Appendix

APPENDIX 1

Microbiological media composition

1. Luria Bertani Medium (Hi-media, India)

LB powder	2.5 g
D/w	100 ml

For preparation of solid medium, 2% agar was added to the media here and following prior to autoclaving and sterilized as above.

2. Pseudomonas Agar medium

Casein hydrolysate	1.0 g
Protease Peptone	1.5 g
K_2HPO_4	0.15 g
$MgSO_4.7H_2O$	0.15 g
Glycerol	1.5 ml

All components were dissolved in 100ml of distilled water and autoclaved.

3. Yeast Extract Mannitol agar (YEMA)

5% K2HPO4	1 ml
2% MgSO4.7H2O	1 ml
1% NaCl	1 ml
Mannitol	1 g
Yeast extract	0.1 g

All the components were dissolved in 100ml of D/w and autoclaved. Congo red (0.025g/L) was added immediately prior to pouring in sterile petri plates.

4. M9 minimal medium

10X M9 salt stock (100ml)

- Na_2HPO_4 6 g
- KH_2PO_4 3 g
- NaCl 0.5 g

- NH_4Cl_2 1 g

M9 broth composition

10X M9 stock	10 ml
1M glucose stock	10 ml
1M $MgSO_4$ stock	0.2 ml
1M $CaCl_2$ stock	0.01 ml

M9 stock solution made for final volume to be 100 ml using D/w and autoclaved. Stock solutions were autoclaved separately and added aseptically. For solid medium, agar was dissolved in 80ml D/w and autoclaved while the stock solutions were added aseptically and the media poured into sterile petri plates.

5. CAS medium

Piperazine-N,N′-bis(2-ethanesulfonic acid) (PIPES)	3.24 g
10X M9 stock	10 ml
Casamino acid solution	3 ml
1M glucose stock	10 ml
Blue dye	10 ml

PIPES was added to deferrated Ddw (65 ml), pH adjusted to 6.3 using (5 N HCl or NaOH], 2g agar was added to the medium and autoclaved. The remaining stock solutions were added along the sides of glass wall, while agitating and the mixture was poured into sterile petri plates.

6. Tris-Rock Phosphate (TRP) medium

Stocks to be prepared and autoclaved separately. (composition for 30ml TRP broth)

1M Tris-Cl (pH 8.0)	5 ml
1M NH_4Cl	1.33 ml
0.1M $CaCl_2$	0.3 ml
0.1M $MgSO_4$	0.3 ml
1M KH_2PO_4	0.009 ml
1M Glucose	3 ml

The glassware was soaked for acid wash, Senegal rock phosphate (1mg/ml) was added in Ddw, autoclaved separately and the above stock solutions were added to it aseptically.

7. Pikovskaya's agar medium (Hi-media, India)

Pikovskaya's agar	3.13 g
D/w	100 ml

8. HCN production

2.4 g Nutrient agar supplemented with 0.44g/L glycine in 100ml D/w was used for HCN production.

9. Ammonia production

Peptone	1 g
NaCl	0.5 g

Dissolved in 100 ml D/w, pH adjusted to 7.2 ± 0.2.

10. Potato Dextrose Agar (PDA)

3.9g of Potato Dextrose agar was suspended in 100ml of distilled water and autoclaved at 15 lb for 15 min.

Reagents

The reagents were stored at room temperature (RT) unless specified.

IAA quantification

Salkowski's Reagent: 2 ml of 0.5M $FeCl_3$ was mixed with 49 ml distilled water and 49 ml of 70% perchloric acid.

Siderophore production

- For deferration of 100 ml media, 1g 8-hydroxyquinoline was dissolved in 50 ml chloroform (for immediate use only). This was followed by chloroform wash (twice) to remove traces of hydroxyquinoline.

  All the stock solutions prepared below were deferrated before autoclaving.

- 1M Glucose stock (sterile) – 18 g D-Glucose dissolved in D/w and autoclaved at 10psi for 10min.
- 25 g of NaOH was dissolved in 150 ml of ddH_2O.
- Casamino acid solution: 3 g of casamino acid hydrolysate dissolved in 27 ml of Ddw and filter sterilized (stored at 4°C).
- CAS Blue dye

  Solution 1: Dissolve 0.06 g of CAS in 50 ml ddH_2O.

