Novel \textit{phacB}-Encoded Cytochrome P450 Monooxygenase from \textit{Aspergillus nidulans} with 3-Hydroxyphenylacetate 6-Hydroxylase and 3,4-Dihydroxyphenylacetate 6-Hydroxylase Activities

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\textit{Aspergillus nidulans} catabolizes phenylacetate (PhAc) and 3-hydroxy-, 4-hydroxy-, and 3,4-dihydroxyphenylacetate (3-OH-PhAc, 4-OH-PhAc, and 3,4-dIOH-PhAc, respectively) through the 2,5-dihydroxyphenylacetate (homogentisic acid) catabolic pathway. Using cDNA subtraction techniques, we isolated a gene, denoted \textit{phacB}, which is strongly induced by PhAc (and its hydroxyderivatives) and encodes a new cytochrome P450 (CYP450). A disrupted \textit{phacB} strain (\textit{ΔphacB}) does not grow on 3-hydroxy-, 4-hydroxy-, or 3,4-dihydroxy-PhAc. High-performance liquid chromatography and gas chromatography-mass spectrum analyses of in vitro reactions using microsomes from wild-type and several \textit{A. nidulans} mutant strains confirmed that the \textit{phacB}-encoded CYP450 catalyzes 3-hydroxyphenylacetate and 3,4-dihydroxyphenylacetate 6-hydroxylations to generate 2,5-dihydroxyphenylacetate and 2,4,5-trihydroxyphenylacetate, respectively. Both of these compounds are used as substrates by homogentisate dioxygenase. This cytochrome P450 protein also uses PhAc as a substrate to generate 2-OH-PhAc with a very low efficiency. The \textit{phacB} gene is the first member of a new CYP450 subfamily (CYP504B).

Most living organisms use a small number of catabolic pathways to obtain energy and structural components from organic materials. However, some microorganisms have been able to organize several catabolic pathways in order to grow by using uncommon compounds (of natural or artificial origin) as a carbon source, thereby contributing to the recycling of many of these compounds.

Phenylacetic acid (PhAc) and its hydroxyderivatives (OH-PhAc) are aromatic compounds catabolized by particular organisms, mostly bacteria and fungi, by the following three different pathways (1, 6, 13, 15): (i) the 2,5-dihydroxyphenylacetic acid (2,5-diOH-PhAc) or homogentisic acid pathway, (ii) the 3,4-dihydroxyphenylacetate (3,4-diOH-PhAc) or homoprotocatechuate pathway, and (iii) the phenylacetate-coenzyme A (PhAc-CoA) pathway.

Usually, the same microorganism catabolizes PhAc and its hydroxyderivatives by using several pathways. For example, \textit{Pseudomonas putida} catabolizes PhAc via a phenylacetyl-CoA pathway, 3-hydroxyphenylacetate via a 2,5-diOH-PhAc pathway, and 4-di-3,4-dihydroxyphenylacetate via a 3,4-dihydroxyphenylacetic acid pathway (2). This implies the production of enzymes for three full pathways to catabolize just three compounds and does not appear to be a very efficient way to use the cellular machinery.

\textit{Aspergillus nidulans} is a filamentous fungus which is able to grow in PhAc and PhAc-hydroxyderivatives as the only carbon source by using the 2,5-diOH-PhAc pathway. There are some microorganisms which catabolize PhAc through the 2,5-diOH-PhAc pathway (mainly via 3-hydroxyphenylacetate) (1, 18, 19), but in \textit{A. nidulans}, this catabolic pathway occurs via 2-hydroxyphenylacetate (6, 7, 10). PhAc is converted to 2,5-diOH-PhAc through two sequential hydroxylations in the aromatic ring, at positions 2 and 5 (Fig. 1). In addition, this fungus is a eukaryotic organism which, unlike bacteria, uses cytochromes P450 to hydroxylate the aromatic rings of these compounds.

The homogentisate pathway is also used to catabolize phenylalanine and tyrosine. Upper pathways are specific for PhAc (and hydroxyderivatives) and phenylalanine/tyrosine, and the lower pathway (from 2,5-diOH-PhAc to fumarate and acetoacetate) is common to PhAc and phenylalanine/tyrosine catabolism (6, 10) (Fig. 1).

