

ORIGINAL PAPER

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Liquid-culture pH, temperature, and carbon (not nitrogen) source regulate phenazine productivity of the take-all biocontrol agent *Pseudomonas fluorescens* 2-79

Received: 12 July 1994 / Received revision: 8 October 1994 / Accepted: 22 November 1994

Abstract Strain 2-79 is a biocontrol agent against take-all, an important disease of wheat caused by *Gaeumannomyces graminis* var. *tritici*. In the rhizosphere, it produces the antibiotic phenazine 1-carboxylic acid (PCA) as the primary means of disease suppression. One barrier to commercial use of phenazine-producing pseudomonads, like strain 2-79, is the lack of liquid-culture technology for mass production. For instance, there is little published research concerning the impact of liquid-culture secondary metabolism on the biocontrol qualities of the cell harvest, i.e., efficacy, phytotoxicity, and storage survival. Yet it is important to know whether the fermentation process should be designed to enhance or eliminate secondary metabolite accumulation. To enable future exploration of this issue, we identified liquid-culture parameters that could be manipulated to control the phenazine productivity of strain 2-79. Our results indicated that PCA accumulation was very sensitive to the culture pH and temperature. It was possible to produce large cell populations with either high or low phenazine productivity by choosing to control culture pH at 7 and 8 respectively. Although high cell accumulations were achieved over the broad 25–34°C range studied, high, moderate, or low PCA productivities were observed at 25–27°C, 29–32.5°C, or 34°C respectively. When pH was controlled at 7, specific PCA productions at 25°C could be modulated by the choice of carbon source supplied. PCA accumulation per unit

biomass reached 0.31 g/g on glucose, 0.16 g/g on glycerol and xylose, and only 0.09 g/g on fructose. Although the nitrogen source was also tested as a variable, it had little influence on culture PCA productivity under controlled pH.

Introduction

Take-all is the most significant root disease of wheat world-wide, and there is no satisfactory commercial product available for its control (Cook and Veseth 1991). However, fluorescent pseudomonads, which have been implicated in the natural biological control phenomenon known as take-all decline, have captured the attention of many researchers because of the potential for developing these bacteria as seed inocula for disease control. When introduced with seed, the pseudomonads can colonize the roots and limit primary infection and/or subsequent secondary spread of the target pathogens (Weller and Cook 1983; Weller 1988; Thomashow and Weller 1990). Secondary metabolism involving phenazine production has been shown to play an active role in disease suppression by pseudomonads. The classic strain *Pseudomonas fluorescens* 2-79, originally isolated from a wheat field in spontaneous take-all decline, produces the antibiotic phenazine 1-carboxylic acid (PCA), which is active against several soil-borne fungal pathogens including the causative agent of take-all, *Gaeumannomyces graminis* var. *tritici* (Weller and Cook 1984; Gurusiddaiah et al. 1986; Brisbane et al. 1987). Phenazine-mediated antibiosis has been shown to be the primary mechanism by which strain 2-79 suppresses take-all in the rhizosphere (Thomashow and Weller 1988; Thomashow et al. 1990; Bull et al. 1991). The *P. aureofaciens* strain 30-84 has since been discovered to produce three phenazines (PCA primarily and smaller amounts of 2-hydroxyphenazine-1-carboxylic acid and

Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

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2-hydroxyphenazine), and in studies paralleling those of 2-79, it too has been shown to suppress take-all via phenazine antibiosis (Thomashow et al. 1990; Mazzola et al. 1992; Pierson and Thomashow 1992). A number of other phenazine-producing soil bacteria have been reported (Haynes et al. 1956; Toohey et al. 1965a; Messenger and Turner 1983), and screening methods targeting phenazine-producing strains for root disease biocontrol have been discussed and used (Thomashow and Weller 1990; Korth et al. 1990).

