

ROLE OF SEED BORNE BACTERIAL PATHOGENS ON RICE SEED HEALTH

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IN PARTIAL FULFILMENT OF THE REQUIREMENTS
FOR THE DEGREE OF**

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(PLANT PATHOLOGY)**

**By
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BHUBANESWAR-751 003, ORISSA
2003**

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Dr. N. K. DHAL

Dedicated
To my
Beloved Parents

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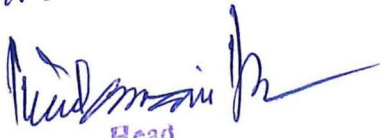
CERTIFICATE-I

This is to certify that the thesis entitled **“ROLE OF SEED BORNE BACTERIAL PATHOGENS ON RICE SEED HEALTH”** submitted in partial fulfillment of the requirement of the Degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** to the **Orissa University of Agriculture and Technology, Bhubaneswar** is a faithful record of bonafide and genuine research work carried out by **Niranjan Chinara** under my guidance and supervision during the **academic session, 2001-2003**. No part of this thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of investigation has been duly acknowledged by him.

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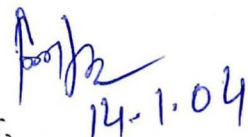

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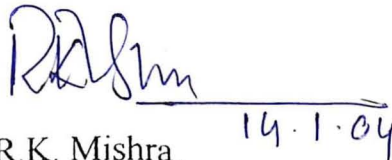
This is to certify that the thesis entitled "**ROLE OF SEED BORNE BACTERIAL PATHOGENS ON RICE SEED HEALTH**" submitted by **Niranjan Chinara** to the **Orissa University of Agriculture and Technology, Bhubaneswar** in partial fulfillment of the requirements for the Award of the Degree of **MASTER OF SCIENCE IN AGRICULTURE (PLANT PATHOLOGY)** has been approved by the Students' Advisory Committee after an Oral Examination on the same in collaboration with an external examiner.

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*Last but not least with heartfelt devotion, I bow down my head before "**Sri Sri Thakur Anukul Chandra**" for his blessing without which I couldn't have been able to complete my thesis.*

Niranjan Chinara.
(Niranjan Chinara)

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ABSTRACT

TITLE OF THESIS : Role of seed borne bacterial pathogens on rice seed health.

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During the 2002 wet season, harvested seeds from 15 popular rice varieties were collected from the experimental farm, OUAT, Bhubaneswar and phyto-pathological analysis of the seeds were carried out to study the association of different bacteria with the test varieties. The isolated bacteria were identified on the basis of morphological, physiological and biochemical characters. Role of seed borne bacteria on seed health with respect to seed germination, reduction in root and shoot growth and their effect on seedling vigour were assessed following paper towel method.

Phytopathological analysis of seed sample revealed the association of five bacterial species, namely, *Xanthomonas oryzae oryzae*, *X. o. oryzicola*, *Pseudomonas syringae panici*, *Erwinia chrysanthemi* and *E. carotovora*. Among the five bacterial species *X. o. oryzae* was detected from most of the rice varieties except Lalat which caused highest per cent of seed infection. The rest of the bacterial species were found to be associated with 2 – 4 per cent of seeds irrespective of varieties.

Among the 15 test varieties, seed infection was maximum in Mahanadi (50.0%) followed by Rambha and Armada. Lowest seed infection with bacteria was recorded in Parijat and Swarna.

Studies on seed health revealed that increase in seed infection resulted decrease in seed germination, decrease in root and shoot length and decrease in seedling vigour. It is interesting to note that seed infection resulted in the development of more number of abnormal seedling to the level of 68.0% in Mahanadi and 52.0% in Annapurna. Development of abnormal seedlings might be due to the association of *X. o. oryzae* as the bacterium synthesized A₁ to A₄ fraction of ethyl acetate which inhibited growth of root and shoot of rice seedlings (Sreeramula *et al.*, 1986). Also, Naeem *et al.* (2002) reported association of *X. o. oryzae* with rotten and abnormal seedlings.

Further studies on the aspect may reveal interesting result which shall be helpful in improving production of quality seeds in rice.

CHAPTER-I

INTRODUCTION

INTRODUCTION

Rice is, unquestionably, an important crop in India, or for that matter, the continent of Asia. India ranked first in the world with regard to area under rice with 42.3 million hectares and second in production with 88.5 million tonnes per annum. Orissa occupies an important position among the leading rice producing states in the country with an area of 4.3 million hectares and production of 6.3 million tonnes per year. However, the average productivity of the state is much below the national average. Disease plays an important role in low production and deterioration in the quality of seeds.

Prevalence of warm and humid climatic condition during most part of the year favours disease development and particularly bacterial diseases about which little is known. On the other hand, plant pathogenic bacteria do not form endospores. Most of the important plant pathogenic bacterial genera are not well fitted for their survival in soil due to high summer temperature. They are rather soil invaders than soil inhabitants and largely depend on their host for survival through the critical periods. Particularly in annual or seasonal host plants like rice the bacterial pathogens more depend on seed for their survival.

Seed being the planting material and the carrier of the genetic potential of the crop, bacterial pathogens play an important role in determining the quality of the seed and ultimately affecting the crop growth and yield. This occurs at a time when the agricultural scientists are trying hard to stabilize, sustain and improve upon the hard earned state of self-sufficiency in food grain production to meet the additional estimated production need of over 100 million tons to sustain self-sufficiency by 2010 A.D.

Little is known about the composition of bacterial communities of rice seed. Characterisation bacterial population on rice phylloplane has commonly been restricted to those bacteria that cause diseases in rice; much in the same way that studies on rice seed microflora have been more confined to seed borne pathogens, mostly seed borne fungi. Several bacterial species, other than those that cause diseases have been reported to be associated with rice seed (Cottyn *et al.*, 2001; Xie *et al.*, 2002). No much attempt has been made to study their role on seed health.

Quality of seed and food grain are also greatly affected by the presence of bacterial pathogens and other microorganisms. Therefore, understanding of bacterial community and their role in deteriorating the seed health would be the first step towards sustainable rice production.

Surprisingly, little effort has been made to study the seed borne bacterial pathogens, besides those that cause disease in rice plant. Envisaging the current global trade and quality consciousness of the consumers, it is imperative to initiate studies on bacteria in determining the seed health standard.

Therefore, the present study was undertaken with the following objectives:

1. To study the association of culturable aerobic bacterial isolates with the rice seeds belonging to early, medium and late maturation groups.
2. To identify the bacterial isolates.
3. To assess their role in regulating seed health in respect of seed germination, root and shoot length and seedling vigour.

CHAPTER-II

REVIEW OF LITERATURE

REVIEW OF LITERATURE

Bacterial plant pathogens cause severe yield losses. Bacterial leaf blight disease in rice caused by *Xanthomonas oryzae* pv. *oryzae* alone lead to 6-74% and sometimes even up to 94% loss in yield (Ahmed and Singh, 1975; Srinivasan, 1982).

Effective disease control measures are predicted basing upon the knowledge of ecology and etiology of the causal agent. It is well-known that plant pathogenic bacteria are carried in or on seed and any bacterial plant pathogen may be potentially transmitted through seeds, which perhaps has not been given due thought by the plant pathologists. On the basis of limited information available in the literature, an attempt has been made in this chapter to present a brief account of the bacterial community associated with rice seed, advances made in their detection methods and their role on seed health.

1. Association of bacteria with rice seed:

A large number of pathogenic bacteria are known to be associated with rice seeds. A few of them produces disease symptoms in plant as *Xanthomonas oryzae* pv *oryzae* causing bacterial leaf blight and *X. oryzae* pv *oryzicola* causing bacterial leaf streak. Some others cause exclusively grain diseases as black rot, cinnamon speck, black eye spot, glume blotch of rice etc. (Ou, 1985).

Fang *et al.*(1956) for the first time found the organism not only inside the glumes but occasionally on the endosperm of the seeds and considered seed as source of infection.

Similar finding relating to seed association of *X.o.oryzae* has also been reported by several workers from India. Srivasthava and Rao(1964); Pal *et al.*(1982); Murty and Devadath(1984); Gnanamanickam *et al.*(1995) and from other countries like Indonesia (Supriaman and Tantera, 1974); Pakistan (Butta and Ahmad, 1994); Japan (Mizukami, 1961); Philippines(Emchit and Ou, 1969 and 1970). Bacterial leaf streak of rice caused by *Xanthomonas oryzae* pv *oryzae* was reported from seed by Devadath and Dath (1985), Shekhawat and Srivastava (1972); Nema and Kulkarni (1973), Huang *et al.* (1994).

Several rice grain diseases caused by bacteria have been reported from countries other than India. Black rot caused by *Erwinia herbicola* was first reported from Japan by Iwadara (1931). Later it was reported from other countries like Korea, Taiwan and Manchuria. However, another grain rot disease caused by *Pseudomonas glumae* was reported from Japan by Goto and Ohata (1956) and Tsushima (1991). The individual spikelets due to the disease turned greenish white, later they became dirty grey and finally looked yellowish-brown in colour. Cother (1974) reported glume blotch disease of rice grain caused by *Pseudomonas syringae* pv. *syringae*.

Several other bacteria reported to cause rice grain diseases. Important among them were cinnamon speck of rice grain caused by *Xanthomonas cinnamona* and black eye spot caused by *Xanthomonas atroviridigenum* (Ou, 1985).

Understanding of bacterial composition of rice seed, barring those which cause diseases, is quite useful in biological control of seed borne organisms, to study microbial diversity in nature and ultimately increase in rice seed production. However, few studies so far has been made in this regard. Bhutta and Ahmad (1996) collected 153 seed samples from different parts of Pakistan and observed maximum seed infection of 11% and 12% due to *X. o. oryzae* in variety IRRI-6 at Lahore and Hyderabad respectively. They also recorded 13.0% seed infection by *Acidovorax avenae* sub. sp. *avenae* in variety B-385 collected from Sahiwal. Percentage of seed infection due to bacterial pathogens varied from cultivar to cultivar.

Kim and Song (1996) from Korea detected 51 strains of six major bacterial pathogens associated with rice seeds using PCR- amplification technique. They were *Pseudomonas avenae*, *Pseudomonas glumae* pv. *fuscovaginae*, *P. syringae* pv. *syringae* *X. oryzae* pv. *oryzae*, *X. o. oryzicola* and *Erwinia herbicola*.

Xio *et al.* (1999) made a field survey of rice bacterial disease in the Zhejiang province of China and Luzon Island of the Philippines during 1993-98. They isolated 280 pathogenic bacteria from 116 rice seed samples. Isolation frequency of pathogenic bacteria was about 9.0% and 6.0% in the tropics and subtropics respectively. Eleven bacterial species, namely *X.o.oryzae*, *X.o. oryzicola*,

Pseudomonas fuscovaginae, *P. fluorescens*, *Acidovorax avenae*, pv. *avenae*, *Burkholderia glumae*, *Erwinia chrysanthemi* pv. *zuae*, *Pantoea agglomerans*, *Pseudomonas aeruginosa*, *Pseudomonas fulva* and *P. putida*. Most of the isolates of four species of *Pseudomonas* did not induce any symptoms in rice plants. Some unknown bacteria resulted poor germination and seedling growth.

Subha *et al.* (1992) isolated *X.o. oryzae*, *Pseudomonas castaneae*, *P. gladioli* pv *alliicola*, *P. syringae* pv *dysoxylia*, *Bacillus cereus* alongwith some pathogenic bacteria associated with rice seed.

