

# Exopolysaccharides of the plant pathogens *Pseudomonas corrugata* and *Ps. flavescens* and the saprophyte *Ps. chlororaphis*

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W.F. FETT, P. CESCUTTI AND C. WIJEY. 1996. The rRNA–DNA homology group I pseudomonads *Pseudomonas asplenii*, *Ps. corrugata*, *Ps. flavescens* (plant pathogens), *Ps. alcaligenes*, *Ps. pseudoalcaligenes* subsp. *pseudoalcaligenes* (opportunistic human pathogens), *Ps. aureofaciens* and *Ps. chlororaphis* (saprophytes) were examined for their ability to produce exopolysaccharides (EPSs) when cultured on various solid and liquid complex media with glucose, glycerol or gluconate as primary sources of carbon. All three strains (388, 717 and ATCC 29736) of *Ps. corrugata* produced alginate, a polyuronan. An EPS composed of glucose, fucose, mannose and an unidentified uronic acid substituted with lactic acid was produced by one (B62) of two strains of *Ps. flavescens*. Of four strains of *Ps. chlororaphis* tested, only strain NRRL B-2075 produced EPS. The extracellular material purified by anion-exchange chromatography appeared to be a mixture of alginate plus an acidic hexosamine-containing polymer(s). Production of EPS by the other pseudomonads was not supported by any of the media tested.

## INTRODUCTION

The rRNA–DNA homology group I pseudomonads, or the 'true' pseudomonads (Palleroni 1993), encompass an important assemblage of bacteria due to their pathogenicity towards humans and plants, their possible use for bioremediation of toxic waste or spill sites (due to their extreme catabolic diversity) and their potential use as biological agents for the control of plant diseases. The most studied human pathogen of this group is *Pseudomonas aeruginosa* which often causes chronic lung infections of cystic fibrosis patients (Russell and Gacesa 1988). Many important plant pathogens such as *Ps. syringae* belong to this taxonomic grouping as well as bacteria such as *Ps. aureofaciens* which are promising biological control agents for a variety of plant fungal diseases (Palleroni 1984; Harrison *et al.* 1993; Ryder and Rovira 1993).

Bacteria produce exopolysaccharides (EPSs) in their natu-

ral habitats where they can be found as planktonic cells, microcolonies or as part of bacterial consortia called biofilms (Costerton *et al.* 1994). EPSs are important in the general ecology of the producing bacterium as well as for pathogenicity of human and plant pathogenic bacteria. In natural habitats, EPSs have numerous functions such as protectants against desiccation and predation, as mediators of biofilm and microcolony formation, and as adhesins mediating attachment to the surfaces of inert and living entities. Production of EPSs by human and plant pathogens is known to play a critical role in the host–pathogen interaction (Moxon and Kroll 1990; Leigh and Coplin 1992). Bacterial EPSs also have found industrial uses. Currently, five such polymers (cellulose, dextran, curdlan, xanthan and gellan) are employed for a variety of applications in food and non-food industries. The commercial success of these polymers, especially xanthan gum which is produced by the plant pathogen *Xanthomonas campestris*, has led to the search for additional bacterial EPSs with unique physical properties.

Previous studies in this laboratory as well as by others (Gross and Rudolph 1987; Russell and Gacesa 1988; Fett *et al.* 1995) have indicated that as a group, the group I

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pseudomonads are capable of producing a variety of acidic EPSs including alginate (a polyuronan) as well as the neutral polymer levan (a fructan). The term alginate encompasses a group of structurally-related linear, acidic polysaccharides containing varying amounts of  $\beta$ -1,4-linked D-mannuronic acid and its C-5 epimer  $\alpha$ -L-guluronic acid. Alginates comprise a major structural polymer in brown algae and alginates isolated from algae harvested from the open sea have a variety of food and non-food industrial uses (Russell and Gacesa 1988). Bacterial alginates are being studied as possible substitutes for algal alginates for certain commercial applications. Levan is an unusual bacterial EPS in that it is produced extracellularly from sucrose by the action of the enzyme levansucrase (Han 1989).