Previously, by using cDNA subtraction, we were able to clone several genes induced by PhAc from \textit{A. nidulans} and use them to clone the corresponding human and mouse genes (4–8, 10). One of them, \textit{phacA}, was the first member of a new family of cytochromes P450 (CYP504A1) and catalyzes the 2-hydroxylation of PhAc. Loss-of-function mutation of \textit{phacA} results in residual growth on PhAc as the only carbon source, but the fungus retains some capacity to produce 2-hydroxyserylactic acid, which indicates that more than one gene is responsible for 2-hydroxylation of PhAc. We found a second gene involved in 2-hydroxylation of PhAc (10). This second gene, previously denoted \textit{pshA} and now called \textit{phacB}, also encodes a new cytochrome P450, which defines a new subfamily (CYP504B). In this work, we show that \textit{phacB} codifies a 3-hydroxy-PhAc and 3,4-dihydroxy-PhAc 6-hydroxylase and catalyzes the 6-hydroxylation of 3-hydroxyphenylacetate to produce 2,5-dihydroxy-PhAc (homogentisic acid) and of 3,4-dihydroxy-PhAc to produce 2,4,5-trihydroxy-PhAc. This new cytochrome P450 can also catalyze, to a lesser extent, the 2-hydroxylation of PhAc to produce 2-hydroxy-PhAc. Here we also report gas chromatography-mass spectrometry (GC-MS) characterization of the 2,4,5-trihydroxyphenylacetate synthe-
FIG. 1. Catabolic pathways of phenylalanine, tyrosine, phenylacetate, and mono-, di-, and trihydroxyphenylacetate derivatives in *A. nidulans*. Enzymes involved in the degradation of phenylalanine to fumarate and acetoacetate are present in *A. nidulans* and humans. Enzymes required to catabolize phenylacetate and hydroxyderivatives to homogentisate are present in *A. nidulans* (and some microorganisms). Enzymes: 1, *phacA*-encoded phenylacetate 2-hydroxylase (also catalyzes, to a lesser extent, 3-hydroxyphenylacetate 6-hydroxylation); 2, 2-hydroxyphenylacetate 5-hydroxylase; 3, phenylalanine hydroxylase; 4, tyrosine aminotransferase; 5, 4-hydroxyphenylpyruvate dioxygenase; 6, homogentisate dioxygenase; 7, maleylacetoacetate isomerase; 8, fumarylacetoacetate hydrolase; 9, *phacB*-encoded 3-hydroxyphenylacetate 6-hydroxylase (also catalyzes, to a lesser extent, phenylacetate 2-hydroxylation); 10, 4-hydroxyphenylacetate 3-hydroxylase. Note the change in carbon numbers after hydroxylation.
sized in vitro by the cytochrome P450 product of the \textit{phacB} gene.

Functional characterization of the \textit{phacB} gene is not only important for the catabolism of phenylacetic acid but also for penicillin biosynthesis, because PhAc is a moiety of the penicillin G molecule. \textit{Penicillium chrysogenum}, the industrial producer of penicillin, transforms PhAc into 2-hydroxyphenylacetate, which is not suitable for penicillin production in the fermentation broth, decreasing the available PhAc for penicillin biosynthesis. For this reason, a PhAc hydroxylation mutant of \textit{P. chrysogenum} was long sought but never found. After the cloning of \textit{phacA}, we showed that by decreasing the PhAc hydroxylation capacity, we could increase penicillin production in \textit{A. nidulans} (10). Recently, it was shown that \textit{P. chrysogenum}, after having lost its PhAc hydroxylation capacity, also increases penicillin production fivefold (14).

For these reasons, the characterization of \textit{phacB} has academic, industrial, and ecological interest.