Ashura *et al.* (1999) examined 60 rice varieties in Tanzania and found the association of *Acidovorax avenae* sub sp. *avenae* causing brown stripe disease in 63.0% seed samples. Other pathogens identified were *Pantoea agglomerans*, *X.o. oryzae* and *Burkholderia glumae*.

Cottyn *et al.*(2001) reported several bacteria associated with rice seed in the tropical environment, during 1995 wet season. Harvested rice seed samples were collected from farmers fields at different locations in Iloilo, Philippines. Bacterial isolations from crushed seed yielded 428 isolates. The isolates were characterized by BOX-Polymerase chain reaction finger printing of total genomic DNA and represented 151 finger printing types (FPT). The predominant bacteria associated with rice seed were *Enterobacteriaceae* (28%), *Bacillus* sp. (22%) and *Pseudomonas* sp. (17%). Other bacteria regularly present were identified as *Xanthomonas* sp., *Cellulomonas flavigena*, *Clavibacter michiganense*. Of the total

number of isolated bacteria, 4% exhibited in-vitro antifungal activity against *Rhizoctonia solani*. Two per cent of the isolates were identified as *Burkholderia glumae* and *Burkholderia gladioli*. Five percent of isolates included *Pseudomonas* spp. and *Pantoea* sp.

Hong *et al.* (2002) found that the virulent strains of *Pantoea agglomerans* caused rice seed discolouration and influenced the development and maturation of rice seeds.

Xie *et al.* (2002) reported that among 11 bacteria only *X. o. oryzae*, *P. fuscovaginae* and *Acidovorax avenae* pv. *avenae* were seed borne and seed transmitted using both artificially inoculated and naturally infected seeds.

2. Period of survival of the pathogen in rice seed:

Many workers from different parts have worked on the survival of the pathogen with rice seeds during storage and reported that the pathogens remained viable for few days to several months.

Hsieh (1973, 1978) and Hsieh and Buddenhagen (1975) reported that *X. campestris* (oryzae) pv. *oryzae* could not survive for long under high relative humidity and temperature conditions. According to them bacterium survived for 40 days after harvest when dried under sun and 90 days when dried under shade in Taiwan. Kauffman and Reddy (1975) found the bacterium to remain viable for two months on infected seeds in India when it was stored at 25-30°C. temperature. He also observed decrease in bacterial population while the bacteriophage population increased rapidly.

Mizukami (1961) believed the seeds as an important source of harbouring the pathogen. However, the bacterial population rapidly decreased during June due to high temperature condition in storage. During the course of seed soaking for sowing, the bacterial population in seeds reduced by about 99% within 24 hours and could not be detected after 5 days (Tagami *et al.*, 1963).

Singh and Rao (1977) could find viable bacteria in seeds stored at room temperature upto 11 months after harvest. Pal *et al.* (1982) reported the pathogen to survive upto 3 months in seeds stored in plastic container.

Singh *et al.* (1980) reported the pathogen to survive up to 10 months at room temperature and it could retain enough infection (9%) until the next season to cause an epidemic under favourable conditions.

Murty and Devadath (1984) observed that the seeds harvested in Kharif season carried an infection of 54% and the pathogen survived for 170-180 days. However, the seeds harvested during rabi season recorded seed infection by 45% and the pathogen remained viable for 120-130 days in storage.

Durgapal (1985) conducted a field trial with selfsown rice plant and reported that infected seeds burried at a depth of 25 cm in soil could retain viable inoculum through the rabi season under both cultivated and un-cultivated conditions.

Rao (1987) reported that the disease was transmitted through infected seeds from one summer season to the next, but the disease cycle was broken when the summer season seed was sown in winter season.

Raj and Pal (1988) observed no evidence of the pathogen to overwinter through seeds collected from diseased rice crop.

Tsushima *et al.* (1989) observed that seed, from plants inoculated at the booting stage, stored at room temperature as a possible source of infection in field resulting in seedling rot and grain rot.

3. Methods of detection of seed borne bacteria:

Considering the potential threat of infected seeds in disease epidemiology, caused by phytopathogenic bacteria, it is of utmost importance to test seeds for seed borne bacterial pathogens in rice. A number of methods are available for the detection of seed borne bacteria. It ranged from simple growing on test to highly advanced DNA probes as briefly summarized here.

i) Symptoms on Plant:

This is the simplest but one of the reliable method which has been practised since long. In this method germinated seeds are provided with suitable conditions for the expression of the symptoms by the pathogen. However, this method has been tried by few scientists to detect pathogenic bacteria in rice seeds, may be due to the fact that not many of the seed borne bacteria produce disease symptoms on rice plants.

Srivastava and Rao (1964) reported that infected seeds transmitted the disease to the seedlings when raised under condition of high humidity. Reddy (1983) also observed upward movement of the bacterium in rice seedling grown from infected seeds in petridish containing sterile water and in open field conditions. He observed both the symptom development in the seedling including the wilting phase of the disease and bacterial streaming under the microscope.

Singh *et al.* (1983) reported that the seeds of TN-1 collected from the fields heavily infested with *X. campestris* pv. *oryzae* (*X.o. oryzae*) in Kupurthala produced infected seedlings when raised in growth chambers at the seed pathology institute, Copenhagen.

Singh and Rao (1977) used roll towel method to detect *X.o. oryzae* in paddy. The test procedure involved germinating the seed between 2 moistened papers, incubated at 28°C and an alternate cycle of 12 hr light and 12 hr dark was provided by means of fluorescent tubes. Seedlings were observed for development of pale browning and necrotic lesion on plant parts. Bacterial ooze from such regions confirmed the infection.

Thind and Barar (1992), while raising the seedlings in moist blotter flooded with nitrogen solution observed bacterial blight symptoms on the seedlings raised from the infected seeds. He confirmed his finding by observing bacterial streaming from coleoptile, leaf sheath and leaves.

Tsushima *et al.* (1991) observed that *P. glumae* reproduced on the lower to upper leaf sheath played an important role in outbreaking bacterial grain rot and sheath rot in rice.

ii) Growth on Agar media:

Many workers preferred to use growth media for the detection of Pathogenic bacteria associated with seeds and plants. Several selective, semi-selective and general purpose media are being used to isolate the bacterial pathogens directly from seed husks, endosperm or indirectly by liquid assay.

Zeigler *et al.* (1989) reported that with addition of bromothymol blue and arginine mineral salts to the agar medium, they could successfully differentiate between *Pseudomonas avenae*, *P. glumae*, *P. fuscovaginae*, *P. syringae* pv. *syringae*.

Tsushima *et al.* (1986) used S-PG. medium containing KH_2PO_4 (1.3g), Na_2HPO_4 (1.2g), $(\text{NH}_4)_2\text{SO}_4$ (5g) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.25g), $\text{Na}_2\text{M}_6\text{O}_4 \cdot 2\text{H}_2\text{O}$ (24g), EDTA-Fe (10mg), Amplification sodium (10 mg), Centrimide (10mg), Methylviolet (1 mg), Phenol red (20mg), Agaragar (15g) and 1000 ml distilled water for detection of *Pseudomonas glumae*.

Summathy and Gananamanickam (1985) reported that the ability of the rice bacterial blight pathogen to digest starch could help to detect the presence of the pathogen in seeds by means of SX. agar. Zone of starch hydrolysis appeared around seeds associated with *X.o. oryzae* within 48 hrs. after planting. They used the method to monitor the extent of seed infection and to examine the viability among the pathogenic strains.

Gananamanickam *et al.* (1994) evaluated four selective media that varied with respect to carbon source, amino acids and antibiotics for the growth of *X.o. oryzae*. Planting efficiency in pure culture ranged from 40 – 90% on those media and colonies developed in 3 – 7 days depending on the strains.

Gananamanickam *et al.* (1995) evaluated several growth media and reported that XOS, semi-selective medium developed by Di *et al.*(1991) was most useful for detection and isolation of *X.o.oryzae* associated with rice seeds. However, highest recovery of pure cultures of the strains, PX-086 and Xi-5 occurred on Tic agar.

Lin *et al.* (1994) reported that Wakimoto as the basic medium for detecting *X.o. oryzicola* in rice seeds. For improving formulation methyl green and methyl violet were added to Wakimoto medium.

Kawaradani *et al.* (2000) developed a new selective medium (CCNT) for *Burkholderia glumae* which showed greater selectivity in comparison to Tsushima's S-PG medium. It contained Yeast extract (2g), Polypepton (1 g), Inositol [myo-inositol] (4 g), Cetrimide (10 mg), Chloramphenicol (10mg) Novobiocin (1 mg), Chlorotharonyl (100mg) Agaragar (18g/lit) and pH was adjusted to 4.8. *B. glumae* produced a yellowish white colony with a diffusible yellow pigment on CCNT medium.

Ming *et al.* (1991) developed a semi-selective medium (XOS) containing sucrose, peptons, monosodium glutamate, calcium nitrate, potassium phosphate, iron EDTA, cycloheximide, cephalixin, kasugamycin, methyl violet 2B and agar, to isolate *X.o.oryzae* and *X.o. oryzicola*.

(iii) Phage Plaque method:

Bacteriophage are very useful in detection and identification of Plant pathogenic bacteria. Many of them are highly specific and can differentiate even the strain. The method involves addition to a known amount of phage to a sample of seed extract. An increase in phage titre after incubation indicates the presence of pathogen. False negative result may be obtained due to deficiency in a specific phage-bacterium interaction or due to the presence of phage inhibitors in the sample (Billing and Garreett, 1980).

Unamalai *et al.* (1988) reported to have isolated both free and absorbed phages from infected rice seeds. Free phages isolated after 10 hr of multiplication found to be far greater in husk than the whole seed suggesting that the bacteria survived more in or on husk.

Kauffman and reddy (1975) observed decreased in seed borne bacterial population and increase on phage population when the seed was soaked for 2 days. But within 10 hr of soaking, 40% seeds showed extensive increase in phage population indicating the presence of *X. oryzae* in seed.

Serological and molecular tests:

Among the various molecular tests such as immuno-fluorescent technique (IF), enzyme linked immunosorbent assay (ELISA) and more recently, polymerase chain reaction (PCR) are being tried for the detection of seed borne bacteria.

Unamalai *et al.* (1988) found indirect IF technique to be a sensitive method to detect upto 10² cfu/ml of *X.o. oryzae*. He use the technique to detect the pathogen in rice seeds. The seeds stored for 7 months at 28°C showed a positive result. However seeds collected in dry season showed negative results.

Dong *et al.* (1982) used 32p antiserum proportion for detection *X.o. oryzae* present in trace in inoculated seeds by radio-immuno assay. According to them the method was highly specific and gave good result even with contaminated samples and in the presence of other bacteria.

Li *et al.* (1992) developed monoclonal antibodies and used following DAS-ELISA for detection of *X.o. oryzae* in rice seeds. He could detect the pathogen in 93% of infected seed samples collected from Jaingsu and Zhejian of China.

Gananamanickam *et al.* (1995) demonstrated bacterial colonies which reacted positively to monoclonal antibodies (mabs) specific to *X.o. oryzae* in ELISA. They also examined by indirect Immuno fluorescens technique and observed characteristic epifluorescens with mabs *X.o.* 0-2. According to them, monoclonal antibodies that have been developed for *X.o. oryzae* facilitated quick identification of the pathogen isolated from seed samples as the conventional method of pathogenicity test takes a minimum 7-10 days for symptoms development. They also conducted PCR-amplification using primary from 181113 for detection of low levels of bacteria in plant tissue. According to them, advancement of mabs, DNA probes and PCR amplification assays for bacterial DNA, it should now be possible to address the controversial issue that relates to the importance of seed borne inoculum of *X.o. oryzae* in the perpetuation of bacterial blight in rice.

