigments (Williams *et al.*, 1989):

Plates with glycerol aspragine agar (Shirling and Gottlieb, 1966) were streaked with test organisms and incubated at 37°C.

Diffusible pigment productions were determined after 14 days and were recorded.

Determination of melanoid pigments (Williams *et al.*, 1989):

Plates with peptone-yeast extract iron agar medium were streaked with test organisms and incubated at 37°C.

A tube of deep glucose agar medium was inoculated in fluid condition approximately at 45°C. The tube was rotated to mix the inoculum with the medium and was allowed to solidify.

After incubation at 37°C for 7 days, observation was made to find out whether the organisms grew on the surface and in the upper layer of the medium (strict aerobes), or the organisms grew just a few millimeters below the surface (microaerophiles), or the organisms grew deeper in the medium(strict anaerobes).

Degradation of Tyrosine (Sneath *et al.1986):*

Tyrosine agar plates were inoculated and were incubated for 7 days at 37°C.

Clearing of tyrosine crystals around and below the growth revealed degradation of tyrosine.

Egg-yolk lecithinase test (SAB,1957):

For this test, egg-yolk was added to the medium in aseptic condition prior to pouring in plates. These plates were inoculated and incubated at 37°C.

After 7 days incubation, the appearance of a clear zone around the colony of the isolates indicated the positive result i.e. the organisms produced lecithinase enzyme.

Esculin hydrolysis test (Collins and Lyne 1984):

Many *Streptomyces* spp. have the capability to hydrolyze esculin. Esculin agar (0.1% esculin) slants were inoculated by streak method and incubated at 37°C for 5 days.

Blackening of the medium indicated that the organisms were capable of hydrolyzing esculin. Uninoculated slant tubes were used as control.

Hydrogen sulfide (H_2S) production test:

To detect H_2S production, Peptone-yeast extract iron agar (Shirling & Gottlieb, 1966) deeps were inoculated with test organisms by stabbing each organism with a straight inoculating needle.

After incubation at 37°C for 5 days, culture tubes were examined for H_2S production.

Any blackening of the medium due to the formation of ferrous sulfide (FeS), shows positive reaction while the absence of black color demonstrates negative result.

Hydrolysis of casein (Collins and Lyne 1984):

Casein is a milk protein. Casein comprises about 85% of the total protein in milk. Many microorganisms have the capacity to hydrolyze casein. This test demonstrates the ability of microbes to degrade casein into soluble peptides and amino acids by the enzyme caseiase.

1 ml of sterilized milk was taken in a sterilized Petri–plate and then melted agar medium was poured and mixed thoroughly. After solidifying, the plates were inoculated and incubated at 37°C for 72 hrs.

Formation of a clear, transparent zone around the growth indicated hydrolysis of casein.

Hydrolysis of Gelatin (SAB1957):

Gelatin is a protein formed by boiling an animal protein called collagen in water or an acid solution. Many microorganisms produce and release extra cellular proteases that hydrolyze gelatin into short peptides and individual amino acids which results in the liquefaction. This liquefaction helps to identify microorganisms.

In this test duplicate tubes of nutrient gelatin broth were inoculated with test organisms and incubated at 37°C for 72 hrs.

After incubation, the experimental tubes along with a control tube were kept in a refrigerator. In a few minutes, the medium in the control tube become solidified. Solidification of the inoculated tubes indicated the absence of the enzyme gelatinase in those organisms. If however the inoculated tubes would remain liquefied, that would indicate the hydrolysis of gelatin and hence the presence of the enzyme gelatinase.

Hydrolysis of Strach (Claus 1995):

Organisms capable of hydrolyzing starch to form monosaccharide or disaccharide possess the enzyme amylase. This test revealed the presence or absence of enzyme amylase in the organisms.

For this test, starch-agar plates were inoculated with test organisms and incubated at 37°C for 96 hrs.

After incubation, the surface of these plates was flooded with iodine solution. Development of clear zone around the growth indicated starch hydrolysis.

Nitrate reduction test (SAB 1957):

Nitrate reduction is evident by complete or partial disappearance of nitrate accompanied by appearance of nitrite, ammonia or free nitrogen.

The test was performed to see if the organisms were capable to reduce nitrate to nitrite. The formation of nitrite indicated the presence of the enzyme nitrate reductase in the organisms.

The following three reagents were required for this test:

Reagent A: Sulfanilic acid –acetic acid solution:

Sulfanilic acid - 8.0 g

5N Acetic acid - 1000ml(1 part chemically pure acetic acid , 2.5 parts distilled water)

Sulfanilic acid was dissolved in Acetic acid and stored in brown glass bottle.

Reagent B: Dimethyl-α-napthalamine solution:

Dimethyl-α-napthalamine- 6.0 ml

5N Acetic acid - 1000ml

stored in glass bottle.

Reagent C: Zinc dusts

The tubes of nitrate broth in duplicates were inoculated with test organisms and then incubated at 37°C for 72 hrs. after incubation, 1ml of reagent A was added to the incubated tube and shaken well.

Formation of a distinct red or pink color indicated the reduction of nitrate to nitrite. Absence of nitrite may be due to complete conversion of nitrate as well as no reduction at all. A pinch of zinc dust was then added to the tube showing absence of nitrite and it was allowed to stand for a few minutes. Any remaining nitrate (in case) would be reduced to nitrite by zinc dust and the characteristic pink or red color would appear and no color indicates complete reduction.

Pectinolytic activity(Hankin *et al.*1971):

Pectin agar plates were inoculated with a single streak and were incubated at 37°C. after incubation, the surface of these plates was flooded with aqueous solution of hexadecyl trimethyl ammonium bromide(1% w/v).

Development of clear zone or hydrolytic zone around the growth indicated hydrolysis of pectin. The observation was made after 7 days.

Proteolysis test (SAB 1957):

Proteolysis is the breaking up of large protein molecules into smaller peptides. For this test, the tubes of alkaline egg broth tubes in duplicates were inoculated with test organisms and then incubated at 37°C for 5 weeks.

Progressive clearing of the opaque, whitish medium during growth indicated proteolysis.

Determination of carbon source utilization by the selected isolates:

The selected isolates were tested for their ability to utilize different carbon sources. For this purpose 1% carbon source was added to mineral salt agar medium (ISP-9). ISP-9 was used as basal medium. Glucose, Rhamnose, Cellobiose, Arabinose, Mannose, Mannitol, etc. were used as carbon source and added to the medium prior to autoclave for 10 min. The medium was poured into the sterilized Petri plates and allowed to solidify. Inoculation was done by streak method and incubated at 37°C for 1 week. The results were compared with two controls: growth on basal medium without any carbon source and growth on basal medium with only glucose. The results were recorded as following categories:

Strongly positive utilization: (3), when growth on tested carbon in basal medium is equal to or greater than growth on basal medium plus glucose.

Positive utilization: (2), when growth on tested carbon is significantly better than growth on basal medium without carbon, but somewhat less than on glucose.

Weak utilization: (2), when growth on tested carbon is slightly poor than growth on positive control medium.

Utilization doubtful: (Trace), when growth on tested carbon is slightly better than growth on basal medium without carbon and significantly less than with glucose.

Not utilized: (-), when growth is similar to or less than growth on basal medium without carbon source.

Acid production from carbohydrate (Gordon 1966):

The selected strains were tested for their ability to production of acid from different carbon sources. For this purpose inorganic nitrogen base agar medium (Ayers, Rupp and Johnson 1919) was used. Bromocresol purple was added to this medium as an indicator. 1% carbon source was added to this medium. Glucose, Mannose, Fructose, Trehalose, Sucrose, Galactose, Adonitol, Mannitol, Cellobiose, Rhamnose, Arabinose were used as carbon source. All these were and added to the medium prior to autoclave for 10 min. The medium was poured into the sterilized Petri plates and allowed to solidify. Inoculation was done by streak method and incubated at 37°C for 1 week.

Acid production from carbohydrate was determined by the acid color (yellow) of the indicator around the colony.

Physical properties of the selected isolates:

Growth Response at Different Temp.:

In order to find out the optimum growth temperature of the isolates, ISP-2 was prepared and inoculated with the isolates by streak method and were allowed to grow at different temperature such as 4°C, 10°C, 37°C and 45°C for 3 days. The growth was then compared with the control kept at room temperature (30°C).

Growth Response at Different pH (Sneath *et al.*1986):

The p^H requirement of selected isolates was determined by growing them at different p^H viz. 4.5, 6.5 and 8.5 p^H. Peptone water broth (in 4:1 ratio) was adjusted to the

concerned pH. Buffer solutions used were of the following composition(Williams *et al.* 1971):

pH of the Buffer	Name and Quantity of the Chemical(s) used (g)	Volume of Deionized Water(ml)
4.5	KH2PO4-5.45	200
6.5	KH2PO4-2.72 K2HPO4-1.74 Na2HPO4-1.39	200
8.5	K2HPO4-3.48 Na2HPO4-2.78	200

pH of the buffer solutions was checked after preparation. At each step, medium was adjusted with N/10 HCl or N/10 NaOH solution, as required. The medium was inoculated with the selected isolates and incubated at 37°C. growth of the selected isolates in these p^H were observed and recorded after 7days.

Growth Response at Different Concentration of NaCl (Sneath *et al. 1986*):

To test the salt tolerance of selected strains peptone water with different concentration of NaCl *viz.* 0, 4, 7, 10 and 13% were used. The inoculated test tubes were incubated at 37°C. The growth was recorded after 7 and 14 days.

Test for pathogenic activities of the selected isolates:

Point inoculation method:

The test was done by pricking the healthy potato tuber with a sharp needle containing spore of isolates. The potato tubers were incubated at 35°C for 10 days. The results showed that 26 strains were pathogenic and 6 were saprophytic strains among 33 strains isolated from lesions of common, netted and pitted scab of potato, beet and sweet potato. The rate of disease development is proportionate with successive day's incubation. The results were recorded for 5 successive days.

Etiolated potato sprout test:

The pathogenecity of scab causing organisms was assessed using etiolated potato sproutings. The cut sproutings (6-8cm long) were wrapped individually with a piece of tissue paper containing a concentrated spore suspension of isolates. After incubation for seven days at 25°C and high humidity in the dark, the sproutings were examined for necrotic and raised lesion formation (Tashiro, 1999).

Traditional soil inoculation method :

The test organisms were inoculated on YME medium and incubate for 10-14 days. Then mix each plate of the organism into steam sterilized garden soil. The soil is put into polythene bags and put into plastic pots the contents of one Petri plate are used per pot. Holes punched in the bottoms of bags allow for drainage and bags are partially filled with infested potting mix. Seed tubers which are free of scab lesions was surface sterilized with 0.5% NaOCl for 10 min. then planted 5cm. below the surface of the soil. Plants of the pot was irrigated with tap water (Loria & Davis, 1988).

Pathogenecity test on radish seedlings:

Three isolates viz. B3 (from beet), PB-12 (from potato) and SP-8 (from sweet potato) were tested for seedling pathology according to the method of Flores-Gonzalez(2008). Disinfected radish seedlings were placed onto 8 day old *Streptomyces* culture on OMA plates and incubated for 4 days at 37°C . *Streptomyces* isolates were considered pathogenic when they inhibit germination of radish seeds or caused dark lesions on germinated seedlings (Flores- Gonzalez, 2008).

Results

Isolation of Organisms:

From the collected Samples 35 isolated organisms were screened and out of these 33 were finally selected for detail study.

Colonial characteristics of the selected isolates:

Growth of the selected isolates was observed on modified Yeast Malt Extract agar (YME) medium. All of the colonies were grey or white in color. (Loria & Davis, 1988). Colonial characteristics of the selected isolates are shown in Table-4 and Fig.-8,9,10,11)

Microscopic observations of the selected isolates:

The selected isolates were microscopically studied. (Fig. –12,13,14).
All the isolates were filamentous, branched, coenocytic and in a few cases fragmented.

Growth responses of the selected isolates on different ISP media:

Growth responses of the selected isolates on different ISP media viz.ISP-2, ISP-3, ISP-4, ISP-5, ISP-6, ISP-7, ISP-9 and MBA were studied. The growth pattern, amount of growth, sporulation, aerial mass color, reverse color and diffusible pigment of the selected isolates on different ISP media were recorded. (Table-5,6,7,8 and Fig- 15).

Cultural characteristics of the selected isolates in broth media:

Selected isolates were studied in 3 different borth media viz. ISP-1, ISP-2 and ISP-8. Growth responses with other characteristics were observed (Table-9).