  Solution 2: Dissolve 0.0027 g of $FeCl_3.6H_20$ in 9 ml of 10mM HCl.

  Solution 3: Dissolve 0.073 g of HDTMA in 40 ml ddH_2O.

Solutions were mixed in the same order and autoclaved.

Siderophore characterization

0.1M $FeCl_3$ dissolved in 0.1N HCl was used for the development of TLC plate.

Biofilm Estimation

Sterile PBS Buffer (10X)

NaCl	16 g
KCl	0.4 g
$Na_2HPO_4.7H_2O$	2.3 g
KH_2PO_4	0.4 g

Dissolve in Ddw, make the volume up to 200 ml and autoclave. Store at RT

1% Crystal Violet

Crystal violet	0.5 g
Methanol	5 ml

The dye was dissolved in methanol, the volume made up to 50 ml using D/w and kept on shaker overnight for dissolution. The dye concentration was diluted $1/10^{th}$ immediate before use with sterile PBS.

HCN production

Picric acid solution: 0.5% picric acid dissolved in 2% sodium carbonate.

Ammonia production (Nesseler's reagent)

Potassium iodide (KI)	2.5 g
$HgCl_2$	1.25 g
NaOH	2 g

KI was dissolved in least possible amount of D/w (10ml). A saturated solution of $HgCl_2$ (20ml) was added to it until excess was indicated by formation of ppt. 10ml of 5M NaOH was added and the volume made up to 50ml using D/w.

DNA Extraction

TES buffer (50ml)

1M Tris-Cl	1.25ml
0.5M EDTA	2.5ml
1M Sucrose	15ml
Ddw	31.25ml

The components were autoclaved separately and mixed under sterile conditions.

10% SDS

1g SDS dissolved by inverting gently in 10ml sterile D/w and stored at RT.

10% CTAB

1g Cetyltrimethylammonium bromide dissolved in 10ml of 0.7M NaCl solution.

Alkaline lysis – Plasmid DNA extraction (miniprep)

Alkaline Lysis Solution (ALS) – I

1 M Tris base (pH 8.0)	0.5 mL (v/v)
0.5 M EDTA (pH 8.0)	0.4 mL (v/v)
Glucose	0.18g (w/v)

The components were mixed & the volume made up to 20 mL using D/w & autoclaved at 15 lbs. for

20 mins.

ALS – II (freshly prepared & used at room temperature RT)

10 N NaOH	8 mL (v/v)
10 % SDS	2 mL (v/v)

The components were mixed & volume made up to 20 mL using D/w & autoclaved as above.

ALS – III

5 M K-acetate 12 mL (v/v)

Glacial Acetic acid 2.3 mL (v/v)

The components were mixed & volume made up to 20 mL using D/w & autoclaved as above.

Potassium phosphate buffer (pH 7.6)

KH_2PO_4 (1M stock)	2.72g / 20ml D/w
K_2HPO_4 (1 M stock)	3.48g / 20ml D/w

Plant Extract Buffer (PEB - 100ml)

1M KH_2PO_4	1.6ml
1M K_2HPO_4	2.1ml
D/w	96.3ml
PVPP	2g/L
4mM EDTA	40mg

Anthrone's reagent

Anthrone's powder (Merck, India)	2g
Concentrated H_2SO_4	100ml

The Anthrone's reagent was added to concentrated acid and stored in dark, freshly prepared before use.

Bradford's reagent

Solution I

Coomassie Blue G250	50mg
Methanol	50ml

Solution II

85% H_3PO_4	100ml

Components from solution I were dissolved to mix, Solution II added to solution I. 500ml D/w added to the above mixture and filtered to remove the precipitates. The solution was made up to 1L and stored in a cool place in dark.

