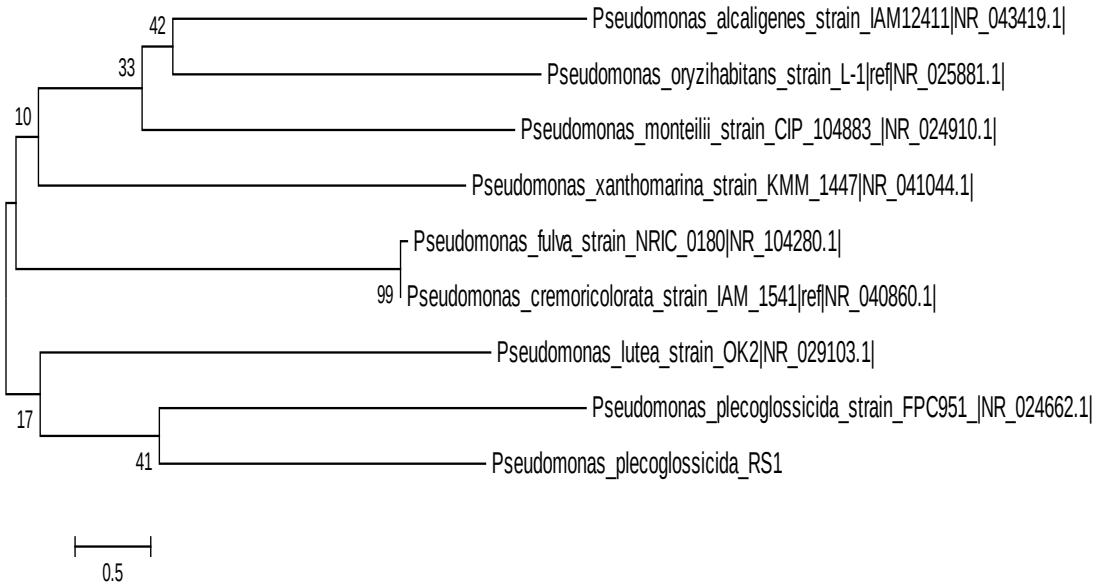
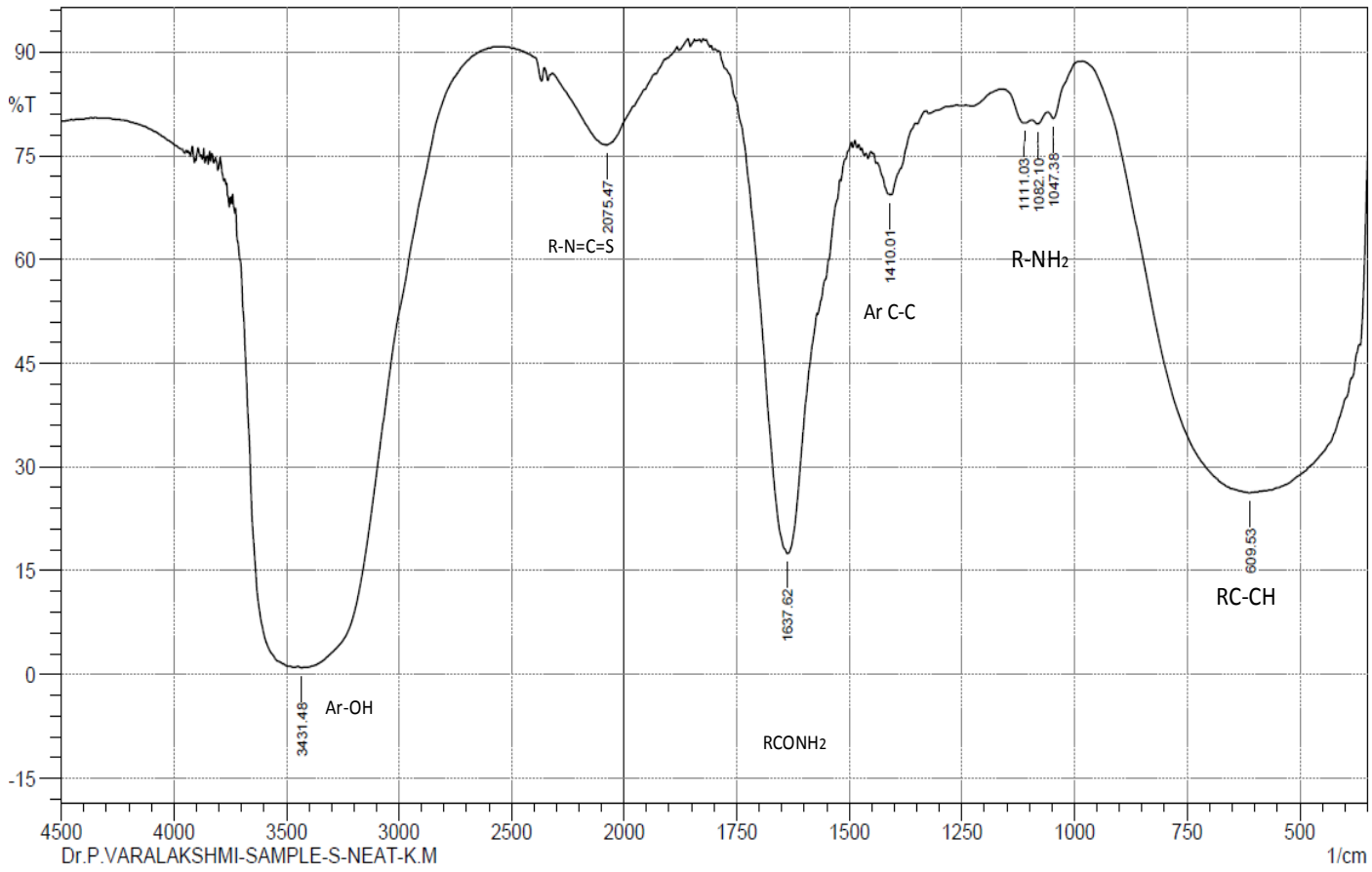
Electronic Supplementary Material (ESI) for RSC Advances.

