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Scientific Note

AN ENDOPHYTE, REVERSING MDR IN *PSEUDOMONAS* STRAIN

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The endosymbiotic gram positive and negative endophytic bacteria that originate from epiphytic bacterial communities colonize and build up a barrier against pathogenic organisms (Kloepper *et al*., 1997; Rosenblueth and Martinez- Romero, 2006; Higgins *et al*., 2007; Panchal and Ingle, 2011). Influenced by the biotic and abiotic factors in host plants they produce bio active and growth competitive interactions (Owen *et al*., 2004; Ting *et al*., 2010; Hallmann *et al*., 2011; Sheng Qin *et al*., 2011). These bacteria obstinately survive in nature by a wide variety of microbe-microbe interactions (Hallmann *et al*., 1997; Zinniel *et al*., 2002; Rosenblueth and Martinez-Romero, 2006; Benardi-Wenzel *et al*., 2010; Saunders *et al*., 2010; Joseph and Mini, 2011).

Mechanisms of microbial interaction include pili, secretion systems, cell surface recognition, vesicles, aerosols, small molecules, transported via efflux pumps or diffusion, phages or viruses and bio films (Phelan *et al*., 2012). A novel type of interaction was observed where multidrug resistant (MDR) *Pseudomonas* progressively loses its antibiotic resistance at various incubation periods to gentamicin, ciprofloxacin and erythromycin in Disc Diffusion test when co cultured with an endophyte isolated from Asian Spiderwort plants.

The root and rootlets of the plant were washed with water followed by surface sterilization with 70% ethanol for 30 seconds. It was treated with sodium hypochloride (3%-5% available chlorine) for 3 minutes. Samples were exhaustively rinsed with sterile water. The rootlets were cut vertically into pieces approximately 0.5cm long and placed on sterile Starch Casein Agar (SCA) [Starch 10g, Casein 2g, KNO_3 2g, NaCl 2g, K_2HPO_4 2g, $MgSO_4$ 0.05 g, $CaCO_3$ 0.2g, $FeSO_4$ 0.01g, Bacto agar 18g, distilled water 1L (pH 7.4)] This was incubated for a period of 20days to observe for natural antimicrobial activity against other naturally occurring bacteria and fungi on the sterile media from other rootlets that was simultaneously inoculated from the plant.

The antibiosis producing endophyte was isolated on SCA. This was again sub cultured on Muller Hinton Agar (MHA, Hi media). This strain was tested against an MDR *Pseudomonas* for antibiotic sensitivity and was found sensitive. The media used for the culturing of the bacteria were MHA for antibiotic sensitivity test and Muller Hinton Broth (MHB, Hi media) for broth culture.

MDR *Pseudomonas* used for the study was obtained from a laboratory in Kozhikode. The antibiotic discs to which Pseudomonas were resistant while performing Kirby Bauer disc diffusion test were vancomycin 30mcg, gentamicin 10mcg, ampicillin 10mcg, ciprofloxacin 5 mcg, erythromycin 15mcg, cloxacillin 1mcg, tetracycline 10mcg, mupirocin 5mcg and oxacillin 1mcg. The endophyte was found sensitive to all the above antibiotics except Cloxacillin 1mcg.

2.5ml of MDR *Pseudomonas* and endophyte cultured in Muller Hinton Broth were aerobically incubated overnight at room temperature. It was subsequently mixed to obtain a total volume of 5ml into a 25ml conical flask. The flask was aerobically incubated at room temperature.

After 4 days of incubation at room temperature, the co-culture was lawn cultured on Sterile Muller Hinton Agar plates. Antibiotic discs were placed over the lawn culture. Then the plate was incubated at room temperature for 24 hours

On the 7th day, the co-culture was again performed antibiotic sensitivity test by following the procedure described for the 4th day. On the 9th day the same culture was again performed antibiotic sensitivity test following the procedure described for the 4th day.

After one month the culture was again performed antibiotic sensitivity test following the procedure described for the 4th day.

