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Effect of salt concentration on siderophore production by *Rhizobium strains* nodulating *Macrotyloma uniflorum* (Lam.) verdc.

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Thirty two *Rhizobium* strains isolated from root nodules of *Macrotyloma uniflorum* (Lam.) Verdc. showed siderophore production on Chrome-Azurol S agar medium. Ten *Rhizobium* strains which showed varying siderophore production at control (without salt) were selected to study the effect of salt concentration on siderophore production. These strains showed low siderophore production at control and it increased with increasing salt concentration up to 1000mM by the strain HGR23 (80μg/mL^{-1}) while two strains HGR16 and HGR30 showed at 400mM concentration. Salt stress enhanced maximum siderophore production (90 μg/mL^{-1}) by the strains HGR5 and HGR8 at 600mM salt concentration over control. Later, the siderophore production decreased with increasing salt concentration. The strains HGR1, HGR4, HGR7, HGR12 and HGR18 showed maximum production at 800mM salt concentration (60.7 μg/mL^{-1} to 70.8 μgmL^{-1}). Paper Electrophoresis of the siderophore extract showed the presence of trihydroxamate type of siderophores.

Key words : Siderophore production, Salt concentration, *Rhizobium* species, *Macrotyloma uniflorum*.

INTRODUCTION

Macrotyloma uniflorum (Lam.) Verdc. is an important pulse and green manure crop of India. It is extensively cultivated on light red and gravel soils of peninsular India. Thirty two *Rhizobium* strains were isolated from the fresh healthy root nodules of *M. uniflorum* plants grown in thirty two soil samples collected from various parts in Andhra Pradesh. The *Rhizobium* strains associated with *M. uniflorum* were found to be highly salt tolerant (Prabhavati and Mallaiah, 2007) and most of them produced siderophores. Siderophore production by *Rhizobium* has been of special interest in view of their prominent role in chelation and accumulation of ferric iron, and importance of iron at several stages of nitrogen fixation and assimilation process (Neilands, 1986). There are very few studies on the effect of salt concentration on siderophore production in bacteria. Hence, the present work was taken up to study the effect of salt concentration on siderophore production.

MATERIALS AND METHODS

The *Rhizobium* isolates were preliminarily screened for their ability to produce siderophores using Petri plates with Chrome-Azurol S (CAS) agar medium (Schwyn and Neilands, 1987). The *Rhizobium* isolates were grown in conical flasks containing YEM broth and the flasks were incubated for 24 h on a rotary shaker (120 rpm) at room temperature (28±2°C). After incubation, the culture was centrifuged and the cell free supernatant was applied to CAS plates in which wells made with cork borer.

The nature of the siderophores was detected by Neiland's spectrophotometric assay (Neilands, 1981) at control to 1000mM salt concentrations where a peak between 420-450 nm on addition of 1 ml of 2% aqueous $FeCl_3$ to 1 ml of cell free culture filtrate indicated the presence of hydroxamate type of siderophores. It was also confirmed by electrophoretic method described by Jalal and Helm, 1950. The tests for catechol type siderophore production were negative for all the isolates.

The estimation of hydroxamate type of siderophores was carried out Atkin's method (Atkin *et al.*, 1970). Ten isolates were selected to study the effect of salt concentrations on siderophore production. For this, basal medium with different salt concentrations in the range control to 1000mM were prepared separately and inoculated with *Rhizobium* sp. to test the effect of various salt concentration levels on siderophore production.

In these ten strains, the strain HGR8 showed maximum siderophore production. This strain was selected to study the production of siderophore along with the growth. For this, the culture was grown in 50 ml of basal medium at various salt concentrations *i.e.*, control to 1000mM with constant shaking on rotary shaker at room temperature for 24 h. Samples were withdrawn from each salt concentration and measured for growth (optical density at 610 nm) and siderophore production.

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RESULTS AND DISCUSSION

Screening for siderophore production:

All the 32 isolates of *Rhizobium* are positive for siderophore production. When culture supernatant was applied to the wells of the CAS plates containing various salt concentrations (control to 1000mM), orange to yellow colour halo produced around the well indicating the production of siderophores. Siderophore production was not indicated in control.

Type of siderophores:

Neiland's assay showed that all isolates produced hydroxamate type of siderophores. Electrophoretic mobilities suggested trihydroxamate nature of siderophores were produced at all the salt concentrations tested.

Effect of salt concentration:

Ten *Rhizobium* isolates *i.e.*HGR1, HGR4, HGR5, HGR7, HGR8, HGR12, HGR16, HGR18, HGR23 and HGR30 with varying levels of siderophore production over control were selected to study the effect of salt concentration on siderophore production. These strains showed significant production of siderophores at all the salt concentrations tested.