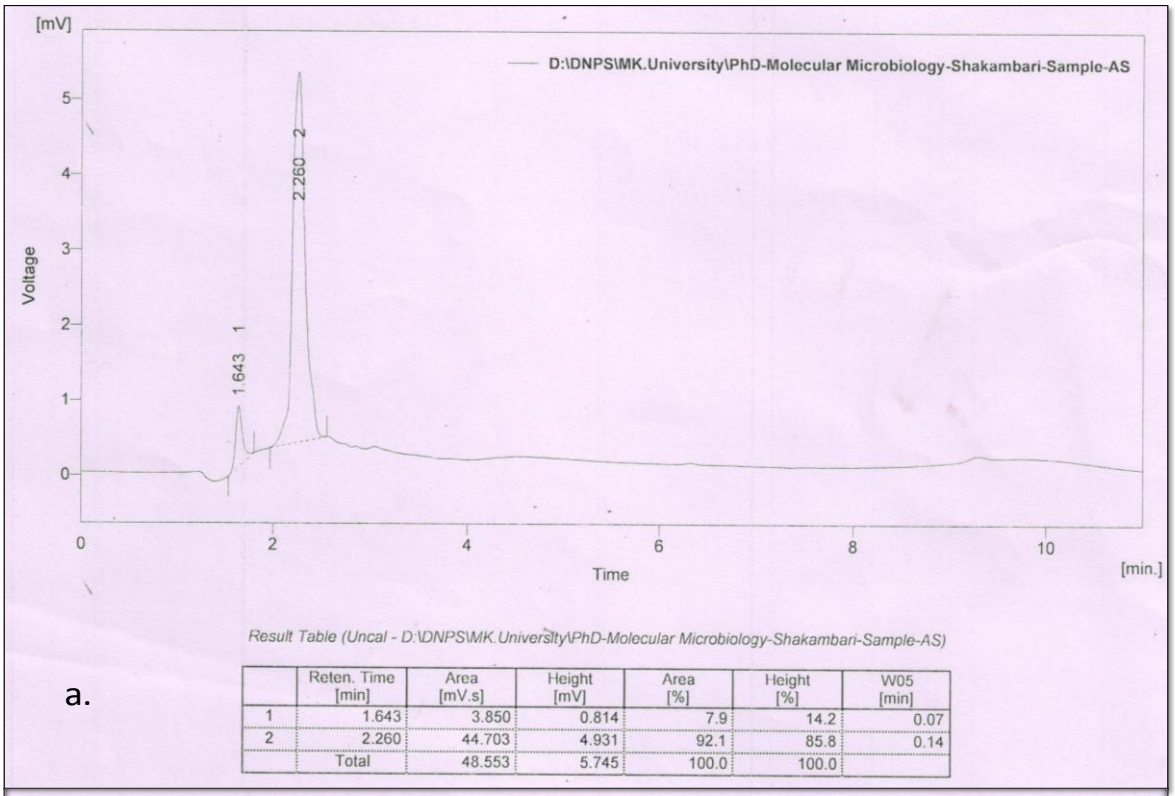


Supplementary Fig. A Phylogenetic analysis of *Pseudomonas pleccoglossicida strain RS1* using MEGA 6 software. Bootstrap analysis with 1000 replications showed its relation closer to *Pseudomonas pleccoglossicida strain FPC951*.



No.	Peak	Intensity	Corr. Intensity	Base (H)	Base (L)	Area	Corr. Area
1	609.53	26.268	0.441	650.03	576.74	42.311	0.303
2	1047.38	80.404	2.032	1058.96	983.73	5.357	0.008
