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Studies on growth and colony morphology of different fluorescent pseudomonads

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An extensive survey had been made for recording, occurence of such bacteria in five agroclimatic zones of West Bengal, namely, hill, terai, laterite, new alluvial and saline alluvium. For naming the isolates collected from different sources first letter from the name of the season, second one for source crop and last one for respective zone had been used. Sometimes extra letters were used for the different growing situation of a specific crop under same zone and in same season. Out of the 39 isolates, 11 isolates (KMH, KKH, KSH, KEH, KCA, KCFH, KBH, KCH, KTA, KSA, KAA) were noted as early grower, only 4 isolates (KBA, KRGA, KRGI, KBA) required maximum time for colony appearance. The isolates like KMA, KRL, KRGA, KRGL, KRGT, KBGS, KCH, KTA and KSEL grew with pink membranous surface whereas BRSA, BRBA, BSA, KKA, KBGA, BBA, KCFH, BSOA and BSEA showed no membranous growth on surface.

Key words: Fluorescent pseudomonads, colony morphology, growth

INTRODUCTION

The most important and highly explored characteristic of fluorescent bacteria are their inhibitory properties against plant pathogenic bacteria or fungi. The scope of utilizing such strains in biological control of plant diseases has been studied earlier. (Shaktivel *et al.*, 1988; Parke *et al.* 1991). Some of the isolated strains of these groups of bacteria have been used successfully for control of some diseases and commercial products from these bacteria have arrived in the market too.

The characteristics of enhancing growth of plants by these bacteria have also been investigated. The findings of Freitas and Pizzinatto (1997) and Viswanathan *et al.* (2000) have reported encouraging results on growth enhancing effect, of these bacteria in cases of crops like sugarcane and others. Presence of such bacteria is reported to increase seed germination, vegetative growth and also yield of such crops. Some of the findings from these bacteria have also indicated its possible role in some chemical transformations in the soil (Clay *et al.*, 1995, Stenstrom *et al.*, 1990). The present study has been made for recording and characteriztion of the identity of the fluorescent pseudomonads bacteria of five agroclimatic zones of West Bengal.

MATERIALS AND METHODS

To study the bacterial isolates, standard bacteriological procedures were followed.

The time requirement for appearance of bacterial colony of total thirty nine isolates was determined. One loopful of bacterial mass (24-48 hrs old) was taken and suspended in 9 ml sterile saline solution (1%) and diluted it up to 10^{-7} dilution. From 10^{-7} dilution, 0.05 ml bacterial suspension was taken and spread on the Petriplates containing King's medium. The test plates were incubated at 28° – 30°C temperature. Colony appearance of different isolates after 72 hrs incubation was observed. Depending upon the time of colony appearance the isolates were grouped in 4 categories. Namely, (1) very early grower i.e., 8-12 hrs, for colony appearance; (2) moderately early growere i.e. 10-12 hrs for colony appearance; (3) early grower i.e. 12-14 hrs for colony appearance, and (4) slow grower i.e. 14-16 hrs for colony appearance.

The isolates were named by their origin like the zones, from where they were collected. For naming of isolates, collected from different sources, first letter from the name of the season, second one for source crop and last one for respective zone has been used. Sometimes extra letters were used for the different growing situation of a specific crop under same zone and in same season.

To study the colony morphological characteristics, the plants were inoculated with different bacterial isolates as the above mentioned procedure and simultaneously the bacterial isolates were also inoculated in tubes containing 5 ml Kings B in sterilized broth for 72 hrs at 28-30°C.

The King's B broth enriched with cetrimide was prepared, distributed in tubes and sterilized at 121°C for 30 minutes. The different isolates of the bacteria were inoculated in separate tubes from 24 hrs old culture of the bacteria.

