Conclusions and perspectives

The cDNA *VupatI* encodes a patatin-like protein that has acylhydrolase activity. This gene is stimulated by drought stress, especially in the sensitive cultivar. Contrary to potato patatin, which acts preferentially on phospholipids, *VUPAT1* exhibits hydrolysing activity essentially towards glycolipids. This fact might be explained by the existence of a protein family composed of different isozymes with different substrate specificities, which would be expressed in response to particular stimuli and in different tissues or organs. The question of whether the proteins responsible for galactolipase activities purified from leaves [6–9] are members of the patatin family or not remains to be investigated. Research concerning the *VUPAT1* participation in lipolytic processes induced by stress will contribute to elucidating the importance of this enzyme.

References


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Microbial degradation of the plant sulpholipid

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Abstract

Five bacterial strains capable of using sulphoquinovose (6-deoxy-6-sulpho-D-glucopyranose) as a sole source of carbon have been isolated and partially characterized.

Introduction

Although it has long been known that the plant sulpholipid (sulphoquinovosyldiacylglycerol) is a major component of the biological sulphur cycle [1], rather little is known of its catabolism. The generally accepted view [2] is that the sulpholipid is scarcely metabolized by higher plants, apart from hydrolysis by plant acylhydrolases and glycosidases. On the other hand, Lee and Benson did show many years ago [3] that leaves of alfalfa, *Medicago sativa*, and of the coral tree, *Erythrina crista-galli*, metabolized glyceryl α-sulphoquinovoside to sulpholactate and sulphoacetate, respectively. Nevertheless, the production of sulphate from sulphoquinovose (6-deoxy-6-sulpho-D-glucose), formed from the sulpholipid by the action of plant enzymes, is held to be brought about by micro-organisms in soil [4–6]. For example, Martelli and Benson [4] showed that a possible *Flavobacterium* from soil could grow on methyl α-sulphoquinovoside and accumulate cysteate, sulphoacetate and sulphate intracellularly. As a part of ongoing studies in this laboratory of the biochemistry of the plant sulpholipid, and of other sulphur-containing compounds, we have isolated and provisionally characterized five bacterial strains that can utilize sulphoquinovose as a sole source of carbon.

Key words: bacterial degradation, Klebsiella, Pseudomonas, sulphoquinovose.

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Materials and methods

The bacteria were isolated by enrichment culture from two sources, a sample of forest leaf mould and a sample of activated sewage sludge. A standard basal salts medium, which did not contain any added sources of carbon or sulphur (although no precautions were taken to ensure that it was sulphur-free), was supplemented with approx. 0.1 % (w/v) of the potassium salt of sulphoquinovose \(^7\) (about 3.3 mM). Batches of medium (100 ml) were inoculated with either approx. 4 g of leaf mould or 10 ml of activated sewage sludge, and incubated at 30 °C with shaking (150 revs/min). After growth for 3 weeks, transfers (10 %, v/v) to fresh media were made and subsequent transfers (1 %, v/v) were made at approx. 5 day intervals. Bacteria from the fifth round of subculture were isolated on nutrient agar plates. Isolates were checked for ability to grow axenically in the basal salts/sulphoquinovose medium: five of those isolates were selected for further study and characterization by analysis of 16 S rRNA gene sequences \(^8\). They were identified as three strains of *Pseudomonas* (one with a high degree of identity with *Pseudomonas putida*) and one of *Agrobacterium* from the leaf mould, and one strain of *Klebsiella* from the sewage sludge. These isolates have been maintained on nutrient agar slopes in the absence of sulphoquinovose.

For biochemical studies, starting cultures were grown for 3 days in the basal salts medium containing sulphoquinovose and then samples containing approximately equal numbers of bacteria, as judged by the \(A_{580}\) of the starting cultures, were used to inoculate further batches of the medium. These were grown, with shaking at 30 °C, and samples removed at intervals for analysis. Growth was estimated by measuring the \(A_{580}\) of the cultures and, after removal of the cells by centrifugation, sulphoquinovose and sulphate in the media were determined as reducing sugar by a 3,5-dinitrosalicylic acid method \(^9\) and by the turbidimetric procedure of Sörbo \(^10\), respectively.

Results and discussion

In all isolates the maximum \(A_{580}\) (0.25–0.52) of the cultures was reached after growth for 24–30 h. The results of the other analyses are given in Table 1, from which it is clear that the apparent reducing-sugar concentration in the media had fallen considerably by this time. In isolates ABR1, ABR2 and ABR6 no reducing sugar was detected but in isolates ABR9 and ABR11 it had apparently fallen to only about 30 % of its initial value. However, no sulphoquinovose, or any other reducing sugar, was detected by TLC \(^7\) in any of the media after growth for 55 h, so the results obtained with media from isolates ABR9 and ABR11 must show the presence of compounds therein that interfere with the analytical method used.

Table 1 also shows that only isolates ABR2 and ABR11 (*Agrobacterium* sp. and *Klebsiella* sp. respectively) accumulated \(\text{SO}_4^{2-}\) in the growth media. No detectable \(\text{SO}_4^{2-}\) was produced by any of the three strains of *Pseudomonas*. However, TLC of the latter media (55 h of growth) showed the presence of significant quantities of a compound reacting with alkaline KMnO\(_4\), which was not seen in the media from the sulphate-producing isolates.

**Table 1**

The apparent reducing-sugar and \(\text{SO}_4^{2-}\) concentrations in the media of bacterial isolates growing in basal salts medium with added sulphoquinovose as a sole carbon source

| Isolates ABR1, ABR6 and ABR9 are *Pseudomonas* spp. isolate ABR2 is an *Agrobacterium* sp. and isolate ABR11 is a *Klebsiella* sp. Apparent reducing sugar determined by a dinitrosalicylic acid method \(^9\); n.d., not detected. All concentrations are given as mM. |
|---|---|---|---|---|---|---|---|
| **ABR1** | **ABR2** | **ABR6** | **ABR9** | **ABR11** |
| **Time (h)** | **RS** | **SO_4^{2-}\** | **RS** | **SO_4^{2-}\** | **RS** | **SO_4^{2-}\** | **RS** | **SO_4^{2-}\** |
| 0 | 2.9 | n.d. | 3.4 | n.d. | 3.6 | n.d. | 3.7 | n.d. | 4.1 | n.d. |
| 7 | 2.9 | n.d. | 3.3 | 0.6 | 2.8 | n.d. | 3.3 | n.d. | 3.7 | n.d. |
| 24 | n.d. | n.d. | 0.8 | 2.8 | n.d. | n.d. | 0.6 | n.d. | 1.0 | 1.5 |
| 31 | n.d. | n.d. | n.d. | 3.3 | n.d. | n.d. | 1.0 | n.d. | 1.2 | 2.7 |
| 48 | n.d. | n.d. | n.d. | 3.3 | n.d. | n.d. | 1.0 | n.d. | 1.3 | 2.6 |
| 55 | n.d. | n.d. | n.d. | 3.2 | n.d. | n.d. | 1.1 | n.d. | 1.3 | 3.0 |
| 72 | n.d. | n.d. | n.d. | 3.3 | n.d. | n.d. | 1.2 | n.d. | 1.1 | 2.3 |
isolates. This compound has not yet been identified and although it is tempting to assume that it will prove to be a sulphonate, an intermediate in the formation of sulphate from sulphoquinovose, there is no evidence for this. It has been shown by TLC to be neither 2,3-dihydroxypropanesulphonate, sulpholactate nor sulphoacetaldehyde.

References

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