Commercialization of *Pseudomonas* inocula for take-all control requires the development of appropriate liquid-culture technology for mass production. Preliminary studies, reviewed in Bothast et al. (1993), have linked the quality of biocontrol agent formulations to metabolic activities occurring during the preceding liquid-cultivation step. The specific role of liquid-culture phenazine productivity in the performance of resulting pseudomonad formulations is of particular interest with respect to development of take-all biocontrol agents, but is as yet unknown. Liquid-culture growth conditions are expected to influence phenazine production (Messenger and Turner 1983), and the ability to manipulate culture phenazine productivity is expected to impact the design of commercial-scale fermentation and formulation processes that optimize biocontrol agent quality, i.e., efficacy, phytotoxicity, and storage survival. In the research presented here, our objective was to identify liquid-culture environmental conditions that could be manipulated to control PCA productivity of *P. fluorescens* strain 2-79 in fermentation processes. Experiments were devised to study the dependence of phenazine productivity on temperature, pH, and sources of carbon and nitrogen—variables commonly manipulated to control fermentation yields and costs. The carbon and nitrogen sources chosen were typical components of industrial media (Zabriskie et al. 1980). Culture responses were analyzed in terms of both cell and PCA accumulations to identify conditions that caused variations in secondary metabolism while high cell yields were conserved. The outcome of these experiments will aid future investigations concerning the impact of liquid-culture phenazine productivity on biocontrol agent quality.

Materials and methods

Stock cultures

Lyophilized *Pseudomonas fluorescens* 2-79 was obtained from the Agricultural Research Service Patent Culture Collection (National Center for Agricultural Utilization Research, USDA Peoria, Illinois) where it is deposited as NRRL B-15132. Glycerol stocks were stored at -80°C (Slininger and Jackson 1992) and used to seed nutrient broth/yeast extract (NBY) slants (prepared according to Vidaver 1967) for preculture inocula.

Media

Flask culture studies

Sources of carbon and nitrogen were varied in a defined medium, which contained the following ingredients per liter: 1 g each of buffers K_2HPO_4 and KH_2PO_4 , 0.01 g each of purines and pyrimidines adenine, cytosine, guanine, uracil, thymine, 0.5 mg each of vitamins thiamine, riboflavin, calcium pantothenate, niacin, pyridoxamine, thioctic acid, 0.05 mg each of vitamins, folic acid, biotin, and B_{12} , 0.1 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.01 g NaCl , and 0.01 g $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ (Zabriskie et al. 1980). Details of the medium preparation have been given in a related paper (Slininger and Jackson 1992). Amino acids (15 g Difco vitamin-free casamino acids, 0.15 g tryptophan, 0.6 g cysteine) and nitrogen-source equivalents (1.26 g/l urea, 3.57 g/l sodium nitrate, 2.77 g/l ammonium sulfate) were supplied on the basis of 0.042 M amino nitrogen. The amino nitrogen content of the vitamin-free casamino acids averaged 6.5% with a range of 4%–9% observed from lot to lot (personal communication with Nancy J. Laskowski, Technical Service Representative, Difco Laboratories, Detroit, Michigan). Our 0.042 M amino nitrogen basis corresponds to 15.75 g/l casamino acids with an amino nitrogen content of 4% by weight. The carbon content of the casamino acids was 50% by weight.

Fermentor studies

In order to accentuate potential biomass and PCA accumulations and increase the dynamic range of variation in fermentor experiments, the mineral and growth-factor components of the above medium were optimized in studies built around the findings of Slininger and Jackson (1992). The buffer strength was also doubled to reduce pH shifts. The optimized fermentor medium composition per liter was as follows: 2 g each of K_2HPO_4 and KH_2PO_4 , 0.03 g cytosine, 0.01 g adenine, 0.01 g thymine, 4.4 mg $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 11.0 mg $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 10 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 2 mg $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 2.4 mg H_3BO_3 , 0.05 g/l EDTA, plus the same vitamins, Mg^{2+} , Na^+ , and Fe^{2+} source concentrations as above, and C and N sources as specified. Concentrated stock solutions of buffer, $\text{Mg}^{2+}/\text{Na}^+$, Fe^{2+} , trace minerals, purines/pyrimidines, and vitamins were prepared to compose 0.8%, 0.2%, 1.0%, 1.5%, 0.6%, and 2% of the total medium volume, respectively. These were each added gradually with stirring to the fermentor, which contained 83.9% of the total medium volume as HCl-acidified water (pH 2–3). After the fermentor contents had been sterilized for 15 min at 121°C , a filter-sterilized carbon/nitrogen source stock solution, comprising the remaining 10% of the medium volume, was added to the vessel, and the medium pH adjusted to 7 by addition of 4 M NaOH. Note that iron and trace mineral stocks were prepared on the day of use and that concentrated purines and pyrimidines could be dissolved with HCl acidification and heating. Hodag FD-62 silicone-based antifoam was added initially at 1 g/l, and then a 50% (w/v) solution was supplied automatically as required to control foam.