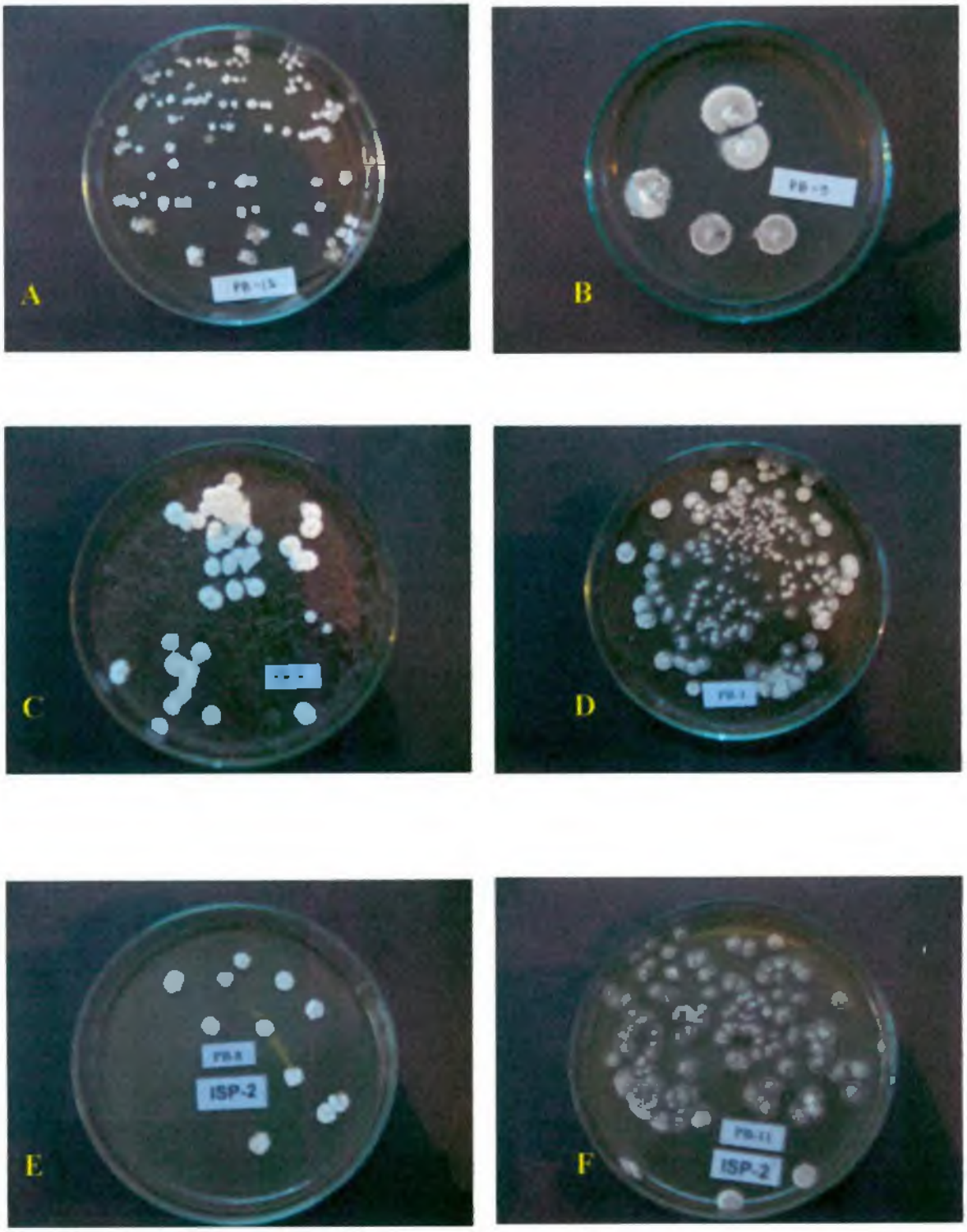
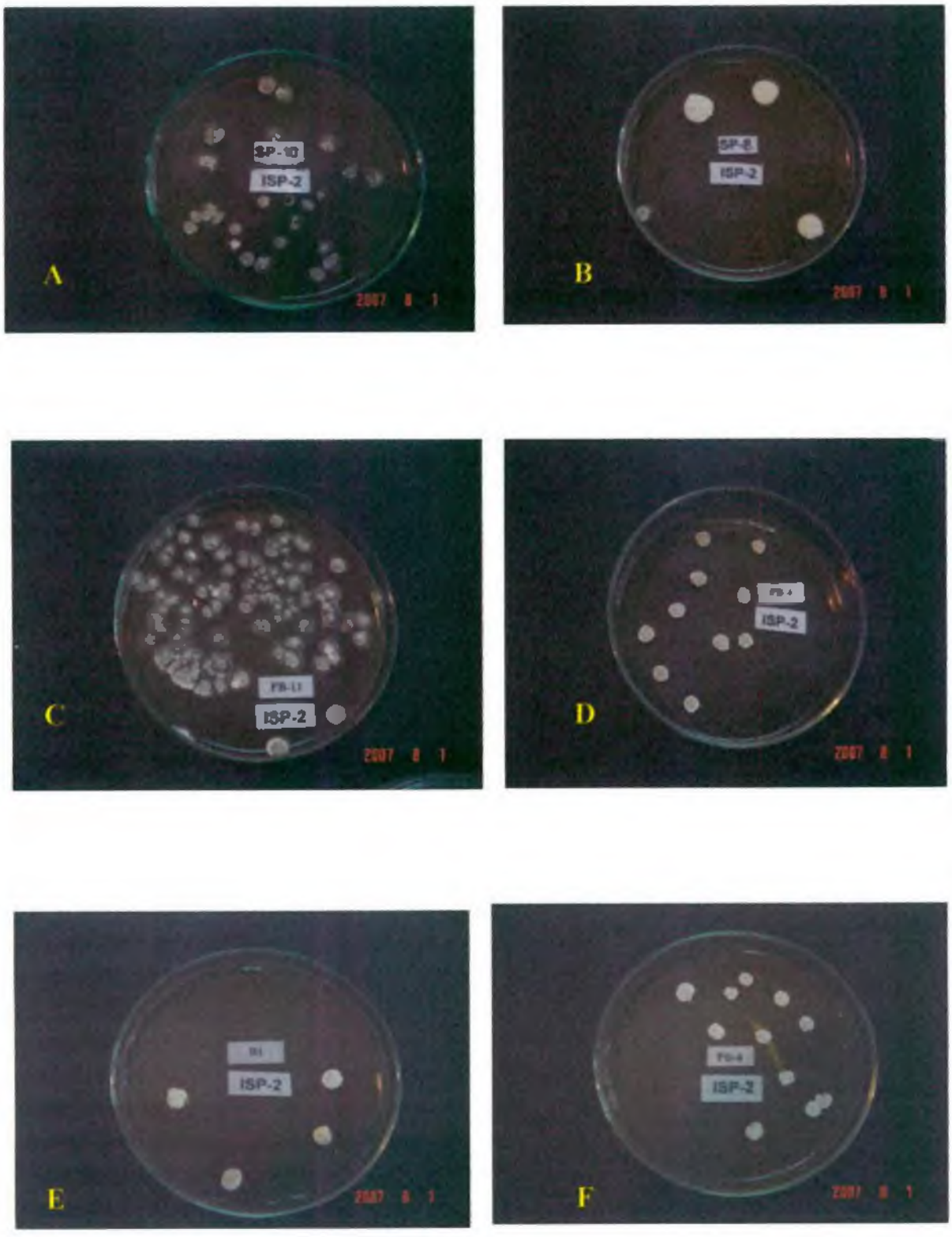


Fig- 9 : Organisms showing colonial characters. (A). PB-12, (B) PB-9, (C) PR-4, (D) PR-1, (E) PB-8, (F) PB-11 on ISP-2 medium.



Fig– 10: (A-F), Organisms showing colonial characteristics. Plates showing the colonies of strain No. (A) SP – 10, (B) SP – 8, (C) PB – 11, (D) PB – 4, (E) B1 and (F) PB -8 on ISP 2 medium.

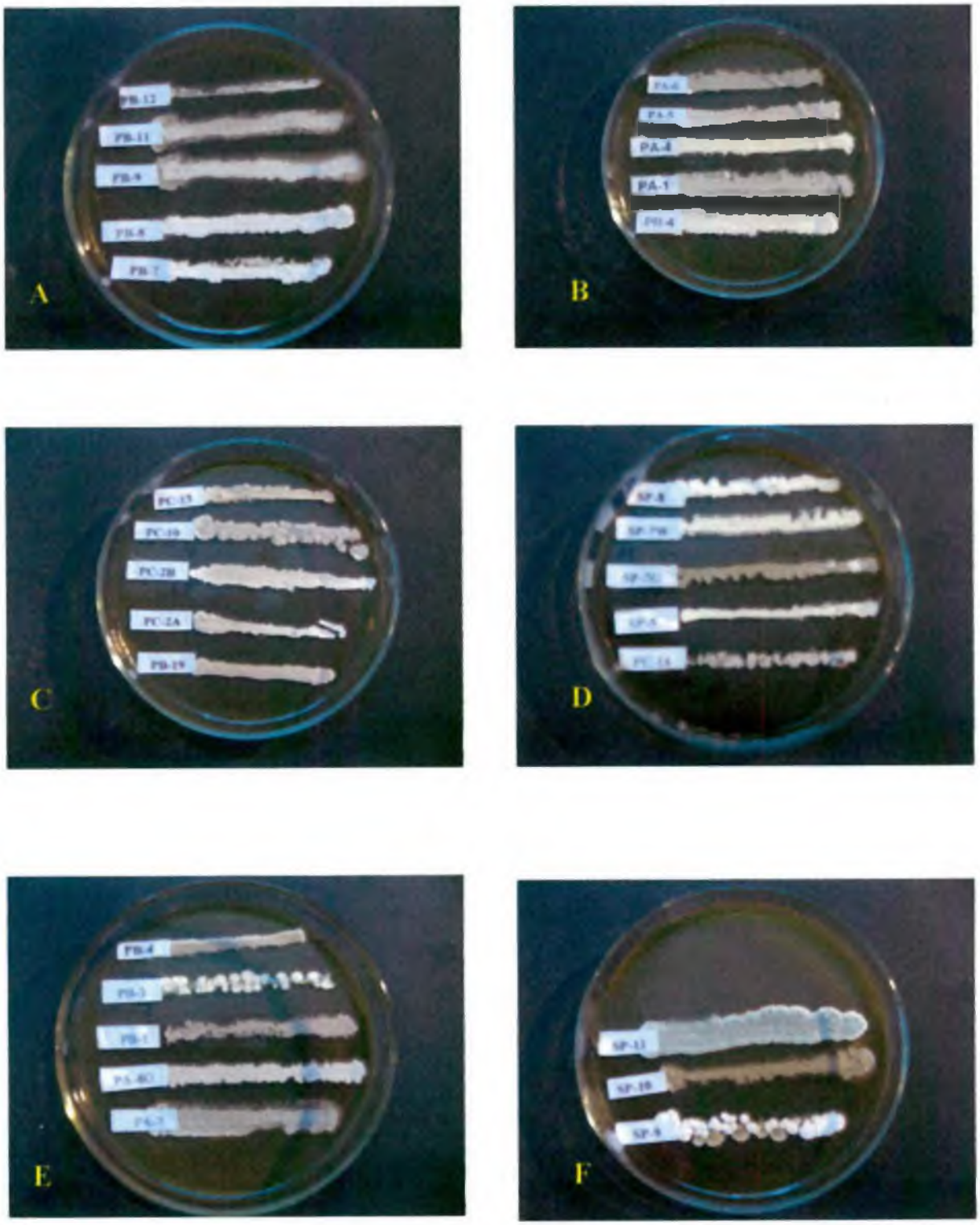


Fig- 11 : (A-F) Organisms Grown on ISP 2 Medium to compare their Colonial characteristics

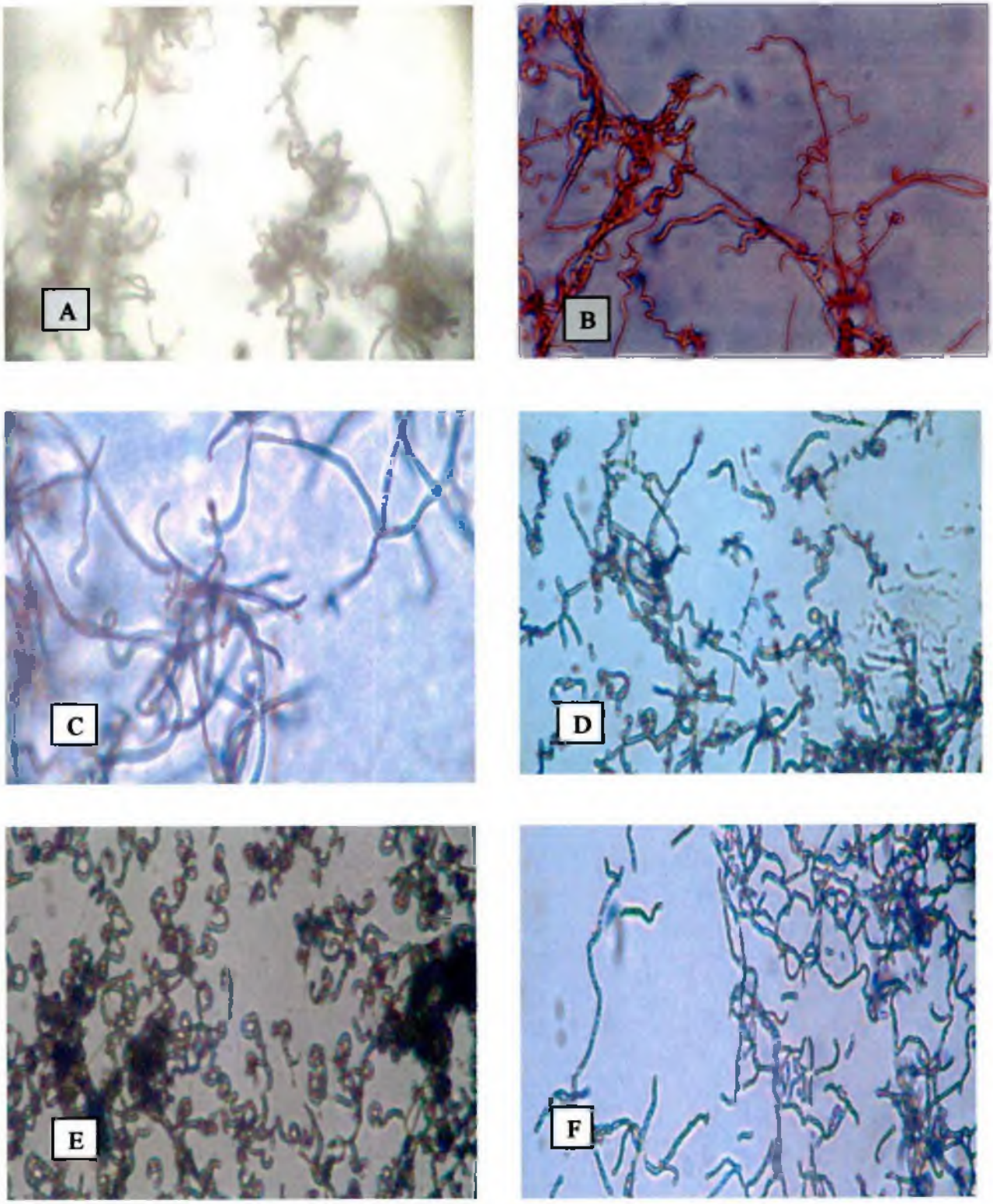


Fig- 12 : Photomicrographs showing vegetative mycelium and spore chains of strain No. DN-10. A & B under compound microscope. A) PR-2, B) PR-4, C) PA-4, D) PA-6, E) PB-9, F) PB-11.