Loreti *et al.* (2002) used PCR-amplification for detection of *Pseudomonas avellanae*. According to them specific oligonucleotides based on HRPW (hypersensitive response and pathogenicity) gene sequence encoding harp protein in phytopathogenic bacteria was designed to detect and identify virulent strain of *P. avellanae* by PCR-amplification.

Adachi *et al.* (2000) detected *X.o. oryzae* by PCR-amplification of 16S-23S RNA spacer region sequence. The method was highly specific for *X.o. oryzae*, the pathogen responsible for causing bacterial blight of rice.

Cottyn *et al.* (2001) used BOX-PCR finger printing to evaluate 428 bacterial isolates belonging to *Enterobacteriaceae* and *Pseudomonadaceae* and other families including *Cellulomonas* and *Clavibacter* groups.

Rott *et al.* (1999) used 3 types of diagnostic tests to identify 34 strains of *P. fuscovaginae* from 136 strains of fluorescent Pseudomonads isolated from diseased rice samples from 5 different countries.

Sakthivel *et al.* (2001) developed a PCR technique for detecting the presence of *X.o. oryzae* in rice seed and transmission from seed to plant.

Sridhar (2002) focused in his paper on the various molecular approaches and molecular markers used in the study of virulence, population structures, molecular characterization, identification and classification of *X.o. oryzae*.

Babu *et al.* (2003) reported increased activities of chitinase and β -1, 3 glucanase due to *X.o. oryzae* in rice plant by Western blot analysis.

Kim *et al.* (1996) was able to differentiate 6 major rice bacterial pathogens by PCR-amplification of ribosomal DNA of the intergenic spacer region, using ribosomal primers R16-1 and R23-2R. He also found that differentiation of PCR-amplified spacer polymorphisms by means of the R16-1 and R23-2R primers to 16S and 23r DNA may be used for rapid differentiation of seed-borne bacteria of rice.

4. Role of bacteria on rice seed health:

Association of bacterial plant pathogen with the plant system and seed, result in easy transmission of disease through seed and sever reduction in yield due to blockage of vasular system. Quality of seed and food grain is also greatly affected by the presence of bacteria and other microorganism with seed.

(i) Loss of yield:

Subash *et al.* assessed yield loss 17.6 – 22.1 per cent due to bacterial leaf blight following yield loss simulation model.

Mandal *et al.* (1996) reported 8.1 per cent and 14.6 per cent yield loss when inoculated with *X.o. oryzae* at maximum tillering and panicle initiation stage respectively.

Morita *et al.* (1990) reported over 80 per cent of grain damage due to infection of *Pseudomonas glumae* within 8 days after flowering.

Srinivasan (1982) reported that the loss in yield under conditions of natural infection by *X.c. oryzae* was related to severity of the kresek phase. This resulted in increase in chaffy grains by 55-59% and reduction in 1000 grain weight by 49-62% which accounted to loss in yield up to 94.16% per hill.

Srivastava and Kapoor (1982) reported that the yield losses in the rice cultivar Jaya caused by BLB (*X.c. oryzae*) varied from 6.3 per cent at infection grade 1 to 36.8 per cent at Grade-9. But many workers suggested that loss in yield depended on growth stage of the crop and disease severity.

Reddy *et al.* (1979) found a significant, linear relationship between disease severity at the soft dough stage of the rice plant and grain yield. They constructed a critical point model to predict crop losses associated with bacterial blight disease of rice. Disease appeared at late stage of crop growth had no effect on grain yield. Severe incidence of the disease at panicle initiation stage reduced grain yield, panicle fertility and grain weight.

Rao and Kauffman (1977) found an insignificant loss in IR-20, 10 per cent in IR-8 and 56 per cent in Karuna.

Singh *et al.* (1977) reported that four cultivars differed greatly in loss in grain yield varying from 14.7 per cent in cultivar Saket-4 to 81.3 per cent in IR-24. They also reported that grain quality of diseased crop was least affected in Saket-4 and most affected in IR-24.

(ii) Effect of bacteria on rice seed health:

Report on bacterial pathogens associated with rice seed has increased in last five years. Some of them produce disease symptoms either in plant or in seed. However, little is known about their effect on seed health.

Sreeramula *et al.* (1986) observed the effect of ethyl acetate extract fraction (A₁ – A₅) of the culture filtrate of *X. campestris* pv. *oryzae* on growth of rice seedlings and reported that fraction (A₁ – A₄) were growth inhibitory to root and shoot and fraction (A₅) was less inhibitory. He also found that phenyl acetic acid Purified from fraction (A₂) was inhibitory to root but not to shoot growth.

Chien *et al.* (1987) found that the rice seeds which were dipped in a water suspension of 10⁸ cfu/ml of *Pseudomonas glumae* for 30 min. before sowing caused no effect on germination but 98 per cent of seedlings died. Artificial inoculation of bacterial suspension by injection caused infection and discolouration of panicles and sheaths. Inoculation at booting or heading by spraying caused infection of panicles but not sheaths resulting more than 60% unfilled grain.

Sato *et al.* (1989) reported that two compounds (*fervenulin* and *texoflavin*) from *P. glumae*, causal agent of bacterial grain rot of rice which produced a chlorotic spots on leaves and also reduced the growth of leaves and roots of rice seedlings.

Kadota *et al.* (1990) reported that the causal agent of bacterial brown stripe of rice *Pseudomonas avenae* which has also been reported from rice seed affected rice plant at seedling stage in 4 ways: inhibiting germination, brown stripes on leaf, curving of leaf sheath and abnormal elongation of mesocotyl. However, the symptoms were marked when infected seedlings were transplanted to paddy fields.

Mew *et al.* (1990) reported that some pathogens infected seeds while others contaminated the seed which was based on seed health testing at IRRI. No direct relationship was observed between disease of mother plants in the fields and the pathogens detected by blotter tests on harvested seeds.

Dey (1992) reported that an increase in foliar bacterial blight disease intensity resulted in the corresponding increase in percentage of chaffy grains, seed discolouration due to seed infection of *X.o. oryzae*. He detected the pathogen both from husk and endosperm. Moreover, an increase in seed infection reduced seed germinability.

Naeem *et al.* (2001) identified 2 bacteria (*X.c. oryzae* and *P. avenae*) from rotten rice seed and abnormal seedlings, which reduced seed germination of associated cultivars.

Anil Kumar *et al.* (2002) reported that *Pseudomonas marginalis* at 10^6 cfu/ml concentration induced highest disease incidence and significantly inhibited seed germination.

Hong *et al.* (2002) reported 4 virulent strains of *P. agglomerans* (FDQ1, FDSN4, XD2, XSH4) from rice seed which, influenced the development and maturation of rice seeds and inhibited their sprouting at a concentration of $\geq 10^8$ cfu/ml.

Xio *et al.*(1999) isolated 280 pathogenic bacteria from 116 rice seed samples. Among them most of the isolates of *Pseudomonas* did not induce any symptoms in rice plants but some unknown bacteria resulted in poor seed germination and seedling growth.

Ashura *et al.*(1999) examined 60 rice varieties in Tanzania and found association of *Acidovorax avenae avenae* causing brown stripe disease in 63 per cent seed samples.

Srivastava *et al.* (1964) reported that seedlings raised from seeds which infected by *X.c. oryzae* were killed in 3 to 5 weeks indicating that the disease was systemic and transmitted internally.

Reddy (1983) found that wilting of rice seedlings started to appear 15 to 20 days after sowing of infected seeds and the number of wilted seedling increased gradually within 30 days of sowing due to the presence of *X.c. oryzae*.

Tsushima *et al.* (1989) observed that seed, from plants inoculated by *P. glumae* at the booting stage, stored at room temperature as a possible source of infection in field resulting in seedling rot and grain rot.

Rao (1987) reported that the rice bacterial blight disease was transmitted through infected seeds from one summer season to the next. However, the disease cycle was broken when the summer season seed was sown in winter season.

Srivastava and Kapoor (1982) observed reduction in panicle fertility and grain weight due to infection of *X.o. oryzae*.

Singh *et al.* (1977) reported that reduction in grain quality due to infection of *X.o. oryzae* depended on the varieties and also absorbed least effect on the grain quality in Saket-4 which is a resistant variety and most in IR-24.

Sarada *et al.*(1990) reported that bacteria isolated from seed were responsible for sprouting of seed but stimulatory or inhibitory effects on root and shoot growth and vigour did not follow any particular pattern.

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CHAPTER-III

MATERIALS AND METHODS

MATERIALS AND METHODS

A. LABORATORY REAGENTS, GLASSWARES, MEDIA ETC.:

1) Cleaning and Sterilization of glasswares:

Petridishes, culture tubes, conical flasks, beakers, pipettes and other glasswares used during present studies, were immersed in dilute solution of potassium dichromate which not only cleaned the glasswares but also destroyed the spore forming organisms present on the slide.

Potassium dichromate solution was prepared by dissolving 60g of the chemical in 1000 ml of distilled water and by slowly adding 60 ml of concentrated sulphuric acid into it.

After keeping the glasswares in the cleaning solution for 4-6 hours, they were washed with distilled water 3-4 times and air dried. The glasswares were wrapped in 3 to 4 layers of newspaper sheets and sterilized in hot air oven for 2 hours at 160°C and used as and when required.

Microscope slides and cover slips after cleaning were stored in suitable containers containing 95% ethyl alcohol and were used at the time of need.

2. Preparation of growth media:

Nutrient sucrose agar medium was found to be suitable for growth of most of the bacterial pathogens associated with rice seed kernels. The medium was prepared out of following items.

Beef extract	-	3g
Peptone	-	5g
Sucrose	-	20g
Agaragar	-	17g
Water	-	1000 ml.

For preparation of the medium, 1000 ml of water was boiled and all the ingredients were added one after another and was allowed to boil until all the ingredients were completely dissolved in water.

For preparation of slants, 8-10 ml aliquots of the prepared medium was poured into each culture tube. For preparation of agar plates the same was taken in suitable conical flasks. The tubes and conical flasks containing the media were sterilized at 10 lbs. psi for 30 minutes in an autoclave. The petri plates and slants were kept inside a dry chamber until further use.

For the present study, the petriplates having dried surface used in order to avoid surface mixture of bacterial colonies by motile bacteria which swim in surface moisture resulting mixing of bacterial colonies.

3. Sterilized moist chamber:

Sterilized moist chambers were used for detection of seed borne microflora following moist blotter test. It was prepared by placing three layer of filter paper with a piece of cotton inside a glass petridish. The filter paper and the cotton were moistured with water. It was sterilized at 15 lbs psi for 20 minutes in an autoclave.

4. Sterilization of other materials:

For sterilization of small equipments like inoculating needle, cork borer, forcep etc. were dipped in 95% ethyl alcohol and then flamed to red hot.

5. Maintenance of aseptic conditions:

In order to maintain aseptic conditions all the operations were carried out in the transfer chamber fitted with laminar flow available in the laboratory of the Department of Plant Pathology, O.U.A.T., Bhubaneswar.

B. PHYTOPATHOLOGICAL ANALYSIS:

I. Collection of seed samples:

Fifteen varieties of matured paddy seeds, five each from early, medium and late duration groups, as listed in table-1, were collected from Central Research Farm, O.U.A.T. during November-December, 2002.

The seed of each variety was cleaned, sun dried for two days and kept in separate polythene packet in a refrigerator. They were taken out from time to time and used for various studies.

II. Detection of bacteria associated with rice kernels:

Detection of bacteria associated with seed kernels of fifteen test rice cultivars was done following the technique developed by National Seed Project, ICAR (Anon, 2001). Seed kernels of more than 200 seeds from each cultivar were separated by removing the husk and used for detection of bacterial association.