Culture conditions

Precultures

Bacteria were transferred by loop from NBY slants to 125-ml flasks containing 50 ml medium like that used in subsequent experimental cultures. Flasks were closed with Bellco silicone sponge plugs (no. 1924). All precultures were shaken at 250 rpm (2.5 cm eccentricity) during incubation at 25°C in a New Brunswick Psychrotherm. Cells from 24-h precultures were pelleted, separated from broth, resuspended to 4.1 g/l in buffer (1 g/l each K_2HPO_4 , KH_2PO_4), and used

to inoculate all experiment cultures to 0.041 g dry biomass/l (0.1 absorbance unit).

Flask culture studies

In the presence of either urea or amino acids as nitrogen source, carbon source and concentration were varied in 200-ml cultures shaken at 150 rpm. In all cases duplicate cultures were carried out in 500-ml flasks with Bellco silicone sponge caps (no. 2004).

Fermentor studies

Controlled-pH studies of carbon and nitrogen source utilization for growth and PCA production were carried out in B. Braun Biostat 2ER fermentors operated at 25°C with a 2-l working volume, 1000 ml/min air flow, and 300 rpm agitation rate (providing an oxygen transfer coefficient of 0.37 min⁻¹). Fermentor pH was monitored using an Ingold probe and could be maintained within ± 0.1 unit of each set point by the automated dosing of either 6 M NaOH or 2 M HCl. Product concentration data were corrected to account for additive dilutions which ranged from 0 to 10% of the working volume. The influence of temperature on metabolism was examined in fermentors controlled at pH 7. During logarithmic-growth studies, dissolved oxygen was maintained in excess at 95% saturation using an Ingold polarographic probe and controlled to actuate agitation and stirring changes.

Sampling and analyses

Time courses of substrate, biomass, PCA, and pH were monitored via twice-daily sampling. Studies of the initial growth rate required hourly or half-hourly absorbance measurements during the first 10 h of culture progress.

Biomass

Dry weight accumulation was determined by filtering and drying known volumes of culture on preweighed, dry cellulose nitrate filters. Filter disks were dried at 100°C for 1.5–2.5 h until constant weight ± 0.0005 g. Dry weight concentrations (*b*) were linearly correlated with culture absorbance at 620 nm (*A*); i.e., $b = kA$, where $k = 0.408$ g/l with correlation coefficient $r^2 = 0.992$, provided that samples were diluted to give readings of 0.05–0.5 absorbance unit. Absorbance contributions from fluorescent pigments were negligible.

Specific growth rate

Logarithmic growth was observed between 0 and 10 h after inoculation, and the specific rate was calculated from the slope of the ln(*b*) time course, $d[\ln(b)]/dt$.

Carbohydrates

Samples were centrifuged, passed through a 0.45-μm syringe filter, and stored at -20°C until analysis. Carbohydrate concentrations were measured using a Waters high-performance liquid chromatography (HPLC) system equipped with a refractive index detector, and a BioRad HPX-87H Aminex ion-exclusion column. The column was operated at room temperature with acidified water (0.85 mM H₂SO₄) as mobile phase.

Phenazine 1-carboxylic acid (PCA)

Whole-culture samples (2.5 ml) were adjusted to pH 4.0 with 1 M HCl and then extracted twice into methylene chloride (1.25 ml each). After the pooled solvent layers had been evaporated at 25°C overnight, PCA residues were reconstituted in 2.5 ml 5% NaHCO₃ and assayed using a previously described reversed-phase HPLC procedure (Thomashow et al. 1990; Slininger and Jackson 1992).