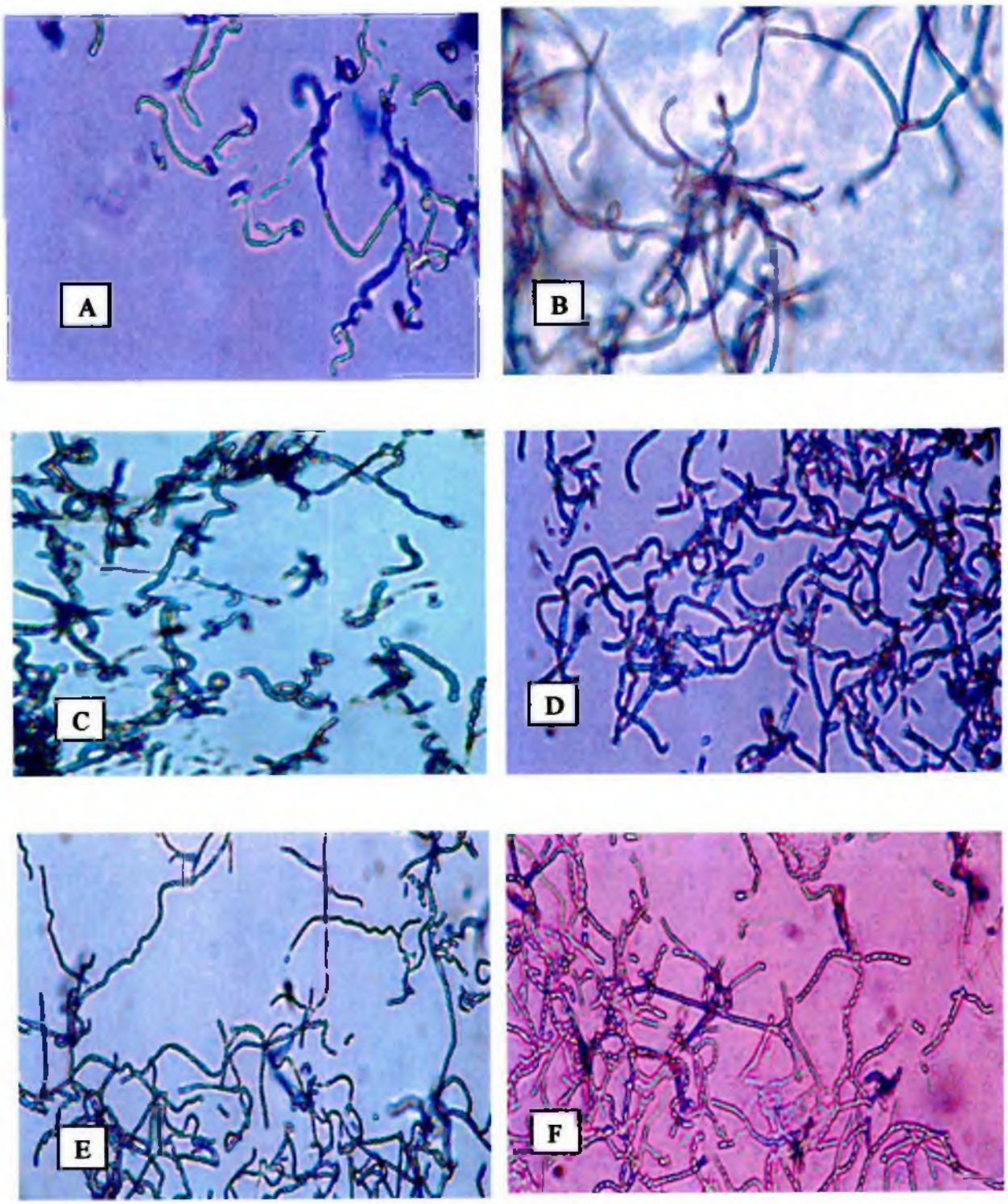


Fig- 13 : Organisms showing colonial characters. Streak plate showing the colonies of strain No. (a). B-1, (b) B-3, (c) PR-3, (d) PA-5, (e) SP-5, (f) SP-7G.

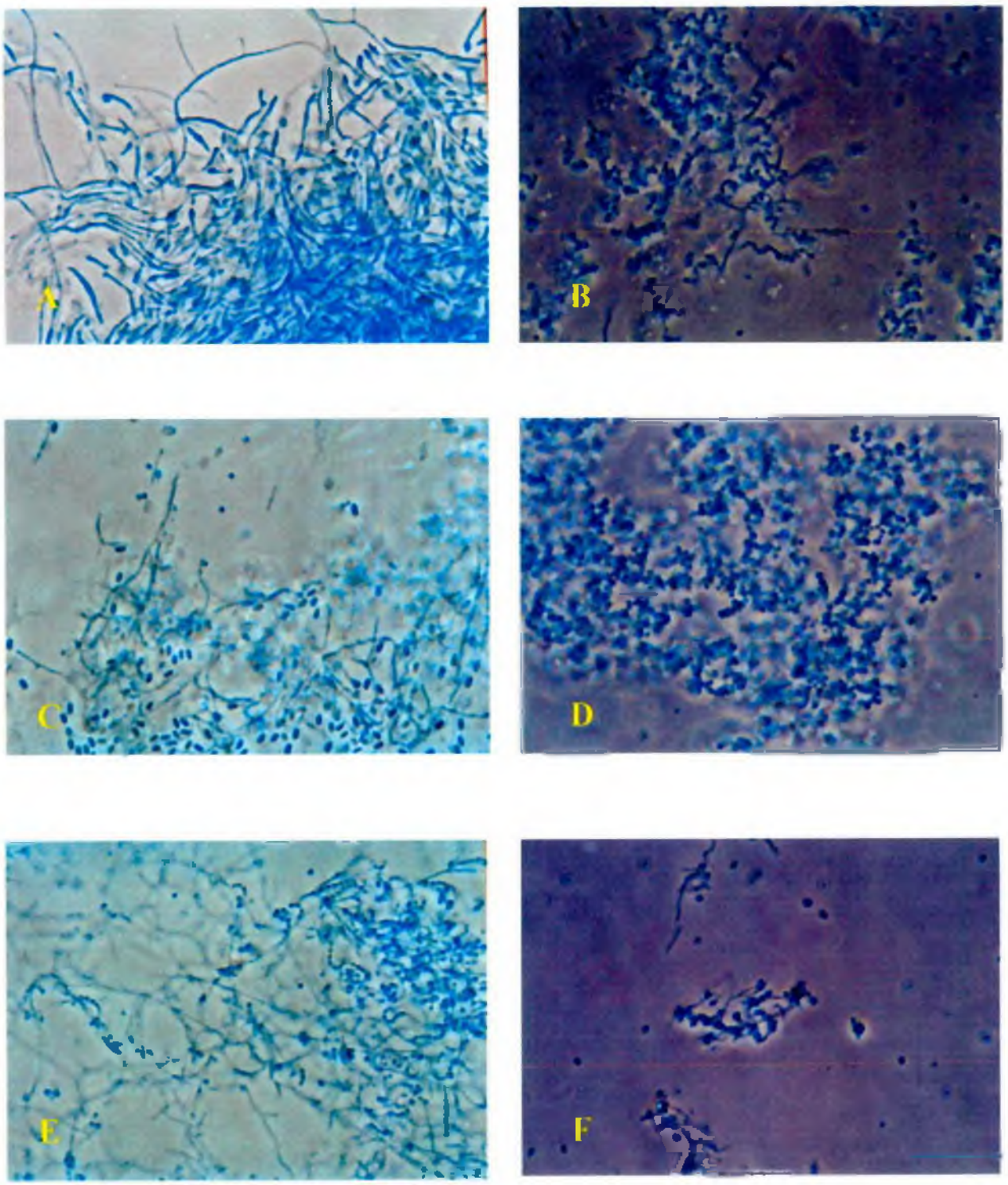


Fig- 14 : Photomicrograph of this organisms A) PA-5, B) PB-7, C) PB-12, D) PC-10, E) PC-14, F) SP-7G

Table:4. Colonial characteristics of selected strain on YME

Isolate No.	Color	Reverse Pigment	Form	Elevation	Margin	Surface
B-1	Greyish white	Brown	Irregular	Pulvinate	Curled	Rugose
B-3	White	Brown	Irregular	Umbonate	indulate	Concentric
PR-1	White	Brown	Circular	Pulvinate	Entire	Concentric
PR-2	White	Brown	Circular	Pulvinate	Entire	Rugose
PR-3	Grey	Brown	Circular	Umbonate	Filamentous	Concentric
PR-4	White	Brown	Circular	Pulvinate	Entire	Concentric
PA-1	White	Brown	Irregular	Umbonate	Curled	Rugose
PA-4	White	Brown	Circular	Umbonate	Entire	Rugose
PA-5	Grey	Brown	Circular	Umbonate	Entire	Concentric
PA-6	Grey	Brown	Irregular	Umbonate	Entire	Rugose
PA-7	Grey	Brown	Irregular	Umbonate	Curled	Rugose
PA-8G	Grey	Brown	Irregular	Pulvinate	Irregular	Rugose
PB-1	Grey	Brown	Circular	Pulvinate	Irregular	Rugose
PB-3	Grey	Dark brown	Irregular	Raised	Filamentous	Concentric
PB-4	Grey	Brown	Circular	Umbonate	Entire	Concentric
PB-7	White	Brown	Irregular	Umbonate	Entire	Rugose
PB-8	White	Brown	Circular	Umbonate	Entire	Rugose
PB-9	Grey	Black	Circular	Pulvinate	Irregular	Concentric
PB-11	Grey	Brown	Irregular	Umbonate	Curled	Concentric
PB-12	White	Brown	Irregular	Umbonate	Irregular	Rugose
PB-19	White	Brown	Irregular	Umbonate	Irregular	Rugose
PC-2A	White	Brown	Circular	Umbonate	Entire	Rugose
PC-2B	White	Brown	Circular	Umbonate	Circular	Rugose
PC-10	White	Brown	Circular	Pulvinate	Circular	Concentric
PC-13	White	Brown	Circular	Umbonate	Circular	Rugose
PC-14	Grey	Brown	Circular	Umbonate	Curled	Concentric
SP-5	White	Brownish grey	Circular	Umbonate	Entire	Concentric
SP-7G	Grey	Brownish grey	Irregular	Pulvinate	Irregular	Rugose
SP-7W	White	Brown	Circular	Umbonate	Entire	Concentric
SP-8	White	Dark brown	Circular	Convex	Curled	Radiate
SP-9	Grey	Brown	Circular	Umbonate	Entire	Concentric
SP-10	Grey	Black	Irregular	Pulvinate	Curled	Concentric
SP-11	Grey	Brown	Circular	Pulvinate	Entire	Concentric

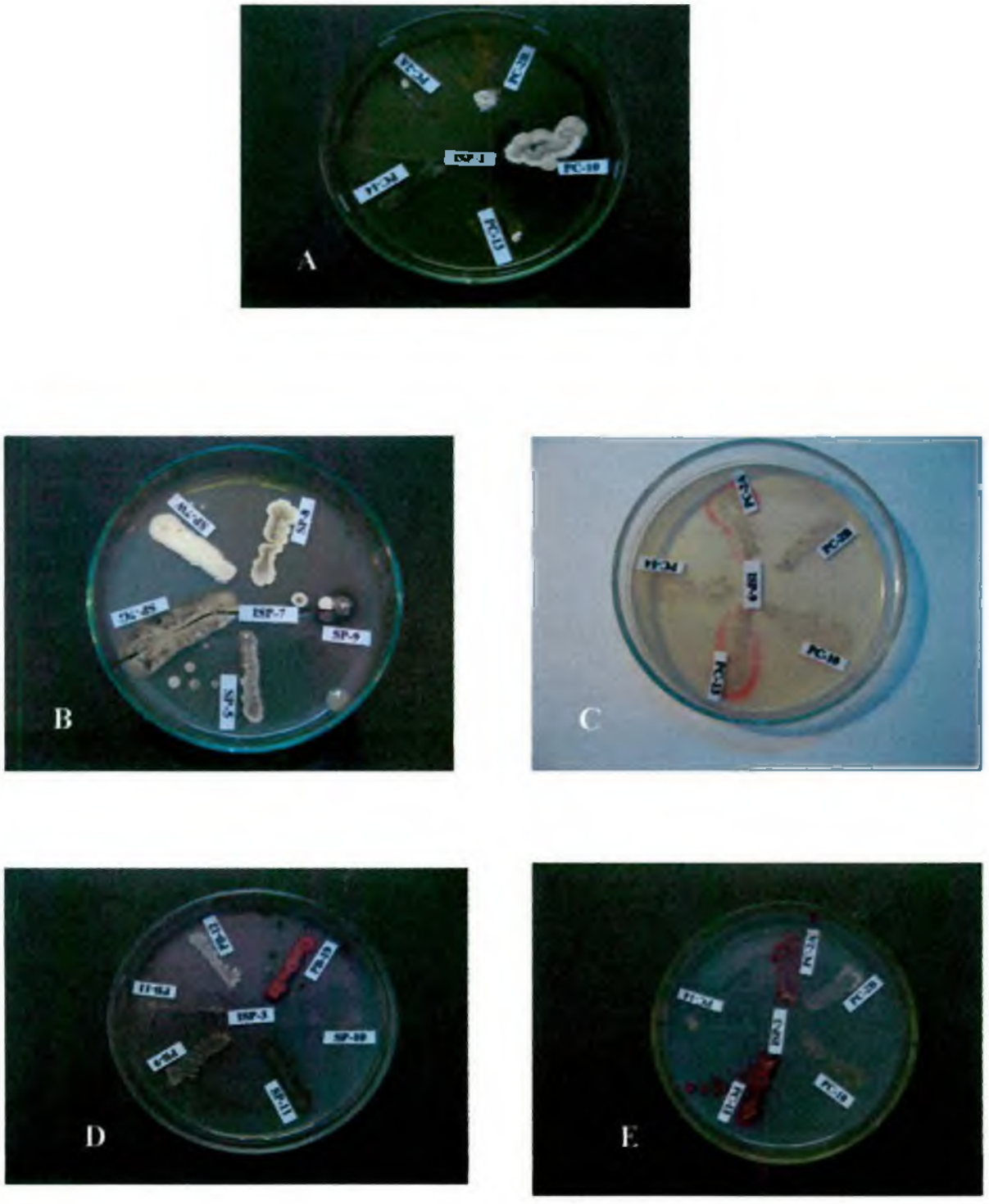


Fig- 15 : (A-E) Growth response of Organisms on Different ISP Medium.

Table-5.Growth responses of the selected isolates on different ISP media

Isolate No.	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6	ISP-7	ISP-9	MBA
B-1	5	3+	5+	4	3+	5	5+	5
B-3	3	1	1	3+	3	3	1	4
PR-1	2+	2	1	4	3	3	1	3
PR-2	5	1+	-	2	3	2	-	3
PR-3	3	2	4+	5	4	3	5	4
PR-4	4	1	1	4	3+	3+	1	4
PA-1	3	2	2+	4	5+	3	2	3
PA-4	4	2	2	3	3+	4	1	2
PA-5	2	2	3	3	1	3+	3	3
PA-6	3	2	3	3+	5	4+	2	4
PA-7	3	2	2	3+	4	3	2	4
PA-8G	3	1	3	1+	-	4+	2	2
PB-1	ND	ND	ND	ND	2+	2	ND	2
PB-3	ND	ND	ND	ND	3	2	ND	5+
PB-4	ND	ND	ND	ND	5+	3	ND	5
PB-7	ND	ND	ND	ND	-	1+	ND	4
PB-8	ND	ND	ND	ND	-	2+	ND	5
PB-9	5+	3	2	3	5	2	1	4++
PB-11	3+	1	2	4	4	3	1	3
PB-12	3	2	2+	3	1	3+	1	4
PB-19	1	3(red)	2	3	3	3+	1	3
PC-2A	3	5	3	3	4+	4	5+	3
PC-2B	3	4	4	2	4	4	4+	4
PC-10	5++	3	5++	5	-	5	5+	5++
PC-13	3	4	5+	4	5	3	5+	3
PC-14	2	1	5	4	3	3+	5+	5+
SP-5	2	ND	ND	2	3	3	2	3
SP-7G	3	ND	ND	4	1	5++	1	3
SP-7W	4	ND	ND	3	4	5	1	4
SP-8	5	ND	ND	5	1	4	5+	4
SP-9	3	ND	ND	4	-	1	5	4
SP-10	5+	5+	1	3+	1	5++	1	3
SP-11	4	4	5	4	2	3	2	4

Note: 1, 2, 3, 4 & 5 represents the amount of growth with 5 as maximum (Visual estimation)."-" represent no growth.