Table-1: Duration and source of fifteen test cultivars.

Sl. No.	Variety	Duration (Days)	Source	
I.	Early variety:			
01.	Udayagiri	98	Central Research Farm, O.U.A.T.,Bhubaneswar.	
02.	Khandagiri	90 – 95		
03.	Lalitagiri	95		
04.	Annapurna	90		
05.	Parijat	95		
II.	Medium Variety			
01.	Bhoi	130		
02.	Annada	130-140		
03.	Sravani	130-135		
04.	Pratap	135-140		
05.	Lalat	130		
III.	Late Variety			
01.	Manika	155-160		
02.	Rambha	150-155		
03.	Jagannath	150		
04.	Mahanadi	150		
05.	Swarna	150-155		

a

b

Fig. 1 **Photograph showing bacterial growth on rice seed kernel. [(a) yellow bacterial growth, (b) white bacterial growth]**

For detection of bacterial association with rice kernels, 25 numbers of kernels were placed at equi-distance on the moistened blotting paper of sterilized moist chambers as described earlier in this chapter.

Petridishes were kept under normal room temperature for 12 hours. Then, they were transferred into a freezer (-20°C) and kept for another 12 hours to inhibit germination of the kernels. Finally, the plates were incubated at $\pm 27^{\circ}\text{C}$ for the next 48 hours. Within the next 24 – 48 hours of incubation they were examined under sterio- binocular microscope for the growth of bacteria on the kernels (Fig.1). Per cent of kernel infection by different coloured colony forming bacteria were recorded. The bacterial colonies were then isolated into pure culture and identified.

III. Isolation, characterization and identification of bacteria:

1) Isolation of Bacteria:-

Different bacterial colonies developed on rice kernels following the modified moist blotter technique developed by NSP were selected on the basis of difference in colour of the colonies were isolated into pure culture following streak plate method. For the purpose, loopful of bacterial growth on rice kernel was transferred into 1 ml of sterile water taken in a test tube and mixed thoroughly. The bacterial suspension was taken with the help of an inoculating needle and 5-6 parallel streaks were drawn on well dried surface of sucrose nutrient agar (SNA), medium. The inoculating needle was then flame sterilized and a second set of parallel streaks were drawn at right angle to the first set of lines. A third set of lines were drawn from second set of lines in the similar way. Then the plates were incubated at $27 \pm 1^{\circ}\text{C}$ for

a



b

Fig. 2 Isolation of bacteria by streak plate method [(a) *X. o. oryzae*, (b) *P. s. panici*]

48 hours in upside down position. Well isolated, pinheaded bacterial colonies were picked up separately on the basis of their colony morphology and purified following the same procedure as was done for isolation.

Purified colonies of different bacteria were transferred separately into slants containing nutrient sucrose agar slants as stab culture. The slants and stab inoculated tubes were kept in a refrigerator after development of bacterial growth for further use.

2) Characterization of bacterial isolates:

Different identification tests were carried out in order to identify different bacteria isolated from rice seed kernels.

a) Capsule staining:

For capsule staining, 24 hours old cultures were used. A thin smear of bacterial suspension was taken on a clean, dry glass slides and allowed to air dry. It was then flooded with crystal violet solution for 5 minutes followed by washing with copper sulphate solution and blot dried. The slides were then examined under microscope using oil immersion lens. Capsules appeared blue to violet and cell was stained dark blue in colour.

b) Flagella staining:

Staining for flagella of the bacterial isolates were done following Schaad, 1992 with tannic acid solution and ammoniated silver nitrate solution.

Tannic acid solution was prepared by adding tannic acid (5.0g); ferric chloride(1.5g); 15% formalin (2.0 ml), 1% sodium hydroxide (1.0 ml) and the volume was brought to 100 ml with addition of distilled water.

Ammoniated silver nitrate solution was prepared by dissolving 2.0g of silver nitrate in 100 ml distilled water from which, 10 ml solution was taken out and kept separately. To the rest of 90 ml of silver nitrate solution, ammonium hydroxide was added, till the heavy precipitate was dissolved. The separated 10 ml of silver nitrate solution was back titrated until slight clouding appeared. The pH of the solution was adjusted to 10.0 with ammonium hydroxide and silver nitrate.

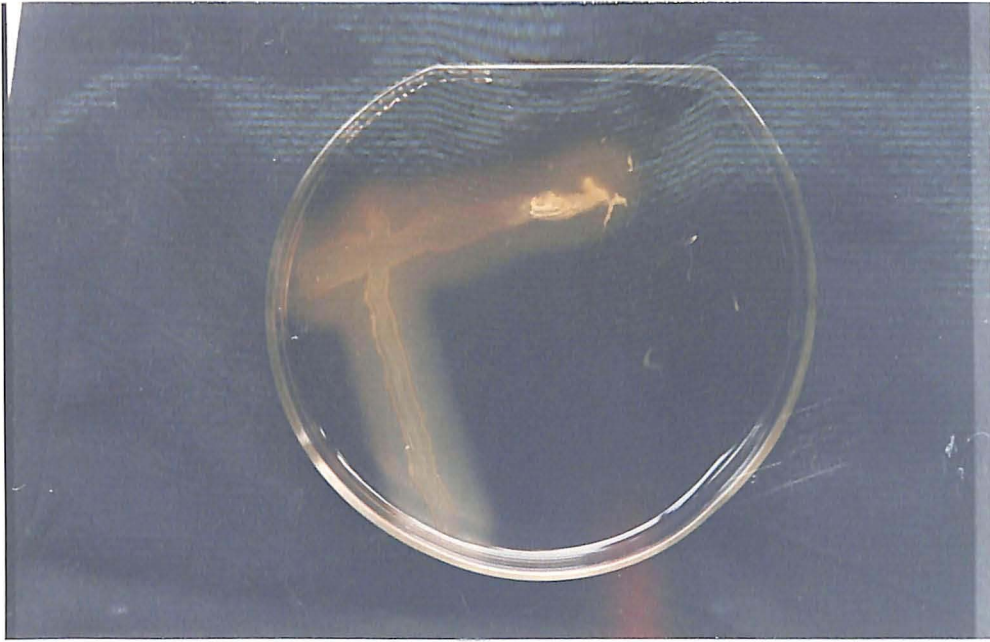
A faintly cloudy bacterial suspension was prepared by slowly adding 1 ml of distilled water to the butt of 24 hour bacterial growth on a slant. A loopful of bacterial suspension and water placed on the slide by just touching each other. The slide was air dried. The bacterial smear was flooded with tannic acid solution for 4-5 minutes. Then it was rinsed in distilled water. Finally, the ammoniated silver nitrate solution was added to the smear and kept for 30 seconds. It was washed immediately in distilled water and air dried. The slide was examined under the microscope using oil immersion lens. The flagella looked as light brown coloured fillaments attached to bacterial cell.

c) Gelatin liquification test:

Bacteria have been differentiated on the basis of their pectiolytic potentialities such as the ability to liquify gelatin. The gelatin liquification test was conducted by using nutrient medium containing 12 per cent of gelatin. The medium was dispensed into tubes, sterilized and cooled. The tubes were inoculated separately by different bacterial isolates and incubated at $27\pm 1^{\circ}\text{C}$. After 3rd and 7th days of incubation, the tubes were placed at 4°C for 30 minutes in a refrigerator. The medium which flowed down readily on gentle tapping of the tubes indicated hydrolysis of gelatin and denoted as positive reaction for the test.

d) Nitrate reduction test:

The nitrate reduction test was conducted by using the nutrient medium containing potassium nitrate (1.0g), peptone (5.0g); yeast extract (3.0g); agar (3.0g). All the ingredients were dissolved in 1000 ml of warmed distilled water. The pH of the medium was adjusted to 7.0 by adding 40% NaOH. 8 ml of the medium was dispensed into each culture tubes and sterilized. Each bacterium was inoculated to separate tubes in triplicate and incubated at $27\pm 1^{\circ}\text{C}$. Observation for nitrate reduction was taken at 3rd, 5th and 7th day after inoculation. For the purpose, 1 ml of 0.85% solution of sulphanilic acid and 1 ml of 0.6% of dimethyl – alphanaphthylamine, each was made in 5N acetic acid, were added. Red colouration indicated the presence of nitrate and no change in colour indicated denitrification (Fig. 4).



a



b

Fig. 3 Photograph showing hydrolysis test.
[(a) Positive reaction with clear zone,
(b) Negative reaction]

e) Starch hydrolysis test:

Nutrient agar medium containing 1.0 per cent soluble starch was prepared and sterilized. The starch enriched medium was transferred into sterilized petridishes. After solidification, it was streak inoculated with different bacterial isolates separately and incubated for 48 hours. Iodine solution containing iodine (5.0g) and potassium iodide (1.0g) in 100 ml of distilled water was prepared. The petriplates were flooded with iodine solution. Dark colouration of the medium with clear zone around bacterial growth indicated hydrolysis of starch (Fig.3).

f) Potato soft rot test:-

Non-sprouted potato tubers were washed and surface sterilized with the help of 70.0% alcohol. Then tubers were cut into slices of 7-8 mm thick. The slices were immersed in 5.25% sodium hypochloride solution for 10 min followed by air drying for 30 min. Three slices were placed in each of the moist petriplates. A triangular depression of 3-4 mm deep with a sterile scalpel was made at the centre of each slice and filled with several drops of cell suspension (10^8 cfu/ml) from a 24 hr growth culture of each bacterium. The plates were incubated at $27 \pm 1^\circ\text{C}$ for 48-72 hours. Non-inoculated potato slices kept in moist blotter served as control. Soft rotting of potato tubers beyond the point of inoculation recorded as positive action. A discolouration only at the point of inoculation recorded as negative reaction for this test.

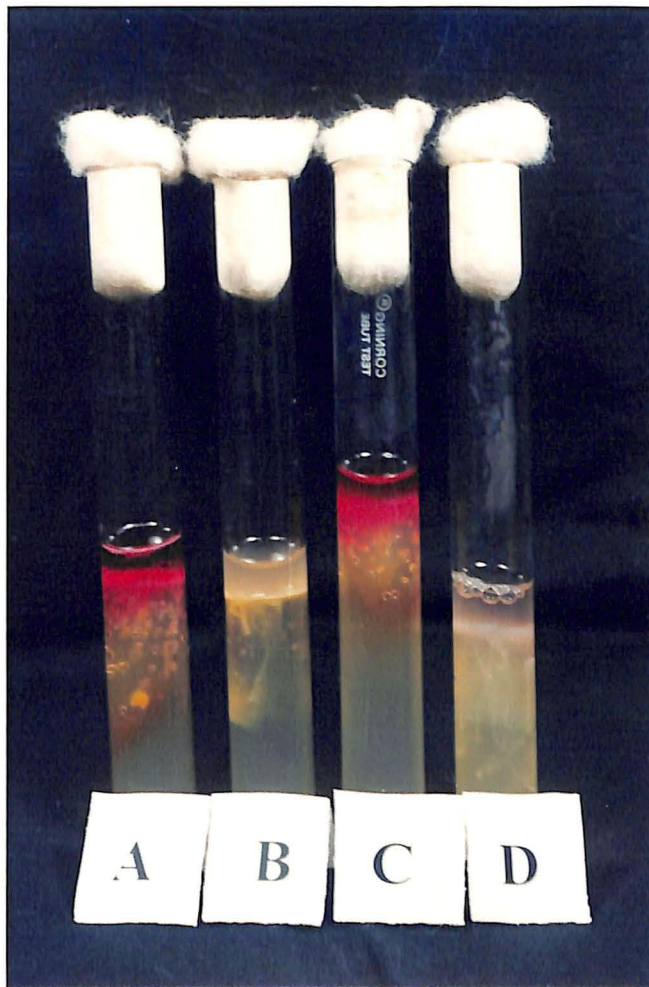


Fig. 4

Photograph showing reduction of nitrate to Nitrite (Red colouration indicates negative & No colouration indicates positive)

g) Pectate degradation test:

For this purpose, 12.53g of commercially available crystal violet pectate medium was taken and dissolved in 1000 ml water followed by sterilization. 20 ml of sterilized media was transferred into each sterilized petridishes and allowed to cool. The petri plates were stab inoculated by different bacteria at 4 places and incubated at $27\pm 1^{\circ}\text{C}$. After 3rd day of inoculation, cavity filled with bacterial growth recorded as positive for pectate degradation.

h) Fluorescence test:

King's B medium containing protease peptone (20g); K_2HPO_4 (1.5g), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (1.5g), Glycerol (1.5g); Agar (17g) in 1000 ml water was used to study the fluorescence bacteria under UV radiation. Different isolated bacteria were streaked on the surface of King's B medium separately and incubated for 24 – 48 hours. They were then observed under UV light. The colonies looking brighter under UV light than normal light indicated fluorescence producing bacteria.

i) Oxidase test:

A loopful of isolated colony of 24 hours old culture was taken on the commercially available oxidase discs. Development of purple colour within 10 seconds was rated as oxidase positively. Colouration within 10 seconds rated as positive and 10 – 60 seconds was rated as delayed positive. No colour development even after 60 seconds was rated as oxidase negative.

