In *Pseudomonas aeruginosa* the extracellular metabolite and siderophore pyochelin is synthesized from two major precursors, chorismate and L-cysteine via salicylate as an intermediate. The regulatory role of isochorismate synthase, the first enzyme in the pyochelin biosynthetic pathway, was studied. This enzyme is encoded by *pchA*, the last gene in the *pchDCBA* operon. The PchA protein was purified to apparent electrophoretic homogeneity from a PchA-overexpressing *P. aeruginosa* strain. The native enzyme was a 52-kDa monomer in solution, and its activity strictly depended on Mg$^{2+}$. At pH 7.0, the optimum, a $K_m = 4.5 \mu M$ and a $k_{cat} = 43.1$ min$^{-1}$ were determined for chorismate. No feedback inhibitors or other allosteric effectors were found. The intracellular PchA concentration critically determined the rate of salicylate formation both in vitro and in vivo. In cultures grown in iron-limiting media to high cell densities, overexpression of the *pchA* gene resulted in overproduction of salicylate as well as in enhanced pyochelin formation. From this work and earlier studies, it is proposed that one important factor influencing the flux through the pyochelin biosynthetic pathway is the PchA concentration, which is determined at a transcriptional level, with pyochelin acting as a positive signal and iron as a negative signal.

In bacteria, biosynthetic pathways are regulated, as a rule, by their end products, which can cause feedback inhibition of early key enzymes as well as repression of some or all enzymes of the pathway (1). For example, in histidine biosynthesis of *Salmonella enterica*, histidine allosterically inhibits the first enzyme and represses, by an attenuation mechanism, the expression of all enzymes of the pathway (2). In arginine biosynthesis of *Pseudomonas aeruginosa*, arginine inhibits the first and the second enzyme and represses the sixth enzyme, involving the transcriptional regulator ArgR (3–5).

A more complicated situation arises in branched biosynthetic pathways where the end products may exert control functions at several checkpoints. For instance, in aromatic biosynthesis of *P. aeruginosa* (Fig. 1), tryptophan inhibits one isoenzyme carrying out the first reaction (*AroA*) and, in addition, inhibits the first tryptophan-specific enzyme, anthranilate synthase (*TraP*). Tyrosine causes feedback inhibition of the second *AroA* isoenzyme and two tyrosine-specific enzymes. Furthermore, tyrosine activates and phenylalanine inhibits one key enzyme (*AroQ-PheA*) in the phenylalanine biosynthetic branch (Fig. 1). Repression plays a relatively minor role in aromatic biosynthesis of *P. aeruginosa* and appears to be limited to three steps in the tryptophan biosynthetic branch (6–11). It is important to note that, in the examples cited, all end products are intracellular metabolites. The question which concerns us here is whether the same general rules also apply to the bacterial production of extracellular metabolites. As an example, we will consider the siderophore pyochelin and its biosynthetic precursor salicylate (Fig. 1), which are produced and excreted by *P. aeruginosa* during iron limitation (12). Pyochelin synthesis starts from chorismate (13–15), a branch point intermediate in aromatic biosynthesis, and uses two molecules of L-cysteine (Fig. 1). Interestingly, pyochelin causes induction rather than repression of its biosynthetic enzymes (16). The mechanism of this autinduction is not entirely clear but probably involves an initial interaction of pyochelin with its outer membrane receptor, FptA, followed by activation of the transcriptional regulator PchR, which turns on the transcription of the pyochelin biosynthetic operons *pchDCBA* and *pchEFGHI* (16–18). In this signal transduction pathway, the end product pyochelin is unlikely to accumulate in the cytoplasm. A similar regulatory mechanism has been observed for another siderophore of *P. aeruginosa*, pyoverdin (19). When cells have accumulated excess iron the Fur repressor is activated, which switches off the expression of the pyochelin and pyoverdin biosynthetic genes (15, 16, 20).

