Pak. j. sci. ind. res., vol. 36, nos. 6-7, June-July 1993

ISOLATION OF *SERRATIA MARCESCENS* SENSTITIVE TO 6-AMINOPENICILLANIC ACID FROM LOCAL ENVIRONMENT

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(Received January 12, 1992; revised April 24, 1993)

A strain of *Serratia marcescens* has been obtained from water of irrigation channel. The minimum inhibitory concentration for 6-aminopenicillanic acid is nearly 250 µg/ml in nutrient agar medium and the strain could grow well in solid medium in petriplate having 10 mg/ml of penicillin G. The strain is designated *Serratia marcescens* SS91, and is useful in screening penicillin G acylase producing *Escherichia coli* isolates.

Key words: *Serratia marcescens*, 6-Aminopenicillanic acid.

Indtroduction

The cells of *Escherichia coli* having penicillin G acylase or the enzyme purified there from can be used to produce 6-aminopenicillanic acid (6-APA), which is further utilized for producing semisynthetic penicillins and cephalosporins [1,2]. A bacterial strain, *Serratia marcescens* ATCC 27117, is used to screen penicillin G acylase producing natural *E. coli* isolates [3] and also *E. coli* recombinants produced though genetic engineeering [4]. As it was found difficult to acquire or purchase *S. macescens* ATCC 27117, therefore, an attmept was made to isolate *S. marcescens* from local environment. Here we describe a method to isolate *S. marcescens* from water of irrigation channel, that proved sensitive to 6-APA and highly resistant to penicllin G, and can be used to screen penicillin G acylase producing *E. coli* isolates.

Materials and Methods

Medium composition. The medium (Peptone - glycerol agar) used for the isolation of *S. marcescens* was composed of Difco peptone, 5 g; glycerol, 10 ml; Difco agar, 20 g; volume adjusted to 1 litre with distilled water [5].

Isolation. Water samples of irrigation channel were collected around NIAB Campus. Freshly collected samples were diluted, 2 ml of it to 5 ml, by addition of sterile distilled water. The water was passed through a millipore prefiltration pad remove the heavy particles. The pre-filtrate 2–3 ml was passed through sterile millipore filters (0.45 µm). The filter which retained the bacteria was placed on peptone - glycerol agar, incubated at 28°. Pink colonies were picked and again streaked on peptone - glycerol agar. Pink colonies appeared after overnight incubation at 28°. The bacterial strain isolated was seemed to be *Serratia marcescens*. It was maintained on nutrient agar slant, and was furhter studied for its morphological, physiological and biochemical characteristics. The bacterial isolate was confirmed as *S.marcescens* by morphological, physiological and biochemical tests, and its sensitivity to 6-aminopenicillanic acid.

Morphological characterization. Microscopic slide was prepared for observation under micorscope and gram reaction was studied [6].

Agar colonies. Bacterial strain was streaked on nutrient agar medium. It was incubated at 28° for 18 hr. and colony characteristics were observed.

Agar slants. The isolate was grown on nutrient agar slants for 18 hr. at 28° and it growth characteristics on slants were observed.

Physiological characterization. Differential physiological characteristics were studied according to Prokaryotes Vol. II [7] and Bergey'sManual [8] and are summarized in Table 1.

Biochemical characterization. Biochemical reactions, indole, methyl red, Voges-Proskauer, citrate utilization, DNase and carbon source utilization were made to confirm the isolate as *Serratia marcescens* [7], and it was maintained on nutrient agar slant.

Sensitivity towardds 6-aminopenicillanic acid. To determine the susceptibility of *S. marcescens* SS91–6-APA, different concentrations of 6-APA were added in a series of plates containing nutrient agar. One plate was kept as control. The concentration used were 25, 50, 75, 100, 125, 150, 175, 200, 225, 250 and 300 µg/ml. Plates were streaked for *S. marcescens* SS91, and incubated overnight for 18 hr. at 28° and growth inhibition was observed.

Screening of penicillin G acylase producing E. coli. Overlay test was performed for screening of pencillin G acylase producing *E. coli* that was point inoculated from nutrient agar onto petriplates containing Bacto peptone (Difco), 0.5%; beef extract (Difco), 0.3%; phenylacetic acid, 0.15% and incubated for 18 hr. at 37°C and then overlayed with 5 ml of nutrient agar containing 5 mg of penicillin G per ml. About 0.2 ml of an 18 hr. culture of *S. marcescens* SS91, grown in liquid broth con-

taining 1% peptone and 0.5% sodium chloride, was spread over the hardened upper layer. The test plates were incubated overnight at 28°C and inhibitory zones were scored.

Results and Discussion

An isolate of *S. marcescens* was obtained from water of irrigation channel. Habitats, generally, described for *S. marcescens* are water, soil, milk, food, silkworms and other insected [7,8].

The medium (Peptone-glycerol-agar) used for the isolation of *S. marcescens* is described in Materials and Methods. Pigmented (pink) colonies were isolated on Peptoneglycerol agar after 18 hr. incubation at 28°. A low phosphate agar without glucose such as peptone-glycerol is the best way to demonstrate pigmentation [5]. Pigmented *Serratia* isolated from terrestrial waters most often are *S. marcescens* and *S. plymuthica* and less frequently *S. marinorubra* [9].