3	1082.1	79.581	0.994	1095.6	1058.96	3.499	0.091
4	1111.03	79.709	1.472	1163.11	1095.6	5.84	0.155
5	1410.01	69.357	6.573	1438.94	1352.14	11.632	1.691
6	1637.62	17.461	46.171	1790	1572.04	76.386	40.726
7	2075.47	76.545	0.284	2079.33	1955.88	12.405	0.764
8	3431.48	0.959	0.141	3448.84	3421.83	53.654	0.86

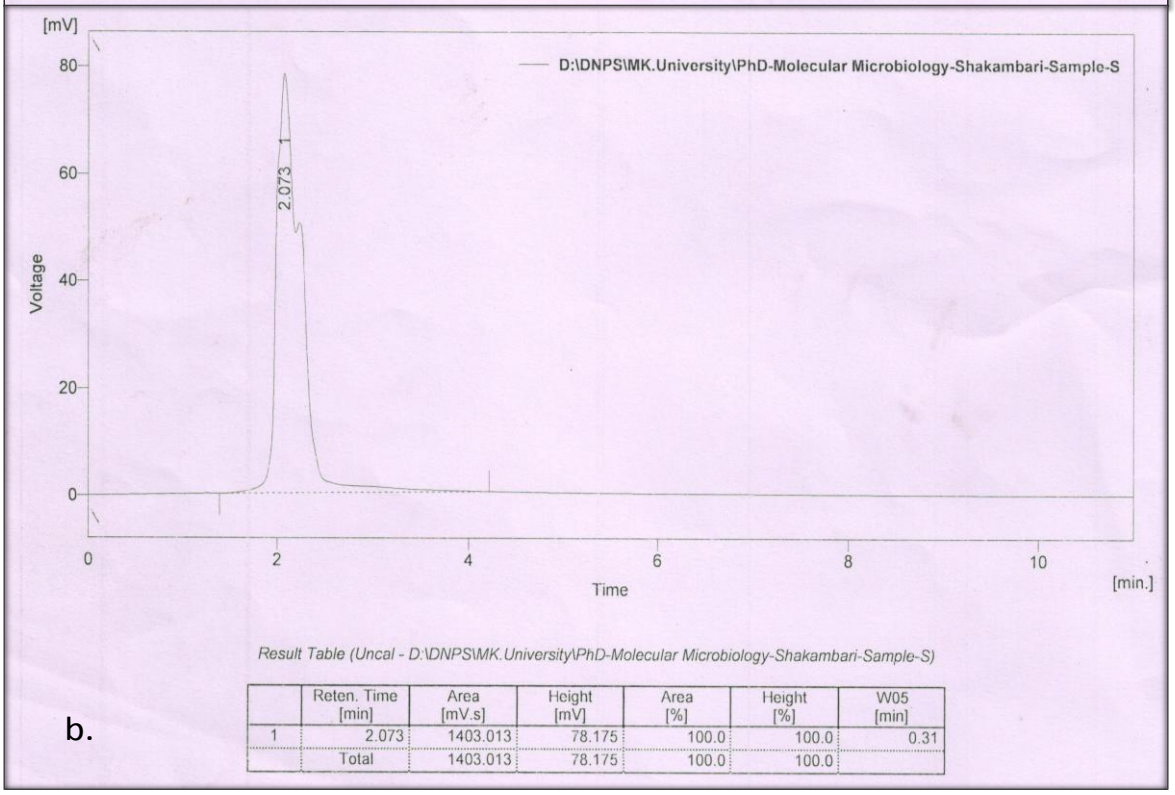
Supplementary Fig. B FT-IR analysis of effluent sample with evidence of organic nitrogen compounds.