Proline Estimation

Solution I

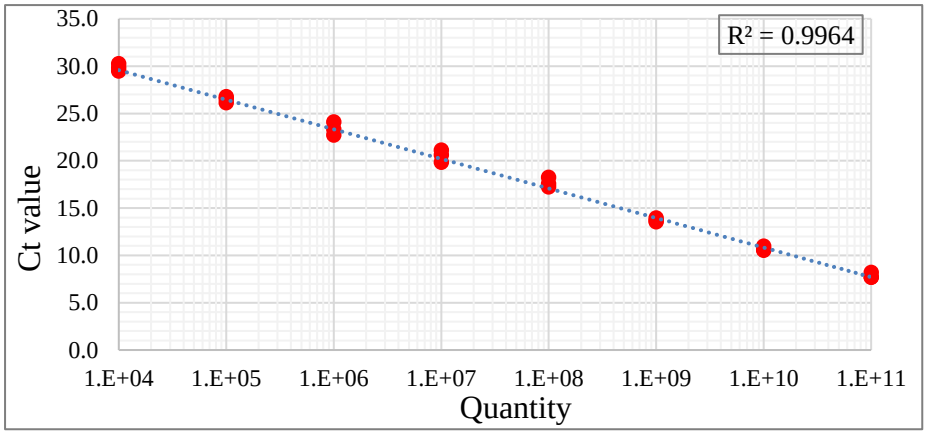
100ml 3% Sulphosalicyclic acid (HiMedia Labs)	5ml
Solution II - Acid Ninhydrin reagent	10ml
Ninhydrin (SRL Pvt. Ltd, India)	2.5g
Glacial acetic acid	30ml
6M Orthophosphoric acid	
Solution III – Glacial acetic acid	10ml

The components of solution II were mixed by gentle vortex in water bath (60°C). All the solutions were mixed immediately before use and maintained in a cool place.