Seed germination test by paper towel method:

The seed germination test was carried out by paper towel method. 100 seeds were placed at equidistant between two moistened paper towel which were rolled alongwith an outer covering of wax paper and incubated for 7 days in upright position. The rolled paper towels were moistened from time to time to maintain sufficient moisture inside. After an incubation period of 7 days, the seedlings were examined and on the basis of seed germination and seedling growth, observation on per cent of normal germination, abnormal germination and no germination were recorded. The seedlings showing equal and normal shoot and root growth were recorded as normal germinated seeds. Germinated seeds having pale sickly appearance, poor and unequal growth of root and shoot and various degrees of coleoptile browning were separated and recorded as abnormally germinated seeds. The seeds which failed to germinate were recorded as ungerminated seeds.

The presence of bacteria with abnormal seedlings and with ungerminated seeds were isolated following the methods described earlier.

Seedling vegour index of different varieties were calculated using the following formula.

Vigour index of seedlings = (Length of root + Length of shoot) x germination %

- 0 -

CHAPTER-IV

EXPERIMENTAL RESULTS

EXPERIMENTAL RESULTS

4.1 Taxonomy of bacteria isolated from rice seed kernel:

Phytopathogenic bacteria that were found to be associated with rice seed kernel were isolated into pure culture. A total of seven bacterial isolates frequently occurring on the rice kernels of the 15 test varieties were analysed with regard to their morphological, physiological and biochemical differentiation (Table-2). On the basis of the said characters five bacterial species were tentatively identified as described below:

a) *Xanthomonas oryzae* pv. *oryzae* (*X. o. oryzae*):

Morphological observations indicated that the bacterial cells appeared as short rods with rounded ends. It was gram-negative, non-spore forming and capsulated with one polar flagellum. Number of cells joined to form an aggregated mass. On nutrient agar medium it produced minute yellow colonies and the growth was slow. However, more rapid growth was observed on peptone-sucrose-agar medium and produced colonies which were circular, convex and waxy-yellow in colour. The yellow pigment was insoluble in water.

Physiological and biochemical studies indicated that the bacterium was aerobic, did not liquified gelatin, did not reduce nitrate and not hydrolysed starch. On oxidase discs it produced no colour within 60 seconds and showed negative reaction. It found glucose and sucrose as the best carbon source alongwith other carbon sources except fructose. When the pathogen was inoculated to healthy rice plant, produced typical blight symptom on the leaf.

Table-2: Morphological, Physiological and Biochemical characters of different isolates.

Sl. No	Shape	Flagellation	Colony type	Colour	Gelatin Liquification	Nitrate reduction	Starch Hydrolysis	Potato soft rotting	Oxidation reaction	Identified organism
1.	Rods	Monotrichous	Circular, convex, entire	Waxy yellow	-ve	-ve	-ve	-ve	-ve	<i>Xanthomonas oryzae pv. oryzae</i>
2.	Rods	Monotrichous	Circular, convex, entire	Pale yellow	+ve	-ve	-ve	-ve	-ve	<i>X. o. oryzicola</i>
3.	Rods	Cephalotrichous	Circular, convex, entire	Creamy white	+ve	+ve	+v	V	± Ve	<i>Pseudomonas syringae pv. panici</i>
4.	Rods	Peritrichous	Circular, convex, amoeboid	Gray-white	+ve	+ve	-ve	+ve	-	<i>Erwinia chrysanthemi</i>
5.	Rods	Peritrichous	Circular, convex, amoeboid	Grayish-white	-ve	-ve	+ve	+ve	+ve	<i>Erwinia carotovora</i>

+ve : Positive, -ve: Negative, V: Variable, ± Ve: Slow reaction.

b) *Xanthomonas oryzae* pv. *oryzicola* (*X. o. oryzicola*):

Morphological and physiological observations indicated that individual cells appeared as short rods with rounded ends, occasionally remained in pairs without having either spore or capsule. Colonies of nutrient agar plates looked circular, smooth, convex with entire margin and pale yellow in colour. It liquified gelatin but did not produce nitrite from nitrate and not hydrolysed starch. Negative result was produced on oxidase test. The isolate readily utilized dextrose, sucrose, xylose and manose. Spray inoculation of pathogen resulted production of typical streaks symptoms, on rice leaves.

c) *Pseudomonas syringae* pv. *panici* (*P. s. panici*):

Morphological and physiological studies revealed that the bacterium appeared as rods without having capsule or spore. On nutrient agar plates the colonies were circular, raised, glistening producing creamy white to yellow colonies. Abundant slimy growth of the isolate was observed on nutrient sucrose agar medium with production of nitrite from nitrate. Gelatin liquification, starch hydrolysis and oxidase tests recorded positive reactions. The isolate readily utilized glucose, fructose, xylose, manitol, lactose and sucrose.

d) *Erwinia chrysanthemi* (*E. chrysanthemi*):

Isolated bacterial cells were of rod shaped with peritridous flagellation. Colonies on nutrient sucrose agar medium were grayish-white, circular, amoeboid and butyrous. The bacterium liquified gelatin within 48 hours of growth. Starch could

not be hydrolyzed by the bacterium. However, it caused reduction of nitrate to nitrite. Oxidase reaction was negative. The bacterial isolate produced gas from glucose, galactose, arabinose, sucrose, glycerol and xylose. It caused soft rotting of potato slices.

e) *Erwinia carotovora* (*E. carotovora*):

The bacterium isolated from rice seed kernel was rod shaped with no capsule or endospore. It was motile by peritrichous flagella. Colonies on nutrient sucrose agar were circular, grayish-white and butyrous. Gelatin liquification test and nitrate reduction test showed negative reaction. However, the bacterium readily hydrolyzed starch. It produce gas from glucose, galactose, sucrose, fructose, xylose, lactose and glycerol. It caused rapid soft rotting of potato slices.

4.2 Association of different phytopathogenic bacteria with rice seed kernels:

Five popular rice varieties each from three duration groups, namely, early, medium and late, were analysed for association of different plant pathogenic bacteria. The result presented in table -3 revealed the association of five bacterial species, namely, *E. chrysanthemi*, *X. o. oryzae*, *X. o. oryzicola*, *E. carotovora* and *P. s. panici*. Among the five bacterial species, *X. o. oryzae* was found to be associated with 24.70 per cent of the total seeds by taking all the test varieties of rice into account. Also, maximum per cent of seed was found to be infected by *X. o. oryzae*. Average seed infection of 3 – 4% was found to be caused by *E. chrysanthemi*, *E. caratovora* and *P. s. panici*. Less than 3.0 per cent seed was infected with *X. o. oryzicola*.

Table-3: Association of different bacteria with seed kernels of popular rice varieties:

Duration Group	Variety	Duration (days)	% of seed infection by different bacteria					Total
			<i>E. chrysanthemi</i>	<i>X. o. oryzae</i>	<i>E. carotovora</i>	<i>X. o. oryzae</i>	<i>P. s. panici</i>	
Early Duration Group	Udayagiri	98	4.00	2.00	0.0	2.00	0.0	8.00
	Khandagiri	90-95	8.00	16.00	2.00	2.00	8.00	36.00
	Lalitagiri	95	4.00	2.00	4.00	0.0	0.0	10.00
	Annapurna	90	4.00	18.00	10.00	2.00	4.00	38.00
	Parijat	95	0.0	4.00	0.0	0.0	2.00	6.00
		Mean		4.00	8.40	3.20	1.20	2.80
Medium Duration Group	Bhoi	130	2.00	4.00	2.00	0.0	4.00	12.00
	Annada	130-140	4.00	20.00	6.00	4.00	8.00	42.00
	Sravani	130-135	0.0	2.00	0.0	0.0	2.00	4.00
	Pratap	135-140	2.00	8.00	4.00	2.00	0.0	16.00
	Lalat	130	0.0	0.0	2.00	0.0	4.00	6.00
		Mean		1.60	6.80	2.80	1.20	3.60
Late Duration Group	Manika	155-160	8.00	16.00	2.00	2.00	4.00	32.00
	Rambha	150-155	4.00	24.00	3.00	8.00	4.00	48.00
	Jagannath	150	0.0	8.00	0.0	2.00	0.0	10.00
	Mahanadi	150	8.00	28.00	6.00	8.00	4.00	54.00
	Swarna	150-155	0.0	2.00	2.00	0.0	4.00	8.00
		Mean		4.00	15.60	3.60	4.00	3.20
	Grand Mean		3.20	10.27	3.53	2.13	3.20	24.70

Among the early duration varieties, highest total seed infection of 38.00 per cent by different bacteria was observed in rice variety, Annapurna which was closely followed by Khandagiri. Lowest seed infection of 6.0 per cent was observed in Parijat. The per cent of seed infection, irrespective of varieties was high by , *X. o. oryzae*. It was also found to be high in Annapurna and Khandagiri. *E. chrysanthemi* and *P. s. panici* each resulted in 8.0 per cent seed infection in Khandagiri. However, *E. carotovora* also was found to be associated with 10.0 per cent of seed in Annapurna. *P. s. panici* in the seeds of Udayagiri and Lalitagiri; , *X. o. oryzicola* in Lalitagiri and Parijat; *E. carotovora* in Udayagiri and Parijat; and *E. chrysanthemi* in Parijat were not found to be associated with the seeds.

In medium duration rice varieties, maximum of 20.00 per cent of seed infection was caused by , *X. o. oryzicola* in Annada which also recorded highest total seed infection of 42.00 per cent by different bacteria. Rice varieties, Bhoi and Pratap recorded 12.00 and 16.00 per cent seed infection respectively. Minimum seed infection by different bacteria was recorded in Sravani which was also found to be free from infection by most of the bacterial species. However, maximum of only 4.00 per cent seed infection was caused by *P. s. panici*. It was also the only variety found to be free from infection of *X. o. oryzae*.

Among the five long duration rice varieties, Mahanadi recorded highest seed infection of 50.00 per cent with different bacteria and it was closely followed by Rambha and Manika with 48.0 and 32.0 per cent respectively. Lowest bacterial

seed infection of 8.00 per cent was recorded in Swarna. Maximum seed infection was caused by *X. o. oryzae*. Mean seed infection by rest of the four bacterial species was ranged between 3.00 to 4.00 per cent.