Result Table (Uncal - D:\DNPS\MK.University\PhD-Molecular Microbiology-Shakambari-Sample-AS)

a.

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	1.643	3.850	0.814	7.9	14.2	0.07
2	2.260	44.703	4.931	92.1	85.8	0.14
	Total	48.553	5.745	100.0	100.0	

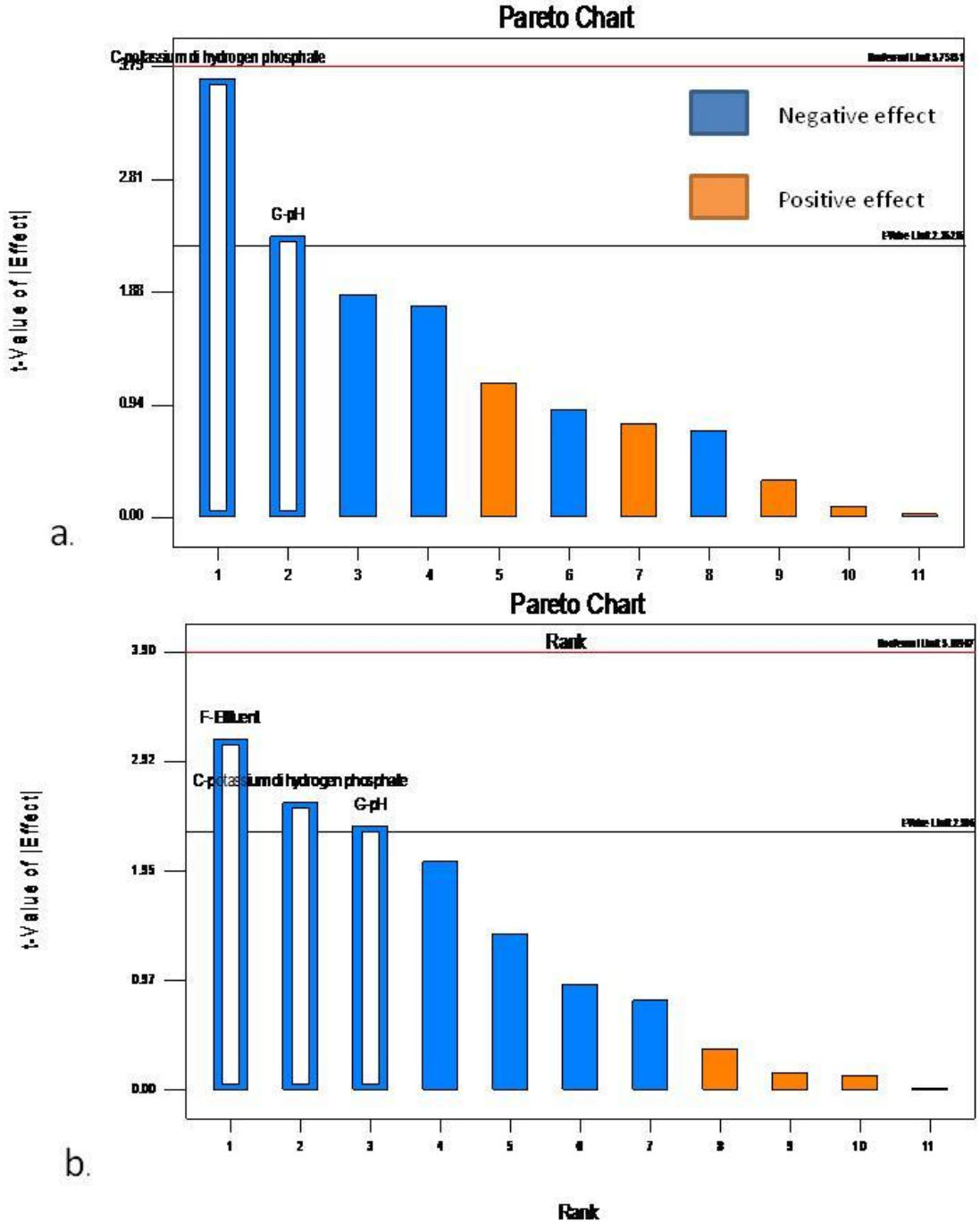


Result Table (Uncal - D:\DNPS\MK.University\PhD-Molecular Microbiology-Shakambari-Sample-S)

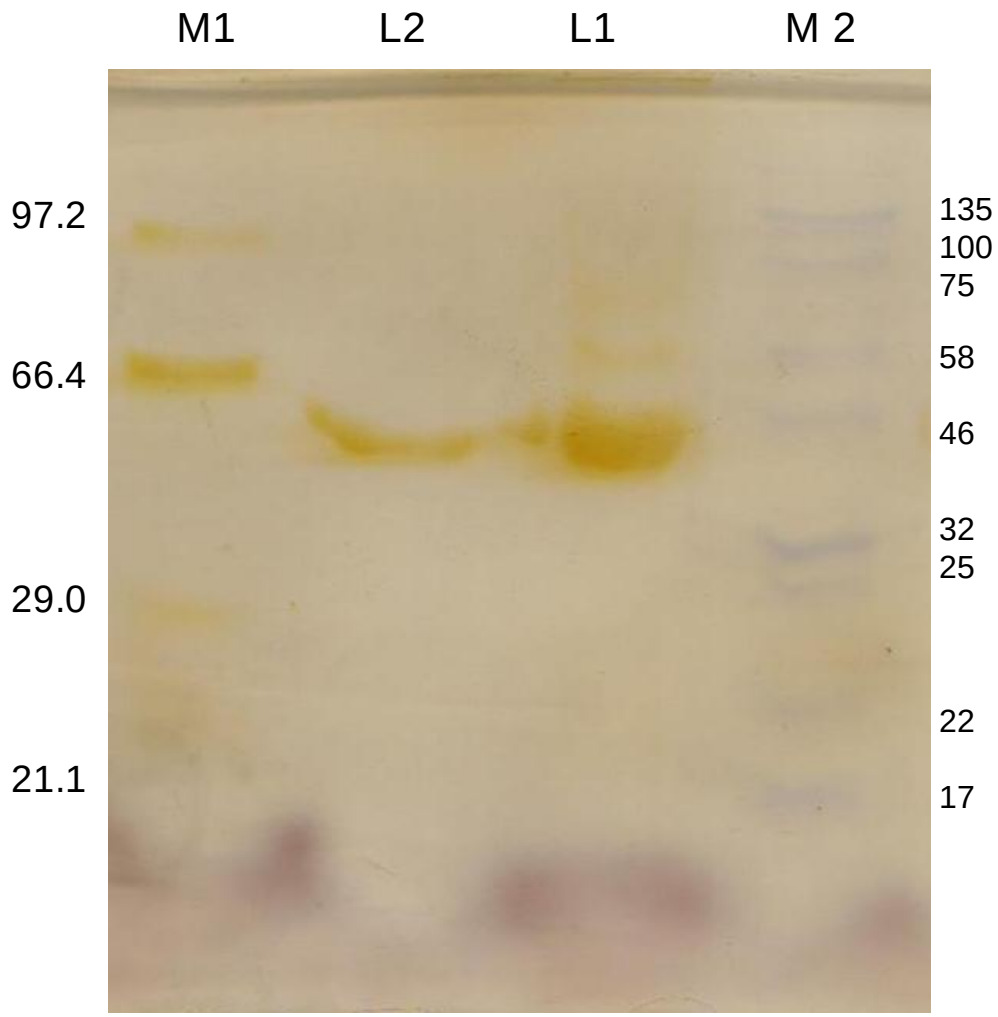
b.