Results

pH and temperature optima

Specific growth rate and biomass accumulation remained optimal over relatively broad pH ranges of 7–8 and 6–8 respectively (Fig. 1). In contrast, PCA accumulation reached a narrow optimum of 0.92 g/l PCA and then abruptly fell an order of magnitude to less than 0.09 g/l at pH 8. Similarly, the temperature optima for growth rate and biomass production were broad, ranging from 25°C to 32.5°C and 34°C respectively, though PCA production dropped to 0.03 g/l as the temperature was raised to 34°C. The 29–32.5°C range provided an intermediate level of PCA accumulation averaging 0.3 g/l. These data indicate that it is possible

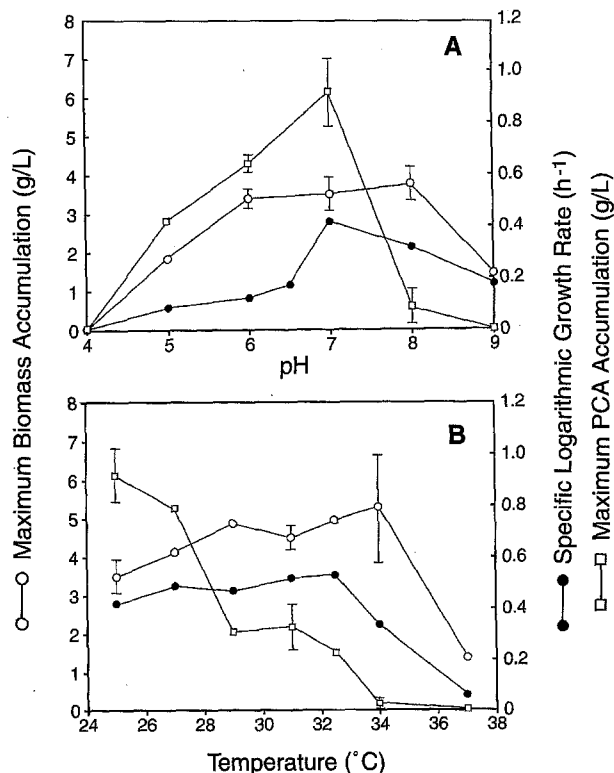


Fig. 1A, B Optimum pH (A) and temperature (B) for growth and phenazine 1-carboxylic acid (PCA) accumulation in acid/base-controlled fermentors supplied with 20 g/l glucose and 0.042 M N as urea. Error bars standard deviations in data taken from multiple batches—two at 25°C pH 6, seven at 25°C pH 7, six at 25°C pH 8, and four at 31°C and 34°C pH 7

to design fermentations to produce large cell populations with high, moderate, or very low PCA productivities depending on the choice of temperature and pH.

Nitrogen sources and carbon-to-nitrogen ratio

Given sufficient glucose, flask cultures accumulated similar concentrations of biomass and antibiotic regardless of whether nitrogen was supplied as amino acids or urea (Fig. 2). When urea was supplied, it functioned only as a source of nitrogen and not of carbon, such that no growth occurred unless carbohydrate was added (Fig. 2B, C). Casamino acids served as a source of both carbon and nitrogen, supplying 7–8 g/l carbon. In control cultures with no added carbohydrate, casamino acids supported moderate growth and low-level (0.02 g/l) PCA production (Fig. 2A, C). Maximum productions with complete sugar utilization were accomplished by adding 10 g/l glucose to cultures in amino acid media or 20 g/l to those in urea media. The difference in carbohydrate addition required for optimal productions on the two nitrogen sources was consistent with the carbon contributed by the amino acids. The combination of 20 g/l glucose and 1.26 g/l urea represents the stoichiometric molar carbon:nitrogen ratio of 16:1. The optimal glucose-casamino acids combination corresponded to an estimated molar C:N ratio of 10–22:1, which is consistent with the glucose:urea ratio but less exact owing to the uncertainty in usable nitrogen content. Both cell and PCA yields were optimized by the same C:N ratio. Although these results indicate that exogenous amino acids were not a strict requirement of cell and PCA production, they supported more rapid initial growth than urea at all C:N ratios tested (Fig. 2C).