Table-6. Sporulation of the selected isolates on different media

Isolate No.	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6	ISP-7	ISP-9	MBA
B-1	5+	3	4	5	-	5+	2	5
B-3	5	1	1	4	2	5	1	4
PR-1	5	1	1	4	1	5	1	4
PR-2	5	1	-	3	-	4	-	4
PR-3	5	1	2	3	-	-	2	4
PR-4	5	1	1	4	3	5	1	4
PA-1	3	-	-	2	4	1	1	2
PA-4	5	1	2	1	3	5	1	1
PA-5	2	1	3	3	-	3	1	2
PA-6	3	1	-	2	2	6	1	2
PA-7	3	-	-	1	3	1	1	1
PA-8G	3	-	2	-	-	4	1	1
PB-1	ND	ND	ND	ND	-	2	ND	2
PB-3	ND	ND	ND	ND	-	3	ND	3++
PB-4	ND	ND	ND	ND	4+	3	ND	3++
PB-7	ND	ND	ND	ND	-	2	ND	3
PB-8	ND	ND	ND	ND	-	2	ND	3+
PB-9	3	1	1	3	-	-	1	4
PB-11	3	1	1	2	1	-	1	3
PB-12	3	2	2	3	1	3	1	4
PB-19	1	1	1	2	4	3	1	3+
PC-2A	1	3+	3+	-	3	2	1	4
PC-2B	2	4	4	-	3	3	1	5
PC-10	5	5+	5+	-	-	5	1	5++
PC-13	1	4+	4+	1	3	3	2	4
PC-14	-	5	5	-	-	2	1	5++
SP-5	2	ND	ND	-	3	2	2	1
SP-7G	3	ND	ND	2	-	5++	3	1
SP-7W	4	ND	ND	-	4	4	4+	1
SP-8	5	ND	ND	5	-	3	3+	1
SP-9	3	ND	ND	1	-	2	4	1
SP-10	4	-	-	2	-	2	1	2
SP-11	3	2	5	1	1	2	2	5

Note: 1,2,3,4 & 5 represents the amount of sporulation with 5 as maximum (Visual estimation). "-" represent no sporulation.

Table-7. Reverse pigment of the selected isolates on different media

Isolate No.	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6	ISP-7	ISP-9	MBA
B-1	Brown	Cream	Yellowish grey	Brown	Brown	Blackish brown	Cream	Dark brown
B-3	Dark brown	Greenish yellow	-	Light brown	Dark brown	Light brown	-	Light brown
PR-1	Brown	Greenish yellow	-	Light brown	Brown	Light brown	-	Light brown
PR-2	Brown	Greenish yellow	-	Light brown	Brown	Light brown	-	Light brown
PR-3	Dark brown	Light brown	Off White	Brown	Brown	Light brown	-	Brown
PR-4	Dark brown	Greenish yellow	-	Brown	Dark brown	Brown	-	Brown
PA-1	Light brown	Off White	Off White	Cream	Dark brown	Light Brown	Cream	Cream
PA-4	Dark brown	Yellowish green	Yellowish white	Light cream	Brown	Blackish brown	-	Cream
PA-5	Light brown	Cream	Cream	Cream	-	Black	Light Grey	Cream
PA-6	Brown	Cream	Off White	Cream	Dark brown	Brown	Cream	Cream
PA-7	Brown	Off White	Off White	Cream	Dark brown	Light brown	Cream	Cream
PA-8G	Light brown	-	Cream	Light cream	-	Black	Cream	Cream
PB-1	ND	ND	ND	ND	Light brown	Dark brown	ND	Pink
PB-3	ND	ND	ND	ND	Brown	Light brown	ND	Dark grey
PB-4	ND	ND	ND	ND	Brown	Light brown	ND	Grey
PB-7	ND	ND	ND	ND	-	Whitish brown	ND	Cream
PB-8	ND	ND	ND	ND	-	Brown	ND	Light brown
PB-9	Brown	Greyish brown	Pinkish brown	Blackish brown	Brown	Brown	Grey	Dark brown
PB-11	Brown	Light grey	Cream	Light brown	Light brown	Light brown	Grey	Cream
PB-12	Brown	Grey	Cream	Cream	-	Brown	Grey	Cream
PB-19	-	Pink	Pink	Cream	Dark brown	Maroon	Pinkish grey	Pink
PC-2A	Brown	Pink	Pinkish white	Cream	Brown	Blackish grey	Grey	Dark brown
PC-2B	Brown	-	Grey	Cream	Brown	Blackish grey	Light grey	Grey
PC-10	Black	Brown	Grey	Cream	Dark brown	Blackish grey	Light grey	Grey
PC-13	Brown	Dark pink	Dark pink	Cream	Dark brown	Blackish grey	Grey	Dark brown
PC-14	Brown	-	Grey	Cream	Brown	Blackish grey	Light grey	Brown
SP-5	Dark brown	ND	ND	Cream	Brown	Brownish grey	White	Yellowish brown
SP-7G	Black	ND	ND	Cream	Cream	Brownish black	Grey	Brown
SP-7W	Brown	ND	ND	Cream	Brown	Brownish grey	Cream	Brown
SP-8	Dark brown	ND	ND	Yellowish cream	Brown	Brownish grey	Grey	Cream
SP-9	Brown	ND	ND	Cream	-	Black	Grey	Black
SP-10	Black	Cream	-	Brownish ash	-	Brown	-	Brown to black
SP-11	Brown	Black	Grey	Light brown	Ash	Brown	Grey	Pink to grey

Note: "-" represents no pigmentation

Table-8. Diffusible pigment of the selected isolates on different media

Isolate No.	ISP-2	ISP-3	ISP-4	ISP-5	ISP-6	ISP-7	ISP-9	MBA
B-1	-	-	-	-	-	-	-	-
B-3	-	-	-	-	-	-	-	-
PR-1	-	-	-	-	-	-	-	-
PR-2	-	-	-	-	-	-	-	-
PR-3	-	-	-	-	-	-	-	-
PR-4	-	-	-	-	-	-	-	-
PA-1	-	-	-	-	-	-	-	-
PA-4	-	-	-	-	-	-	-	-
PA-5	-	-	-	-	-	Black	-	-
PA-6	-	-	-	-	-	-	-	-
PA-7	-	-	-	-	-	-	-	-
PA-8G	-	-	-	-	-	Black	-	-
PB-1	ND	ND	ND	ND	-	-	ND	-
PB-3	ND	ND	ND	ND	-	-	ND	Pink
PB-4	ND	ND	ND	ND	-	-	ND	-
PB-7	ND	ND	ND	ND	-	-	ND	-
PB-8	ND	ND	ND	ND	-	-	ND	-
PB-9	-	Brown	-	Brown	-	-	-	-
PB-11	-	Brown	-	-	-	-	-	-
PB-12	-	Pink	-	-	-	Pink	-	-
PB-19	-	-	-	-	-	-	-	-
PC-2A	-	-	-	-	-	-	-	-
PC-2B	-	-	-	-	-	-	-	-
PC-10	Black	-	-	-	-	-	-	-
PC-13	-	-	-	-	-	-	-	-
PC-14	-	-	-	-	-	-	-	-
SP-5	-	ND	ND	-	-	-	-	-
SP-7G	-	ND	ND	-	-	-	-	-
SP-7W	-	ND	ND	-	-	-	-	-
SP-8	-	ND	ND	-	-	-	-	-
SP-9	-	ND	ND	-	-	Black	-	-
SP-10	-	-	-	-	-	-	-	-
SP-11	-	Brown	-	-	Black	-	-	-

Note: "-" represents no diffusible pigment.

Table-9. Growth response in different ISP broth.

Isolate No.	Isp media	Amount of growth	Surface growth	Sub surface growth	Sediment
B-1	Isp-1	1	Ring	None	Flaky
	Isp-2	3	Ring	None	Flaky
	Isp-8	1	Ring	Small colonies submerged with the medium	Flaky
B-3	Isp-1	3	Pellicle	None	Flaky
	Isp-2	3	Pellicle	None	Flaky
	Isp-8	3	Pellicle	None	None
PR-1	Isp-1	3	Pellicle	None	Flaky
	Isp-2	3	Pellicle	None	Flaky
	Isp-8	4	Pellicle	None	None
PR-2	Isp-1	3	Pellicle	None	Flaky
	Isp-2	3	Pellicle	None	Flocculer
	Isp-8	ND	-	None	None
PR-3	Isp-1	2	Flocculent	Small colonies attached to the tube	Floccular
	Isp-2	2	Flocculent	None	Flaky
	Isp-8	1	Ring	None	Flaky
PR-4	Isp-1	3	Pellicle	None	None
	Isp-2	3	Pellicle	None	Flocculent
	Isp-8	4	Pellicle	None	Viscid
PA-1	Isp-1	2	Pellicle + Ring	None	Flaky
	Isp-2	2	Pellicle + Ring	None	Flaky
	Isp-8	3	Pellicle + Ring	Small colonies submerged with the medium	Floccular
PA-4	Isp-1	3	Pellicle	None	Flaky
	Isp-2	3	Pellicle	None	Floccular
	Isp-8	3	Pellicle	None	None
PA-5	Isp-1	2	Pellicle + Ring	None	None
	Isp-2	1	Flocculent	None	Flaky
	Isp-8	1	Small colony floating on the surface and attached to the wall	None	Viscous
PA-6	Isp-1	3	Pellicle + Ring	None	Flaky
	Isp-2	2	Ring	None	Flaky
	Isp-8	4	Pellicle + Ring	Small colonies submerged with the medium	Flaky
PA-7	Isp-1	2	Pellicle	None	None
	Isp-2	2	Ring	None	Flaky
	Isp-8	4	Ring	Small colonies attached to the wall	Viscid
PA-8G	Isp-1	1	Powdery	None	Viscid
	Isp-2	2	Ring + Flocculent	None	Flaky
	Isp-8	1	Flocculent	Small colonies submerged & attached to the wall	Flaky

Table-9. (Cont) Growth response in different ISP broth.

Isolate No.	Isp media	Amount of growth	Surface growth	Sub surface growth	Sediment
PB-1	Isp-1	2	Pellicle + Ring	Small colonies attached to the wall	Flaky
	Isp-2	1	Ring	None	Flaky
	Isp-8	1	Small colony floating	None	Flaky
PB-3	Isp-1	1	Ring	None	Floccular
	Isp-2	2	Ring	None	Flocculent
	Isp-8	2	Flocculent	None	Flocculent
PB-4	Isp-1	3	Ring + Flocculent	None	None
	Isp-2	2	Ring + Flocculent	None	Flocculent
	Isp-8	4	Pellicle + Ring	None	Viscid
PB-7	Isp-1	1	Powdery	None	Membranous
	Isp-2	3	Pellicle	None	Flaky
	Isp-8	1	Flocculent	None	Flaky
PB-8	Isp-1	2	Ring	None	Flaky
	Isp-2	3	Pellicle + Ring	None	Flaky
	Isp-8	3	Flocculent	None	Flaky
PB-9	Isp-1	3	Pellicle + Ring	None	None
	Isp-2	2	Ring	None	Viscid
	Isp-8	2	Pellicle	None	Flocculent
PB-11	Isp-1	3	Pellicle	colonies attached to the pellicle	None
	Isp-2	2	Ring	None	Flocculent
	Isp-8	1	Flocculent	None	Flaky
PB-12	Isp-1	2	Membranous	None	Flaky
	Isp-2	3	Pellicle	None	Flocculent
	Isp-8	2	Membranous	None	Viscid
PB-19	Isp-1	3	Pellicle + Ring	Small colonies attached to the tube	Floccular
	Isp-2	2	Ring	None	Flaky
	Isp-8	3	Pellicle	None	Flaky
PC-2A	Isp-1	3	Pellicle	None	None
	Isp-2	2	Ring	None	Flaky
	Isp-8	3	Pellicle + Ring	None	Flocculent
PC-2B	Isp-1	2	Pellicle + Ring	None	Viscid
	Isp-2	2	Ring	None	Flaky
	Isp-8	2	Flocculent	None	Granular
PC-10	Isp-1	ND	Ring	ND	ND
	Isp-2	2	Ring	None	Flocculent
	Isp-8	2	Ring	None	Flaky
PC-13	Isp-1	2	Ring	None	Floccular
	Isp-2	2	Ring	None	Flaky
	Isp-8	2	Ring	None	Flaky
PC-14	Isp-1	-	ND	None	ND
	Isp-2	2	Ring	None	Flaky
	Isp-8	2	Ring	Small colonies submerged with the medium	Flocculent

Table-9. (cont) Growth response in different ISP broth.

Isolate No.	Isp media	Amount of growth	Surface growth	Sub surface growth	Sediment
SP-5	Isp-1	3	Pellicle	Small colonies attached to the wall	Flaky
	Isp-2	2	Ring	None	Flaky
	Isp-8	4	Pellicle	Small colonies submerged with the medium	Flocculent
SP-7G	Isp-1	2	Membranous	None	Flaky
	Isp-2	3	Pellicle	None	Flaky
	Isp-8	1	Pellicle	None	Flocculent
SP-7W	Isp-1	3	Pellicle	None	None
	Isp-2	3	Pellicle + Ring	None	Flaky
	Isp-8	3	Pellicle	None	Flocculent
SP-8	Isp-1	2	Pellicle	None	None
	Isp-2	3	Pellicle	None	Flaky
	Isp-8	1	Small colony floating on the surface	None	None
SP-9	Isp-1	ND	ND	ND	ND
	Isp-2	2	Flocculent	None	Flocculent
	Isp-8	1	None	None	Flocculent
SP-10	Isp-1	2	Powdery	None	Viscid
	Isp-2	3	Pellicle	None	None
	Isp-8	2	Flocculent	None	Viscid
SP-11	Isp-1	1	None	None	Viscid
	Isp-2	2	Ring	None	Viscid
	Isp-8	2	Ring	None	Flaky

Note: "+" indicates Positive Result, "++" indicates super positive,"- "indicates Negative result & ND indicates not done.