	Reten. Time [min]	Area [mV.s]	Height [mV]	Area [%]	Height [%]	W05 [min]
1	2.073	1403.013	78.175	100.0	100.0	0.31
	Total	1403.013	78.175	100.0	100.0	

Supplementary Fig. C HPLC Analysis of a.L-Asparagine monohydrate (Himedia) conc. 0.5mg/mL methanol, b. Effluent sample (diluted 1:10) in methanol.

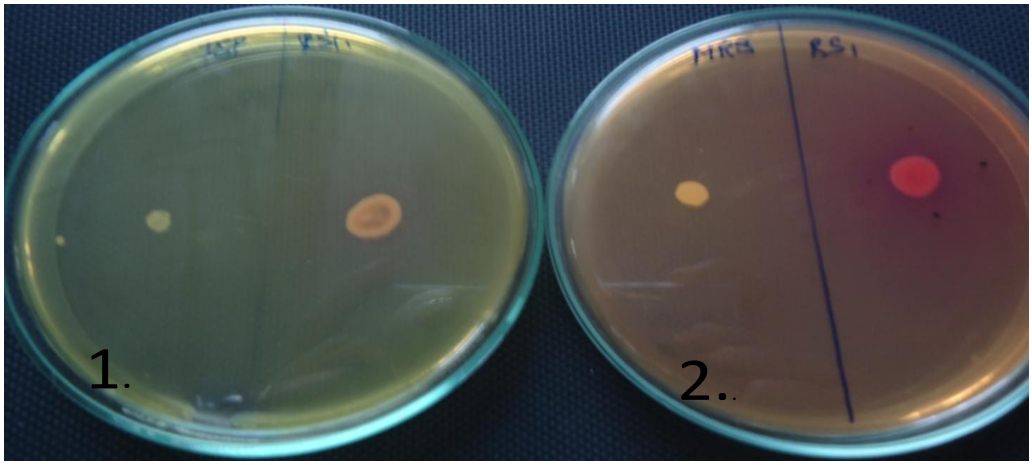


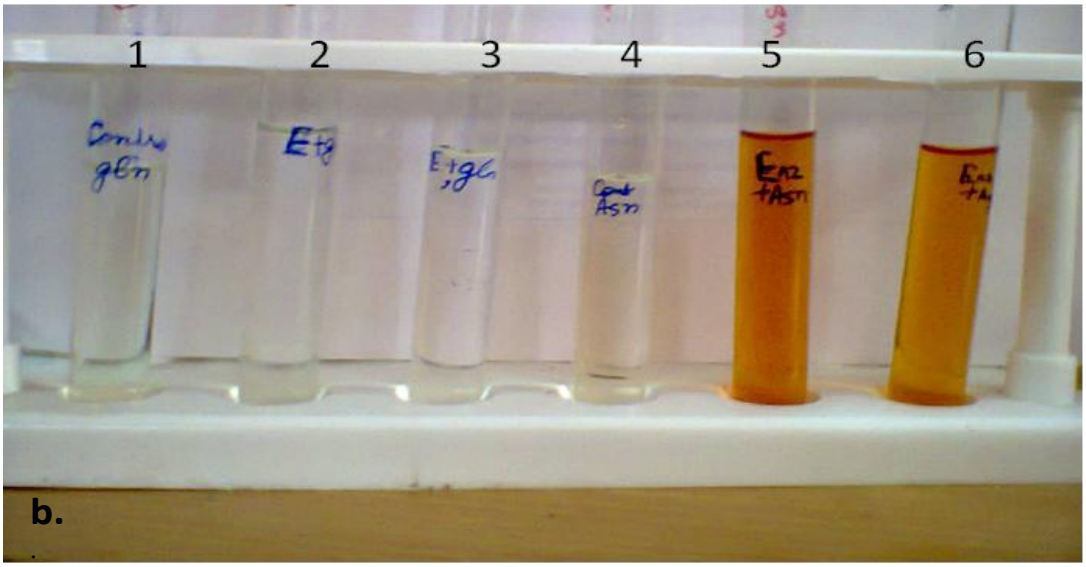
Supplementary Fig. D Paretos Chart of the effects of the factors on the L-asparaginase production using sugar industry effluent as substrate in M-9 medium, by *Pseudomonas plecoglossicida* strain RS1 in terms of a. Enzyme activity (IU/ml) and b. Specific activity (IU/mg)