Morphological characteristics were studied and bacterial isolate was found gram-negative, in the form of short rods, occuring singly and occasionally in chains of 5 or 6 elements. Agar colonies were circular, thin, granular, white becoming red. Growth on agar slant was observed as white, smooth, moist layer becoming red. All the morphological characteristics were found same as described in Bergey's Manual [8] and are shown in Table 1.

Physiological characteristics studied, Table 1, showed the optimal growth temperature between 25–30° after 18 hr. incubation. The bacterial growth was also observed at 17°. At this low temperature white colonies appeared after 48 hr., which were turned pink after 72 hr. No growth was observed at 5° and 37°. Growth range described for *S. marcescens* is 15 –30° [8]. Best results are obtained when *Serratia* cultures are incubated at 30°[7]. The isolate was tolerent to 4% NaCl at 17°and 30°. The salt tolerence and relatively low minimal growth temperature of all *Serratia* species may help devise a means of enriching them. Bacterial isolate was well grown on nutrient agar medium at pH 9, incubated at 30°. Grwoth at pH 9 is a characteristic of genus *Serratia* [10].

Further, biochemical reactions, indole (-), methyl red (-), Voges-Proskauer (+), citrate utilization (+), DNase (+), and different carbon source utilization tests i.e. sorbitol (+), sucrose (+), inositol (+), mannitol (+), xylose (-) and arabinose (-) confirmed the isolate being *Serratia marcescens* [7,8] as shown in Table 1.

The major and the important characteristics of *S. marcescens* SS91 are its sensitivity against 6-APA and resistance against penicillin G. Various concentrations of penicillin G were used to confirm the bacterial resistance. Initially 50–900 µg/ml concentration of penicillin G were used. The bacterial isolate was found resistant to these concentrations.

Table 1. Differential Morphological, Physiological and Biochemical Characteristics of Isolate *Serratia marcescens* SS91.

Tests	Results
Morphological	
(i) Gram reaction	Gram negative
(ii) Microscopic observation	Short rods, occurring singly or occasionally in chains of 5 or 6 elements
(iii) Agar colonies	Circular, thin, granular, white becoming red.
(iv) Agar slant	White, smooth, moist-layer becoming red.
Physiological	
(i) Growth	
At 25-30°	Optimum
At 17°	Positive (after 72 hrs)
At 5°	Negative
At 37°	Negative
At pH 9	Positive
(ii) Tolerence	To 4% NaCl
(iii) Resistance	To 10 mg/ml penicillin G.
(iv) Sensitivity	To 6-APA at 250 µg/ml.
Biochemical	
(i) Indole	Negative
(ii) Voges - Proskauer	Positive
(iii) Methyl red	Negative
(iv) Simmon's citrate	Positive
(v) DNase	Positive
(vi) Carbon source utilization:	
(a) Sorbitol	Positive
(b) Sucrose	Positive
(c) Inositol	Positive
(d) Mannitol	Positive
(e) Xylose	Negative
(f) Arabinose	Negative

and when these were increased from 1–10 mg/ml even then it showed resistance for penicillin G.

The *S. marcescens* is resistant to penicillin G but susceptible to 6-APA. Minimum inhibitory concentration of 6-APA was found nearly 250 µg/ml. An overlay test was performed as described in Materials and Mehtods. Failure of growth of *S. marcescens* SS91 resulted in clear inhibition zones around colonies of test isolate, due to breakdown of penicillin G into

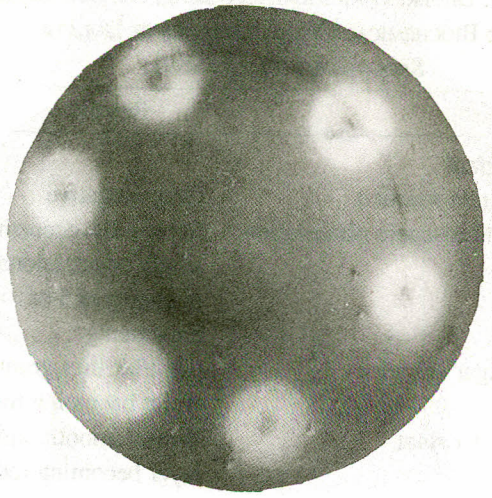

Fig. 1. Penicillin G acylase producing *E. coli* colonies showing zones of inhibition around them when lawn of *S. marcescens* SS91 was grown of penicillin G containing medium by overlaying technique.

6-APA and provided presumptive evidence for penicillin acylase activity (Fig. 1). So the bacterial strain isolated, *S. marcescens* SS91 is useful in screening penicillin G acylase producing *E. coli*

We have not screened different habitats for isolating and scoring of *S. marcescens*. The main aim was to acquire a strain of *S. marcescens* to screen penicillin G acylase producing *E. coli* and water of local irrigantion channel, part of vast irrigation channel system of Pakistan, proved a promising habitat. As we could not get *S. marcescens* ATCC 27117, therefore it was not possible to make any comparison with that strain.

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