\section*{MATERIALS AND METHODS}

\subsection*{Strains, media, and growth conditions.} The \textit{Aspergillus nidulans} strains used in this work are as follows. The \textit{hek1} strain, a bacteri auxotroph, was used as a wild type (6, 7). The \textit{hek1} (argB\textsuperscript{B}:\textsuperscript{A}cm) \textit{biA1 methG1} mutant has a disrupted \textit{bmgA} gene, is homoglutamine dioxygenase deficient, and is a bacteri and methionine auxotroph (7). The \textit{phacA} (argB\textsuperscript{B}:\textsuperscript{A}cm) \textit{biA1 methG1} mutant has a disrupted \textit{phacA} gene, is PhAc 2-hydroxylation deficient, and is a bacteri and methionine auxotroph (10). The \textit{phacB} (argB\textsuperscript{B}:\textsuperscript{A}cm) \textit{biA1 methG1} mutant has a disrupted \textit{phacB} gene and is a bacteri and methionine auxotroph created for this study. The \textit{phacA} \textit{phacB} \textit{biA1 methG1} argB2 strain is a bacteri, methionine, and arginine auxotroph and was used as a recipient for \textit{phacB} gene disruption (6, 7, 10). The \textit{hek1} \textit{methG1} strain is a bacteri and methionine auxotroph (6, 7, 8, 10) and was used as control in some experiments. The \textit{argB2 riboB2} strain was used to construct the double mutant strain by sexual crosses.

\textit{Aspergillus} standard media (3) were used for the maintenance of the strains and for growth test and transformation experiments. A defined liquid medium was used to obtain mycelia, protein extracts, and microsomes and contained the following (in g/liter): KPO\textsubscript{4}H\textsubscript{2}, 13.2; (NH\textsubscript{4})\textsubscript{2}SO\textsubscript{4}, 2; MgSO\textsubscript{4} \textsubscript{5H\textsubscript{2}O}, 0.005; and CuSO\textsubscript{4} \textsubscript{5H\textsubscript{2}O}, 0.001. The pH was adjusted to 7 with KOH, and glucose (0.3%) was added after autoclaving. Supplements were added when necessary.

Liquid cultures and mycelium harvesting were carried out as previously described (6). To summarize, 10\textsuperscript{5} conidiospores/ml were inoculated in minimal media for 18 h. The mycelium was harvested by filtration, washed, and transformed in a vacuum. Solid residue was resuspended in 25 ml of pyridine and 40 ml of BSTFA [N,O-bis(trimethylsilyl)]trifluoroacetamide] as a derivatizing reagent. The mixture was incubated for 30 min at 80°C and was injected into a chromatograph (Varian CP-3800) with a Varian VF-5MS column (30 m \times 0.25 mm [internal diameter] \times 0.25-\mu m film thickness). The temperature program was 70°C (0 min), an increase to 200°C at 10°C/min, a hold at 200°C (7 min), and an increase to 300°C (4 min). The carrier gas was helium at 1 ml/min, and the injector temperature was 250°C. The detector was a Saturn 2200 ion trap (Varian), and the ion range was from 40 m/z to 550 m/z. Compounds were identified by comparing the obtained spectrum with the NIST/EPAnIH mass spectrum library and a previously published spectrum of 2,4,5-trihydroxyphenylacetate (20).

\section*{RESULTS AND DISCUSSION}

Cloning, sequencing, and expression of the \textit{phacB} gene. The \textit{phacB} gene was isolated in a differential subtractive cDNA screen to isolate cDNAs induced by PhAc (6, 7, 8, 10). The \textit{phacB} gene encodes a 517-amino-acid protein that contains the consensus sequence GXGXXRCXXG (heme group binding site), which indicates it is a cytochrome P450. The gene has been classified as the first member of a new cytochrome P450 subfamily (CYP504B) by David Nelson (http://drnelson.utmem.edu/CytochromeP450.html). Genomic DNA sequencing showed that the \textit{phacB} gene has three introns (71, 66, and 85 nucleotides). The sequence has GenBank accession number DQ217596. A search through databases reveals similar genes in other fungi, with very high identities. The highest identity is found for protein 1397 (GenBank accession number XP 659001) from \textit{Aspergillus nidulans} (99%), but without identity in the last 13 amino acids, which is probably the result of an error in the sequence of this protein. Moreover, we found a very high identity (84%) with a putative phenylacetate 2-hydroxylation (accession number EAL90209) from \textit{Aspergillus fumigatus}. However, the protein from \textit{A. fumigatus} has only 40% identity with the phenylacetate 2-hydroxylase (encoded by the \textit{phacA} gene) from \textit{Aspergillus nidulans}. For this reason, we think that it is probably the 3-hydroxyphenylacetate 6-hydroxylase from \textit{Aspergillus fumigatus}. A protein (XP 748171) from \textit{A. fumigatus} which displays 43% identity with the protein encoded by the \textit{phacB} gene and 85% identity with the \textit{phacA}-encoded protein should be the real phenylacetate 2-hydroxylase from \textit{A. fumigatus}. Proteins from \textit{Gibberella zeae} and
at 37°C, and then RNAs were isolated. Transferred cultures were incubated for 1 h PhAc; and 11, acetate. All compounds were added at a concentration equivalent to 5 mM PhAc.  