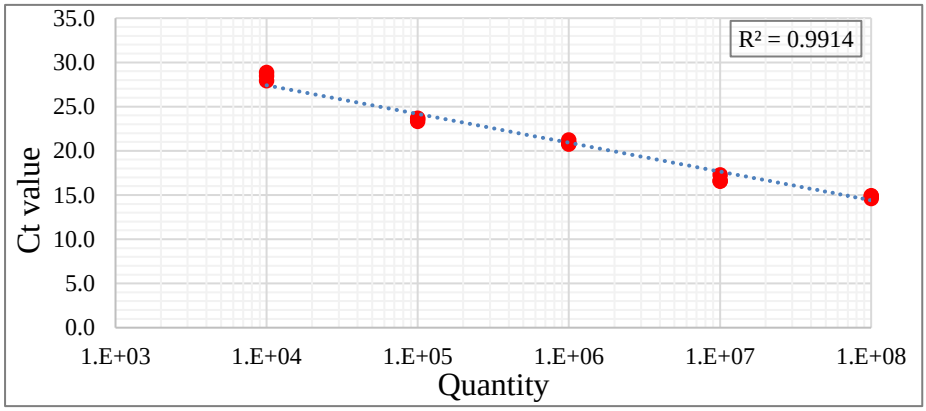
APPENDIX II

Q-PCR Studies – Ct value graph for copy number determination

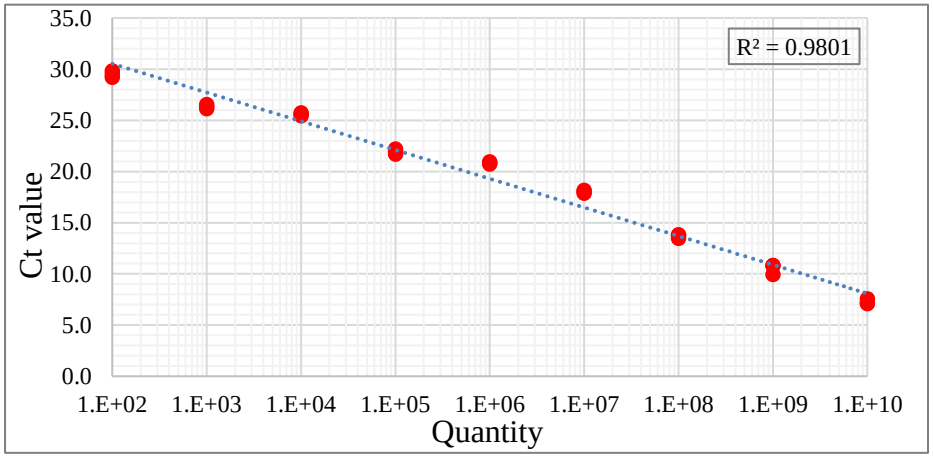
***Enterobacter* sp.**



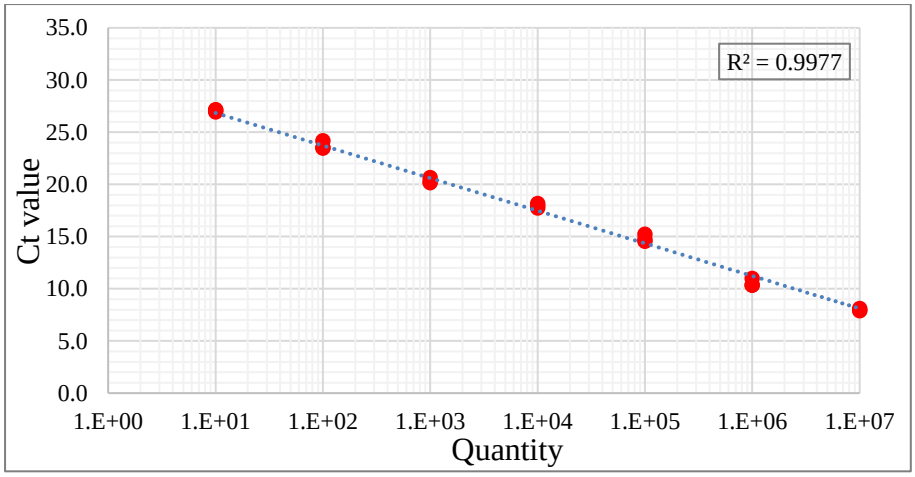
***Pseudomonas* sp.**



***Rhizobium* sp.**

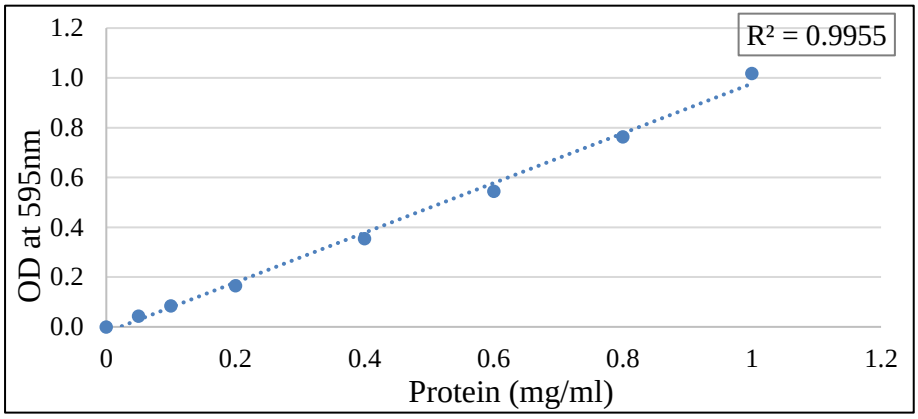


NGR234

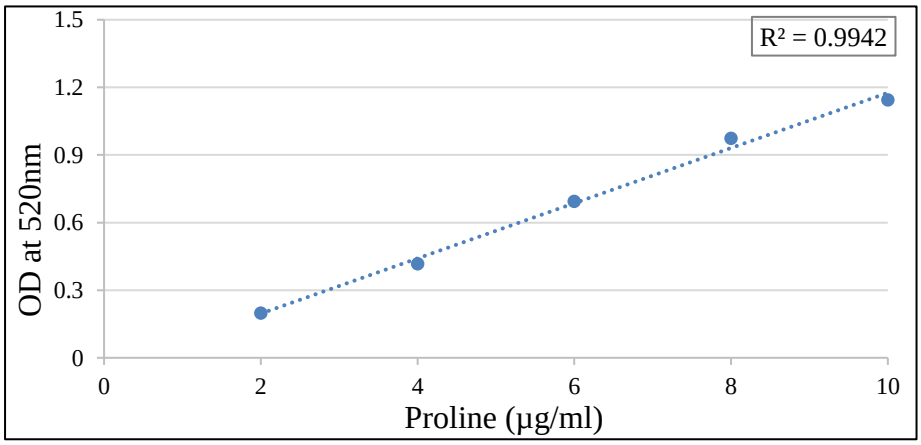


Standard Graphs

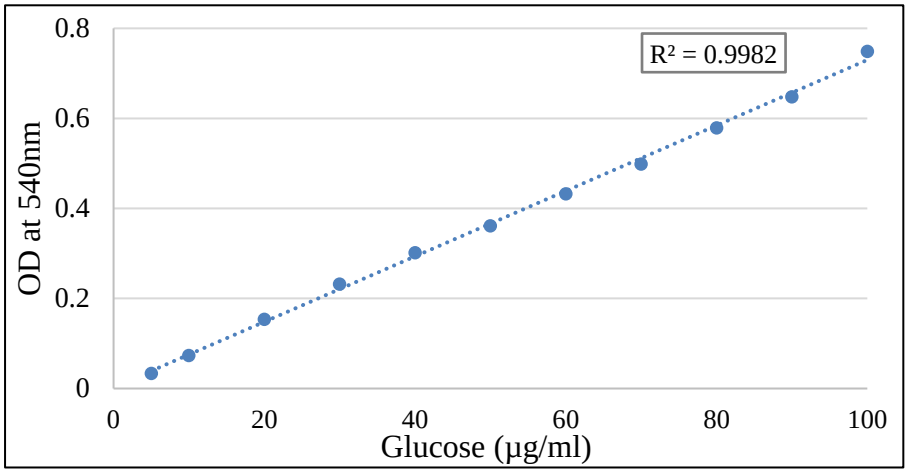
Total Protein



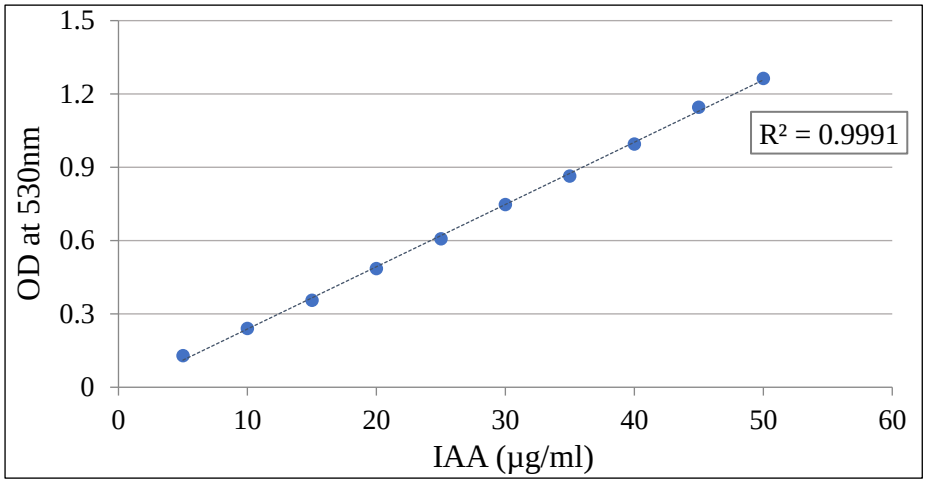
Proline



Total sugars



IAA Production



APPENDIX III

Table A1: Shoot/Root growth parameters in plants treated with individual strains under salt stress.

		Salt (%)	PGPR Consortia								
			C1S	C2S	C3S	C1I	C2I	C3I	C1N	C2N	C3N
Shoot parameters	Length (cm)	0	16.76 ± 2.70	17.07 ± 2.29	16.50 ± 2.67	16.00 ± 2.16	18.29 ± 3.25	18.44 ± 3.48	12.91 ± 2.59	16.39 ± 2.38	17.33 ± 3.20
		0.1	15.63 ± 2.28	14.00 ± 3.84	11.13 ± 2.30	11.13 ± 1.04	16.15 ± 2.85	16.00 ± 2.09	9.50 ± 2.11	9.36 ± 1.50	10.50 ± 2.07
		0.2	9.30 ± 2.45	11.82 ± 1.33	7.00 ± 7.07	7.00 ± 2.56	9.61 ± 3.66	8.00 ± 3.79	3.50 ± 1.78	6.50 ± 2.43	6.36 ± 3.20