Although observed pH shifts were similarly mild in flask cultures supplied with urea and amino acids (7 to 6 at C:N ≤ 16:1), this was not the case with the inorganic nitrogen sources tested. Sodium nitrate and ammonium sulfate resulted in pH shifts (to approx. 9 and approx. 5 respectively) that were severe enough to influence product accumulations significantly. This dependence of pH shift pattern on nitrogen source is common

(see for example, Zabriskie et al. 1980). When pH was automatically controlled at 7 in fermentors, both urea and inorganic nitrogen sources supported similar growth rates and product yields (Table 1).

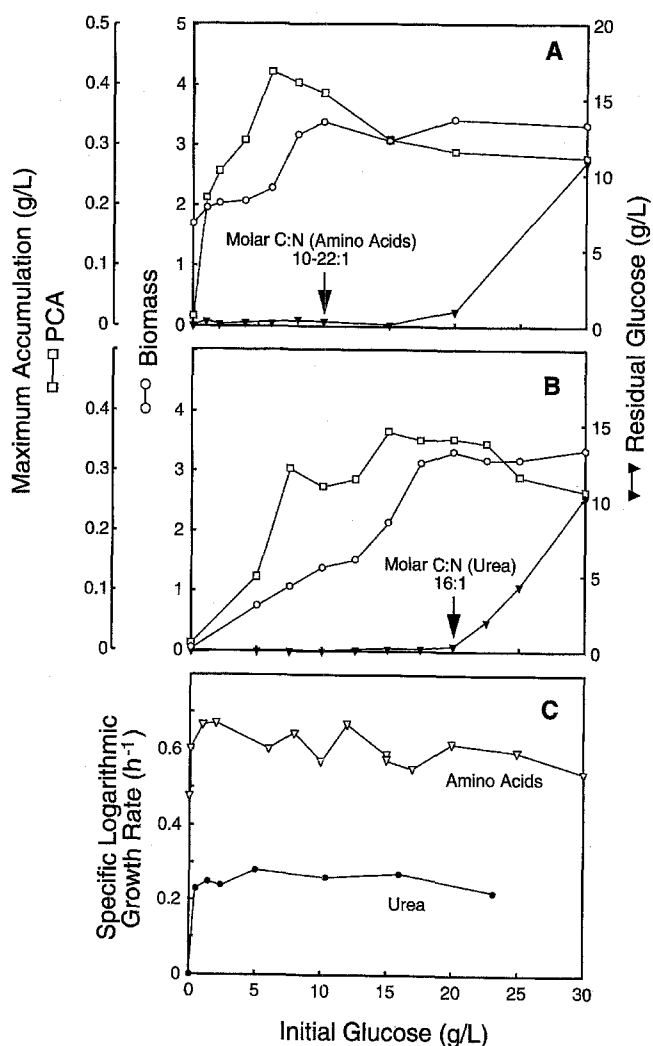


Fig. 2A–C Optimum C:N ratio for product accumulations and substrate utilization in flask cultures supplied with 0.042 M N as (A) amino acids or (B) urea. C Rapid growth advantage of amino acids over urea (initial pH 6.8 ± 0.1 in all cases)

Table 1 Minimal impact of nitrogen source on growth and secondary metabolism in fermentors supplied with 20 g/l glucose and controlled at pH 7, 25°C. All values indicate the standard deviation about the average measurements taken from duplicate cultures. PCA phenazine 1-carboxylic acid

Nitrogen source (0.042 M N)	24-h biomass (g/l)	Concentrations (g/l)			Specific production PCA/biomass (g/g)
		Maximum biomass	Maximum PCA	Residual glucose	
Urea	2.33 ± 0.53	3.21 ± 0.36	1.00 ± 0.15	0.0	0.31 ± 0.08
$(\text{NH}_4)_2\text{SO}_4$	2.51 ± 0.33	2.94 ± 0.39	1.11 ± 0.14	0.0	0.38 ± 0.10
NaNO_3	1.70 ± 0.43	2.98 ± 0.19	0.95 ± 0.11	0.0	0.32 ± 0.06

Table 2 Variation of PCA productivity with carbon source in fermentors supplied with 0.042 M N as urea and controlled at pH 7, 25°C. All values indicate the standard deviation about the average measurements of replicate samples taken from each culture. The residual carbon source concentration (g/l) was zero for all sources except glycerol and xylose which were in the range 0.5–2 g/l, and maltose which remained at 20 g/l