To date, acidic EPS production by several group I pseudomonads has not been reported. In this study we examined seven such species for their ability to produce EPS under a variety of culture conditions. Included in the study were strains representative of the human opportunistic pathogens *Ps. alcaligenes* and *Ps. pseudoalcaligenes* subsp. *pseudoalcaligenes* (Palleroni 1984), the plant pathogens *Ps. asplenii*, *Ps. corrugata* (Bradbury 1986) and *Ps. flavescens* (Hildebrand *et al.* 1994) and the saprophytic bacteria *Ps. aureofaciens* and *Ps. chlororaphis* (Palleroni 1984). After purification, compositions of the EPSs produced were determined.

## MATERIALS AND METHODS

### Bacterial strains

The strains of *Pseudomonas* spp. used in this study are listed in Table 1. The bacteria were maintained on *Pseudomonas* agar F (Difco Laboratories, Detroit, MI) at 4°C with bimonthly transfers to fresh media. Long-term storage was in trypticase soy broth (BBL, Becton Dickinson, Cockeysville, MD) with 20% glycerol at -80°C.

### Screening studies and isolation of EPS

All media components were obtained from Difco Laboratories or Sigma Chemical Company (St Louis, MO). The following agar media were tested for their ability to support EPS production: nutrient agar-yeast extract (0.5%, w/v)-dextrose medium (NDYA) with 1 or 5% (w/v) dextrose; modified Vogel and Bonner's medium (Chan *et al.* 1984) containing 3 mmol l<sup>-1</sup> Mg<sup>2+</sup> and 214 mmol l<sup>-1</sup> gluconate as the sole carbon source (MVBm); yeast extract-dextrose-CaCO<sub>3</sub> medium (Takahashi and Doke 1984); *Pseudomonas* agar F (PAF) with glycerol at 1 or 5% (w/v); yeast extract (0.2%, w/v)-glycerol (2%, w/v) medium (Sage *et al.* 1990); and casamino acids-peptone-glucose medium (Duvick and

Species	Strain	Source	Origin
<i>Ps. alcaligenes</i>	ATCC 14909 <sup>T</sup>	ATCC	Swimming pool water
<i>Ps. asplenii</i>	ATCC 10855	ATCC	Unknown
	ATCC 23835 <sup>T</sup>	ATCC	Unknown
<i>Ps. aureofaciens</i>	NRRL B-1482	NRRL	Soil
	NRRL B-1576	NRRL	Clay
	NRRL B-1681	NRRL	Soil
	ATCC 13985 <sup>T</sup>	ATCC	River clay
<i>Ps. chlororaphis</i>	NRRL B-1869	NRRL	Soil
	NRRL B-2075	NRRL	Caterpillar
	NRRL B-14869	NRRL	Sewage sludge
	ATCC 9446 <sup>T</sup>	ATCC	Plate contaminant
<i>Ps. corrugata</i>	388	F. L. Lukezic	Alfalfa
	717	F. L. Lukezic	Raspberry
	ATCC 29736 <sup>T</sup>	ATCC	Tomato
<i>Ps. flavescens</i>	B62 <sup>T</sup>	M. Hendson	Walnut
	B62-D5	M. Hendson	Walnut
<i>Ps. pseudoalcaligenes</i> subsp. <i>pseudoalcaligenes</i>	ATCC 17440 <sup>T</sup>	ATCC	Sinus drainage

**Table 1** List of *Pseudomonas* strains studied and their origin

<sup>T</sup> Designates type strain.

ATCC, American Type Culture Collection, Rockville, MD, USA; NRRL, USDA, ARS, National Center for Agricultural Utilization Research, Peoria, IL, USA; F. L. Lukezic, Department of Plant Pathology, Pennsylvania State University, University Park, PA, USA; M. Hendson, Department of Plant Pathology, University of California, Berkeley, CA, USA.

Sequeira 1984). Bacteria were streaked onto the agar media and incubated for up to 7 d at 20 and 28°C. *Pseudomonas alcaligenes* and *Ps. pseudoalcaligenes* subsp. *pseudoalcaligenes* were also tested at 37°C.