Deep Glucose agar test:

On the basis of Oxygen requirement 32 were aerobic and only PB-1was facultative anaerobic (Table-10 and Fig.- 17-C).

Hydrolysis of starch, Casein, Gelatin and Esculin by the selected isolates:

Out of 33 strains 24 were amylase positive i.e. capable of hydrolyzing starch and 25 strains were able to hydrolyze casein. Gelatin was not hydrolyzed by a single strain. In case of esculin Hydrolysis, 6 strains showed negative result. Rest showed positive reaction (Table-11 and Fig.-17-B).

Test for Melanoid pigment, Hydrogen Sulfide by the selected isolates:

(Table- 12) Out of 33 strains only PA-5,PA-8g, SP-9, SP-11 were able to produce melanin pigment (Fig- 16) and B-1, PA-5, PA-8G, PB-8, PC-10, PC-14, SP-9 and SP-11 produced hydrogen sulfide (Fig- 17-A)

Test for decomposition of Urea, tyrosine and Nitrate reduction:

(Table-13 and Fig.-17-D) shows results of urease tests. From a total of 33 strains 19 could decompose urea.

Test for degradation of tyrosine:

24 strains were able to degrade tyrosine (Table-13).

Test for Nitrate reduction:

22 strains out of 33 possessed nitrate reductase enzyme, capable of reducing nitrate to nitrite (Table-13 and Fig- 17-E).

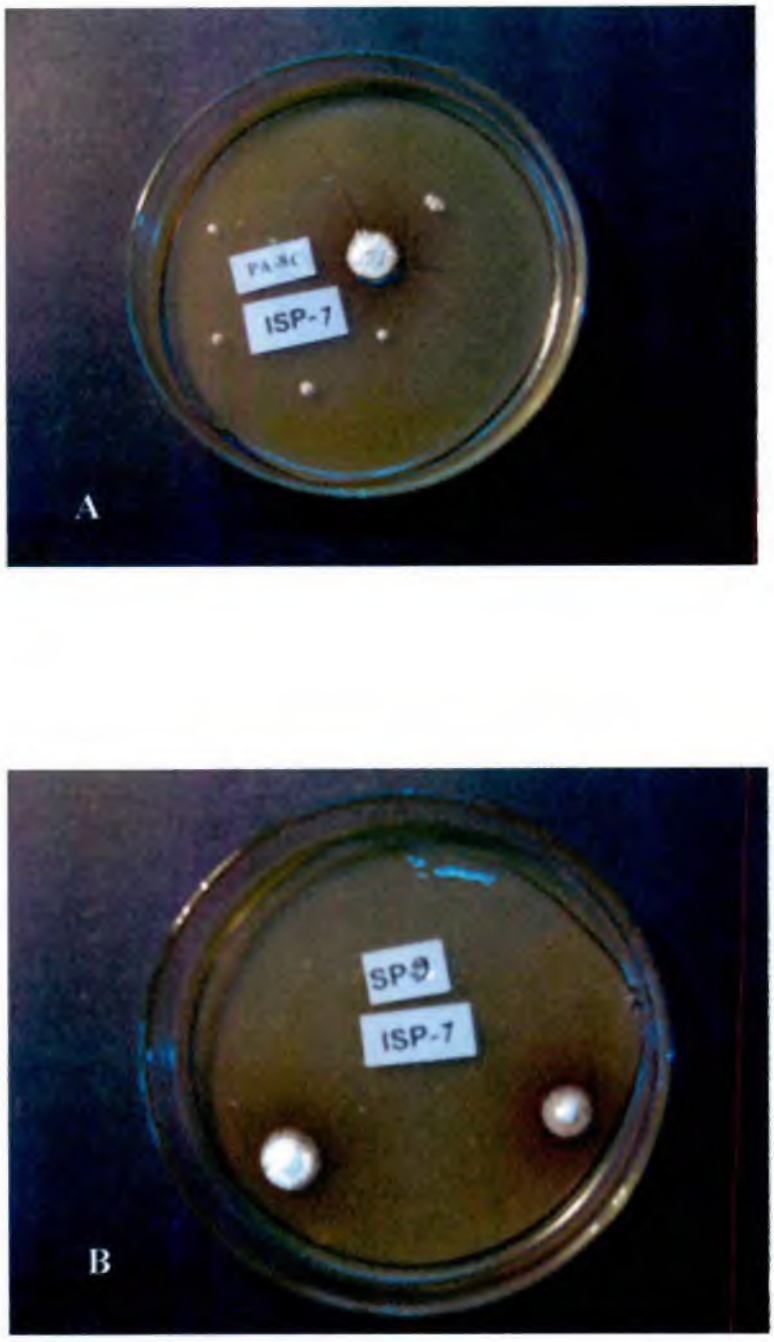


Fig- 16 : Production of Melanin Pigment by Isolates A) PA-8G, B) SP-9 on ISP 7 Medium.

Fig- 17 : Culture tubes showing results for A) H_2S production (blackening of medium indicates H_2S production), B) Esculin (blackening of medium indicates Esculin degradation), C) Deep glucose agar test, D) Urease test (Pink positive), E) Nitrate Reduction (Pink, Red positive).

Table-10. Deep Glucose Agar test.

Strain No.	Observation	Decision
B-1	Surface growth	Aerobic
B-3	Heavy surface growth	Aerobic
PR-1	Heavy surface growth	Aerobic
PR-2	Heavy surface growth	Aerobic
PR-3	Surface growth	Aerobic
PR-4	Heavy surface growth	Aerobic
PA-1	Surface growth	Aerobic
PA-4	Heavy surface growth	Aerobic
PA-5	Surface growth	Aerobic
PA-6	Heavy surface growth	Aerobic
PA-7	Heavy surface growth	Aerobic
PA-8G	Surface growth and growth upto 1/2 inch from surface	Aerobic
PB-1	Surface growth and also through out the tube, gas was produced	Facultative
PB-3	Surface growth	Aerobic
PB-4	Heavy surface growth	Aerobic
PB-7	Heavy surface growth	Aerobic
PB-8	Surface growth	Aerobic
PB-9	Surface growth	Aerobic
PB-11	Heavy surface growth	Aerobic
PB-12	Surface growth	Aerobic
PB-19	Heavy surface growth	Aerobic
PC-2A	Surface growth	Aerobic
PC-2B	Surface growth	Aerobic
PC-10	Surface growth	Aerobic
PC-13	Surface growth	Aerobic
PC-14	Surface growth	Aerobic
SP-5	Surface growth	Aerobic
SP-7G	Surface growth	Aerobic
SP-7W	Heavy surface growth	Aerobic
SP-8	Heavy surface growth	Aerobic
SP-9	Surface growth	Aerobic
SP-10	Surface growth	Aerobic
SP-11	Heavy surface growth	Aerobic

Table: 11. Hydrolysis of Starch,Casein,Gelatin and Esculin

Isolate No.	Starch	Casein	Gelatin	Esculin
B-1	+	+	+	+
B-3	-	+	+	+
PR-1	-	+	+	-
PR-2	-	+	-	+
PR-3	+	+	+	+
PR-4	-	+	+	+
PA-1	+	+	+	-
PA-4	-	+	+	-
PA-5	+	+	+	+
PA-6	+	+	+	+
PA-7	+	+	+	+
PA-8G	+	+	+	+
PB-1	+	+	+	+
PB-3	+	+	+	+
PB-4	+	+	+	+
PB-7	-	-	+	+
PB-8	+	+	+	+
PB-9	+	+	+	+
PB-11	+	+	+	-
PB-12	-	+	+	+
PB-19	+	+	+	+
PC-2A	-	ND	+	+
PC-2B	-	ND	+	+
PC-10	+	ND	+	+
PC-13	+	ND	+	+
PC-14	+	ND	+	+
SP-5	+	+	+	-
SP-7G	+	-	+	+
SP-7W	+	ND	+	+
SP-8	+	-	+	+
SP-9	+	ND	+	+
SP-10	+	ND	+	-
SP-11	+	ND	+	+

Note: "+" indicates Positive Result, "- "indicates Negative result & ND indicates not done.

Table-12. Production of melanoid pigment and hydrogen sulphide by the selected isolates on different media

Isolate No.	ISP-6	ISP-7	H_2S
B-1	-	-	+
B-3	-	-	-
PR-1	-	-	-
PR-2	-	-	-
PR-3	-	-	-
PR-4	-	-	-
PA-1	-	-	-
PA-4	-	-	-
PA-5	-	+	+
PA-6	-	-	-
PA-7	-	-	-
PA-8G	-	+	+
PB-1	-	-	-
PB-3	-	-	-
PB-4	-	-	-
PB-7	-	-	+
PB-8	-	-	-
PB-9	-	-	-
PB-11	-	-	-
PB-12	-	-	-
PB-19	-	-	-
PC-2A	-	-	-
PC-2B	-	-	-
PC-10	-	-	+
PC-13	-	-	-
PC-14	-	-	+
SP-5	-	-	-
SP-7G	-	-	-
SP-7W	-	-	-
SP-8	-	-	-
SP-9	-	+	+
SP-10	-	-	-
SP-11	+	-	+

Note: "+" indicates Positive Result, "- "indicates Negative result.

Table-13. Decomposition of Urea, Nitrate reduction and degradation of Tyrosin

Strain No.	Urease	Nitrate reductase	Tyrosin
B-1	-	+	-
B-3	+	+	-
PR-1	+	+	-
PR-2	+	+	-
PR-3	-	+	-
PR-4	+	+	-
PA-1	-	-	-
PA-4	+	+	-
PA-5	-	+	-
PA-6	-	-	-
PA-7	-	-	-
PA-8G	-	+	-
PB-1	+	+	-
PB-3	+	-	+
PB-4	+	-	-
PB-7	-	+	-
PB-8	+	+	-
PB-9	-	+	-
PB-11	-	+	-
PB-12	-	+	-
PB-19	+	+	+
PC-2A	+	+	-
PC-2B	+	+	-
PC-10	+	+	-
PC-13	+	+	-
PC-14	-	+	-
SP-5	+	-	-
SP-7G	-	-	-
SP-7W	+	+	-
SP-8	ND	-	-
SP-9	+	-	-
SP-10	+	-	-
SP-11	+	-	-

Note: "+" indicates Positive Result, "- "indicates Negative result & ND indicates not done.

Test for Lecithinase Proteolysis, Pectionolytic activity:

2 strains were lecithinase positive and rest were negative. Organisms varied in their ability to disintegrate egg albumin, which was indicative of their proteolytic activity. Except PB-12 and SP-11, all strains were unable to produce protease enzyme. All strains showed negative result in pectinolytic activity test i.e. no strain possessed pectinase (Table-14).

Determination of carbon source utilization by selected isolates:

The isolates were tested for their carbon utilization properties. It was done in ISP-9 agar plate medium (Table- 15).

Acid production from carbohydrate by selected isolates:

The isolates were tested whether they could produce acid from carbohydrate.
10 different carbohydrates were used as carbon source for this test. Most of the strains produced acid from glucose, galactose and cellobiose. Only strain No. PB-3 produced acid from all tested carbon sources but others did not(Table- 16).

Growth response at different concentrations of NaCl:

Effects of salt concentration on the selected isolates are shown in table-17, The result showed that the organisms had a wide range of tolerance towards NaCl concentration. The maximum growth of the organisms were in between 0 to 4% NaCl. Growth reduced drastically at higher concentration of salt.

Strain No. PB-19 showed red Pigmentation in between 4-10% NaCl. But this pigmentation was absent without NaCl. Strain SP-9 produced red pigment in the absence of NaCl. Sp-11 showed pigmentation in 4% NaCl.

Strain SP-5, PB-2A, PC-2A, PA-4, PR-1 grew in 13% NaCl. Most of the strains grew better in the absence of NaCl but PA-5 grew better in 7% & 10% NaCl.

Table-14. Test for Lecithinase production, Proteolysis and Pectinolytic activity

Strain No.	Lecithinase	Proteolysis	Pectinolytic activity.
B-1	-	-	-
B-3	-	(Tansparent)-	-
PR-1	-	(Tansparent)-	-
PR-2	-	(Tansparent)-	-
PR-3	-	(Tansparent)-	-
PR-4	-	(Tansparent)-	-
PA-1	-	-	-
PA-4	-	-	-
PA-5	-	-	-
PA-6	-	-	-
PA-7	-	(Tansparent)-	-
PA-8G	-	-	-
PB-1	-	-	-
PB-3	+	(Tansparent)-	-
PB-4	-	(Tansparent)-	-
PB-7	-	-	-
PB-8	-	-	-
PB-9	-	(Tansparent)-	-
PB-11	-	(Tansparent)-	-
PB-12	-	+	-
PB-19	+	(Tansparent)-	-
PC-2A	-	-	-
PC-2B	-	(Tansparent)-	-
PC-10	-	-	-
PC-13	-	ND	-
PC-14	-	-	-
SP-5	-	-	-
SP-7G	-	-	-
SP-7W	-	(Tansparent)-	-
SP-8	-	-	-
SP-9	-	-	-
SP-10	-	(Tansparent)-	-
SP-11	-	+	-

Note: "+" indicates Positive Result, "- "indicates Negative result & ND indicates not done.

Table-15. (a) Carbon source utilization pattern of the selected isolates.

Strain No.	Control	Control with Glucose	Mannose	Galactoe	Cellobioe	Mannitol	Arabinoe	Fructose	Maltose	Trehaloe
B-1	+	+	+	+	+	+	+	+	+	+
B-3	+	-	+	-	+	+	+	+	-	+
PR-1	+	+	+	+	+	+	+	-	+	-
PR-2	-	-	+	+	+	+	-	-	-	Trace
PR-3	+	+	+	+	+	+	+	+	+	+
PR-4	+	+	+	Trace	+	+	+	-	+	+
PA-1	+	+	+	+	+	+	+	-	+	+
PA-4	+	+	+	+	+	+	-	-	+	+
PA-5	+	+	+	+	+	+	+	+	+	+
PA-6	+	+	+	+	+	+	+	+	+	+
PA-7	+	+	+	+	+	+	+	+	+	+
PA-8G	+	+	+	+	+	+	+	+	+	+
PB-1	+	+	+	+	+	+	+	+	+	+
PB-3	+	+	+	+	+	+	+	+	+	+
PB-4	+	+	+	+	+	+	+	+	+	+
PB-7	+	+	+	+	+	+	+	+	+	+
PB-8	+	+	+	+	+	+	+	+	+	+
PB-9	+	+	+	+	+	+	+	-	+	+
PB-11	+	+	+	+	+	+	+	-	+	+
PB-12	+	+	+	+	+	+	+	+	+	+
PB-19	+	+	+	+	+	+	+	-	+	+
PC-2A	+	+	+	-	+	+	-	-	-	+
PC-2B	+	+	Trace	-	+	+	Trace	-	+	+
PC-10	-	+	+	+	+	+	-	+	+	+
PC-13	+	+	+	-	+	+	Trace	-	+	+
PC-14	+	+	+	+	+	+	+	-	+	+
SP-5	+	-	Trace	-	-	+	-	-	Trace	-
SP-7G	+	+	+	-	-	+	+	-	+	+
SP-7W	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
SP-8	+	+	+	+	+	+	+	+	+	+
SP-9	+	+	+	+	+	+	+	+	+	+
SP-10	+	+	+	-	+	+	Trace	-	+	+
SP-11	+	+	+	+	+	+	+	-	+	+

Note:"+" indicates Positive Result, "- "indicates Negative result & ND indicates not done.