Mean seed infection by different bacterial pathogen varied from one duration group to the other. Maximum seed infection was recorded in long duration group rice varieties and lowest seed infection was recorded in medium duration varieties.

4.3 Effect of bacterial seed infection on seed germination:

Seed germination test for fifteen rice varieties, five each from early, medium and late duration group was conducted following paper towel method and it was recorded as normal, abnormal and no-germination. Each group was analysed for their bacterial association by plating random seeds from each category on nutrient sucrose agar medium.

The result presented in table-3 revealed that higher seed infection resulted in poor seed germination. Among the five test varieties of early duration group, normal seed germination was recorded in more than 80 per cent of seeds in Udayagiri, Lalitagiri and Parijata. They also recorded low seed infection with different bacterial pathogens which ranged between 6.0 to 10.0 per cent.

On the other hand, poor normal seed germination of 44.0 and 56.0 per cent were recorded in Annapurna and Khandagiri respectively which also showed higher seed infection of 38.0 and 36.0 per cent respectively. Poor seed germination in Annapurna was mainly due to development of higher percentage (52.0) of abnormally seeds. In comparison to Annapurna, Khandagiri recorded 28.0 and 16.0 per cent of abnormal and no germination respectively. Accordingly, the per cent of bacterial association with abnormal and no germinated seeds increased in both the varieties.

Among the medium duration varieties more than 90.0 per cent of normal seed germination was recorded in Sravani and Lalat with low seed infection by bacteria. Lowest seed germination of 52.0 per cent was recorded Annada which was mainly due to development of higher percentage of abnormal seedlings and due to non-germination of seeds. Both the non-germinated and abnormally germinated seeds showed high percentage of bacterial association of 16.0 and 18.0 respectively.

Similar trend of result was also recorded among the varieties of late duration group. Mahanadi showed poor seed germinability (24.0%) with high bacterial seed infection which resulted due to development of 68.0 per cent of abnormally germinated seeds and only of 8.0 per cent of non-germinated seeds.

In all the duration groups, bacterial association was also recorded in normally germinated seedlings. However, it was more in late duration group in comparison to non-germinated seeds.

4.4 Effect of bacterial seed infection on the growth of seedling of 15 different rice varieties:

Seeds of 15 rice varieties belonging to early, medium and late duration groups were tested for their germinability and seedling growth following paper towel method. The result presented in table-4 revealed that the average lengths of root and shoot were more in short duration varieties than medium and long duration varieties. Among all the varieties, Udayagiri recorded highest root and shoot length of 7.7 and 3.6 cm respectively and germination per cent of 92.00 with low seed infection by bacteria. Lowest root and shoot growth of 0.18 and 0.9 cm were recorded in Khandagiri of short duration variety which was closely followed by Mahanadi, a long duration variety. Both the varieties recorded low seed germination and high seed infection by different bacteria.

Among the late duration varieties, Manika and Rambha recorded 76.00 and 64.00 per cent seed germination with 32.00 and 48.00 per cent of seed infection by different bacteria respectively. However, there was no much reduction in root length. Rather, the root length (3.2 cm) was more in Rambha than (2.9 cm) in Manika. Again, the root and shoot lengths in Jagannath were found to be less than in Manika and Rambha, though Jagannath recorded a high seed germination of 92.00 per cent. The result contradicted with the findings of other rice varieties and needed further studies for confirmation.

Table-4: Effect of seed borne bacteria on seed germination of different rice varieties:

Duration Group	Variety	Seed germination (%)			Bacterial Association (%)			Total
		Normal germination	Abnormal germination	No germination	Normal germination	Abnormal germination	No germination	
Early Duration Group	Udayagiri	92.0	4.0	4.0	0.0	2.0	0.0	8.00
	Khandagiri	56.0	28.0	16.0	4.0	12.0	20.0	36.00
	Lalitagiri	84.0	8.0	8.0	0.0	2.0	8.0	10.00
	Annapurna	44.0	52.0	4.0	12.0	24.0	2.0	38.00
	Parijat	80.0	8.0	12.0	2.0	0.0	4.0	6.00
	Mean		71.2	20.0	8.8	3.6	8.0	8.00
Medium Duration Group	Bhoi	84.0	4.0	12.0	2.0	2.0	8.0	12.00
	Annada	52.0	28.0	20.0	8.0	18.0	16.0	42.00
	Sravani	96.0	4.0	0.0	2.0	2.0	0.0	4.00
	Pratap	72.0	16.0	12.0	4.0	8.0	4.0	16.00
	Lalat	92.0	8.0	0.0	0.0	6.0	0.0	6.00
	Mean		79.2	12.0	8.8	3.2	7.2	5.5
Late Duration Group	Manika	76.0	4.0	20.0	12.0	2.0	18.0	32.00
	Rambha	64.0	24.0	12.0	16.0	24.0	8.0	48.00
	Jagannath	92.0	8.0	0.0	4.0	4.0	0.0	10.00
	Mahanadi	24.0	68.0	8.0	8.0	40.0	2.0	50.00
	Swarna	96.0	4.0	0.0	2.0	2.0	0.0	8.00
	Mean		70.4	21.6	8.0	8.4	14.4	5.6

4.5 Effect of bacterial seed infection on seedling vigour:

Seedling vigour from infected seeds used in the study was compared with apparently healthy seeds of the same variety to study the effect of bacterial seed infection on seedling vigour. The result presented in table-6 indicated that average seedling vigour of healthy seeds decreased with increase in maturation duration of the varieties. Maximum seedling vigour of 968.00 was recorded in early maturing varieties which gradually decreased to 738.00 and 638.00 as the maturation period increased to medium and late duration groups respectively which may be a varietal character. However, decrease in seedling vigour was quite evident in infected seed samples.

Percentage of seedling vigour increased with increase in seed infection with bacterial pathogens. Among the early duration rice varieties, the seedling vigour decreased by 92.4 per cent in variety, Khandagiri which had 36.00 per cent seeds infected with bacterial pathogen. However, Annapurna with 38.00 per cent infected seeds recorded 63.7 per cent decrease in seedling vigour. Minimum of 28.7 per cent decrease in seedling vigour was recorded in Udayagiri which had 8.0 per cent seeds infected with different bacterial pathogen. The rate of decrease in seedling vigour always could not be correlated with rate of increase in seed infection as in case of Parijat and Lalitagiri, which recorded 45.00 to 49.00 per cent decrease in seedling vigour with seed infection of 6.0 and 10.0 per cent respectively.

Table-5: Effect of Bacterial seed infection on the growth of rice seedlings:

Duration Group	Variety	Normal germination	Seedling growth(cm)		Bacterial seed infection
			Root	Shoot	
Early	Udayagiri	92.0	7.7	3.6	8.00
	Khandagiri	56.0	0.18	0.9	36.00
	Lalitagiri	84.0	5.1	3.1	10.00
	Annapurna	44.0	1.6	0.8	38.00
	Parijat	80.0	3.0	1.7	6.00
	Mean	71.0	4.5	2.4	19.6
Medium	Bhoi	84.0	1.9	1.4	12.00
	Annada	52.0	1.8	1.8	42.00
	Sravani	96.0	4.1	2.9	4.00
	Pratap	72.0	2.8	2.6	16.00
	Lalat	92.0	3.7	3.0	6.00
	Mean	79.2	2.8	2.3	16.00
Late	Manika	76.0	2.9	2.4	32.00
	Rambha	64.0	3.2	2.0	48.00
	Jagannath	92.0	2.7	2.1	10.00
	Mahanadi	24.0	0.2	1.9	50.00
	Swarna	96.0	2.7	2.5	8.00
	Mean	70.4	2.3	2.1	29.6

Table-6: Effect of bacterial seed infection on seedling vigour:

Duration Group	Variety	Seedling vigour index (%)		Decrease in vigour index (%)	Total seed infection(%)
		Healthy seed	Infected seed sample		
Early	Udayagiri	1460.4	1039.6	28.7	8.00
	Khandagiri	200.0	15.1	92.4	36.00
	Lalitagiri	1270.0	688.0	45.8	10.00
	Annapurna	1140.0	413.6	63.7	38.00
	Parijat	770.0	376.0	48.8	6.00
	Mean	968.0	506.4	55.8	19.6
Medium	Bhoi	550.0	277.2	49.6	12.00
	Annada	650.0	187.2	71.2	42.00
	Sravani	920.0	672.0	26.9	4.00
	Pratap	770.0	388.8	49.5	16.00
	Lalat	800.0	616.4	22.9	6.00
	Mean	738.0	428.3	44.0	16.00
Late	Manika	780.0	402.8	48.3	32.00
	Rambha	740.0	332.8	55.0	48.00
	Jagannath	650.0	441.6	32.0	10.00
	Mahanadi	190.0	50.4	73.4	50.00
	Swarna	830.0	499.2	39.8	8.00
	Mean	638.0	345.3	49.7	29.6

Among the medium duration varieties, maximum of 71.2 per cent decrease in seedling vigour was recorded in Arnada with 42.00 per cent seed infection and minimum of 22.9 per cent decrease was recorded in Lalat with 6.0 per cent seed infection.

Among the late duration group, maximum of 73.4 per cent decrease in seedling vigour was recorded in Mahanadi with 50.0 per cent seeds infected with bacterial pathogens. The variety, Swarna which had the lowest per cent (8.00) of infected seeds recorded 39.8 per cent decrease in seedling vigour.

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CHAPTER-V

DISCUSSION

DISCUSSION

Rice is the primary food grain consumed by almost half of the world's population, making it the most important food crop currently produced. Focussing on the increase in world's population, there is need to increase in rice production to meet the challenge of food scarcity. Seed, being the primary planting material and carrier of the genetic potential of the crop, is considered the starting point for achieving sustainable high yields. This has resulted in an increased movement of elite germplasm and planting material all over the globe which has resulted a significant international threat in terms of dissemination of destructive pathogen through seeds. Envisaging the current global trade and quality consciousness of the consumers, it is imperative to initiate studies on bacterial pathogens associated with rice seeds as little is known about the rice bacterial flora and their role in the deterioration of seed health for which the present study was undertaken.

Seeds of fifteen popular rice varieties, five each from early, medium and late maturing groups were collected and used for the present study. The seeds of each variety were analyzed for association of phytopathogenic bacteria with the rice kernel. The bacteria growing on the rice seed kernel placed in moist blotter plates (Anon, 2001) were isolated into pure culture and tentatively identified on the basis of morphological, physiological and biochemical characters.

Five bacterial species were found to be associated with the rice seed kernels. They were identified as *Xanthomonas oryzae oryzae*, *X. oryzae oryzicola*, *Pseudomonas syringae panici*, *Erwinia chrysanthomi* and *E. carotovora*. Seed association of *X. o. oryzae* and *X. o. oryzicola* are known as the two important disease causing pathogens of rice plant and have been reported to be associated with rice seed by several workers from India (Srivastava and Rao, 1964; Devdath and Dath, 1970; Pal *et al.*, 1982; Murthy and Devdath, 1984; Dey, 1992; Sethi, 1994; Gnanmanikam *et al.*, 1995 and many others) and from other countries (Fang *et al.*, 1956; Mizukami, 1961; Emchit and Om, 1969; Supriaman and Tantera, 1974; Buta and Ahmad, 1994; Huang *et al.*, 1994; Xio *et al.*, 1999; Xie *et al.*, 2002). The present study confirms their association with rice seed kernel.