RESULTS AND DISCUSSION

Growth and colony morphological characteristics of different isolates of fluorescent pseudomonads from Table 1, data showed that BRSA, KRSA, KMA, KRL, BKA, BSA, BAA, BJA, KKA, KBGA, KRGL, KPL, KBGL, KBGS, KRGS, BBA, KBS, BSOA, KEA, KPS, KSEL and BSEA were categorized under the first group, very early grower i.e. 8-12 hrs for colony appearance whereas only one isolate BRBA was under the second one, moderately early grower i.e. 10-12 hrs for colony appearance. Eleven isolates i.e. KMH, KKH, KSH, KCA, KBH, KCH, KCFH, KTA, KSA, KAH and KEH were noted as early grower which required 12-14 hrs for colony appearance. Only four isolates (KBA, KRGA, KRGT, KBA) required maximum time for colony appearance (14-16 hrs) among the total isolates tested.

The colony form of maximum number of isolates as showed in Table 1 were circular except KRSA and KSH i.e. with non circular form. Surface of colonies of all isolates were smooth, lusture of all isolates were bright. Colony of maximum isolates were slightly elevated whereas KRSA, KCH, and KCFH produced flat surface colonies. It was shown that optical character of all isolates were opaque.

Further studies on growth characteristics of the bacteria in liquid KBC medium was studied and the data are presented in Table 2. The isolates like KMA, KRL, KRGA, KRGL, KRGT, KBGS, KRGS, KCH, KTA and KSEL grew with pink membranous surface whereas KPS, KSH, KEH, KAH, KSA, KBS, KBH, KBA, KCA, KPL, BJA, KRBA, KKH, KMH, and KRSAK with white membranous surface. Only the isolates BKA, BAA, KBGL, and KEA grew with brown membranous surface whereas some isolates like, BRSA, BRBA, BSA, KKA, KBGA, BAA, KCFH, BSOA and BSEAK showed no membranous growth on surface.

Some isolates like BRSA, KMA, KRL, BKA, BRBA, BSA, BAA, BJA, KKA, KBGA, KRGA, KBGL, KBGS, KRGS, BBA, KCFH, BSOA, KEA and BSEA grew rapidly in heavy (H) clouding in medium was recorded. But the isolates i.e. KSEL, KPS, KTA, KCH, KBS, KCA, KRGL, KPL, KRGL, KSH, KRBA and KRSA showed medium turbidity whereas KMH, KKH, KBA, KBH, KSA, KAH, KEH and KSH showed light turbidity in the medium.

All the isolates produced pungent odour. Sedimentation was recorded at the floor of the tube. The colour of the sediment varied from isolate to isolate. Bluish green sediment was recorded in case of KMA, KBGS, and KRGS whereas the isolates like BRSA, KRL, BJA, KKA, KBGA, KBGL, BBA, BSOA, KEA and BSEA produced dark green sediment. Only the isolates BKA, BSA and KRGA, were recognized by their green pigment but KRSA, KCFH, and KSA produced brown pigment. The isolate BRBA only produced yellowish green pigment. Brownish white pigment was produced by KMH, KKH, KRBA, KPL, KCA, KBA, KBH, KBS, KTA, KAH, KEH, KSH and KPS isolates whereal KSEL, KCH, KRGT, and KRGL deposited pinkish white mass at the bottom of the tube.

In regard to crystal formation the isolates were able to produce white crystal in the medium and their quantity varied from isolate to isolate. The isolates like BRSA, KMA, BJA, KKA, KBGA, BBA, BSOA, KEA and BSEA produced such crystals, whereas the isolates like KSEL, KPS, KSH, KEA, KAH, KTA, KCH, KBS, KBH, KCA, KRGT and BRBA did not have the ability to produce such crystal. Some isolates had the ability to produce moderate amount