Table-15. (b) Carbon source utilization pattern of the selected isolates.

Strain No.	Control	Control with Glucose	Xylose	Rhamnose	Raffinose	Adonitol	Sucrose
B-1	+	+	-	+	-	-	-
B-3	+	-	-	+	+	+	+
PR-1	-	+	-	-	-	-	-
PR-2	-	-	-	+	+	+	+
PR-3	+	+	-	-	+	-	+
PR-4	-	-	-	+	+	+	+
PA-1	+	+	-	-	-	-	-
PA-4	-	-	-	-	-	-	-
PA-5	+	+	-	+	-	+	+
PA-6	-	-	-	-	-	-	+
PA-7	-	-	-	-	-	-	+
PA-8G	-	+	-	-	+	+	+
PB-1	-	+	-	-	-	-	-
PB-3	+	+	+	-	+	+	-
PB-4	+	+	+	+	-	+	-
PB-7	+	-	+	+	+	+	-
PB-8	-	+	+	-	-	-	-
PB-9	+	+	+	-	-	-	-
PB-11	-	+	+	+	-	-	-
PB-12	+	+	+	-	+	+	+
PB-19	+	+	+	+	+	+	+
PC-2A	+	+	+	+	+	+	+
PC-2B	+	+	+	-	-	+	-
PC-10	-	+	+	-	-	-	-
PC-13	+	+	+	-	-	+	-
PC-14	+	-	+	+	-	+	-
SP-5	+	-	+	-	+	+	-
SP-7G	-	+	-	-	-	-	-
SP-7W	+	-	+	+	+	+	+
SP-8	+	+	+	-	-	-	-
SP-9	+	+	+	-	-	+	-
SP-10	+	+	+	+	+	+	+
SP-11	+	+	-	-	-	-	-

Note: "+" indicates Positive Result, "- "indicates Negative result

Table-16. Acid production from carbohydrates.

Strain No.	Glucose	Mannose	Fructose	Trehalose	Sucrose	Galactose	Adonitol	Mannitol	Cellobiose	Rhamnose	Arabinose
B-1	-	-	+	+	+	-	-	-	-	+	+
B-3	+	+	-	-	-	+	+	+	+	-	-
PR-1	+	+	-	-	-	+	+	+	+	-	-
PR-2	+	+	-	+	-	+	+	+	+	-	-
PR-3	+	+	-	+	-	+	-	+	+	+	+
PR-4	+	+	-	-	-	+	+	+	+	-	-
PA-1	+	+	-	+	-	-	-	-	+	+	+
PA-4	+	+	-	-	-	+	+	+	+	-	-
PA-5	+	+	+	-	+	+	-	+	+	+	+
PA-6	+	+	+	+	-	+	-	+	+	+	+
PA-7	+	+	+	+	-	+	-	+	+	+	+
PA-8G	+	+	+	-	+	-	-	-	+	-	+
PB-1	+	+	+	+	+	+	-	+	+	+	+
PB-3	+	+	+	+	+	+	+	+	+	+	+
PB-4	+	+	+	+	-	+	+	+	+	+	+
PB-7	+	ND	+	-	ND	+	+	+	+	-	+
PB-8	+	ND	+	+	ND	+	+	+	+	+	+
PB-9	+	ND	+	+	ND	+	-	+	+	+	+
PB-11	+	+	+	+	-	+	+	+	+	+	+
PB-12	+	ND	+	+	ND	+	+	+	+	+	+
PB-19	+	ND	+	+	ND	+	-	+	+	+	+
PC-2A	+	ND	+	+	ND	+	-	+	+	+	+
PC-2B	+	ND	+	+	ND	+	-	+	+	+	+
PC-10	+	ND	-	+	ND	+	-	+	+	+	+
PC-13	+	ND	+	+	ND	+	-	+	+	+	+
PC-14	+	ND	-	-	ND	+	-	-	-	+	-
SP-5	+	ND	+	-	ND	+	+	+	+	-	+
SP-7G	+	ND	+	-	ND	+	+	+	+	-	+
SP-7W	+	ND	-	-	ND	+	+	+	+	-	+
SP-8	+	ND	+	+	ND	+	-	+	+	+	-
SP-9	-	ND	-	-	ND	-	-	-	-	-	-
SP-10	+	ND	+	+	ND	+	-	+	+	-	-
SP-11	+	ND	-	+	ND	+	+	+	+	+	-

Note: "+" indicates Positive Result, "++" indicates super positive,"- "indicates Negative result & ND indicates not done.

Table-17. Growth response at different conc. of Nacl.

Strain No.	Nacl %				
	0	4	7	10	13
B-1	2	-	-	-	-
B-3	5	4	3	2	-
PR-1	5	4	3	2	Trace
PR-2	4	3	2	1	-
PR-3	4	3	-	-	-
PR-4	4	3	2	-	-
PA-1	4	3	2	1	-
PA-4	5	4	3	2	1
PA-5	-	Trace	5	5	-
PA-6	3	4	2	1	-
PA-7	4	2	1	-	-
PA-8G	2	1	-	-	-
PB-1	1	Trace	Trace	-	-
PB-3	2	2	1	Trace	-
PB-4	3	2	1	-	-
PB-7	1	1	1	-	-
PB-8	2	2	1	Trace	-
PB-9	5	3	2	-	-
PB-12	2	2	1	-	1
PB-19	4 (no pigment)	3 (red)	2 (red)	1(red)	-
PC-2A	3	2	1	Trace	1
PC-2B	3	2	1	Trace	-
PC-10	2	Trace	-	-	-
PC-13	3	2	2	Trace	-
PC-14	2	1	-	-	-
SP-5	2	4	3	4	3
SP-7G	1	1	1	2	-
SP-7W	ring 3	ring 3	ring 2	-	-
SP-8	2	-	-	-	-
SP-9	1(pigment)	-	-	-	-
SP-11	1(pigment)	2(pigment)	1	-	-

Note: "+" indicates Positive Result, "++" indicates super positive,"- "indicates Negative result & ND indicates not done.

Growth response of the selected isolates at different pH:

Selected isolates were tested for their growth response at different pH levels i.e. pH 4.5, 6.5 and 8.5). Growth of PR-1, PB-11, PB-4 were indifferent in different pH (Table-18, Fig- 20)

Growth response of the selected isolates at different temperature:

Selected isolates were tested for their growth response at different temperatures. The result showed that the isolates can grow at a wide range of temperature. No growth was found at 4°C. Only SP-10 showed growth at 10°C. However, the optimum temperature of most of the isolates was 37°C (Table-19, Fig- 21).

Pathogenic activities of the selected isolates:

Point inoculation method:

Out of 33 strains, 26 strains were pathogenic and 6 were saprophytic (Table-20, Fig-18(C-F).

Etiolated potato sprout test:

8 strains were taken for this test and 6 shown positive result while 2 shown negative result (Table-21, Fig-18(B).

Traditional soil inoculation method :

All 8 strains found pathogenic.

Pathogenecity test on radish seedlings:

Despite the diversity of scabbed samples, the pathogenic isolates obtained were similar to Phenotypic traits traditionally attributed to *S. scabies* The 3 strains considered pathogenic as (Table-22, Fig-19) they inhibited growth of seedlings (SP-8) or caused dark lesions on the hypocotyls (B3,PB-12) of the germinated seedlings. The seedlings germinated in the control media found spotless and fresh.

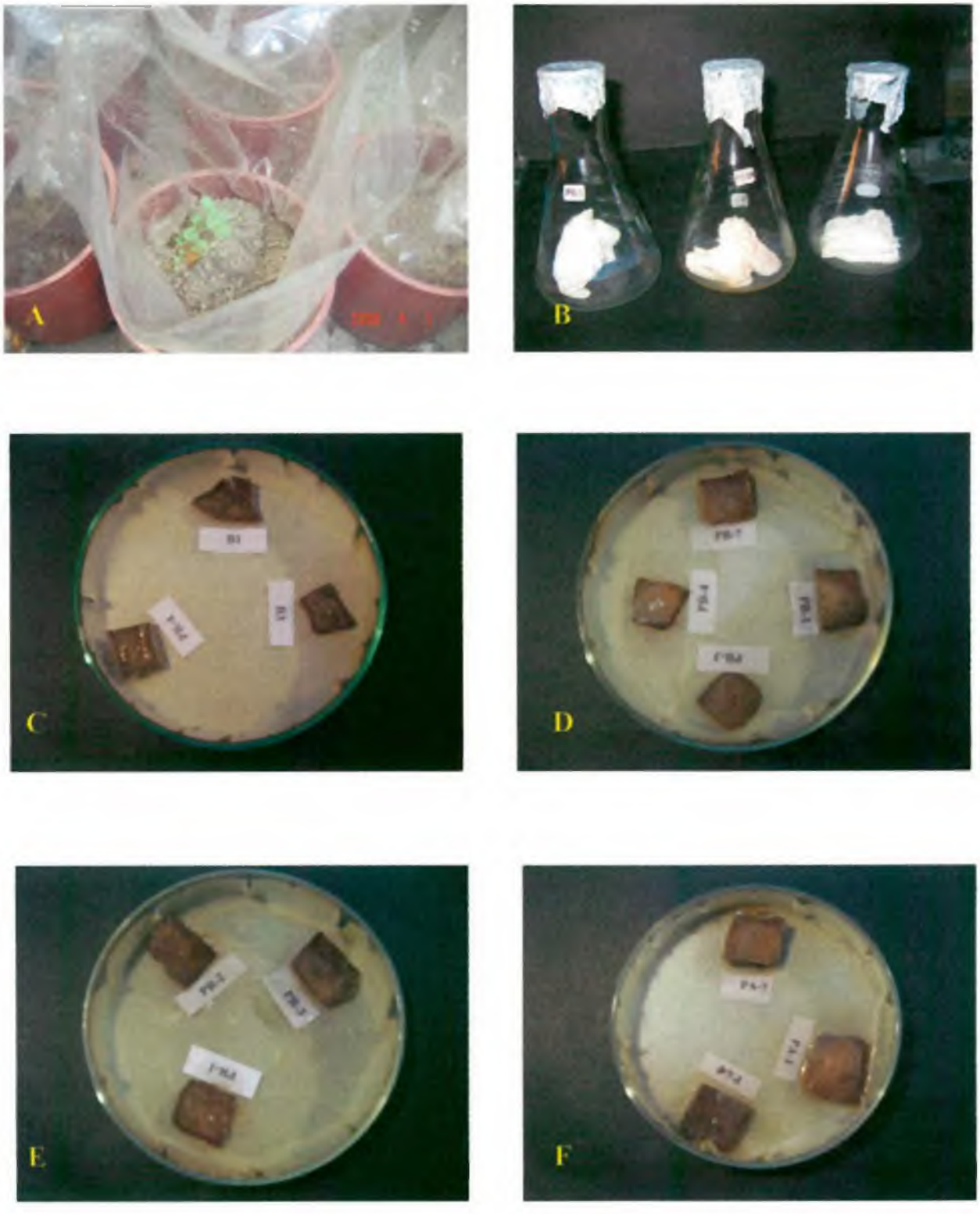


Fig- 18 : Pathogenesity test by A) Soil inoculation method, B) Tissu paper method (C-F) Point inoculation in Tuber.

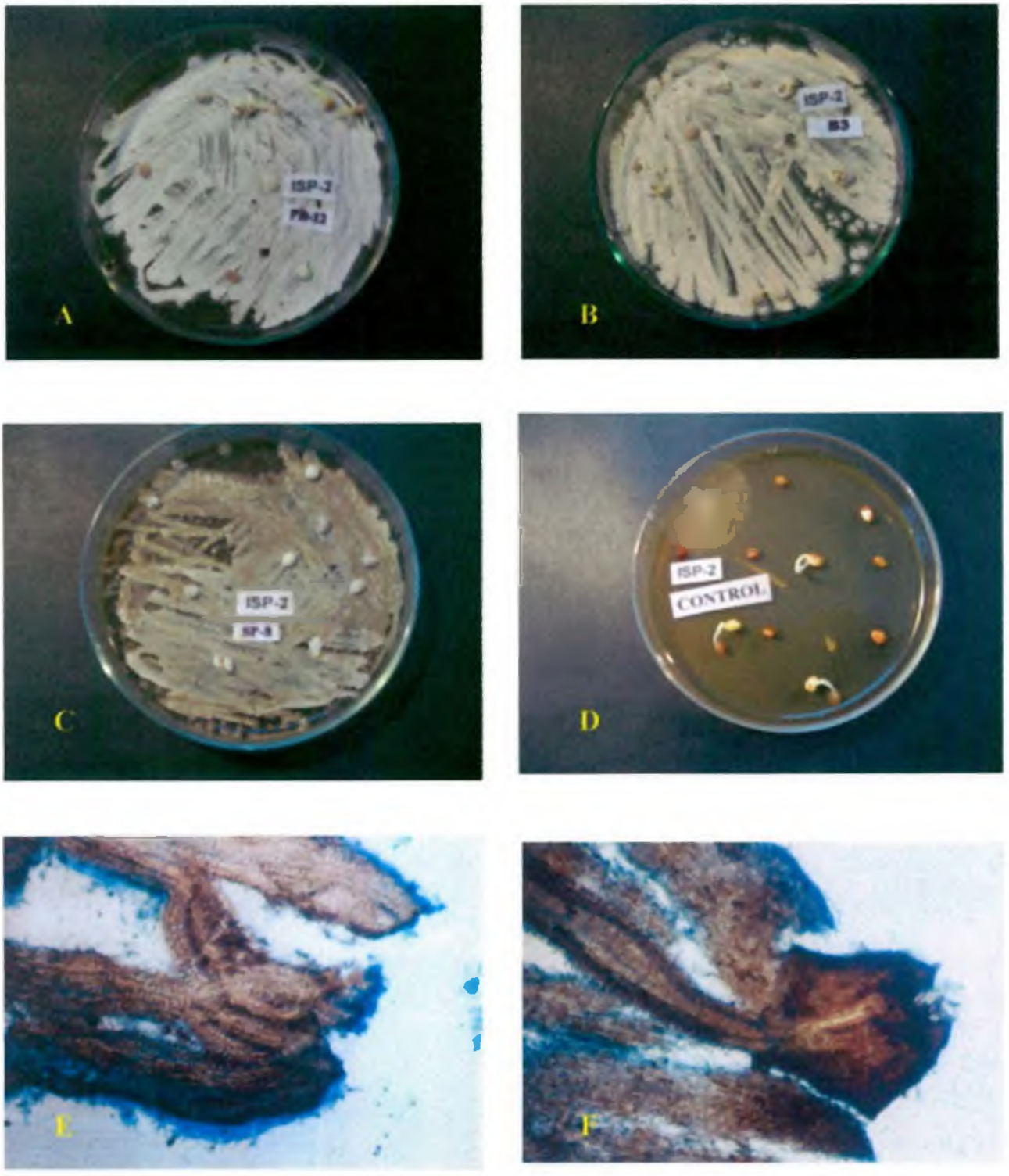


Fig- 19 : Pathogenecity test by seedling method. A,B,C) Seedlings grown on Culture Plates inoculated with strain PB-12, B-3, SP-8, D) Control, E) Photomicrograph showing the normal anatomy of a growing root tip. F) Photomicrograph showing blackening of the root tip indicating pathogenic effect of strain no. B3.