Strains which did not exhibit mucoid growth on solid media were also examined for EPS production in three liquid media. The media used were King's medium B (King *et al.* 1954) with tryptone substituted for proteose peptone, NDYA without addition of agar and with dextrose at 5% (w/v) and MVBW without addition of agar. The protease Alcalase 2.4L (Novo Industri A/S, Copenhagen, Denmark) was added to all media to a final concentration of 0.005% (w/v) in order to inhibit any EPS depolymerases produced. Seed cultures were grown overnight in nutrient broth (0.8%, w/v)–yeast extract (0.2%, w/v) at 24–37°C (determined by the optimal growth temperature of the strain) with shaking (250 rev min<sup>-1</sup>). Inoculum (0.5 ml) was added to 25 ml of liquid medium contained in 250-ml Erlenmeyer flasks with duplicate flasks per strain per medium. Incubation was as for the seed cultures except that incubation temperatures were 24°, 28° and 37°C. After 5 d, bacterial cells were removed by centrifugation (16 300 g, 30 min) and cold (4°C) isopropanol (3 vol) was added to the culture supernatant fluids to precipitate any EPS present.

Bacterial strains which produced EPS were then grown on the appropriate media for maximum EPS production and the EPS analysed. The three strains of *Ps. corrugata* were streaked on culture dishes (100 × 15 mm) containing PAF with the glycerol content increased from 1 to 5% (w/v). Cultures were incubated at 28°C for 2–4 d. *Pseudomonas flavescens* B62 was cultured as above except that incubation was at 20°C for 5 d. *Pseudomonas chlororaphis* NRRL B-2075 was cultured on NDYA with incubation at 28°C for 4–5 d. After incubation, bacterial growth and EPS were harvested from the solid media with a bent glass rod and distilled water. After being stirred, bacterial cells were pelleted by centrifugation (16 300 g, 30–60 min), and the supernatant fluids were collected. A concentrated aqueous solution of KCl (25% w/v) was added to the supernatant fluids to give a final concentration of 1% (w/v) to aid in the precipitation, and the EPS was then precipitated by the addition of 3 volumes of cold isopropanol. After sitting at 4°C for at least 2 h, the precipitated EPS was collected by centrifugation (10 000 g, 20 min) and re-dissolved in distilled water. Precipitation with isopropanol was repeated twice for a total of three times, the final pellet taken up in Milli-Q purified water, freeze-dried and weighed. This material is referred to as partially purified EPS. A minimum of two separate, partially purified EPS samples were prepared for each bacterial strain.

#### Anion-exchange chromatography

Partially purified EPS samples (40–80 mg) were dissolved in 0.05 mol l<sup>-1</sup> Tris-HCl buffer (pH 6.5) (1–2 mg EPS per ml

buffer) and loaded onto a column of DEAE-Sephacrose CL-6B (Pharmacia, Piscataway, NJ) (2.5 by 18 cm). After the sample had entered into the bed, the column was eluted with 300 ml of buffer alone followed by a 500-ml linear 0 to 2 mol l<sup>-1</sup> NaCl gradient prepared in buffer. Finally, the column was washed with 2 mol l<sup>-1</sup> NaCl in buffer. Fractions (6 ml) were collected and tested for the presence of neutral sugar and uronic acids as described below. Peak material fractions were combined, dialysed against distilled water, and freeze-dried.

#### Analytical methods

All reagents and standards were obtained from Sigma Chemical Company. Total neutral sugar was determined by the method of Dubois *et al.* (1956) with D-glucose as standard. The uronic acid content was determined by the method of Blumenkrantz and Asboe-Hansen (1973) with either a commercial preparation of algal alginate or manuronolactone as standard. Acetate content was determined by the method of McComb and McCready (1957) with glucose pentaacetate as standard. The presence of pyruvate was determined by use of an enzyme assay kit according to the manufacturer's (Sigma) instructions. Content of hexosamine was determined by the method of Johnson (1971) with D-glucosamine as standard. Before analysis for hexosamine, samples were hydrolysed in 6 mol l<sup>-1</sup> HCl for 4 h at 100°C, neutralized with concentrated NaOH solution, and dried under a stream of nitrogen. Ketose sugar content was determined by the method of Dische (1962) with fructose as standard. Proton-NMR analyses were done as previously described (Cescutti *et al.* 1995).