Carbon ^c source (20 g/l)	24-h biomass (g/l)	Maximum concentrations (g/l)		Specific production PCA/biomass (g/g)
		Biomass	PCA	
Glycerol	0.28	3.23 ± 0.33	0.54 ± 0.03	0.17 ± 0.03
Xylose	1.85	3.74 ± 0.37	0.57 ± 0.01	0.15 ± 0.02
Glucose ^a	2.33 ± 0.5	3.23 ± 0.36	1.01 ± 0.15	0.26 ± 0.06
Fructose	0.55	3.13 ± 0.36	0.29 ± 0.06	0.09 ± 0.03
Galactose	0.93	2.46 ± 0.22	0.74 ± 0.09	0.30 ± 0.06
Mannose	1.06	2.03 ± 0.22	0.38 ± 0.01	0.19 ± 0.02
Maltose	0.05	0.14 ± 0.04	0	0

^a Number reported corresponds to the average of seven runs

Carbon source

In flask cultures, strain 2-79 consumed significant amounts of all added carbon sources except the disaccharides maltose, lactose, and sucrose. Biomass and antibiotic accumulations varied with carbon source, but the assessment of relative performances was precluded by the variety of pH shifts that also occurred in these cultures. To limit pH variation and isolate carbon source effects on product formations, we controlled the pH at 7 in fermentors by doubling the buffer strength over that used in flask culture media and by automatically dosing with acid and base. Under these conditions, high cell concentrations (3–4 g/l) were obtained on various sources of carbon, including glucose, xylose, glycerol, and fructose (Table 2). Galactose and mannose supported moderate cell production at 2–2.5 g/l (Table 2). When pH was tightly controlled at 7, specific PCA productivities were highest on glucose and galactose (0.26 and 0.30 g PCA/g biomass respectively), intermediate on mannose, glycerol and xylose (0.19, 0.17, and 0.15 g/g, respectively), and lowest on fructose (0.09 g/g). Maltose utilization did not improve with pH control.

Discussion

Since the pH optimum for PCA productivity of strain 2-79 was sharp around 7, there was strong potential for phenazine accumulations to be influenced by even small uncontrolled pH shifts. Large shifts below pH 6 or above 8 were sometimes observed in flask cultures, and studies in pH-controlled fermentors allowed us to isolate the effects of carbon and nitrogen on product accumulations. Results from our pH-controlled culture studies showed that growth of strain 2-79 is optimized by a variety of sources of carbon (including glucose,

xylose, glycerol, and fructose) and of nitrogen (including amino acids, urea, ammonium, and nitrate) and by broad operating ranges of pH at 6–8 and temperature at 25–34°C. Within this set of conditions allowing prolific growth, specific selections of temperature, pH and carbon source can be used to control the PCA productivity of the cell population. Maximum PCA productivity is achieved on glucose at 25–27°C and pH 7. Process conditions leading to moderate PCA productivities include temperatures in the 29–32.5°C range, pH 6, or carbon sources such as glycerol or xylose. PCA productivity can be minimized by operating at 34°C or pH 8, or by providing fructose as carbon source. Just as for maximum biomass accumulation, the choice of nitrogen source did not significantly affect PCA productivity when pH was controlled at 7.

