Hydrogen Cyanide Production Ability by *Pseudomonas Fluorescence* Bacteria and their Inhibition Potential on Weed Germination

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Abstract

This research was undertaken for the purpose of isolation and purification of indigenous Pseudomonas spp. and evaluating its ability in hydrogen cyanide synthesis and also evaluating the potential of super-strains on seedling growth inhibition in weeds. According to this, the research was carried out in laboratory tests. 136 strains (obtained from rhizosphere soil of 62 weed species) and 27 strains of Pseudomonas fluorescence were sub-cultured, purified and refreshed. Then these strains were evaluated for the capability in cyanide synthesis by a qualitative method and at last 4 super-strains of cyanogenic Pseudomonas were selected and used in further experiments. The effects of these strains on stem length, root length and stem length/root length rate in rye, wild barley, and wheat were evaluated in three different in vitro tests examining the effects of gas and liquid metabolites produced by the bacteria. The results showed that the abundance and probability of the bacteria isolation was low (about 3.6%). About 37 percent of Pseudomonas isolates were capable of HCN production and this capacity was different among the strains. Gas metabolites reduced more than 90 percent of root and shoot growth in weeds. In this case gas metabolites had greater inhibitory effects rather than other metabolites on plants. However these influences were different in every bacteria treatment. Also wheat had less growth reduction in comparison with weeds which probably mean that the bacteria are plant specific. In conclusion, achievements proved that cyanogenic pseudomonas fluorescence had the potential of biological weed control. However the further studies about their application in natural conditions like greenhouse, and field seem to be necessary.

Introduction:

Weeds cause more economic losses in agricultural lands. Currently, the most effective means of managing weeds are herbicides (6). Some widely used herbicides have been implicated in contamination of groundwater, soils, and food products, which may threaten public health and safety (6).

Today, biological methods have known as effective and appropriate ways in weed control. One group of microorganisms largely overlooked as biocontrol agents of weeds include the Deleterious Rhizobacteria (DRB) that can colonize plant root surfaces and able to suppress plant growth (11). Many DRB are plant specific (9). A major group of rhizobacteria with potential for biological control is the *Pseudomonades* (6). A secondary metabolite produced commonly by rhizosphere *pseudomonads* is Hydrogen Cyanide (HCN), a gas known to negatively affect root metabolism and root growth (10). Begonia proved that the *pseudomonads* isolated from velvetleaf (*abutilon theophrasti*) roots were able to reduce velvetleaf viability and emergence significantly. Also in other experiment *pseudomonas fluorescent* inhibited bean growth by cyanide production (2). This project aimed to investigate the qualitative and quantitative capability of rhizobacteria isolates in HCN production and determining the super strain effects on root and shoot growth of Rye grass (*Secale cereale*) , Wild Barley (*Hordeum spontaneum Koch)* and also wheat (*Triticum aestivum*) under in vitro condition.

Method and materials:

Collection of weeds and bacteria isolation:

Seedling and mature weeds species were collected. Standard microbioligical methods were applied to isolate bacteria from the rhizosphere and rhizoplane (obtained from rhizosphere soil of 62 weed species) using nutrient agar (NA) medium. 136 non-identified isolated and stored 4◦c.

Assessment of HCN potential:

Qualitative cyanide determination were carried out by Lorck method (7) modified by Alstrom (2). Isolates sub cultured on NA medium were supplemented with glycine (4/4 gl-1). The production of cyanide was detected 48h after inoculation, using picrate/Na2Co3 paper fixed to the under side of the Petri-dish lids which where scaled with parafilm before incubation at 28◦c. A change from yellow to orange, red, brown, or reddish brown was recorded as an indication of weak, moderate, or strongly cyanogenic potential, respectively. Then, cyanogenic isolates were determined and identified. Among those, 4 pseudomonas fluorescent strains were selected for further experiments.

Effects of *p. fluorescent* volatile metabolites on root and shoot growth:

Pre-germinated surface sterilized wild barely, rye and wheat seeds (10 per plate) were placed equidistantly apart on the surface of 1/0% agar. Rhizobacterial isolates were streaked on NA medium containing glycine and incubated for 24h. After incubation each inoculated plate was paired with a plate containing the pre-germinated seeds (2).

Effects of *p. fluorescent* liquid metabolites on root and shoot growth:

A: 30µl of bacterial inoculations (the suspension of bacteria, nutrient broth medium, and glycine) were added to the pre germinated and surface sterilized seeds which were placed on the surface of 1/0% agar (1).

B: cultures of each isolated strain of bacteria, grown for one day in nutrient broth medium (amended with glycine) were centrifuged at 10000 rpm for 6 min and 2ml of supernatant was added to the surface of 9/0% water agar plates. Fifteen pre germinated surface sterilized seeds of each weed species and wheat were then placed on each plate (8).