#### Gas-liquid chromatography

Samples were hydrolysed with 2 mol l<sup>-1</sup> trifluoroacetic acid at 120°C for 1 h or with 1 mol l<sup>-1</sup> H<sub>2</sub>SO<sub>4</sub> at 100°C for 1.5 h. The released sugars were identified as their aldonitrile acetate (Varma *et al.* 1973) or alditol acetate derivatives (Albersheim *et al.* 1967) by gas-liquid chromatography (GLC). The model 5995B gas-liquid chromatograph (Hewlett-Packard, Wilmington, DE) was fitted with a 15-m SP-2330 capillary column (Supelco, Inc., Bellefonte, PA) with temperature programming from 150 to 250°C at 4°C min<sup>-1</sup>. Before hydrolysis and derivatization, samples containing significant amounts of uronic acid as determined by colorimetric assay were also reduced with sodium borohydride via the carbodiimide adduct (Taylor and Conrad 1972). Sugars were identified by co-chromatography with derivatized commercial standards.

## RESULTS

Under the variety of cultural conditions tested, EPS production was evident only for *Ps. corrugata* (all three strains), *Ps. flavescens* (strain B62 only) and *Ps. chlororaphis* (strain NRRL B-2075 only). Based on the results of the initial screening studies, EPSs of *Ps. corrugata* and *Ps. flavescens* were isolated after growth on PAF with 5% glycerol while *Ps. chlororaphis* was grown on NDYA with 5% dextrose. The appearance of these cultures after 4–5 d of incubation ranged from mucoid for the three strains of *Ps. corrugata* to slightly mucoid for *Ps. flavescens* B62 and *Ps. chlororaphis* NRRL B-2075. Yields of EPS per culture dish after isopropanol precipitation were 2–4 mg for *Ps. corrugata*, 5–9 mg for *Ps. flavescens* and 2–3 mg for *Ps. chlororaphis*. None of the strains tested in broth media produced significant amounts of EPS as indicated by either the complete lack of, or paucity of stringy precipitate upon addition of isopropanol to culture supernatant fluids.

Colorimetric analyses of the partially purified EPSs isolated from the three strains of *Ps. corrugata* indicated that they all contained high levels (greater than 40%, w/w) of uronic acid. No significant amounts of other sugars were detected. Further purification by anion-exchange chromatography of a sample (40 mg) of partially purified *Ps. corrugata* 388 EPS resulted in the elution of a single, small peak of neutral sugar-positive material with buffer alone and a single, large uronic acid-containing peak eluting between 0.7 and 0.9 mol l<sup>-1</sup> NaCl. After dialysis and freeze drying of the retentates, only 3 mg of material eluted with buffer alone was recovered. By colorimetric assays, this material contained 13% (w/w) of neutral sugar and no uronic acid and was not evaluated further. For the material eluted during the salt gradient, a total of 28 mg was recovered. Colorimetric assays indicated that this material was composed solely of uronic acid (Table 2). Reduction and subsequent GLC analyses of

the column purified sample from strain 388 and also of the partially purified EPSs (not purified by anion-exchange chromatography) obtained from all three of the *Ps. corrugata* strains demonstrated the presence of mannose alone. This indicates that the samples consisted of alginate (polymannuronic acid). Colorimetric assays of the column-purified sample indicated that the alginate was acetylated (10%, w/w).