The expression pattern of the phacB gene resembles that of some other genes involved in PhAc catabolism in A. nidulans (6, 7, 8, 10). It is strongly induced by PhAc and by all monohydroxyderivatives and dihydroxyderivatives, phenylalanine, and tyrosine. Glucose and acetate are not inducers (Fig. 2, lanes 2 and 11), and glucose (lane 3) repressed expression of the phacB gene.

Phenotype of A. nidulans strains on PhAc and PhAc hydroxyderivatives. Aspergillus nidulans mutants in minimal medium, with PhAc and phenylalanine as the only carbon sources, is shown in Table 1. The ΔthgA strain is not able to grow on PhAc, 2-hydroxy-, 3-hydroxy-, 4-hydroxy-, 2,5-dihydroxy-, and 3,4-dihydroxy-PhAc, which indicates that the catabolism of all these compounds is via 2,5-diOH-PhAc (homogentisic acid). The ΔphacA strain does not grow on PhAc (10), and the ΔphacB strain does not grow on 3-OH-PhAc, 4-OH-PhAc, and 3,4-diOH-PhAc. This shows that the phacB gene is involved in catabolism of 3-OH-PhAc, 4-OH-PhAc, and 3,4-diOH-PhAc. The ΔphacB strain was sexually crossed with an arginine- and riboflavin-deficient strain as the first step towards obtaining a double mutant strain (ΔphacA ΔphacB). No arginine-independent progeny were able to grow on 3-OH-PhAc, 4-OH-PhAc, or 3,4-diOH-PhAc. Auxotroph colonies requiring arginine were able to grow on these hydroxyderivatives. This shows that the argB” allele disrupting the phacB gene is the only event which affects the use of 3-OH-PhAc, 4-OH-PhAc, and 3,4-diOH-PhAc.

Secretion of 2-OH-PhAc is reduced in ΔphacA and ΔphacB mutants. Aspergillus nidulans secretes 2-OH-PhAc into the culture medium when it is grown on PhAc as the carbon source (10). The accumulation of 2-OH-PhAc in culture supernatants of some mutants after the addition of PhAc is shown in Fig. 3. ΔphacA and ΔphacB strains showed a reduced accumulation of 2-OH-PhAc. The double mutant strain (ΔphacA ΔphacB) accumulated no 2-OH-PhAc. This shows that the phacA and phacB genes are involved in the conversion of PhAc to 2-OH-PhAc and that these two genes are solely responsible for 2-hydroxylation of PhAc. We know that the phacA gene encodes a phenylacetate 2-hydroxylase (10), and the growth of the ΔphacB mutant in hydroxyderivatives (Table 1) indicates that this gene is involved in the catabolism of 3-OH-PhAc, 4-OH-PhAc, and 3,4-diOH-PhAc. The decrease in 2-OH-PhAc secretion into the medium by the ΔphacB strain suggests that this mutant could be affected in ortho-hydroxylation of PhAc to produce 2-OH-PhAc. From this information, we can hypothesize that the phacB gene encodes a 3-hydroxyphenylacetate 6-hydroxylase which produces 2,5-diOH-PhAc from 3-OH-PhAc but which is also able to catalyze the 2-hydroxylation of PhAc to 2-OH-PhAc, probably due to structural analogy between PhAc and 3-hydroxy-PhAc and the lack of specificity of the enzyme encoded by the phacB gene (Fig. 1 and 3; see below).