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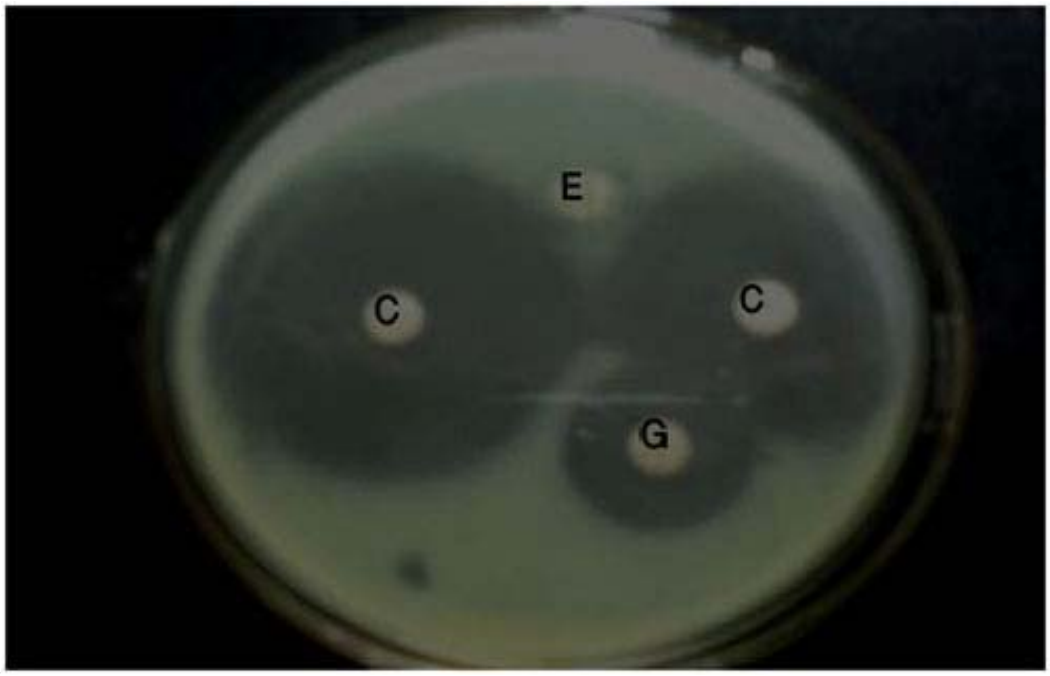

Fig. 1. 9th day MDR *Pseudomonas* strain becomes sensitive to Gentamicin and Ciprofloxacin when cultured with endophytic bacteria at room temperature (E. **Erythromycin**, C. **Ciprofloxacin**, G. **Gentamicin**).

Results obtained as follows:

4th day: Gentamicin sensitivity zone appeared with a diameter of 0.8cm.

7th day: Gentamicin sensitivity zone appeared with a zone of 1.5cm diameter and ciprofloxacin sensitivity zone appeared with a diameter of 2cm (see Fig. 1).

9th day: The zone diameter improved to 2cm for gentamicin and 2.5 - 3cms for ciprofloxacin.

1 month: A further increase was observed in the gentamicin sensitivity zone. The sensitivity zone reached a size of 2.6cm. Similarly, the ciprofloxacin sensitivity zone measured 3.5cm. In addition to these, another amino glycoside, erythromycin and tetracycline discs demonstrated sensitivity zones of 1.8 and 2cm respectively.

Pseudomonas showed pigmentation from Day 1 onwards in the co culture.

The endophytic bacteria was able to progressively induce sensitivity to a multi drug resistant *Pseudomonas* while co- cultured at room temperature at various incubation periods showing re-emergence of antibiotic sensitivity. It may be inferred that multiple drug resistance is *Pseudomonas* may not be a permanently acquired character. The interactive mechanism by which some of the endophytes progressively induce antibiotic resistance in multidrug resistant bacteria requires rigorous study.

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