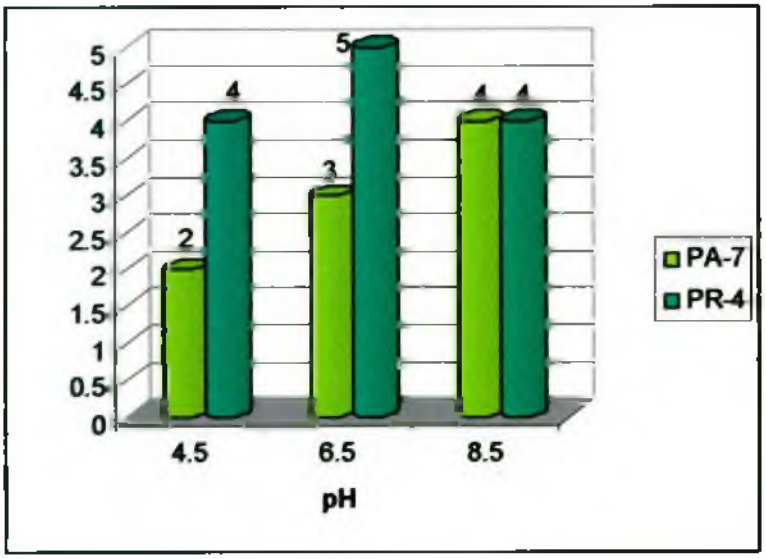


Fig- 20 : Graphic representation of the growth response of PA-7 & PR-4 at different pH level.

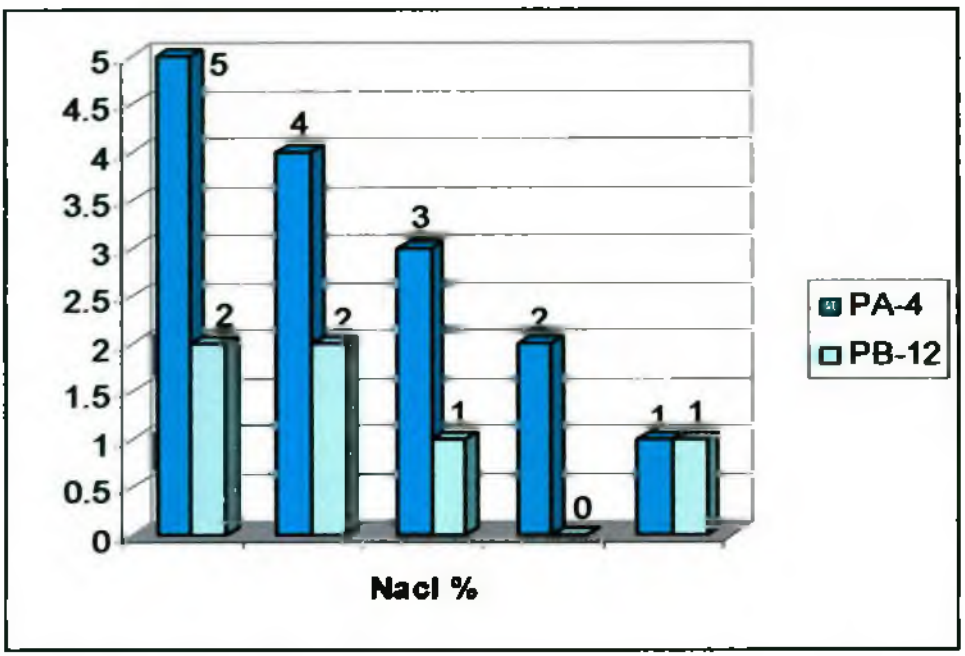


Fig- 21 : Graphic representation of the growth response of PA-4 & PB-12 at different concentrations of NaCl.

Table-18. Growth response at different pH levels.

Strain No.	pH		
	4.5	6.5	8.5
B-1	4	3	3
B-3	4	5	5
PR-1	5	5	5
PR-2	2	3	4
PR-3	3	5	2
PR-4	4	5	4
PA-1	2	4	3
PA-4	3	4	5
PA-5	3	5	4
PA-6	3	5	4
PA-7	2	3	4
PA-8G	2	4	3
PB-1	3	2	2
PB-3	3	2	2
PB-4	2	2	2
PB-7	3	4	4
PB-8	3	2	2
PB-9	5	4	3
PB-11	4	4	4
PB-12	3	3	3
PB-19	3	4	5
PC-2A	4	3	2
PC-2B	2	3	4
PC-10	4	3	2
PC-13	2	3	4
PC-14	3	4	2
SP-5	3	4	5
SP-7G	5	3	2
SP-7W	ND	ND	ND
SP-8	5	3	1
SP-9	5	4	2
SP-10	3	2	3
SP-11	1	4	3

Note: "+" indicates Positive Result, "++" indicates super positive,"- "indicates Negative result & ND indicates not done.

Table-19. Growth response at different Temp

Strain No.	Tempareture			
	4 °C	10 °C	37 °C	45 °C
B-1	-	-	5	5
B-3	-	-	3	3
PR-1	-	-	4	5
PR-2	-	-	3	3
PR-3	-	-	4	4
PR-4	-	-	3	5
PA-1	-	-	1	2
PA-4	-	-	3	5
PA-5	-	-	3	-
PA-6	-	-	2	2
PA-7	-	-	4	2
PA-8G	-	-	4	-
PB-1	-	-	ND	ND
PB-3	-	-	5+	5
PB-4	-	-	ND	ND
PB-7	-	-	4	-
PB-8	-	-	3	3
PB-9	-	-	3	5
PB-11	-	-	3	4
PB-12	-	-	4	-
PB-19	-	-	2	-
PC-2A	-	-	ND	ND
PC-2B	-	-	2	-
PC-10	-	-	4	4
PC-13	-	-	2	-
PC-14	-	-	3	4
SP-5	-	-	3	1
SP-7G	-	-	4	5+
SP-7W	-	-	5	4
SP-8	-	-	3	3
SP-9	-	-	5	3
SP-10	-	1	5	5+
SP-11	-	-	5	2

Note: "+" indicates Positive Result, "++" indicates super positive,"- "indicates Negative result & ND indicates not done.

Table-20. Pathogenesity test of the selected isolates in point inoculation method.

Isolate No.	Days after inoculation				
	10th	11th	12th	13th	14th
B-1	-	-	-	-	-
B-3	+	+	+	+	+
PR-1	+	+	+	+	+
PR-2	-	-	-	-	-
PR-3	-	-	-	-	-
PR-4	-	-	-	-	-
PA-1	+	+	+	+	+
PA-4	+	+	+	+	+
PA-5	+	+	+	+	+
PA-6	+	+	+	+	+
PA-7	+	+	+	+	+
PA-8G	+	+	+	+	+
PB-1	+	+	+	+	+
PB-3	+	+	-	+	+
PB-4	++	++	++	++	++
PB-7	+	+	+	+	+
PB-8	-	-	-	-	-
PB-9	-	+	+	+	+
PB-11	+	+	+	+	+
PB-12	+	+	+	+	+
PB-19	ND	ND	ND	ND	ND
PC-2A	-	+	+	+	+
PC-2B	+	+	+	+	+
PC-10	ND	ND	ND	ND	ND
PC-13	+	++	++	++	++
PC-14	-	-	-	-	-
SP-5	+	++	++	++	++
SP-7G	+	+	+	+	+
SP-7W	+	+	+	+	+
SP-8	+	+	+	+	+
SP-9	+	+	+	+	+
SP-10	+	+	+	+	+
SP-11	+	+	+	+	+

Note: "+" indicates Positive Result,"- "indicates Negative result & ND indicates not done.

Table-21. Result of Pathogenesity test by potato sprout (Tashiro,1999).

Strain No.	Result
PB-9	+
PB-3	+
PB-12	+
PB-11	+
PC-13	+
PC-2B	+
PC-10	-
PC-14	-

Note: "+" indicates Positive Result, "- "indicates Negative result.

Table-22.Result of Pathogenesity test on radish seedling(Flores- Gonzalez, 2008).

Name of strain	Total no. of seed	Germination	No. of seed germinated	Dark lesion produced
B3	10	+	6	+
PB-12	10	+	3	+
SP-8	10	-	0	-
Control	10	+	3	-

Note: "+" indicates Positive Result, "- "indicates Negative result.

Provisional identification of the selected isolates:

At present time, accurate identification of a microorganism requires much sophistication and all the required facilities are not available in Bangladesh. Under such circumstances, the isolates were provisionally identified with the help of Bergey's Manual of Systematic Bacteriology vol.-4, ed.-9 (Sneath *et. al.*, 1986, Table-24). The 33 selected isolates, belonged to the genus *Streptomyces.*

Table:23. Provisional identification of the selected isolates

B-1	*Streptomyces aureofacience*
B-3	*Streptomyces flaveolus, Streptomyces longisporoflavus, Streptomyces ochraceiscleroticus*
PR-1	*Streptomyces albus*
PR-2	*Streptomyces ochraceiscleroticus*
PR-3	*Streptomyces bikiniensis*
PR-4	*Streptomyces ochraceiscleroticus*
PA-1	*Streptomyces halstedi*
PA-4	*Streptomyces albus, Streptomyces varsoviensis*
PA-5	*Streptomyces griseoruber*
PA-6	*Streptomyces rochei, Streptomyces canus*
PA-7	*Streptomyces lydicus*
PA-8G	*Streptomyces violaceousniger, Streptomyceschattanoogensis, Streptomyces canus*
PB-1	*Streptomyces antibioticus*
PB-3	*Streptomyces rochei*
PB-4	*Streptomyces lydicus*
PB-7	*Streptomyces anulatus, Streptomyces atroolivaceus*
PB-8	*Streptomyces albidoflavus*
PB-9	*Streptomyces pactum*
PB-11	*Streptomyces cellulosae, Streptomyces graminofaciens*
PB-12	*Streptomyces albidoflavus*
PB-19	*Streptomyces ochraceiscleroticus, Streptomyces rimosus*
PC-2A	*Streptomyces ochraceiscleroticus*
PC-2B	*Streptomyces rimosus*
PC-10	*Streptomyces californicus*
PC-13	*Streptomyces rimosus, Streptomyces varsoviensis*
PC-14	*Streptomyces halstedii, Streptomyces bikiniensis*
SP-5	*Streptomyces albus*
SP-7G	*Streptomyces rochei*
SP-7W	*Streptomyces ochraceiscleroticus, Streptomyces fradiae*
SP-8	*Streptomyces albus*
SP-9	*Streptomyces canus, Streptomyces olivaceoviridis*
SP-10	*Streptomyces bikiniensis, Streptomyces ipomoeae*
SP-11	*Streptomyces rochei, Streptomyces pactum*

CHAPTER- 4

Discussion

Discussion

It is reported that only a very small proportion of the described *Streptomyces* species are known to be plant or animal pathogens. Plant pathogenic S. spp cause diseases of underground structures of diverse plant species. *S. scabies*, a cause of common scab of potato and similar diseases of other plant species, was the first of these species described and is the best studied of the pathogenic species. *S. acidiscabies* produces symptoms like those of *S. scabies* on potato and on other taproot crops. *S. ipomoea* causes soil rot of sweet potato. Several other S. spp cause disease, although much less is known about these pathogens than about the three species mentioned above (Loria, 1997).

On the basis of phylogenetic analysis of 16S rRNA gene sequences, most of the isolates were classified as *Streptomyces scabies* (Song et al, 2003). It was also revealed that *S. europaeiscabiei* could be clearly differentiated from *S. scabiei* (Song et al, 2003). *S. europaeiscabiei* was detected as the main cause of potato common scab in Western Europe. (Flores et al. 2008).

3 *Streptomyces* spp. were isolated from potato with common scab disease lesions in Korea (Park et al. 2003) named *S. luridiscabiei,* sp nov., *S. puniciscabiei* sp nov.., and *S. niveiscabiei* sp nov. and were described as novel species. Morphological and physiological properties of these isolates were distinct from those of previously described *Streptomyces* species strain.

Nine Streptomycetes strains were isolated from scab lesions on potato tubers in Egypt. Five of these nine isolates were pathogenic on potato minitubers but four of the pathogenic isolates produced thaxtomin in infected tuber tissues but one didnot (Sayed et al.2001). Thaxtomin is considered as the toxin responsible for producing characteristic scab symptom (Loria, 1997).

In Israel 35 potato scab inducing strains and 12 reference strains of *S. scabies* were physiologically characterized. Overall similarities were determined by numerical taxonomy. The majority of the pathogenic isolates were *S. violaceus* and *S. griseus* (Doering et al. 1992)

Streptomyces strains isolated from potato scab lesions in Japan were characterized by morphological and physiological analysis, DNA homology and RFLPs and at least four *Streptomyces* species including *S. scabies, S. acidiscabies* (Sawada et al. 1995). Identification of 132 potato scab containing samples collected in Hokkido, Japan in 1994. showed the presence of *S. scabies, S. achromogenes, S. turgidiscabies* and *S. acidiscabies* (Tanaka, 2003).

Streptomyces strains isolated from potato scab lesions in USA *S. scabies, S. diastatochromogenes, S. albogriseolus* were identified and one could not be identified (Lorang et al,1995)

A comparative study was done with the isolated organism as follows.

Table: 24. A comparative study of the isolated organisms.