![FIG. 2. Northern analysis of phacB gene expression. A. nidulans was grown in minimal medium with 0.3% glucose for 18 h and then transferred to medium with the following substrates: 1, PhAc; 2, glucose; 3, PhAc plus glucose; 4, 2-OH-PhAc; 5, 3-OH-PhAc; 6, 4-OH-PhAc; 7, phenylalanine; 8, tyrosine; 9, 2,5-diOH-PhAc; 10, 3,4-diOH-PhAc; and 11, acetate. All compounds were added at a concentration equivalent to 5 mM PhAc. Transferred cultures were incubated for 1 h at 37°C, and then RNAs were isolated.](image1)

![FIG. 3. Secretion of 2-hydroxyphenylacetic acid by A. nidulans. A. nidulans was grown in minimal medium with 0.3% glucose for 18 h and then transferred to medium with 5 mM phenylacetate. At the appropriate moment, 1 ml of medium was harvested, filtered, and analyzed by HPLC. Wild-type (■), ΔphacA (■), ΔphacB (▲), and ΔphacA ΔphacB double mutant (●) strains were examined.](image2)

### TABLE 1. Growth of some A. nidulans strains on PhAc and PhAc hydroxyderivatives

| Strain               | Growth on substrate |
|----------------------|---------------------|
|                      | PhAc | 2-OH-PhAc | 3-OH-PhAc | 4-OH-PhAc | 2,5-diOH-PhAc | 3,4-diOH-PhAc |
| Wild type            | +    | +         | +         | +         | +             | +             |
| ΔphacA mutant        | –    | +         | +         | +         | +             | +             |
| ΔphacB mutant        | +    | –         | –         | +         | +             | +             |
| ΔphacA ΔphacB mutant | –    | –         | –         | –         | +             | –             |
| ΔthgA mutant         | –    | –         | –         | –         | –             | –             |

* +, growth; –, no growth.
Phenylacetate is a precursor in penicillin biosynthesis. A lack of PhAc hydroxylation should increase penicillin production and decrease PhAc consumption. For this reason, the industry has been looking for a non-PhAc-hydroxylating strain of *Penicillium chrysogenum* (the industrial producer of penicillin) for many years. It has been shown that *phacA* mutant strains of *Penicillium* and *A. nidulans* increase penicillin production (10, 14). However, Δ*phacB* and double mutant (Δ*phacA* Δ*phacB*) strains of *A. nidulans* did not increase penicillin production compared to that of the Δ*phacA* strain (data not shown), which also suggests a minor role for the *phacB* gene in hydroxylation of PhAc in *P. chrysogenum*.

**In vitro hydroxylation of PhAc and hydroxyderivatives.** To clearly define the roles of the *phacA* and *phacB* genes in hydroxylation of PhAc and its hydroxyderivatives, we assayed the hydroxylating capacity of some *Aspergillus nidulans* strains in vitro by using microsomes. Hydroxyderivatives, the products of the reactions, were analyzed by HPLC. When PhAc was the substrate, microsomes from the wild-type strain were able to catalyze the production of 2-OH-PhAc, and microsomes from the Δ*phacA* strain catalyzed this hydroxylation to a lesser extent (10) (Fig. 4, top panel). Microsomes from the Δ*phacB* strain catalyzed 2-hydroxylation of PhAc to the same extent as the wild-type strain. Double mutant (Δ*phacA* Δ*phacB*) microsomes were not able to produce any 2-OH-PhAc, which again indicates a minor role of the *phacB* gene in 2-hydroxylation of PhAc. However, this is the second described gene to encode a protein with PhAc 2-hydroxylase activity.

When the substrate was 3-OH-PhAc (Fig. 4, bottom panel), microsomes from the wild-type and Δ*phacA* strains generated 2,5-diOH-PhAc. Microsomes of the Δ*phacB* strain produced 2,5-diOH-PhAc to a lesser extent than did those of the wild-type strain, which demonstrates the major role of the *phacB* gene in hydroxylation of 3-OH-PhAc. The double mutant mi-

![FIG. 4. In vitro synthesis of 2-hydroxyphenylacetate from phenylacetate (top) and of 2,5-dihydroxyphenylacetate from 3-hydroxyphenylacetate (bottom) by *A. nidulans* microsomal fractions from wild-type (■), Δ*phacA* (●), Δ*phacB* (▲), and Δ*phacA* Δ*phacB* double mutant (●) strains.](image1)

![FIG. 5. Gas chromatography analysis of in vitro synthesis of 2,4,5-trihydroxyphenylacetate (retention time, 17.770 min) from 3,4-dihydroxyphenylacetate (retention time, 14.775 min).](image2)
crosomes (ΔphacA ΔphacB) produced no 2,5-diOH-PhAc, which also indicates that the phacA gene has a minor role in 6-hydroxylation of 3-OH-PhAc and PhAc. NADPH-cytochrome P450 oxidoreductase was assayed as a control (10, 16) in the wild-type and mutant strains, and all of them showed similar levels of activity with ferricyanide (680 to 810 nmol/min · mg in all assays) and cytochrome c (250 to 290 nmol/min · mg in all assays) as substrates.