Plates in all experiments were sealed with Parafilm and incubated in the dark at 27◦c. Controls were considered for each experiment. Each isolate was tested in four replicates. After 48 hours the root and shoot length were measured. Data were analyzed using a one-way ANOVA. .

Results and discussions:

The results of qualitative test of HCN production showed that low percent of isolated bacteria were capable of producing HCN (about 3.6%). Further tests indicated that the HCN producing isolates were belonging to *Pseudomonas florescent*. 10 *Pseudomonas* strains could produce HCN in different levels (out of 27 strains). Due to the results of semi-quantitative test of HCN, 4 *P. florescent* super strains were identified as high HCN producers.

Analysis of the gas metabolite data showed that isolated bacteria significantly reduced the root and shoot length of weeds ($P<0.01$). According to the table1, root and shoot growth of weed seedlings were inhibited by volatile metabolites during the growth of cyanogenic *Pseudomonas* in the paired-plate assemblies. This growth reduction in root length was 17 to 93.5% and in shoot length was 28 to 93% in comparison with those of controls.

Table 1-Mean comparison of studied characters in wheat, rye grass And wild barley effected by volatile metabolites produced by different *Pseudomonas fluorescent* strains

		studied characters			
	Bacterial treatments	root length	% reduction	shoot length	% reduction
Wheat	*P. fluorescent* 459	23.16 c	45.55	22.84 d	37.18
	P. fluorescent 460	37.80 a	4.82	45.64 a	-25.52
	P. fluorescent 467	26.16 b	32.85	25.40 c	35.14
	P. fluorescent 27-2	17.28 d	55.65	16.92 e	53.47
	Control*	38.96 a	0	36.36 b	0
	LSD 1%	1.996		1.757	
Rye grass	*P. fluorescent* 459	0.72 b	33.76	0.59 d	87.69
	P. fluorescent 460	0.75 b	74.12	0.79 b	78.08
	P. fluorescent 467	0.59 c	84.07	0.60 d	87.52
	P. fluorescent 27-2	0.63 c	81.86	0.71 c	82.62
	Control*	1.28 a	0	1.39 a	0
	LSD 1%	0.0495		0.0549	

Wild Barley	*P. fluorescent* 459	1.65 d	92.52	1.46 c	92.39
	P. fluorescent 460	5.12 b	28.63	4.82 b	17.75
	P. fluorescent 467	1.63d	93.73	1.39 c	93.10
	P. fluorescent 27-2	1.80 c	91.29	1.48 c	92.11
	Control*	6.07 a	0	5.32 a	0
	LSD 1%	0.1346		0.2081	

*Inoculant without bacteria is used in control

Inhibition of seedling growth by cyanide has been previously reported by researches of Adam and Zdor (1), and Begonia and Kremer (4). Kremer and Souissi (5) have reported growth inhibition of lettuce and barnyard grass by volatile metabolites of the cyanogenic rhizobacteria confirmed that HCN was the major inhibitory compound produced.

Table 2-Mean comparison of studied characters in wheat, rye grass And wild barley effected by liquid metabolites (method A) produced by different *Pseudomonas fluorescent* strains

		studied characters			
	Bacterial treatments	root length	% reduction	shoot length	% reduction
Wheat	*P. fluorescent* 459	27 a	-32.14	37.32 a	-10.81
	P. fluorescent 460	24.4 b	-9.62	34.32 b	-1.90
	P. fluorescent 467	20.16 c	1.34	26 d	22.80
	P. fluorescent 27-2	28.04 a	-37.23	27.52 c	18.29
	Control*	23.52 b	0	33.68 b	0
	LSD 1%	1.699		1.516	
Rye grass	*P. fluorescent* 459	23.08 b	1.78	16.88 b	38.12
	P. fluorescent 460	26.80 a	-14.04	17.16 b	37.10
	P. fluorescent 467	17.04 c	27.49	17.24 b	36.81
	P. fluorescent 27-2	17.04 c	27.49	13.80 c	49.41
	Control*	27.20 a	0	27.28 a	0
	LSD 1%	1.631		1.297	
Wild Barley	*P. fluorescent* 459	18.36 c	12.29	25.36 a	-0.16
	P. fluorescent 460	18.36 c	12.29	23.80 b	6
	P. fluorescent 467	19.5 b	6.56	24.76 ab	2.20
	P. fluorescent 27-2	19.64 b	6.17	17.68 c	30.17
	Control*	24.12 a	0	25.32 a	0
	LSD 1%	1.278		1.44	