An unexpected finding was the association of *Pseudomonas syringae panici* with rice seed kernel in the present study. It was earlier reported by Goto and Ohata (1961) to cause bacterial stripe disease in rice in Japan producing symptoms like, longitudinal stripes on leaves, stunting and death of seedlings and bud rotting. Infection on matured plant was not observed and for the first time it was detected from the rice seed kernel and claimed to be a new report. It may be a fact that the bacterium moved systemically from the root through the vascular system or the tubular sheath of the matured plants resulting infection of the seeds. The finding needs confirmation and further studies may give interesting results.

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Erwinia carotovora, the other pathogen found to be associated with the rice seed kernel in the present study was earlier reported from Indonesia to cause withering of sheath and rotting of panicle (Goto, 1965). Cottyn *et al.*(2001) reported the association of *E. carotovora* (now *Pectobacterium carotovorum*) along with several other species with the rice seed. However, association of *E. herbicola* with the rice seeds has been reported by Kim and Song (1996) which was earlier reported from Japan to cause black rot disease of rice grain (Iwadara, 1931). The present study confirmed its association with rice seed from Bhubaneswar.

Erwinia chrysothemis reported from Japan to cause bacterial foot rot of rice (Goto, 1979) and soft rotting of upper nodes and folded leaves resulting death of the plant. It was surprisingly detected from rice seed kernel in the present study. The bacterium differed from *E. carotovora* as it produced diffusible brown pigment into the medium when grown on potato sucrose agar. However, more detail study is required for confirmation of the result.

X. o. oryzae was found to be associated with all the test rice varieties except in Lalat. Also, it was found to be associated with maximum per cent of seed. Rest of four bacterial species, namely, *E. chrysothemis*, *E. carotovora*, *P. s. panici* and *X. o. oryzicola* were detected in 2 to 4 per cent of seeds irrespective of varieties. Highest seed infection was recorded in Mahanadi followed by Rambha and Arnada showing 40 to 50 per cent seed infection. Lowest seed infection with bacterial species was recorded in Lalat and Parijat. All the said pathogens except *P. s. panici* have

earlier been reported from rice seeds by several workers. More recently, it has been reported by Xio *et al.*(1999), Cottyn *et al.*(2001) and Xie *et al.*(2002). The present result confirmed the previous reports.

Studies on seed health indicated that increase in seed infection by the bacterial pathogens resulted in the decrease of seed germination. Maximum inhibition of seed germination was recorded in Mahanadi followed by Rambha and Annada. It is interesting to note that poor seed germination was resulted due to development of more number of abnormal seedlings rather than non-germination of seeds. Development of abnormal seedling was recorded as high as 68.0 per cent in Mahanadi which was closely followed by Annapurna which produced 52.0 per cent abnormal seedlings. Development of more number of abnormal seedlings might be due to the association of *X. o. oryzae* with rice seed kernels as most of the other bacterial species detected from the test varieties were rot causing pathogens and would have resulted in non-germination of seeds. Sreeramula *et al.*(1986) observed that *X. campestris oryzae* (*X. o. oryzae*) inhibited the root and shoot growth due to synthesis of ethyl acetate fraction (A₁ – A₄). However, A₅ fraction of ethyl acetate was not inhibitory to root and shoot growth. Dey (1992) recorded reduction in seed germination by *X. o. oryzae* . Naeem *et. al.*(2002) reported that association of *X. o. oryzae* and *Pseudomonas avenae* with rotten seeds and abnormal seedlings resulted in poor seed germination. However, further studies are required to determine the role of each bacterial species in influencing seed germination.

Increase in seed infection by different bacterial plant pathogens resulted decrease in root and shoot growth and ultimately affected the seedling vigour. Reduction in seedling vigour was found to be maximum (92.4%) in Khandagiri which had 36.0 per cent seed infection. It was closely followed by Mahanadi, Arnada and Annapurna. Reduction of vigour index reflected poor root and shoot growth and poor seed germination. The present findings confirmed the report of Sreeramula *et al.* (1986), Dey (1992) and Neem *et al.*(2002). However, more detail studies on the role of seed borne bacteria may lead to interesting result.

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CHAPTER-VI

SUMMARY AND CONCLUSION

SUMMARY AND CONCLUSION

Bacterial plant pathogens are not well fitted for survival in soil, particularly in the tropical climatic condition where the summer temperature remain around 40°C. They are considered as soil invaders rather than soil inhabitants and depend on their host for survival. Possibility of survival of bacterial plant pathogen through seed appeared to be more common in annual and seasonal crops like rice. However, bacterial community composition on rice seed and their role in deteriorating seed health has not yet been fully studied. Most of the research on rice seed has been confined to those few bacterial pathogens responsible for causing diseases in rice. Therefore, the present study was undertaken highlight on the role of seed borne bacterial pathogen on seed health.

During the 2002 wet season, harvested seeds from 15 popular rice varieties were collected from the experimental farm, OUAT, Bhubaneswar and phyto-pathological analysis of the seeds were carried out to study the association of different bacteria with the test varieties. The isolated bacteria were identified on the basis of morphological, physiological and biochemical characters. Role of seed borne bacteria on seed health with respect to seed germination, reduction in root and shoot growth and their effect on seedling vigour were assessed following paper towel method.

Phytopathological analysis of seed sample revealed the association of five bacterial species, namely, *Xanthomonas oryzae oryzae*, *X. o. oryzicola*, *Pseudomonas syringae panici*, *Erwinia chrysanthemi* and *E. carotovora*. Among the five bacterial species *X. o. oryzae* was detected from most of the rice varieties except Lalat which caused highest per cent of seed infection. The rest of the bacterial species were found to be associated with 2 – 4 per cent of seeds irrespective of varieties.

Among the 15 test varieties, seed infection was maximum in Mahanadi (50.0%) followed by Rambha and Armada. Lowest seed infection with bacteria was recorded in Parijat and Swarna.

Studies on seed health revealed that increase in seed infection resulted decrease in seed germination, decrease in root and shoot length and decrease in seedling vigour. It is interesting to note that seed infection resulted in the development of more number of abnormal seedling to the level of 68.0% in Mahanadi and 52.0% in Annapurna. Development of abnormal seedlings might be due to the association of *X. o. oryzae* as the bacterium synthesized A₁ to A₄ fraction of ethyl acetate which inhibited growth of root and shoot of rice seedlings (Sreeramula *et al.*, 1986). Also, Naeem *et al.* (2002) reported association of *X. o. oryzae* with rotten and abnormal seedlings.

Further studies on the aspect may reveal interesting result which shall be helpful in improving production of quality seeds in rice.

BIBLIOGRAPHY

BIBLIOGRAPHY

- Adachi, N.; Oku, T.(2000). PCR-modified detection of *X.o.pv. oryzae* by amplification of 16S-23S r-RNA spacer region sequence. *Journal of General Plant Pathology*. **66**(4):303-309.
- Ahmed, K.M.; Singh, R.A.(1975). Disease development and yield losses in rice varieties by bacterial leaf blight. *Ibid.* **28**:502-507.
- Anilkumar; Mahendra Prasad(2002). Pathology of a rice disease due to *P. marginalis* stevens reported from Ranchi, *Advances in Plant Sciences*, **15**(1):115-123.
- Annon (2001). Annual report of National seed project, I.C.A.R.
- Ashura, L.K.; Mabagala, R.B., Mortensen, C.N.(1999). Isolation and characterization of seed borne pathogenic bacteria from rice (*Oryza sativa* L.) in Tanzania, *Tanzania Journal of Agricultural Science*, **2**(1):71-80.
- Babu, R.M.; Sajeena, A; Vidyasekharan, P; Seetharaman, K; Reddy, M.S.(2003). Differential induction of chitinase and β -1,3 Glucanase in rice in response to inoculation with BLB pathogen *X.o.oryzae*. *Zeitschrift fiir Pflanzenkrankheiten und Pflanzenschutz*. **110**(2):105-112.
- Bhutta, A.R.; Ahmad, S.I.(1996). Detection of bacterial pathogens in paddy seed lots in Pakistan. *Pakistan Journal of Scientific and Industrial Research*.**37**(9): 382-384.
- Billing and Garrett, C.M.E.(1980). Phases on the identification of plant pathogenic bacteria in microbial classification and identification. *Eds. Good Fellow, M. and Board, R.G. Pub. Academic Press, London*, 318-338.

- Chien, C.C.; Chang, Y.C.(1987). The susceptibility of rice plants at different growth stages and of 21 commercial rice varieties to *P. glumae*. *Journal of Agril. Research of China*, **36**(3):302-310.
- Cother, P.; Lin, C.F; Goto, M.(1974). Identification of *P. syringae*, causal agent of glume blotch of rice. *Plant disease report*. **48**:442-443.
- Cottyn, B.; Regalado, E; Lanoot, B; Cleene, M., MEW, T.W. and Swings, J.(2000). Bacterial population associated with rice seed in the tropical environment. *Phytopathology*, **91**:282-292.
- Devadath, S. and Dath, A.P.(1985). Infected chaffs as a source of inoculum of *Xanthomonas campestris* pv. *oryzae* to the rice crop. *Zeitschrift für pflanzenkrankheiten under Pflanzenschutz*. **92**(5):485-488.
- Dey, K.K.(1992). Studies on leaf to seed transmission of *Xanthomonas campestris* pv. *oryzae* causing bacterial blight of paddy, Post Graduate Thesis, Orissa University of Agriculture and Technology, Bhubaneswar.
- Dong, Y., So., B.H. and Meng, X.J.(1982). Radiomunoassay for detecting the pathogen of rice bacterial leaf blight. *Application of atomic energy in agriculture, Jilin Agriculture University, Jilin, China* No.3:47-51.
- Durgapal, J.C.(1985). Self-sown plants from bacterial blight affected rice seeds a possible source of primary infection in M.W. *India Current Science*. **54**(24):1283-84.
- Emachit, S.; Ou, S.H.(1970). *Phillipp. Agric.* **54**:33-45.
- Fang, C.T.; Lin, C.F.; Chu, C.L.(1956). A preliminary study on the disease cycle of the bacterial leaf blight of rice. *Acta Phytopathologica Sinica*. **2**:173-185.

- Gnanamanickam, S.S.; Sakthivel, N.; Nelson, R.; Alvaraz, A.M. and Leach, J.E.(1995). Evaluation of culture media and molecular probes for detection of *X.o. oryzae* in rice seeds and plants. In *detection of plant pathogens and their management (Eds, J.P. Verma, A. Verma and B. Kumar) Angkor publishers (P) Ltd., New Delhi.* pp.159-172.
- Gnanamanickam, S.S.; Shigaki, T.; Medalla, E.S.(1994). Problems in detection of *X.o. pv. oryzae* in rice seed and potential for improvement using monoclonal antibodies. *Plant Disease.* **78**(2):173-178.
- Goto, K.; Okata, K; Fuktatzu, R.(1953). Over wintering of the causal bacteria of rice blight in the rice plant and grasses. *Agril. & Hort., Japan.* **28**:207-208.
- Hong Yong Cong; Hu Fangping; Huang Yiaonan(2002). Pathogenicity of *Pantoea agglomerans* on rice seeds. *Journal of Fujian Agril. & Fo. University.***31**(1): 32-36.
- Hsieh, S.P.Y.(1973). Ecological Studies on *Xanthomonas oryzae* the causal organism of bacterial blight of rice. Ph.D. Thesis, Univ. of Hawaii, Honolulu, 162 pp.
- Hsieh, S.P.Y.(1978). Ecology of *Xanthomonas oryzae*, the causal organism of bacterial blight of rice plant. *Symp. At Joint Commission for Rural Reconst., Taiwan.*
- Hsieh, S.P.Y.; Buddenhagen, I.W.(1975). Survival of *Xanthomonas oryzae* in relation to substrates, tramp. and humidity. *Ibid.* **65**:513-519.
- Huang, S.H.; Zeng, L.X.; Xu, X.M.(1994). Inspection of *Xanthomonas oryzicola* in rice seeds by McAb technique on selective culture media. *Guangdong Agricultural Sciences.* (1):38-40.
- Iwadara, S.; Dye, D.W.(1931). Identification of *E. herbicola* associated with rice plant. *Annals of Phytopathological society of Japan.* **28**(3):201-202.