		0.3	4.05 ± 1.23	5.38 ± 2.90	3.75 ± 3.35	3.75 ± 1.40	8.19 ± 2.11	8.50 ± 3.52	0.00	0.00	0.00
	Dry weight (mg)	0	830.0	854.0	441.3	225.0	693.2	891.0	593.5	639.0	289.2
		0.1	740.0	738.0	265.0	250.0	698.3	613.4	364.6	375.0	285.7
		0.2	372.0	332.0	41.1	41.1	270.0	143.5	96.2	124.5	108.1
		0.3	98.0	140.0	29.5	29.5	203.0	61.5	0.0	0.0	0.0
Root Parameters	Length (cm)	0	4.50 ± 0.94	4.38 ± 0.59	5.63 ± 0.744	6.50 ± 1.91	6.29 ± 0.95	5.07 ± 0.62	5.09 ± 1.14	5.29 ± 1.04	5.17 ± 0.98
		0.1	3.34 ±0.04	3.93 ±0.90	4.06 ±0.15	4.06 ±1.94	4.25 ±0.97	3.95 ±0.65	4.17 ± 0.25	3.88 ± 0.25	3.33 ± 0.00
		0.2	2.54 ±0.62	2.09 ±0.70	2.50 ±0.71	2.50 ±0.95	3.86 ±0.38	2.40 ±0.55	1.36 ± 0.63	1.67 ± 0.52	3.00 ± 0.82
		0.3	1.54 ± 3.24	1.50 ± 0.30	1.50 ± 5.25	1.50 ± 0.14	3.22 ± 1.27	3.00 ± 2.25	0.00	0.00	0.00
	Dry weight (mg)	0	455.5	467.0	410.9	119.0	298.0	392.6	340.0	484.2	302.0
		0.1	514.0	1037.3	983.1	600.0	402.8	598.0	667.0	934.2	1027.5
		0.2	746.8	418.0	202.4	202.4	555.6	490.0	807.0	604.6	900.0
		0.3	1419.0	546.0	154.9	154.9	618.0	305.0	0.0	0.0	0.0