The partially purified EPS samples from *Ps. flavescens* B62 contained approximately 36% by weight of neutral sugar and 20% by weight of uronic acid. Results of an enzymatic assay for pyruvate and colorimetric assays for hexosamine and ketose sugar were negative. A partially purified sample (80 mg) was then subjected to anion-exchange chromatography. Only a single peak of neutral sugar-containing material was obtained, eluting between 0.2 and 0.4 mol l<sup>-1</sup> NaCl. After dialysis and freeze drying of the retentate, 50 mg of sample was recovered. Colorimetric analyses indicated the presence of 36% (w/w) neutral sugar, 21% (w/w) uronic acid and 3% (w/w) acetate (Table 2). The presence of acetate was confirmed by proton-NMR analysis (resonance at 2.1 ppm) and by the disappearance of this resonance after mild alkaline hydrolysis. Analyses by GLC of the unreduced purified sample indicated the presence of glucose, fucose and mannose in an approximate ratio of 2:1:0.5 based on relative peak areas. The chromatogram obtained after GLC analyses of the reduced purified sample did not differ from that obtained for the unreduced sample except for a peak of material eluting approximately 6.5 min after glucose. The nature of this material is under investigation in these laboratories. Preliminary results indicate that this late eluting material consists of an unidentified uronic acid substituted with lactic acid.

The partially purified EPS samples from *Ps. chlororaphis* NRRL B-2075 appeared to be a mixture of macromolecules. The samples contained approximately 1–14% (w/w) neutral sugar, 12–33% (w/w) uronic acid and 10% (w/w) hexos-

**Table 2** Composition of purified acidic exopolysaccharides produced by *Pseudomonas corrugata*, *Ps. flavescens* and *Ps. chlororaphis*

Bacterium	Strain	Uronic acid % (w/v)	Neutral sugar % (w/v)	Hexosamine % (w/v)	Sugar composition (relative amounts)
<i>Ps. corrugata</i>	388	100	0	0	Mannuronic acid
<i>Ps. flavescens</i>	B62	21	36	0	Glucose, fucose, mannose (2:1:0.5), a uronic acid
<i>Ps. chlororaphis</i>	NRRL				
	B-2075 I*	8	2	16	Not determined
	II*	44	7	5	Mannuronic and guluronic acids (9:1)
	III*	18	1	3	Not determined
	IV*	13	10	2	Not determined

\* Acidic fractions separated by anion-exchange chromatography.

amine and were devoid of ketose sugar. After mild acid hydrolysis, derivatization and GLC analyses, several small peaks corresponding to neutral hexoses and pentoses were noted. When samples were reduced the peak corresponding to mannose was predominant with a small peak corresponding to gulose, indicating that alginate was present. Based on relative peak areas for gulose and mannose, gulose comprised 7% of the polymer. When subjected to anion exchange chromatography, four peaks of neutral sugar-containing material were eluted, three (designated I, II and III) during the 0–1 mol l<sup>-1</sup> salt gradient and one (IV) with 2 mol l<sup>-1</sup> salt (Table 2). When 88 mg of partially purified material was loaded, a total of 65 mg was recovered in the four fractions (I, 12 mg; II, 44 mg; III, 6 mg; IV, 3 mg). Colorimetric assays indicated that all four fractions were low in neutral sugar (10% or less, w/w). All four fractions contained uronic acid; fraction II contained the highest amount (44%, w/w) with fractions I, III and IV having 18% or less. All four fractions also contained hexosamine (I, 16%; II, 5%; III, 3%; IV, 2%; w/w). Analysis by GLC of a reduced sample of fraction II material indicated that alginate was present with a mannuronate to guluronate ratio of 9 : 1 based on relative areas of the peaks corresponding to mannose and gulose. Fractions I, III and IV were not examined further.

## DISCUSSION

Exopolysaccharide production was found for three of the seven group I pseudomonads tested. *Pseudomonas corrugata*, one of the EPS producers, is one of the non-fluorescent pseudomonads which belong to this taxonomic group, others being *Ps. fragi*, *Ps. stutzeri*, *Ps. mendocina*, *Ps. alcaligenes* and *Ps. pseudoalcaligenes* (De Vos *et al.* 1985; Stead 1992; Palleroni 1993). *Pseudomonas corrugata* causes a necrosis and collapse of the pith of infected tomato plants and is unable to produce levan when grown on sucrose-containing media (Bradbury 1986). The bacterium is also being studied as a biocontrol agent targeted at both pre- and post-harvest plant diseases (Ryder and Rovira 1993; Smilanick *et al.* 1993). In a previous study (Fett *et al.* 1986) we were unable to induce EPS production by a strain (NCPPB 2445) of *Ps. corrugata*. Subsequently, however, Southern blot hybridization studies indicated that *Ps. corrugata* strain 388 contained genes with homology to alginate biosynthetic and regulatory genes cloned from the alginate-producing bacterium *Ps. aeruginosa* (Fett *et al.* 1992). Thus, in this study we re-examined strains of this species for the ability to produce EPS. The results demonstrated that *Ps. corrugata* produces alginate as an acidic EPS. The alginates were very low or devoid of guluronic acid and were acetylated; traits common to alginates produced by pseudomonads (Russell and Gacesa 1988; Fett *et al.* 1995). The nature of the acidic EPS produced by two additional non-fluorescent group I pseudomonads has been reported.