- Kadota, I; Ohuchi, A.(1990). Symptoms & ecology of bacterial brown stripe of rice. *Japan Agril. Res. Quarterly*. **24**(1):15-21.
- Kauffman, H.E.; and Reddy, A.P.K.(1975). Seed transmission studies of *Xanthomonas oryzae* in rice *Phytopath*. **65**(6):663-666.
- Kawaradani, M.; Okada, K; Kusakari, S.(2000). New selective medium for isolation of *Burkholderia glumae* from rice seeds. *Journal of General Plant Pathology*. **66**(3):234-237.
- Kim, H.M.; Song, W.Y.(1996). Characterization of ribosomal RNA intergenic spacer region of several seed borne bacterial pathogens of rice. *Seed Science and Technology*, **24**(3):571-580.
- Lin, C.; Lorito, M; Fujii, T.(1994). Improvement of Walkimoto basic medium for detection of *X. o. oryzae*. *Annals of Phytopathological society of Japan*. **60**(5):240-242.
- Loreti, T.; Tantera, D.M; Walker, C.(2002). PCR amplification for detection of *P. avenae*. *Int. J. Syst. Bacteriol*. **37**:144-152.
- Mandal, A.H.; Latif, M.A.(1996). Effects of fertilizers on the incidence of bacterial blight & yield loss in rice. *Bangladesh Journal of Botany*.**25**(2): 121-126.
- Mew, T.W.; Gergon, E.B.; Merca, S.D.(1990). Impact of seed borne pathogens in rice germplasm exchange. *Seed Science & Tech*. **18**(2):441-450.
- Ming, D.; Ye, H.Z.; Chaad, N.W.; Rath, D.A.(1991). Selective recovery of *X. sp.* from rice seed *Phytopathology*. **81**(11):1358-1363.
- Mizukami, T.(1961). Studies on the ecological properties of *Xanthomonas oryzae*, the causal organism of bacterial leaf blight of rice plant. *Ibid*,**13**:1-85.

- Morita, H.; Dhanapala, M.P.(1990). "So called" grain sterility of rice in Sri Lanka in 1985 & 1986 Maha cropping seasons. *Japaneese Journal of Tropical Agl.* **34**(1):20-26.
- Murty, V.S.T.; and Devadata, S.(1984). Role of Seed in survival and transmission of *Xanthomonas campestris* pv. *oryzae* causing BB of Rice. *Phytopathological sche Zeitschrift.* **110**(1):15-19.
- Naeem, C.V. (2002). Toxins produced by *P. glumae*. *Annals of Phytopathological Society of Japan.* **59**(3): 379-386.
- Nema, A.G.; Kulkarni,S.N.(1973). Pectinolytic enzymes produced by *Xanthomonas translucens* f. sp. *oryzicola* (Fang *et. al.*) Bradbury, causing bacterial streak of rice. *JNKVV Research Journal.* (7):186-187.
- Ou, S.H.(1985). Rice diseases. *Common wealth Mycological Institute, Kew, Survey, England,* **380**.
- Pal, V., Rani, N.; Chand, J.N.(1982). Studied on the BLB of rice caused by *Xanthomonas campestris oryzae* (Uyeda & Ishiyama) Dowson. *Haryana Agric. Univ. V.J. Res.***12**:619-623.
- Raj, K.; Pal, V.(1988). Overwintering of *Xanthomonas compestris* pv. *oryzae*. *International Rice Research Newsletter.* **13**(1):22-23.
- Rao, P.S.; Kauffman, H.E.(1977). Potential yield losses in dwarf rice varieties due to bacterial blight in India. *Phytopathologische zeitschrift.* **90**:281-284.
- Rao, P.S.(1987).. Across-Season Survival of *Xanthomonas campestris oryzae*, causal agent of bacteria leaf height. *International Rice Research Newsletter.***12**(2):27.
- Reddy, P.R.(1983). Evidence of seed transmission of *Xanthomonas campestris* pv. *oryzae*. *Current Sci.* **52**(6):265.

- Reddy, A.P.K.; Mackenzie, D.R.; Rouse, D.I.; Rao, A.U.(1979). Relationship of bacterial leaf blight severity to grain yield of rice. *Phytopath.* **69**(9):967-969.
- Rott, P.; Honegger, J.; Notteghem, J.L.(1991). Identification of *P. fuscovaginae* with biochemical serological & Pathogenicity tests. *Plant disease*, **75**(8):843-846.
- Sakthivel, N.; Mortensen, C.N.; Mathur, S.B.(2001). Detection of *X.o. oryzae* in artificially inoculated & naturally infected rice seeds & plants by molecular technique. *Applied Microbiology and Biotechnology.* **56**(3/4):435-441.
- Sato, Z.; Koiso, Y.; Iwasaki, S.; Matsuda, I; Shirata, A.(1989). Toxins produced by *P. glumae*. *Annals of Phytopathological Society of Japan.* **55**(3): 353-356.
- Schaad, N.W.(1992). Initial identification of common genera. Laboratory guide for identification of plant pathogenic bacteria. *International Book Distributing Co.* 3p.
- Sharma, S.; Sthapit, B.R.; Pradhanang, P.M.; Joshi, K.D.(1997). Bacterial sheath brown rot of rice caused by *Ps. Fuscovaginae* in Nepal. *CIRAD-CA*:107-112.
- Sharada, M.S.; Shetty, S.A; Setty, H.S.(1990). Impact of seedborne bacteria on rice seedlings. *International Rice Research Newsl.* **15**(2):13.
- Shekhawat, G.S.; Srivastava, D.N.(1972). Mode of seed infection and transmission of bacterial leaf streak of rice. *Annals of the Phytopathological society of Japan*, **38**:4-6.
- Singh, D.V.; Banerjee, A.K.; Raj, M.; Srivastava, S.S.L.(1980). Survival of *Xanthomonas campestris* pv. *oryzae* in infected paddy seeds in plains of U.P. *Indian Phytopath.* **33**(4):601-602.

- Singh, D.; Vinther, P.; Mathur, S.B.(1983). Seed transmission of bacterial leaf blight in rice. *Seed Pathology Newsl.* No.15:11.
- Singh, G.P.; Srivastava, M.K.; Singh, R.V.; Singh, R.M.(1977). Variation in quantitative and qualitative losses caused by bacterial blight in different rice varieties. *Indian Phytopath.* **30**(2):180-185.
- Singh, R.A., Rao, M.H.S.(1977). A simple technique for detecting *Xanthomonas oryzae* in rice seed. *Seed Science and Technology.* **59**(1):123-127.
- Sreeramula, A.; Nayudu, M.V.(1986). Rice seedling growth retarding activities of ethyl acetate extent fractions of the culture filtrate of virulent isolate of *X.c. oryzae*. In *advances in research on plant pathogenic bacteria based on the proceeding of the national symposium on Phytobacteriology.* 14-15.
- Sridhar, R.(2002). Use of molecular tools for understanding bacterial blight pathogen Population of rice & usefulness of disease resistance genes. *Indian Phytopathology.* **55**(4):417-429.
- Srinivasan. M.(1982). Losses in rice yield due to Kressek(Wilt) phase of bacterial blight. *Madras Agric. J.* **69**(4):247-249.
- Srivastava, D.N. and Rao, Y.P.(1964). Paddy farmers should be aware of bacterial blight, *Indian farming.* **14**(6):32-33.
- Srivastava, M.P. and Kapoor, T.R.(1982). Yield loss due to Bacterial leaf blight. *Int. Rice Res. Newsl.* **7**(3):7.
- Subha, K.; Shetty, S.A.; Shetty, H.S.; Karanth, N.G.K.(1992). Interaction between seed borne bacteria and fungi of paddy. *International Journal of Tropical Plant disease.* **10**(1):125-130.
- Subhash Chander; Agrawal, P.R.; Reddy, P.R.(2002). Assessment of yield loss in rice due to BLB using simulation models. *Annals of Plant Protection Sciences.* **10**(2):277-281.

- Sumati, S.P.; Gnanamanikam, S.S. (1985). Use of starch agar as selective isolation medium for seedborne strains *X. c. oryzae*. *Madras Agril. Journal*. 72(4): 199-2002.
- Supriman, T.; Tantera, D.M.(1974). Detection of *Xanthomonas oryzae* in rice seed samples. *Contr. Central Res. Agr., Indonesia* (1972) 1,1-4.
- Tagami, Y.; Kuhara, S.; Kurita, T.; Fujii, H.; Sekiya, N.; Yoshimura, S.; Sato.; Watanabe, B.(1963). Epidemiological studies on bacterial blight of rice. *Bulletin of the Kyushu Agricultural Experiment Station*. 9:89-122.
- Thind, B.S. and Barar Jasveer S.(1992). A rapid method to prove the seed transmission *Xanthomonas oryzae* pv. *oryzae*. *Indian Phytopathology*. Supplementary Issue of Vol. 45 LXII.
- Tsushima, S.; Magi, S.; Naito, H.; Saito, H.(1991). Population of *P. glumae* on rice plants. *Annals of the Phytopathological society of Japan*.57(2):145-152.
- Tsushima, S.; Magi, S.; Naito, H; Saito, H(1986). Existence of *P. glumae* on the rice seeds and development of the simple method for detecting *P. glumae* from rice seeds. *Bulletin of the Kyushu National Agl. Experiment Station*. 25(3):261-270.
- Tsushima, S.; Naito, H.(1991). Spatial distribution & dissemination of bacterial grain rot of rice caused by *P. glumae*. *Annals of the Phytopathological Society of Japan*. 57(2): 180-187.
- Tsushima, S.; Narimatsu, M.(1994). Cloned DNA probes for detection of *P. glumae* causing bacterial grain rot of rice. *Annals of the Phytopathological society of Japan*. 60(5):576-584.
- Unnamalai, N.; Mew T.W. and Gnanamanickam, J.S.(1988). Sensitive methods for detection of *Xanthomonas compestris* pv. *oryzae* in rice seeds. In *Advances in Research on Plant Pathogenic Bacteria* (Eds. Gnanamanickam S.S. and Mahadevan, A.), Today and Tomorrow Printer and Publ., New Delhi. pp73-82.

- Vidyasekharan, P.; Alvenda, M.E.; Mew, T.W.(1989). Physiological changes in rice seedlings induced by extracellular Polysaccharide produced by *X.c.oryzae*. *Physiological and Molecular Plant Pathology*. **35**(5):391-392.
- Xie Guanlin, Zhu Goonian, Ren Xiaoping (2002). Diversity of Pathogenic bacteria from rice seed. *Acta Phytopathologica Sinica*. **32**(2):114-120.
- Xie-Guanlin; Zheng-Jichi, Mew T.W.; Xie, G.L.; Zheng, J.C.(1999). Pathogenic bacteria associated with rice seed in the subtropic and the tropic areas. *Acta-Agricultural-Zhejjangensis*. **11**:3(127-132).
- Zeigler, R.S.; Alvarez, E.(1989). Grain discoloration of rice caused by *Pseudomonas glumae* in Latin America *Plant Disease*. **73**(4):368(En.).

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