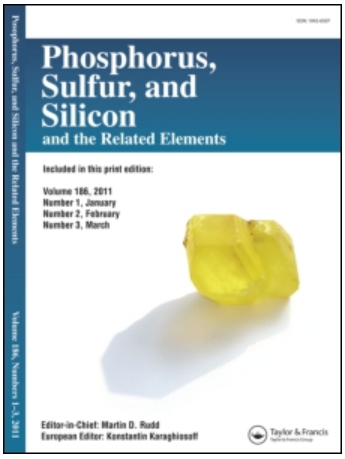

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DEGRADATION OF AMINO-(3-METHOXYPHENYL)-METHANEPHOSPHONIC ACID BY *Alternaria* sp.

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Alternaria *sp. isolated from the surface of carrot (*Daucus carota*) seeds appeared to be able to degrade amino-(4-methoxyphenyl)-methanephosphonic acid using it as a sole source of carbon, nitrogen, and phosphorus for growth.*

Keywords: Aminophosphonic acids; biodegradation; fitopathogenic fungi; P—C bond cleavage

INTRODUCTION

Organophosphonates are a group of both synthetic and biogenic compounds characterized by the presence of covalent carbon-to-phosphorus bond. The conversion of phosphonates to phosphate products by living systems, apart from being essential for the return of carbon-bound phosphorus to the phosphate metabolic pool, is of considerable practical importance since phosphonates have recently found extensive application. Compounds containing C—P bond occur in an increasing number of industrial, agricultural, medical, and housecleaning products. As a consequence, thousands of tones of these xenobiotics are introduced annually into the environment.

Although the C—P bond is resistant to chemical degradation (hydrolytic, thermal, or photochemical), organophosphonates are generally

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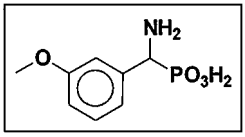
considered to be nonpersistent because a number of microorganisms possess pathways suitable for conversion to nontoxic metabolites or complete mineralization of these compounds. Thus, the ability to catabolize phosphonates is widespread among bacteria, and many soil-borne strains are able to grow on phosphonates as the sole source of phosphorus. Far less is known about the metabolism of these xenobiotics by fungi, although these organisms are supposedly responsible for the biodegradation of organophosphonates in soil. Even though many synthetic organophosphonates may be readily degraded in the environment by biotic transformations, our knowledge of their environmental fate remains limited.[1]

Alternaria are an oligophagous plant pathogen characteristic in that they readily accommodate to changing environmental conditions and are able to use a wide variety of organic compounds as carbon source for growth. On the other hand, amino-(3-methoxyphenyl)methanephosphonic acid is the only one α-aminophosphonic acid readily degraded by most of the studied fungi,[2–5] although the degradation process most probably requires mechanism different from those described so far for other phosphonates.



RESULTS AND DISCUSSION

The studied strain of *Alternaria* sp. was isolated during our studies devoted to the carrot allelochemicals, namely when searching for terpenic substances responsible for seed resistance to fungal infections. Among eight species of *Alternaria* found on the surface of carrot (variety *Perfecta*) seeds, only one strain appeared to be able to utilize amino-(3-methoxyphenyl)methanephosphonic acid as the sole source of carbon, nitrogen, and phosphorus for growth.

This is quite an interesting finding considering that chemical modeling indicated that dephosphonylation of aminoalkylphosphonic acids of this type may also occur as a spontaneous side-reaction after the condensation of aminophosphonate with pyridoxal **3′**-phosphate.[6] Although it represents an unlikely mechanism for C—P lyase (which is believed to be a general mechanism of an enzymatic cleavage of compounds containing P—C bond), existence of such a pathway should not be ruled out. Moreover, these studies indicate the possibility of the

existence of mixed chemico-enzymatic mechanism for degradation of phosphonates. Therefore, we undertook some more detailed studies on the degradation of the title aminophosphonate, with special emphasis on identification of its presumable metabolites by means of NMR and capillary electrophoresis.

Studies were carried out in a standard manner, namely by growing *Alternaria* sp. in Czapek mineral liquid medium containing glucose as a carbon source for growth. Medium deficient in phosphate and supplemented with equimolar amount of aminophosphonate allowed quite significant growth of the tested strain. The morphology of *Alternaria* was strongly altered, however and its color changed from black to creamy-white.

^{31}P NMR studies had undoubtedly shown the presence of a second phosphonate in growth medium, a presumable metabolite of the studied aminophosphonate. Capillary electrophoresis had also shown the presence of starting aminophosphonate and the additional aromatic compound in the growth medium. The strain produced unidentified short peptide(s) composed most likely from valine (or leucine), alanine, and possibly methionine. This is not surprising if considering the fact that *Alternaria* are known to produce short peptides under stress conditions.[7] These peptides serve as antibacterial and antifungal agents signals observed in our case overlap with signals of glucose and phosphonates present in medium and cause that ^{1}H NMR spectra are less useful for the interpretation experimental data.

The strain was also able to grow (to the limited extend) in aqueous solution of amino-(3-methoxyphenyl)methanephosphonic acid, which served as a sole source of phosphorus, nitrogen, and carbon for growth. Additional production of inorganic phosphate released to the growth medium was observed in this case. This is not unusual because the amount of carbon is a limiting factor of growth under these conditions, and the amounts of phosphate obtained from aminophosphonate degradation exceeded significantly the amount of carbon required for the proper growth of the fungi.

Maintaining the growth of our strain in Czapek medium additionally supplemented with amino-(3-methoxyphenyl)methane-phosphonic acid resulted in strain modified in such a manner that it did not produce peptidyl stress metabolites. This variety is also able to utilize studied aminophosphonate using it as the source of carbon, phosphorus, and nitrogen in an identical manner as described above and exhibited the same pattern of activity. Thus, when grown on Czapek medium with aminophosphonate replacing phosphate, it produced metabolite (δ in ^{31}P NMR at 19.6 ppm at pH 5.5) which accompany the substrate ($\delta = 11.4$ ppm at the same pH). The amount of the metabolite did not

exceed 20%. The same strain grown in aqueous solution of amino-(3-methoxyphenyl)methanephosphonic acid had additionally released inorganic phosphate to the medium ($\delta = 1.3$ ppm at acidic pH). We have tentatively assigned the structure of the metabolite as hydroxy-(3-methoxyphenyl)methanephosphonic acid.

The results of our studies seem to confirm an existence of a new mechanism of biodegradation of P—C bond (most possibly red-ox mechanism). However, the detailed understanding of the biodegradation pathway requires more detailed studies with a higher number of structurally related aminophosphonates.

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