Variants of *Ps. mendocina* and transconjugants of *Ps. stutzeri* genetically manipulated to produce EPS were both reported to synthesize alginate (Govan *et al.* 1981; Hacking *et al.* 1983; Goldberg *et al.* 1993). Recent studies in this laboratory demonstrated that the naturally-occurring type strain (ATCC 17588) of *Ps. stutzeri* does not produce alginate, but rather a novel acidic EPS containing glucose, mannose and rhamnose (1 : 1 : 1) substituted with lactic acid (Osman *et al.* 1994).

*Pseudomonas flavescens*, another of the EPS-producing bacteria described here, is a recently described plant pathogenic species which causes cankers on walnut trees and is also unable to form levan from sucrose (Hildebrand *et al.* 1994). The bacterium produces a yellow, cellular pigment in addition to a diffusible fluorescent pigment. The EPS produced by *Ps. flavescens* B62 appears to be novel for a group I pseudomonad in that it contains glucose, fucose, mannose and an unidentified uronic acid substituted with lactic acid. The only other group I pseudomonad so far found to produce a uronic acid-containing EPS is *Ps. 'gingeri'* (Cescutti *et al.* 1995). The *Ps. 'gingeri'* EPS contains glucose, mannose and glucuronic acid (1 : 1 : 1) and is substituted with acetate and pyruvate. Lactic acid was also found to occur as a substituent of the acidic EPS produced by the type strain (ATCC 10844) of *Ps. marginalis* (Osman and Fett 1993).

*Pseudomonas chlororaphis*, the third EPS producer described, is a saprophytic bacterium and is proposed to be synonymous with *Ps. aureofaciens* (Johnson and Palleroni 1989). Both of these fluorescent pseudomonads were at one time considered biotypes of *Ps. fluorescens*. *Pseudomonas chlororaphis* is capable of producing levan from sucrose (Johnson and Palleroni 1989) and in this study we demonstrate that *Ps. chlororaphis* NRRL B-2075 is also capable of producing the acidic EPS alginate along with an acidic hexosamine-containing polymer(s). The various minor sugar peaks that appeared on chromatograms obtained after mild acid hydrolysis may have represented minor contaminants in the EPS preparation as hexosamine-containing polymers are highly resistant to acid hydrolysis. Only one other hexosamine-containing EPS has been reported for the group I pseudomonads (Fett *et al.* 1995). This EPS is produced by a strain of the saprophyte *Ps. fluorescens* and the relationship between the two polymers, if any, is not yet clear.

In summary, we are able to induce acidic EPS production by three of the seven *Pseudomonas* species tested. One species (*Ps. flavescens*) produced a unique acid EPS while two other species (*Ps. corrugata* and *Ps. chlororaphis*) were demonstrated to produce alginic acid in common with several other members of the *Pseudomonas* rRNA–DNA homology group I. In addition, *Ps. chlororaphis* NRRL B-2075 also produced another acidic EPS(s) containing hexosamine. The demonstration of the roles of these EPSs in the natural habitats of the producing bacteria as well as any possible commercial uses of the unique EPSs identified requires further study.

For the bacterial strains from which we were unable to isolate EPSs, further culture manipulations or genetic manipulation may allow expression of normally silent genes, resulting in EPS production *in vitro*. Additional novel EPSs produced by group I pseudomonads most likely are waiting to be discovered.