*Inoculant without bacteria is used in control
The negative symbol shows an increase in the studied character caused by the strains

table 3-Mean comparison of studied characters in wheat, rye grass And wild barley effected by liquid metabolites (method B) produced by different *Pseudomonas fluorescent* strains

		studied characters			
	Bacterial treatments	root length	% reduction	shoot length	% reduction
Wheat	*P. fluorescent* 459	16.40 b	11.26	26.6 a	0.30
	P. fluorescent 460	13.92 c	24.68	25.56 a	1.20
	P. fluorescent 467	12.52 d	32.25	25.92 a	2.85
	P. fluorescent 27-2	17.92 a	3.02	25.92 a	2.85
	Control*	18.48 a	0	26.68 a	0
	LSD 1%	1.235		1.512	
Rye grass	*P. fluorescent* 459	14.70 b	18.26	17.36 b	11.61
	P. fluorescent 460	13.6 b	24.28	16.92 b	13.85
	P. fluorescent 467	8.64 c	51.89	13.16 d	32.99
	P. fluorescent 27-2	14.12 b	21.38	15.44 c	21.39

	Control*	17.96 a	0	19.64 a	0
	LSD 1%	1.093		1.278	
Wild Barley	*P. fluorescent* 459	7.36 e	70.61	10.56 d	60.83
	P. fluorescent 460	13.28 c	46.97	18.76 c	30.42
	P. fluorescent 467	9.28 d	62.94	28.12 ab	-4.30
	P. fluorescent 27-2	16.08 b	35.78	26.84 b	0.44
	Control*	25.04 a	0	26.96 ab	0
	LSD 1%	1.50		1.652	

*Inoculant without bacteria is used in control

The negative symbol shows an increase in the studied character caused by the strains

The results revealed that growth inhibition of wild barley by liquid metabolites was statistically significant (tables2 and 3). Liquid metabolites showed more inhibitory effects in the second method, in non-existence of bacteria. This result indicated that bacteria presence in soil can adjust inhibitory effect.

In addition, the results of *in vitro* tests revealed that gas metabolites had greater inhibitory effects rather than other metabolites on weeds (table1,2 and 3). Kremer and Suissi (5) have reported similarly result. They explained that HCN produced by bacteria in contact with root surfaces may be rapidly dispersed, degraded, or deactivated more so than if roots were exposed to high levels of HCN. This result confirms that despite of cyanogenic bacteria existence in soil weeds are not controlled.

Conclusion

In conclusion, we can declare that:

- Cyanogenic rhizobacteria might have the potential of biological weed control.
- Despite of similar cyanogenic levels, *Pseudomonas* strains showed different growth inhibitory effects on weeds. Strains 467 and 27-2 performed the most and strain 460 showed the least growth inhibition. Probably this difference is caused by different metabolites produced by bacteria.
- The effect of each strain was different due to weed species and method. Growth inhibition was the most in gas metabolite.
- Wheat was influenced less than the weeds, but the growth reduction of about 50% in gas metabolites and 30% in liquid metabolites may cause worse impact on wheat in comparison with those of weed existence in field.
- The effectiveness order of various tests in weed growth inhibition was:

Gas metabolite> Liquid metabolite B > Liquid metabolite A

References:

1. Adam, O., and R. Zdor. 2001. Effect of cyanogenic rhizobacteria on the growth of velvetleaf (*Abutilon theophrasii*) and corn (*Zea mays*) in autoclaved soil and the influence of supplemented glycine. Soil. Biol. Biochem. 33: 801-809.
2. Alstrom, S., and R.G. Burns. 1989. Cyanide production by rhizobacteria as a possible mechanism of plant growth inhibition. Biol. Fertil. Soils. 7: 232-238.
3. Bakker, A.W. and B. Schippers. 1986. Microbial cyanide production in the rhizosphere in relation to potato yield reduction and *Pseudomonas spp.* -Mediated plant growth-stimulation. Soil. Biol. Biochem. 19: 451-457.
4. Begonia, M.F.T., and R.J. Kremer. 1994. Chemotaxis of deleterious rhizobacteria to velvetleaf (*Abutilon theophrasti* Medik.) seeds and seedlings. FEMS. Microbiol. Ecol. 15: 227-236.
5. Kremer, R. J. and T. Souissi. 2001. Cyanide production by rhizobacteria and potential for suppression of weed seedling growth. Curr. Microbiol. 43 : 182-186.
6. Kremer, R. J., and A. C. Kennedy. 1996. Rhizobacteria as biocontrol agents of weeds. Weed Technol. 10: 601-609.
7. Lorck, H. 1948. Production of hydrocyanic acid by bacteria. Physiol. Plant. 1: 142-146.
8. O'hara, Gr. 2005. Biological control of weeds in vineyards. Murdoch University press. West Australia.
9. Schippers, B., A.W. Bakker, and P.A. Bakker. 1987. Interaction of deleterious and beneficial rhizosphere microorganisms and the effect of cropping practices. Annu. Rev. Phytopathol. 25: 339-358.
10. Schippers, B., Bakker, A., Bakker, P., and van Peer, R., 1990. Beneficial and deleterious effects of HCN-producing pseudomonads on rhizosphere interactions. Plant and Soil. 129:75-83.
11. Suslow, T.V., and M.N. Schroth. 1982. Role of deleterious rhizobacteria as minor pathogens in reducing crop growth. Phytopathol. 72: 111-115.