The maximum production of siderophores by different isolates varied with the isolate and salt concentrations. Thus the strains HGR16 and HGR 30 showed maximum siderophore production at 400mM, HGR5 and HGR8 at 600mM and five isolates *viz.* HGR1, HGR4, HGR7, HGR12, and HGR18 at 800mM. One isolate *viz.* HGR23 showed maxium siderophore production at 1000mM. This study suggests that increased salt concentration increased siderophore production over controls.

The maximum siderophore production recorded during this study is 90 μg/mL^{-1} and it occurred at 600mM salt concentration in two isolates *viz.* HGR5 and HGR8. Later, siderophore production decreased with increasing salt concentrations. The strain HGR23 showed maximum production of 80.7 μg/mL^{-1} at 1000mM salt concentration and later decreased. The siderophore production by the strains HGR1, HGR4, HGR7, HGR12 and HGR18 was very low at control. It slowly increased up to 800mM salt concentration (60.7 μg/mL^{-1}, 70.8 μg/mL^{-1}) and later decreased (Table 1). Statistical analysis revealed that there is no significance between salt concentrations and siderophore production.

The growth of the isolate and siderophore production at different salt concentratrions was compared for the strain HGR8 in which highest siderophore production was recorded (Fig. 1.). This isolate produced only 30.2 μg/mL^{-1} of siderophore in control without salt concentration,

Table 1 : Siderophore production at various salt concentrations by *Rhizobium* strains nodulating *M. uniflorum*. Siderophore production («g/mL^{-1})

Strain No.	Salt concentrations (mM)										
	Control	100	200	300	400	500	600	700	800	900	1000
HGR1	20.5	0.03	10.2	16.0	20.3	40.4	50.9	55.0	60.7	52.0	40.9
HGR4	20.5	0.06	5.6	8.8	10.9	40.2	60.8	66.3	70.8	65.0	60.8
HGR5	50.5	40.3	50.4	56.0	60.9	80.4	90.0	70.2	50.8	30.6	20.9
HGR7	0.5	60.4	70.2	70.2	70.2	70.4	70.4	70.4	70.8	65.0	66.8
HGR8	30.2	60.2	70.0	70.2	70.8	80.4	90.0	50.8	40.9	33.0	27.0
HGR12	8.0	50.6	50.9	56.8	60.4	64.6	66.6	68.5	70.8	60.2	53.0
HGR16	40.2	20.1	40.3	50.4	60.8	58.0	50.6	40.8	34.0	30.0	12.0
HGR18	40.5	30.9	40.1	50.2	60.0	64.0	66.0	68.4	70.8	60.2	50.8
HGR23	20.9	30.1	30.3	36.2	40.9	50.3	56.0	64.0	70.2	76.4	80.7
HGR30	17.0	50.3	56.4	60.2	66.5	45.0	38.2	27.4	22.0	22.0	22.7

The variation between rows (Ft=3.835; Fc=2.069) and columns (Ft=2.283; Fc=2.012) is also insignificant.

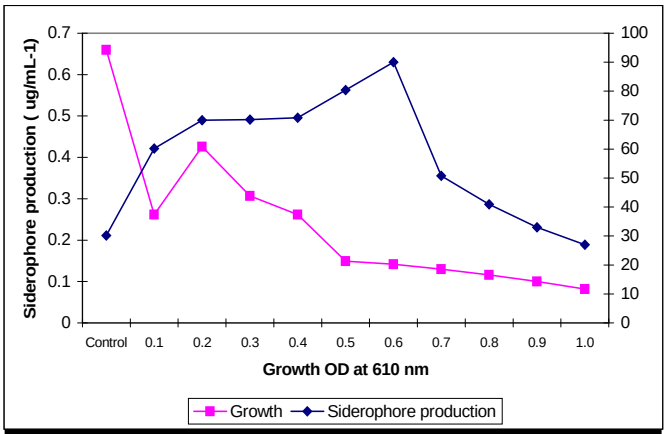

Fig. 1 : Effect of salt concentration on growth and siderophone production by the *Rhizobium* strain HGR8.

even though growth was maximum. The amount of siderophore production gradually increased with increase in salt concentration (90 $\mu g/mL^{-1}$) up to 600mM salt concentration. However, the growth of the isolate decreased gradually with increase in salt concentration. It showed that salt concentration enhanced siderophore production up to certain level even though growth showed decreased trend. Later, the growth and siderophore production decreased with increasing salt concentration. These *Rhizobium* strains showed maximum siderophore production under salt stress conditions over controls. Hence, these *Rhizobium* strains can be useful in reclamation of saline as well as in non saline soils.

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