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Degradation of the phenoxy acid herbicide diclofop-methyl by *Sphingomonas paucimobilis* isolated from a Canadian prairie soil

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***Sphingomonas paucimobilis*, isolated from a soil in Manitoba, Canada, was able to utilize diclofop-methyl, (*R*,*S*)-methyl-2-[4-(2,4-dichlorophenoxy)phenoxy]propionate, as the sole source of carbon and energy. An actively growing aerobic culture completely degraded 1.5 μg diclofop-methyl ml^{-1} to diclofop acid within 54 h, at 25°C. A biphasic growth pattern indicated that this organism was capable of degrading diclofop acid to 4-(2,4-dichlorophenoxy)phenol and 2,4-dichlorophenol and/or phenol. The accumulation of 2,4-dichlorophenol in the growth medium, however, suggested that *Sphingomonas paucimobilis* was unable to utilize this compound as a source of carbon and energy.**

Keywords: diclofop-methyl; biodegradation; herbicide; phenoxyalkanoic acid

Introduction

Diclofop-methyl, (*R*,*S*)-methyl-2-[4-(2,4-dichlorophenoxy) phenoxy]propionate, is the active ingredient of two post-emergence herbicides, Hoegrass 284^{R} and Hoegrass II^{R}, commonly used for the control of wild oats and other annual grasses throughout Canada and the United States [2,17]. This chiral compound is structurally related to a number of other widely used chlorine-substituted phenoxy-acetic acid herbicides including 2,4-D (2,4-dichlorophenoxy-yacetic acid), 2,4,5-T (2,4,5-trichlorophenoxyacetic acid), MCPA (2-methyl-4 chlorophenoxyacetic acid), mecoprop (2-[4-chloro-2-methylphenoxy]propionic acid), and dichlorprop (2-[2,4-dichlorophenoxy]propionic acid).

Laboratory and field studies have shown that, in moist non-sterile soils, indigenous microorganisms rapidly mineralize the commercial formulation containing the racemic mixture of diclofop-methyl to carbon dioxide and water [6,7,14,17–20,24]. Furthermore, the complete mineralization of [^{14}C]diclofop-methyl to [^{14}C]CO_2 by a biofilm consortium of nine bacterial strains and one alga [26–28], confirms that diclofop-methyl degradation in the environment is brought about by microbial metabolic and/or co-metabolic activities [1,3,5,13,16]. Based on these and other observations, Smith [17] proposed a possible degradation pathway of this herbicide (Figure 1).

In 1996, using a soil-enrichment technique with diclofop-methyl as a source of carbon and energy, Smith-Grenier and Adkins reported isolation of a variety of Gram-negative, oxidase-positive bacilli from a herbicide-contaminated agricultural soil in Portage la Prairie, Manitoba, Canada [21]. Based on a number of physiological and biochemical tests, these authors identified *Sphingomonas paucimobilis* as a member of the diclofop-methyl-degrading consortium [21]. Although the role of this organism in the degradation of diclofop-methyl had been alluded to previously [28], our pure culture studies established the precise role of *S. paucimobilis* in the degradation process. The following is a short review of our results; the reader is referred to the actual publication for more detail [22].

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Pure culture degradation studies

When *S. paucimobilis* was grown aerobically in a basal mineral salts medium [22] with technical grade (97% pure) diclofop-methyl (1.5 μg ml^{-1}) as the sole source of carbon and energy, a biphasic growth curve was observed (Figure 2). Growth consisted of a short lag (1 h), followed by two distinct exponential phases of approximately 50 and 100 h, respectively. Gas chromatographic analyses designed to compare significant changes in cell density (indicated by arrows in Figure 2) with changes in the chemical composition of the spent medium, showed that within 54 h post inoculation all of the added herbicide had disappeared from the growth medium (Figure 3), but not from sterile controls (data not shown). The rapid formation of diclofop acid as the major degradation product immediately after onset of growth (Figure 3), suggested hydrolysis of the ester bond of the alkanoic side chain of diclofop-methyl with release of methanol and concomitant growth of *S. paucimobilis*. A buildup of persistently high concentrations of this compound (between 71 and 152 h, Figure 3), signified that the rate of acid production was faster than its conversion to 4-(2,4-dichlorophenoxy)phenol and the subsequent degradation to 2,4-dichlorophenol. Such a trend would account for the biphasic growth pattern of *S. paucimobilis* (Figure 2). The accumulation of 2,4-dichlorophenol in relatively high concentrations (0.5–0.65 μg ml^{-1}) throughout the experiment implied that: (i) dichlorophenol was inherent in the technical grade diclofop-methyl used for preparation of the culture medium; (ii) *S. paucimobilis* was unable to degrade this chemical; and (iii) 2,4-dichlorophenol was the end product of the degradation pathway in this case. Absence of growth in the mineral salts medium