The dependences of cell growth and antibiotic production on environmental factors shown in this work and in previous mineral and growth factor investigations (Slininger and Jackson 1992) are consistent with the regional and seasonal variabilities of biocontrol efficacy that so often plague field studies. For example, the sharpness of the pH optimum for antibiotic production suggests a narrow pH range for optimal biocontrol efficacy. Seasonal and regional variations in rhizosphere temperature, composition (carbon, nitrogen, minerals), and pH could potentially have profound effects on the PCA productivity and take-all suppressiveness of strain 2-79. Ownley et al. (1992) have reviewed the interrelationships of soil composition and pH and have conducted studies of the influence of *in vitro* and *in situ* pH on suppression of *G. g. var. tritici* by a rifampicin-resistant derivative of 2-79 (-RN₁₀). On modified Kanner agar, they observed that inhibition of *G. g. var. tritici* hyphal growth by 2-79RN₁₀ was least at pH 4.9–5.8, greatest at pH 6–6.6, and falling from pH 6 to 8. These ranges roughly correspond with our findings of diminished growth and PCA production at pH values below 6, optimal growth and phenazine production at pH 6–7, and declining PCA production between pH 7 and 8. Certainly agar- and liquid-culture pH optima are not expected to be directly comparable because of the diffusion limitations in agar that are absent in well-mixed liquid cultures. In Ritzville silt loam, Ownley et al. found that soil pH did not appear to be a limiting factor in suppression of take-all by 2-79RN₁₀. Relevant to this finding, Thomashow et al. (1990) discuss how increases in root populations of strain 2-79 coincide with take-all lesion development and how proliferation of strain 2-79 on roots with lesions is thought to occur in response to nutrient leakage. Antibiotic produced in lesions colonized by 2-79 would then be likely to contact the pathogen and limit its progression. Thus the environmental conditions (pH, temperature, nutrients, carbon source) in the lesions and resulting exudate may be more significant in determining antibiotic productivity and the suppressiveness of 2-79 than the bulk soil conditions.

Aside from implications on the variability of take-all suppression observed in the field, the environmental methods of PCA regulation identified in the current and previous (Slininger and Jackson 1992) studies are valuable to the design of fermentation processes that either maximize or minimize metabolisms in accordance with the biocontrol product quality desired. Biocontrol research has been in progress for the last 80 years, and strong efforts at commercializing these products have led to only a few reaching the market in the last 10 years. It has become increasingly evident that research is needed to extend existing fermentation technologies cost-effectively to biocontrol agent production and formulation. Despite the fact that liquid cultivation is a standard industrial method of producing cells and their products, researchers have typically cultured potential biocontrol strains on agar petri plates for greenhouse and small field plot experiments. Recently, Slininger et al. (1994) have demonstrated that the relative efficacies of biocontrol strains grown on agar compared to liquid media are often very different. Studies reviewed by Bothast et al. (1993) further indicate that metabolic activities during cultivation influence the qualities of the resulting biocontrol agent formulation. They showed that liquid-culture metabolites carried along with 2-79 cells in seed coatings were associated with improved biocontrol efficacy but reduced viability retention and wheat germination rates. The specific role of PCA (and perhaps other metabolites) in these observations is not yet defined, but this resultant combination of positive and negative effects of the liquid-culture metabolites is consistent with descriptions of phenazine activities given in the literature. Phenazine compounds (particularly PCA) are known to have antifungal activity, which contributes to the suppression of take-all by rhizobacteria (Gurusiddaiah et al. 1986; Thomashow et al. 1990; Mazzola et al. 1992; Pierson and Thomashow 1992); however, they have also been reported to exert both antibacterial (Gurusiddaiah et al. 1986) and phytotoxic effects (Toohey et al. 1965b). The current identification of nutritional and physical conditions that regulate phenazine metabolism allows us to choose fermentation process designs that minimize, moderate, or maximize PCA productivity. In future research we can use this array of designs to assess the impact of liquid-culture PCA productivity on resulting biocontrol agent qualities and ultimately to determine the fermentation and formulation conditions that are consistent with minimizing early seedling phytotoxicity while maximizing take-all suppressiveness and shelf-life of viable cells in stored formulations.

Acknowledgements We thank Drs. David M. Weller, Linda S. Thomashow, and R. James Cook of the Agricultural Research Service Root Disease and Biological Control Research Unit, Pullman, Washington for useful discussions of our findings and encouragement of continued work.