Table 1. Colony characteristics of isolates of fluorescent bacteria

Isolates (code)	Hours for colony to appear	Colony characters				
		Form	Surface	Elevation	Lusture	Optica character
BRSA	8-12	Circular	Smooth	Flat	Bright	Opaque
KRSA	"	Non circular	"	Slightly elevated	"	"
KMA	"	circular	"	"	"	"
KRL	"	"	"	"	"	"
KMH	12-14	"	"	"	"	"
KKH	"	"	"	"	"	"
BKA	8-12	"	"	"	"	"
BRBA	10-12	"	"	"	"	"
KBA	14-16	"	"	"	"	"
KSH	12-14	Non-circular	"	"	"	"
BSA	8-12	circular	"	"	"	"
BAA	"	"	"	"	"	"
BJA	"	"	"	"	"	"
KKA	"	"	"	"	"	"
KBGA	"	"	"	"	"	"
KRGA	14-16	"	"	"	"	"
KRGL	8-12	"	"	"	"	"
KPL	"	"	"	"	"	"
KBGL	"	"	"	"	"	"
KRGT	14-16	"	"	"	"	"
KBGS	8-12	"	"	"	"	"
KRGK	"	"	"	"	"	"
BBA	"	"	"	"	"	"
KCA	12-14	"	"	"	"	"
KBA	14-16	"	"	"	"	"
KBH	12-14	"	"	"	"	"
KBS	8-12	"	"	"	"	"
KCH	12-14	"	"	Flat	"	"
KCFH	"	"	"	"	"	"
KTA	"	"	"	Slightly elevated	"	"
BSOA	8-12	"	"	"	"	"
KSA	12-14	"	"	"	"	"
KEA	8-12	"	"	"	"	"
KAH	12-14	"	"	"	"	"
KEH	12-14	"	"	"	"	"
KPS	8-12	"	"	"	"	"
KSEL	"	"	"	"	"	"
BSEA	"	"	"	"	"	"

of crystals like KRSA, KRL, BKA, KRBA, BSA, BAA, KPL, KBGL, and KBGS but the isolates like KSA, KBA, KRGS, KRGA, KKH, and KMH produced still less amount of crystals.

Because of their important characteristics like enhancement of growth of plants, inhibitory properties towards different microorganism and some others, these bacteria have been studied

Table 2. Growth of fluoresent bacteria in KBC broth

Isolates	Surface	Growth on KBC-broth for 15 days			
		Clouding	Odour	Sediment	Crystal formation
KRSA	Wm	M	Pungent	Brown	Moderate
BRSA	Nm	H	"	Dark green	Heavy
KMA	Pm	H	"	Bluish green	"
KRL	Pm	H	"	Dark green	Moderate
KMH	Wm	L	"	Brownish white	Less
KKH	Wm	L	"	"	Less
BKA	Bm	H	"	Green	Moderate
BRBA	Nm	H	"	Yellowish green	Nil
KBA	Wm	M	"	Brownish white	Moderate
KSH	Wm	M	"	"	Nil
BSA	Nm	H	"	Green	Moderate
BAA	Bm	H	"	"	
BJA	Wm	H	"	Dark green	
KKA	Nm	H	"	"	
KBGA	Nm	H	"	"	
KRGA	Pm	H	"	Green	
KRGL	Pm	M	"	Pinkish white	
KPL	Wm	M	"	Brownish white	
KBGL	Bm	H	"	Dark green	
KRGT	Pm	M	"	Pinkish white	
KBGS	Pm	H	"	Bluish white	
KRGS	Pm	H	"	"	
BBA	Nm	H	"	Dark green	
KCA	Wm	M	"	Brownish white	
KBA	Wm	L	"	"	
KBH	Wm	L	"	"	
KBS	Wm	M	"	"	
KCH	Pm	M	"	Pinkish white	
KCFH	Nm	H	"	Brown	
KTA	Pm	M	"	Brownish white	Nil
BSOA	Nm	H	"	Dark green	Heavy
KSA	Wm	L	"	Brown	Less
KEA	Bm	H	"	Dark green	Heavy
KAH	Wm	L	"	Brownish white	Nil
KEH	Wm	L	"	Brownish white	"
KPS	Wm	M	"	"	"
KSEL	Pm	M	"	Pinkish white	"
BSEA	Nm	H	"	Dark green	Heavy

Legends : Wm = white membranous; B = Brown; P = Pink; N = No; H = High; M = Medium; L = Low.

extensively in different parts of the world (Clay *et al.*, 1995; Stenstrom, *et al.*, 1990).

Large number of reports on such bacteria from different parts of the world, occurring in soils and rhizospheres of different crop plants and other situations are all most corroborative with the results of the present works.

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