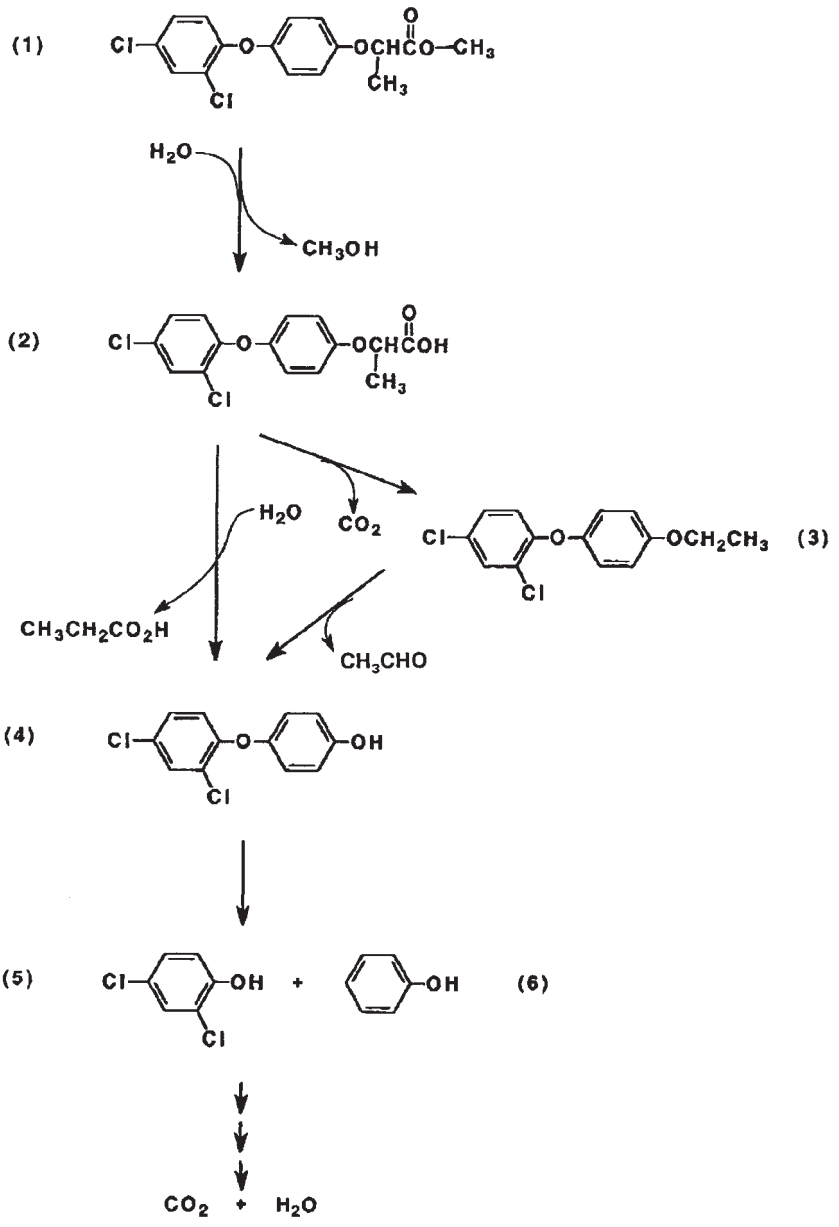

Figure 1 Possible pathway of the microbial degradation of diclofop-methyl (adapted from [17]). (1) Methyl ester, diclofop-methyl (methyl-2-[4-(2,4-dichlorophenoxy)phenoxy] propanoate); (2) acid metabolite, diclofop acid; (3) 4-(2,4-dichlorophenoxy)phenetole; (4) 4-(2,3-dichlorophenoxy)phenol; (5) 2,4-dichlorophenol; (6) phenol.

amended with 2,4-dichlorophenol as the sole source of carbon and energy (Figure 2), verified these assumptions [22].

Although *Sphingomonas paucimobilis* alone was unable to completely mineralize diclofop-methyl to CO_2 and water [22], the initial stages of degradation corresponded to those of the proposed pathway (Figure 1). They also resembled those observed in agricultural soils [9,14,17–20,24], in the water column of an aquatic microcosm [12], in clay field plots seeded to soy beans [6], and by a continuous flow-cell biofilm consortium [26–28].

Partial degradation of the herbicide by *S. paucimobilis* in this study [22] suggests that this organism is similar to *P. paucimobilis* (presumably *S. paucimobilis* after the recent reclassification of these organisms; [4,29]), isolated from a diclofop-methyl-degrading biofilm by Wolfaardt *et al* [27]. In pure culture, with [^{14}C]diclofop-methyl as the sole source of carbon and energy, this organism converted less than 2% of the label to [$^{14}CO_2$] during 7 days incubation in a batch culture reactor. However, when an exogenous carbon source (1% tryptic soy broth) was supplied as co-substrate, 28.3% of the added diclofop-methyl was converted to [$^{14}CO_2$]. These results suggest that degradation of at least part of the molecule depends upon a co-metabolic attack.