Characteristics	Organisms from potato	Organisms from beet	Organisms from sweet potato
1. Number of organisms isolated	1. 23	1. 2	1. 7
2. Provisionally identified species	*2. S. albus.* *S.. ochraceiscleroticus* *S bikiniensis* *S. halstedi* *S. varsoviensis* *S. griseoruber* *S. rochei* *S. canus* *S. lydicus* *S. violaceousniger,* *S. chattanoogensis,* *S. antibioticus* *S. albidoflavus* *S. anulatus,* *S. atroolivaceus* *S. pactum* *S. cellulosae,* *S. graminofaciens* *S. rimosus* *S. californicus*	*2. S. aureofacience* *S. flaveolus,* *S. longisporoflavus,* *S. ochraceiscleroticus*	*2. S. albus* *S. rochei* *S. fradiae* *S. ochraceiscleroticus* *S. canus* *S. olivaceoviridis* *S bikiniensis* *S. pactum* *S. ipomoea*

Characteristics	Organisms from potato	Organisms from beet	Organisms from sweet potato
3. Comparison of organism common in 3 hosts *S. ochraceiscleroticus*.	3. 4 different isolates of *S. ochraceiscleroticus* were isolated from 3 different varieties of potato. These are PR-1, PR-4, PB-12,PC-2A.	3. The isolate was B3.	3. The isolate was SP-7W.
4. Names of Organisms found in specific sample.	*4. S. halstedi* *S. varsoviensis* *S. griseoruber* *S. lydicus* *S. violaceousniger*, *S. chattanoogensis*, *S. antibioticus* *S. albidoflavus* *S. anulatus*, *S. atroolivaceus* *S. cellulosae*, *S, graminofaciens* *S. rimosus* *S. californicus*	*4. S. aureofacience* *S. flaveolus*, *S. longisporoflavus*,	*4. S. fradiae* *S. olivaceoviridis* *S bikiniensis* *S bikiniensis*
5. Organism common in potato and sweet potato	5. Other than *S. ochraceiscleroticus*, the followings are common in potato and sweet potato: *S. albus.* *S bikiniensis* *S. rochei*, *S. canus* *S. pactum*	5. Except *S. ochraceiscleroticus*, *no other organism matches with the others.*	5. Organisms found both in potato and sweet potato: *S. albus.* *S bikiniensis* *S. rochei*, *S. canus* *S. pactum*

Characteristics	Organisms from potato	Organisms from beet	Organisms from sweet potato
6. *S. ochraceiscleroticus*	6. Spore chain spiral or rectiflexibilis. Spore mass white. Reverse color brown, diffusible pigment usually absent but in some cases present. Melanin is not produced. Growth occur in the presence of 7% NaCl and 45° C. Pectinolytic activity don't occur. Reduce nitrate, don't produce H2S. Mannitol, Raffinose, Sucrose are utilized.	6. Characteristics similar to that of potato	6. Characteristics similar to that of potato
7. *S. albus.*	7. Spore chain spiral, Spore mass white. Reverse color brown, diffusible pigment not produced. Melanin is not produced. Growth occurs in the presence of 7% NaCl and 45° C. Pectinolytic and lecithinase activity don't occur. Reduce nitrate, don't produce H2S. Mannitol, cellobiose, galactose, mannose are utilized but Fructose is not.	7. Organism not found.	7. Characteristics matches with that of potato, except carbon source utilization. Mannitol and Mannose is utilized, but cellobiose, galactose and fructose are not always utilized.
8. *S. bikiniensis*	8. Spore chain rectiflexibilis. Spore mass grey. Reverse color brown, diffusible pigment not present. Melanin is not produced. Growth is not occured in 7% NaCl but occurred at 45° C. Pectinolytic activity don't occur. Reduce nitrate, Glucose, Mannose, Galactose, cellobiose, Mannitol, arabinose, Maltose, Trehalose are utilized.	8. Organism not found.	8. It differs in nitrate reduction which gives negative result, and utilization of carbon source. Galactose was not utilized.

Characteristics	Organisms from potato	Organisms from beet	Organisms from sweet potato
9. S. rochei	9. Spore chain spiral. Spore mass grey. Reverse color brown. Diffusible pigment sometimes produced. Melanin is not produced. Lecithinase , pectinase activity didn't occur, nitrate was not reduced, H2S was not produce. Growth occurred at 45°C and 7% NaCl. Mannitol is utilized but Rhamnose is not.	9. Organism not found.	9. Spore chain spiral. Spore mass grey. Reverse color brown to black. Diffusible pigment and Melanin are sometimes produced. Growth occur in the presence of 7% NaCl and 45° C. Pectinolytic, Lecithinase activity don't occur. Nitrate not Reduced, H2S sometimes produced. Mannitol is utilized but Rhamnose is not.
10. S. canus	10. Spore chain spiral. Spore mass grey. Reverse color brown. Diffusible pigment sometimes produced. Melanin is not produced. Lecithinase , pectinase activity didn't occur, nitrate, H2S often produce. Some strains show growth at 45°C and 7% NaCl. Mannitol is utilized but Rhamnose is not.	10. Organism not found.	10. Spore chain spiral. Spore mass grey. Reverse color brown. Diffusible pigment produced. Lecithinase , pectinase activity, didn't occur, nitrate was not reduced, H2S is produced. Growth occurred at 45°C and 7% NaCl. Mannitol is utilized but Rhamnose is not.
11. S. pactum	11. Spore chain spiral. Spore mass grey. Reverse color brown. Diffusible pigment sometimes produced. Melanin is not produced. Lecithinase , pectinase activity didn't occur, nitrate was not reduced, H2S was not produce. Growth occurred at 45°C and 7% NaCl. Mannitol is utilized but Rhamnose is not.	11. Organism not found.	11. Spore chain spiral. Spore mass grey. Reverse color brown. Diffusible pigment sometimes produced. Melanin is not produced. Lecithinase , pectinase activity didn't occur, nitrate was not reduced, H2S was not produce. Growth occurred at 45°C and 7% NaCl. Mannitol is utilized but Rhamnose is not.

Liu et al. (1996) reported about 93 suppressive strains of *Streptomyces*. Of these 22 strains showed greater antibiotic activity against virulent *Streptomyces scabies* these 22 suppressive strains were non pathogenic on leaf bud tubers in the green house. Suppressive strains significantly reduced scab and did not affect tuber yield in a field pot test. All the virulent strains and 54% of suppressive strains were classified as *S. scabies* (Liu et al. 1996). In another study Bowers et al. (1996) reported that the pathogen population was substantially lower in soil from treatments where the suppressive strains were introduced compared with the non-amended control. The inhibition of the pathogen related population was correlated with a reduction of scab symptoms observed in the field plots into which the suppressive strains were introduced.

Control of potato scab disease by lowering soil pH was suggested by Waksman (1967) but this practice was not found preferred and feasible. The relation ship between inoculum density of suppressive Streptomyces spp. and disease severity were investigated by Ryan & Kinkel, (1997).A fundamental shift in the pathogen-related population in response to the introduction of the suppressive strains for long-term biological control of potato scab are encouraging.

On the event of the global awareness about the physiological specialization and evolution of regional pathogenic strains and the indiscriminate use of hazardous pesticides, we should really go for developing effective biological controlling system. For the purpose we shall have to examine a vast number of related organisms and find out their potentiality for practical application for controlling not only scab but also other pathogens of importance.

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* Original not seen

APPENDIX

Composition of the media and reagents used in this study are as follows. In each case pH was adjusted before sterilization.

Deep glucose agar medium: (SAB 1957)

Beef extract----------3.0 g
Pepton ----------5.0 g
Glucose----------10.0 g
Agar----------15.0 g
Distilled water----------1000 ml
pH adjusted to 7.2

Esculin Agar medium: (Collins and Lyne 1984)

Esculin----------0.1 g
Yeast extract----------3.0 g
Ferric ammonium citrate---0.05 g
Agar----------7.5 g
Distilled water----------1000 ml
pH adjusted to 7.2

Gelatin Agar medium: (Collins and Lyne 1984)

Beef extract----------3.0 g
Pepton ----------5.0 g
Sodium chloride----------5.0 g
Gelatin----------25.0 g
Agar----------15.0 g
Distilled water----------1000 ml
pH adjusted to 7.2

Glucose Asparagine Agar :

Glucose----------10.0 g
Asparagine----------0.5 g

K_2HPO_4----------0.5 g

Agar----------15.0 g

Distilled water----------1000 ml

pH adjusted to 7.2

ISP-1 :Tryptone yeast –extract broth (Shirling & Gottlieb 1966)

Trypton----------5.0 g

Yeast-extract----------3.0 g

Distilled water----------1000 ml

pH adjusted to 7.2

ISP-2 :Yeast –extract malt extract (Shirling & Gottlieb 1966)

Yeast-extract----------4.0 g

Malt-extract ----------10.0 g

Dextrose----------4.0 g

Agar----------20.0 g

Distilled water----------1000 ml

Agar----------20.0 g

pH adjusted to 7.2

ISP-3 :Oat meal agar (Shirling & Gottlieb 1966)

Oat meal----------20.0 g

Agar----------18.0 g

Distilled water----------1000 ml

pH adjusted to 7.2

ISP-4 :Inorganic salts-starch agar (Shirling & Gottlieb 1966)

Solution 1: Soluble starch 10.0 g. Make a paste of the starch with a small amount of water and bring the volume of 500 ml.

Solution 2:

K_2HPO_4----------1.0 g

$MgSO_4.7H_2O$----------1.0 g

NaCl----------1.0 g

$(NH_4)_2SO_4$----------2.0 g

$CaCO_3$----------2.0 g

Distilled water----------500 ml

Trace salt solution----------1.0 ml

Agar----------20.0 g

pH adjusted to 7.2

ISP-5 : Glyserol aspargine agar (Shirling & Gottlieb 1966)

L-asparagine----------1.0 g

Glycerol----------10.0 g

K_2HPO_4----------1.0 g

Distilled water----------500 ml

Trace salt solution----------1.0 ml

Agar----------20.0 g

pH adjusted to 7.2

ISP-6 : Peptone yeast extract iron agar (Shirling & Gottlieb 1966)

Bacto peptone iron agar, dehydrated (Difco)--- 36.0 g

Bacto yeast extract----------1.0 g

Distilled water----------1000 ml

pH 7.2-7.4

Bacto peptone iron agar, dehydrated contains the following ingredients when reconstituted as 36.58 g/l of water: Bacto peptone,15.0 g; Proteose peptone,5.0 g;Ferris ammonium citrate,0.5 g; Di potassium Phospate, 1.0 g; Sodium Thio sulphate,0.08 g; Agar 15.0 g.

ISP-7: Tyrosine agar (Shirling & Gottlieb 1966)

Glycerol ----------15.0g

L- Tyrocine----------0.5g

L-Asparagine----------1.0g

K_2HPO_4----------0.5g

$MgSO_4.7H_2O$----------0.5g

NaCl----------0.5g

$FeSO_4.7H_2O$----------0.01g

Distilled water----------1000ml

Trace salt Solution----------1.0ml

Agar----------20.0g

pH – 7.2-7.4

ISP-8: Bacto nitrate broth (Shirling & Gottlieb 1966)

Beef extract----------3.0g

Peptone----------5.0g

KNO_3----------1.0g

pH – 7.2-7.4

ISP-9 :Mineral salt agar medium (Shirling & Gottlieb 1966)

$(NH_4)_2SO_4$----------2.64 g

H_2SO_4----------2.38 g

$MgSO_4$----------1.0 g

Distilled water----------1000ml

Trace salt Solution----------1.0ml

Agar----------20.0g

pH – 7.2-7.4

Modified Bennett's Agar medium (Iwasa et al.1970)

Yeast extract----------1.0g

Beef extract----------1.0g

Casein----------2.0g

Glucose----------5.0g

Maltose----------5.0g

Distilled water----------1000ml

pH – 7.2-7.4

Nitrate Broth Medium (SAB 1957)

Peptone----------5.0g

Beef Extract----------3.0g

Potassium nitrate----------1.0g

Distilled water----------1000ml

pH – 7.2

Nutrient agar medium

Beef extract----------3.0 g

Peptone----------5.0 g

NaCl----------5.0 g

Agar----------15.0 g

Distilled water----------1000ml

pH – 7.2

Pectinase activity medium (Hankin et al. 1971):

(% w/v)

A. KH_2PO_4----------0.4 g

Na_2HPO_4----------0.6 g

B. Pectin----------0.5 g

C. $(NH_4)_2SO_4$----------0.2 g

Yeast extract----------0.1 g

$MgSO_4.7H_2O$----------0.2 g

$FeSO_4.7H_2O$----------0.0001 g

$CaCl_2$----------0.0001 g

Agar----------1.0 g

pH- 7.2

Peptone Iron Agar

Peptone----------15.0 g

Proteose peptone----------5.0 g

Ferric ammonium citrate---------1.0 g

Sodium Glycerophosphate-------1.0 g

Agar----------15.0 g

Distilled water----------1000ml

pH- 6.7

Starch agar medium

Beef extract ----------3.0 g

Soluble starch----------10.0 g

Agar----------15.0 g

Distilled water----------1000ml

pH- 7.2

Tyrosine agar medium (Sneath et al.1986)

L-Tyrosine----------0.5 g

Distilled water----------10 ml

Nutrient agar----------100ml

Tyrisine was sterilized separately and was added to the medium before inoculation

pH- 7.0

Urease broth (Rustigen & Stuart 1941)

K_2HPO_4----------9.1 g

Na_2HPO_4----------9.5 g

Yeast extract----------0.1 g

Phenol red----------0.01 g

Distilled water----------1000ml

pH- 6.8

10 ml of a 15% (w/v) solution of urea sterilized by filtration, was added to 75 ml of sterile urease broth.

Simmon's Citrate Agar medium (Claus 1995):

Magnesium sulphate ----------0.2 g

Mono ammonium di- hydrogen phosphate------1.0 g

Di potassium Hydrogen phosphate---------- 1.0 g

Sodium Chloride---------- 5.0 g

Sodium citrate----------2.0 g

Bromothymol blue----------0.08 g

Agar----------15 g

Distilled water----------1000ml

pH- 6.8

Acid production from carbohydrates (Schaad, 1988)

$NH_4H_2PO_4$----------0.5 g

K_2HPO_4----------0.5 g

$MgSO_4.7H_2O$----------0.2 g

NaCl----------5.0 g

Yeast extract----------1.0 g

Agar----------12 g

Distilled water----------1000ml

pH- 6.8

Bromocresol purple----------0.7 ml of 1.5% alchohol sol.

Carbon Source- ----------0.5%

Egg yolk lecithinase medium (SAB 1957):

Peptone----------40 g

Na_2HPO_4----------5.0 g

KH_2PO_4----------1.0 g

NaCl----------2.0 g

Mg SO_4----------0.1 g

Glucose----------2.0 g

Agar----------25 g

Distilled water----------1000ml

pH- 7.6

Egg yolk was taken aseptically and was diluted with equal volume of 0.85% NaCl.1 ml of this suspension was added to each 9ml of the medium.