Figure 1 shows the hydroxylation activities of PhAc and 3-hydroxy-PhAc mediated by the phacA and phacB genes.

3-Hydroxyphenylacetate 6-hydroxylases from bacteria (Flavobacterium) and fungi (Trichosporon) have been described previously (1, 11, 18, 19), and recently a gene from Pseudomonas putida encoding a two-component enzyme with 3-hydroxyphenylacetate 6-hydroxylase activity was cloned (2). However, Aspergillus nidulans is a eukaryotic microorganism which, unlike bacteria, uses cytochromes P450 to hydroxylate the aromatic rings of PhAc and its hydroxyderivatives. The phacA and phacB genes are examples of genes encoding these fungal hydroxylases.

An A. nidulans strain which is deficient in homogentisate dioxygenase activity (ΔhmgA) is not able to grow on 4-hydroxy- and 3,4-dihydroxy-PhAc as the only carbon source (Table 1), indicating that the catabolism of these compounds is through the homogentisic acid pathway. It is easy to explain the conversion of 3-OH-PhAc to 2,5-diOH-PhAc (Fig. 1) but more difficult to explain the conversion of 4-OH-PhAc and 3,4-diOH-PhAc to a compound similar to homogentisic acid (2,5-diOH-PhAc) which is utilized by homogentisate dioxygenase as a substrate. Anderson and Dagley (1) proposed a catabolic pathway in Trichosporon cutaneum which metabolizes 4-OH-PhAc and 3,4-diOH-PhAc to a trihydroxy derivative (2,4,5-trihydroxy-PhAc), which is converted by homogentisate dioxygenase (or a similar enzyme) to oxalacetylacetacetaetate (Fig. 1). The 3-OH-PhAc 6-hydroxylase enzyme from Flavobacterium is able to convert 3,4-diOH-PhAc to 2,4,5-triOH-PhAc (18, 19), and there is no indication of substrate use for the Pseudomonas enzyme (2). For this reason, we also assayed the formation of 2,4,5-trihydroxyphenylacetate from 3,4-dihydroxyphenylacetate and 4-hydroxy-PhAc. We used GC-MS to analyze these reactions because the absorption spectra of 2,4,5-triOH-PhAc and 2,4,5-triOH-PhAc and their retention times (in our HPLC system) are very similar. Microsomes of the wild-type strain, the ΔphacA mutant, the ΔphacB mutant, and the ΔphacA ΔphacB double mutant catalyze the formation of 3,4-dihydroxyphenylacetate from 4-hydroxyphenylacetate (data not shown). Microsomes of the wild-type strain and the ΔphacA mutant are able to catalyze the formation of 2,4,5-trihydroxyphenylacetate from 3,4-dihydroxy-PhAc (Fig. 5), but microsomes of the ΔphacB mutant strain cannot catalyze the formation of 2,4,5-trihydroxyphenylacetate (Fig. 5). The true nature of this trihydroxyderivative was confirmed by gas chromatography-mass spectrometry (Fig. 6), clearly indicating that the phacB gene is also responsible for the synthesis of 2,4,5-trihydroxyphenylacetate from 3,4-dihydroxyphenylacetate. This trihydroxyderivative has also been found in the urines of patients with Parkinson's disease treated with L-DOPA (3,4-dihydroxyphenylalanine) (20).

We should also note that homogentisate dioxygenase (hmgA) from A. nidulans is the enzyme responsible for opening the aromatic ring of 2,4,5-triOH-PhAc. The lack of growth of the ΔhmgA strain on 4-OH-PhAc and 3,4-diOH-PhAc indicates that homogentisate dioxygenase from A. nidulans is the enzyme responsible for catabolizing 2,4,5-trihydroxy-PhAc and proves that Aspergillus nidulans, unlike other microorganisms, is able to catabolize PhAc and its mono- and dihydroxyderivatives via the same pathway.

Also, phacB could be important in plant metabolism, as homogentisic acid is a precursor of some photosynthetic pigments (plastoquinone and tocopherols) (12) and because there are some genes with identity to phacB in plant genomes.

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