SupplementaryFig.E Electrophoretic analysis of L-asparaginase produced by *Pseudomonas plecoglossicida* RS1: SDS PAGE on 12% resolving gel visualized with silver nitrate staining M1- Marker (Takara ,Japan- unstained low weight protein ladder)M2- Marker (NEB-P7706- pre-stained ladder) , L1- Crude enzyme, L2- Purified enzyme.

a.





Supplementary Fig. F. **a.** Glutaminase free activity of L-asparaginase produced by *Pseudomonas plecoglossicida* RS1on M-9 medium was verified by plate method (modified method of Gulati et al.)with 1. L-glutamine as substrate and 2. L-asparagine as substrate. Pink colour developed is indicative of alkaline condition produced due to enzymatic liberation of ammonia, which was seen in L-asparagine containing plate and not in L-glutamine containing plate.

b. Glutaminase free L-asparaginase activity of the purified enzyme by direct nesslerization. Tube 1- Enzyme blank (L-glutamine without enzyme), 2 &3 purified L-asparaginase with L- glutamine substrate, showing no ammonia liberation when tested with nesslers reagent, indicating negative reaction for L-glutaminase activity. Tube 4- Enzyme blank (L-asparagine without enzyme), 5& 6 purified L-asparaginase with L-asparagine as substrate, showing ammonia liberation when tested with nesslers reagent, indicating positive activity for L-asparaginase.

I.

II. Effect of pH and temperature for L-asparaginase production by *Pseudomonas pleccoglossicida* strain RS1

Condition		Enzyme activity (IU/mL/min)	Protein content (mg/mL)	Specific activity (IU/mg)
pH	6	0.09 ± 0.02	0.34 ± 0.1	0.454 ± 0.3
	6.5	1.89 ± 0.09	1.62 ± 0.19	1.15 ± 0.15
	7	1.03 ± 0.02	0.56 ± 0.03	1.87 ± 0.1
	7.5	0.006	0.80 ± 0.4	0.0075
	8	0	1.10 ± 0.06	0
Temperature (°C)	25	0.39 ± 0	3.24 ± 0.04	0.12 ± 0.02
	37	1.83 ± 0	1.611 ± 0	1.18 ± 0.25
	50	0	0	0

II.

Characterization of sugar cane industry effluent

No.	Property	Units
1	pH	4.85
2	Total Solids	0.1g/ml
3	Total Reducing Sugar (DNSA method)	0.43±0.03mg/ml
4	Total protein (Folin Lowry method)	40.66±3.8mg/ml
5	Total viable count (before complete sterilization for media purpose)	$0.8^{*}\ 10^{13}$ cfu
6	Biological Oxygen Demand	76.6mg/L
7	TLC of 1:10 effluent for amino acids Standard L-Asparagine	Rf=0.84 Rf=0.86

Supplementary Table III

Plackett Burman Design Summary

No.	Parameter	Central value	Low values (-1)	High values (+1)
A	Glucose (%w/v)	0.3	0.2	0.4
B	Na_2HPO_4(%w/v)	0.6	0.5	0.7
C	KH_2PO_4(%w/v)	0.3	0.2	0.4
D	$MgSO_4$ (%w/v)	0.05	0.04	0.06
E	NaCl (%w/v)	0.05	0.04	0.06
F	Effluent (%v/v)	1	0.8	1.2
G	pH	6.8	6.5	7.1

No.	Parameter	Central value	Low values (-1)	High values (+1)	(-α)	(+α)
A	KH_2PO_4(%w/v)	0.3	0.2	0.4	0.13	0.47
B	Effluent (%v/v)	1	0.8	1.2	0.66	1.34
C	pH	6.8	6.5	7.1	6.3	7.3

Central Composite Design Summary