References

- Bothast RJ, Schisler DA, Jackson MA, VanCauwenberge JE, Slininger PJ (1993) Use of pregelatinized starch and other polysaccharides for improved storage and efficacy of biocontrol agents. In: Berger PD, Devisetty BN, Hall FR (eds) Pesticide formulations and application systems, vol 13. ASTM, Philadelphia, Pennsylvania, pp 45–56
- Brisbane PG, Janik LJ, Tate ME, Warren RO (1987) Revised structure for the phenazine antibiotic from *Pseudomonas fluorescens* 2-79. (NRRL B-15132). *Antimicrob Agents Chemother* 31:1967–1971
- Bull CT, Weller DM, Thomashow LS (1991) Relationship between root colonization and suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* strain 2-79. *Phytopathology* 81:954–959
- Cook RJ, Veseth RJ (1991) Wheat health management. APS, St Paul, Minn
- Gurusiddaiah S, Weller DM, Sarkar A, Cook RJ (1986) Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. *Antimicrob Agents Chemother* 29:488–495
- Haynes WC, Stodola FH, Locke JM, Pridham TG, Conway HF, Sohns VE, Jackson RW (1956) *Pseudomonas aureofaciens* Kluyver and phenazine alpha-carboxylic acid, its characteristic pigment. *J Bacteriol* 72:412–417
- Korth H, Taraz K, Benoni H (1990) Isolation of *Pseudomonas fluorescens* producing phenazine derivatives exclusively under strains conditions of iron deficiency. *Zentralbl Bakteriell* 274:433–435
- Mazzola M, Cook RJ, Thomashow LS, Weller DM, Pierson LS III (1992) Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. *Appl Environ Microbiol* 58:2616–2624
- Messenger AJM, Turner JM (1983) Effect of growth conditions on phenazine production by *Pseudomonas phenazinium*. *J Gen Microbiol* 129:1013–1018
- Ownley BH, Weller DM, Thomashow LS (1992) Influence of in situ and in vitro pH on suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2-79. *Phytopathology* 82:178–184
- Pierson LS, Thomashow LS (1992) Cloning and heterologous expression of the phenazine biosynthetic locus from *Pseudomonas aureofaciens* 30-84. *Mol Plant Microbe Interact* 5:330–339
- Slininger PJ, Jackson MA (1992) Nutritional factors regulating growth and accumulation of phenazine 1-carboxylic acid by *Pseudomonas fluorescens* 2-79. *Appl. Microbiol Biotechnol* 37:388–392
- Slininger PJ, Schisler DA, Bothast RJ (1994) Two-dimensional liquid culture focusing: a method of selecting commercially promising microbial isolates with demonstrated biological control capability. In: Ryder MH, Stephens PM, Bowen GD (eds) Improving plant productivity with rhizosphere bacteria. CSIRO Division of Soils, Glen Osmond, South Australia, pp 29–32
- Thomashow LS, Weller DM (1988) Role of a phenazine antibiotic from *Pseudomonas fluorescens* in biological control of *Gaeumannomyces graminis* var. *tritici*. *J Bacteriol* 170:3499–3508
- Thomashow LS, Weller DM (1990) Role of antibiotics and siderophores in biocontrol of take-all disease of wheat. *Plant Soil* 129:93–99
- Thomashow LS, Weller DM, Bonsall RF, Pierson LS (1990) Production of the antibiotic phenazine 1-carboxylic acid by fluorescent *Pseudomonas* species in the rhizosphere of wheat. *Appl Environ Microbiol* 56:908–912
- Toohey JI, Nelson CD, Krotkov G (1965a) Isolation and identification of two phenazines from a strain of *Pseudomonas aureofaciens*. *Can J Bot* 43:1055–1062

- Toohey JJ, Nelson CD, Krotkov G (1965b) Toxicity of phenazine carboxylic acids to some bacteria, algae, higher plants, and animals. *Can J Bot* 43:1151-1155
- Vidaver AK (1967) Synthetic and complex media for the rapid detection of fluorescence of phytopathogenic pseudomonads: effect of the carbon source. *Appl Microbiol* 15:1523-1524
- Weller DM (1988) Biological control of soilborne pathogens in the rhizosphere with bacteria. *Annu Rev Phytopathol* 26:379-407
- Weller DM, Cook RJ (1983) Suppression of take-all of wheat by seed treatments with fluorescent pseudomonads. *Phytopathology* 73:463-469
- Weller DM, Cook RJ (1984) Method for screening bacteria and application thereof for field control of diseases caused by *Gaeumannomyces graminis*. US patent 4456 684
- Zabriskie DW, Armiger WB, Phillips DH, Albano PA (1980) *Trader's guide to fermentation media formulation*. Trader's Protein, Memphis, Tenn, p 58