Table A2: Shoot/Root growth parameters in pants treated with consortia under salt stress.

		Salt (%)	No PGPR	Individual strains						
				EC1D	Pf-5	PG22	PG38	RST1	RIC3109	NGR234
Shoot parameters	Length (cm)	0	18.45 ± 1.97	16.08 ± 2.35	18.53 ± 2.00	17.50 ± 2.24	16.88 ± 2.83	14.75 ± 3.37	17.05 ± 3.20	16.43 ± 1.45
		0.1	14.77 ± 2.17	13.75 ± 3.72	12.95 ± 3.48	10.95 ± 2.33	12.18 ± 2.79	14.78 ± 2.91	13.30 ± 2.67	12.92 ± 3.48
		0.2	10.25 ± 2.25	8.45 ± 5.09	8.70 ± 6.94	5.44 ± 2.79	6.75 ± 3.85	5.40 ± 1.08	9.19 ± 3.54	8.88 ± 3.80
		0.3	0	8.44 ± 3.71	8.33 ±1.04	0.00	5.86 ± 2.34	9.20 ± 5.07	0.00	11.86 ± 3.67
	Dry weight (mg)	0	754	735.6	1097.0	600.0	1012.0	415.1	620.3	960.4
		0.1	737.2	536.5	434.5	333.0	529.5	509.0	536.7	581.2
		0.2	276.5	265.0	65.0	190.0	150.6	101.7	240.1	224.6
		0.3	0	192.7	43.5	0.0	96.0	109.1	0.0	290.6
Root Parameters	Length (cm)	0	5.64 ± 1.29	6.50 ± 1.51	4.83 ± 0.84	5.40 ± 1.12	5.56 ± 1.36	6.25 ± 1.28	4.68 ± 0.75	7.00 ± 1.18
		0.1	5.62 ± 0.96	7.10 ±1.60	3.15 ± 0.58	4.50 ± 0.85	3.41 ± 1.97	4.89 ± 1.05	4.61 ± 0.74	5.73 ± 1.01
		0.2	2.94 ± 0.68	3.56 ±2.07	2.63 ± 0.48	3.14 ± 1.07	2.56 ± 0.73	1.80 ± 0.84	3.29 ± 0.95	3.50 ± 1.87
		0.3	0.00	2.63 ± 1.30	2.33 ± 4.38	0.00	1.71 ± 4.27	2.80 ± 7.25	0.00	4.43 ± 7.31
	Dry weight (mg)	0	310.0	307.0	528.5	497.0	597.0	379.2	415.1	351.4
		0.1	563.0	516.0	727.5	463.6	649.2	652.3	226.7	377.4
		0.2	546.0	387.5	395.0	1015.0	1117.0	483.4	542.9	305.9
		0.3	0.0	636.2	170.0	0.0	1203.5	365.8	0.0	216.7