It should be noted that *S. paucimobilis* is not the only member of this genus capable of mineralizing phenoxy acid herbicides. For example, Zipper and his co-workers demonstrated that a pure culture of *S. herbicidovorans* utilized the chiral herbicides mecoprop [30] and dichlorprop [31], as well as 2,4-D [31], as sole carbon and energy sources. Since

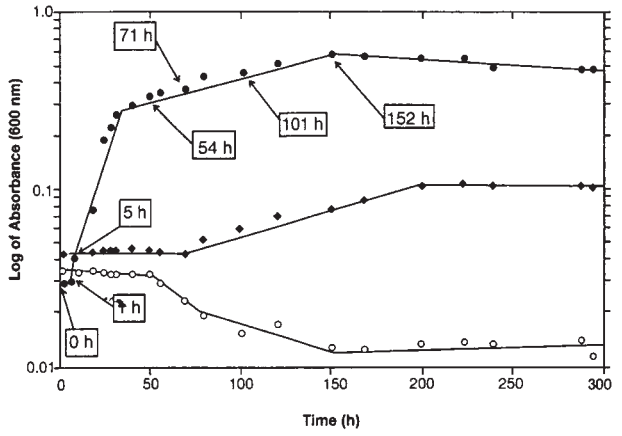

Figure 2 Growth of *Sphingomonas paucimobilis* in basal mineral salts medium amended with either diclofop-methyl (—●—), phenoxyphenol (4-(2,4-dichlorophenoxy)phenol) (—◆—), or dichlorophenol (1.5 μg ml^{-1}) (—○—). Aliquots (10 ml) of a cell suspension containing approximately 10^5 organisms ml^{-1} were incubated aerobically in the dark at 25°C, in 50-ml Erlenmeyer flasks fitted with 16-mm test tube side arms. Cell density was monitored spectrophotometrically at regular intervals. Arrows represent times when samples were removed for gas chromatographic analysis (see Figure 3). The data points are means of triplicate flasks. For additional information refer to Ref [22].

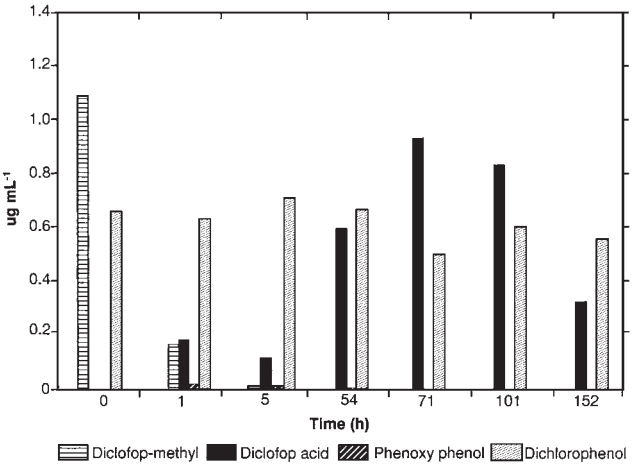

Figure 3 Diclofop-methyl degradation by *Sphingomonas paucimobilis* showing concentrations of indicated compounds present in the growth medium at specific times as indicated by the arrows in Figure 2. Samples (1.0 μl) of a toluene preparation were analyzed using an HP gas chromatograph (model 5890) equipped with a Ni^{63} electron capture detector, and HP autosampler (model 7673), and HPCHEM computer software (Hewlett-Packard, Canada Ltd, Montreal, PQ, Canada). For additional information refer to Ref [22].

this organism is also capable of 2,4-dichlorophenol-degradation, one could hypothesize a co-existence between this organism and *S. paucimobilis* in natural environments. Such an association would support the assumptions of a number of researchers [10,11,26–28], that the complete degradation of diclofop-methyl (as well as many other herbicides) depends upon synergistic enzymatic activities among diverse numbers of microorganisms.

Conclusions

Our 1996 studies [21,22] verified that *S. paucimobilis* is able to utilize at least part of the diclofop-methyl molecule as a source of carbon and energy for its growth. There is unfortunately no comparative information regarding the enantioselectivity in the microbial degradation of the racemic diclofop-methyl as that available for the chiral herbicides meciprop [30] and dichlorprop [31]. This is an area of research that requires further investigation.

In addition to the degradation of diclofop-methyl, *S. paucimobilis* is able to degrade a variety of other compounds; these include: dibenzo-*p*-diozin and dibenzofuran [25], γ-hexachlorocyclohexane [8], chlorinated biphenyls [23], and polyaromatic hydrocarbons [15]. This list is likely to grow as isolates previously classified as *Pseudomonas* or *Flavobacterium* are reclassified as *Sphingomonas*.

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