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# Inhibition of *Salmonella typhimurium* by the products of tartrate metabolism by a *Veillonella* species

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A. HINTON JR AND M.E. HUME. 1996. The inhibition of the growth of *Salmonella typhimurium* by a *Veillonella* species grown on media supplemented with tartrate was examined. Growth of *Salmonella typhimurium* was not inhibited by the concentrations of products metabolized by *Veillonella* cultures on media supplemented with 0 or 50 mmol l<sup>-1</sup> of tartrate, but was inhibited on media supplemented with 100 or 150 mmol l<sup>-1</sup> of tartrate. Inhibition of *Salm. typhimurium* was correlated with the increased production of acetate and propionate from tartrate by the *Veillonella* species.

## INTRODUCTION

*Veillonella* are Gram-negative, anaerobic cocci found in the normal intestinal microflora of many animals (Rogosa 1964, 1984). *Veillonella* produce significant concentrations of volatile fatty acids (VFA) in the intestinal tract (Johns 1951a) and this may be an important factor in inhibiting the colonization of the intestinal tract by enteropathogens (Barnes *et al.* 1976; Bonhoff *et al.* 1979). *Veillonella* metabolize VFA from substrates such as lactate, succinate and fumarate (Rogosa 1964), which are metabolic products of intestinal anaerobes (Holdeman *et al.* 1984). When grown on media supplemented with lactate, succinate or fumarate, *Veillonella* cultures can inhibit the growth of *in vitro* cultures of *Salmonella typhimurium* and *Salm. enteritidis* (Hinton *et al.* 1993).

Tartrate is another metabolite utilized by *Veillonella* to produce VFA (Johns 1951a). Tartrate is found in many fruits and vegetables (Budavar 1964; Strack *et al.* 1987) and is a product of microbial metabolism (Martin and Foster 1955). However, the inhibitory activity of *Veillonella* cultures grown on media supplemented with tartrate has not been previously examined. The purpose of the present study was to determine the effects of the products of tartrate metabolism by *Veillonella* on the growth of *Salm. typhimurium in vitro*.

## MATERIALS AND METHODS

### *Veillonella* cultures

The *Veillonella* isolate used in this study has been previously described (Hinton and Hume 1995). *Veillonella* cultures were

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grown anaerobically in a BBL® GasPak™ Jar System at 37°C for 24 h in Reinforced Clostridial Medium (Difco Laboratories, Detroit, MI). The final cultures contained 4.3 × 10<sup>8</sup> cfu ml<sup>-1</sup>. An agar medium was prepared containing (g l<sup>-1</sup>): tryptose (Difco), 10.0; yeast extract (Difco), 5.0; sodium chloride (Sigma Chemical Co., St Louis, MO), 5.0; beef extract (Difco), 2.4; cystine hydrochloride (Sigma), 0.5; and Bacto agar (Difco), 12.0. The medium was supplemented with either 0, 50, 100 or 150 mmol l<sup>-1</sup> of tartrate (sodium salt; J. T. Baker, Phillipsburg, NJ), and was adjusted to pH 6.7 with 1 mol l<sup>-1</sup> sodium hydroxide. Broth media was prepared by reducing the agar content to 0.06% (w/v). All media were sterilized for 20 min at 121°C.

A 10-µl bacteriological loop (Fisher Scientific, Pittsburg, PA) was used to make a single streak of a 24-h *Veillonella* culture across the middle of agar media contained in Petri dishes and supplemented with the various concentrations of tartrate (see above). The inoculated plates were incubated anaerobically at 37°C for 4 d. The surfaces of the agar media on which the *Veillonella* had grown were covered with 4 ml of melted agar media containing the same concentration of tartrate.

### *Salmonella* culture

*Salmonella typhimurium* ST-10 was cultured for 18–24 h at 37°C in Trypticase Soy Broth (BBL) and diluted in phosphate-buffered water (Association of Official Analytical Chemists 1976) to a final concentration of 10<sup>6</sup> cfu ml<sup>-1</sup>. Sterile cotton-tipped applicators (Puritan Hardwood Products Co., Guilford, ME) were used to spread the *Salm. typhimurium*