Here, we ask how the activity of the first enzyme of pyochelin biosynthesis, isochorismate synthase (ICS) 1; EC 5.4.99.6, is regulated. This enzyme catalyzes the conversion of chorismate to isochorismate and is the product of *pchA*, the last gene of the *pchDCBA* operon in *P. aeruginosa* (14, 15). The subsequent reaction is catalyzed by the *pchB* product, isochorismate pyruvate-lyase (21), which produces salicylate (Fig. 1). The *pchA* gene is strictly co-expressed with the upstream *pchB* gene; without *pchB* being present in cis no expression of *pchA* can be observed (14), suggesting that ICS and isochorismate pyruvate-lyase are produced in proportional amounts by the cells under all circumstances. Here, we report that purified ICS of *P. aeruginosa* is insensitive to end products of aromatic biosynthesis, in particular to salicylate, and that salicylate formation is determined essentially by the concentration rather than by allosteric control of the first enzyme. This also has implications for the productivity of the pyochelin biosynthetic pathway.

**EXPERIMENTAL PROCEDURES**

**Materials**—*P. aeruginosa* strains PAO1 (wild type) and ADD1976 (PAO1 with the T7 RNA polymerase, chromosomally expressed from the lac promoter) (25) as well as plasmid pmE3359 (P$_{\text{T7-pchBA}}$) have

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1 The abbreviations used are: ICS, isochorismate synthase; Dha, dihydroaeruginosate; DTT, dithiothreitol; IPTG, isopropyl-β-D-thiogalactopyranoside.
been described previously (14). The construction of plasmid pME3295 is detailed in the legend of Fig. 2. Media and culture conditions for growth of *P. aeruginosa* have been given elsewhere (14–16). Sodium isochorismate, used as a reference, was a kind gift from E. W. Leistner (University of Cologne) or was prepared and purified as described previously (21). Racemic dihydroaeruginoate (Dha) and chorismate were prepared and purified as described previously (21). Protein concentrations were determined by the methods of Serino et al. (26), and Favre (28) using the low molecular weight calibration kit from Amersham Biosciences as a standard, by Ferguson plot analysis (29). The slopes obtained from plots of the logarithm of relative mobility versus molecular mass concentration of the protein were used to estimate the apparent molecular mass of PchA. The molecular mass of native PchA was also estimated from PAGE in non-denaturing gels of 7.5, 10, 12, 15, and 20% polyacrylamide, with the low molecular weight calibration kit from Amersham Biosciences as a standard, by Ferguson plot analysis (29). The slopes obtained from plots of the logarithm of relative mobility versus molecular mass concentration of the protein were used to estimate the apparent molecular mass of PchA.

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**PURIFICATION OF THE PCHA ENZYMES—Crude cell extracts were prepared from *P. aeruginosa ADD1976 harboring the T7 promoter construct pME3359 (Fig. 2) and grown in 750 ml of nutrient yeast broth with isopropyl-β-D-thiogalactopyranoside (IPTG) induction, as described for the extraction of PchB from a similar strain (25). Extracts contained 8 mg of protein per milliliter of buffer A (50 mM potassium phosphate buffer, pH 7.5, containing 10% (v/v) glycerol and 1 mM dithiothreitol (DTT)). Crude extract (15 ml) was applied to a DEAE-Sepharose CL-6B column (1.6 x 20 cm) equilibrated with 10 volumes of buffer A. PchA was eluted by washing the column with 300 ml of buffer A at a flow rate of 1 ml/min. The fractions containing PchA (180 ml) were combined and loaded onto a column of phenyl-Sepharose CL-4B (1.6 x 10 cm) equilibrated with buffer A. Most of the contaminant proteins, including PchB, were eluted by washing the column with 200 ml of buffer A at 1 ml/min. More hydrophobic proteins were eluted with a step gradient of ethylene glycol as follows: 25% (v/v), 60 ml; 25–40% (v/v), 60 ml; and 40% (v/v), 65 ml. PchA was eluted with about 40 ml of 40% (v/v) ethylene glycol. This fraction was diluted five times, resulting in a buffer of 10 mM potassium phosphate, 8% (v/v) ethylene glycol, 2% (v/v) glycerol, and 1 mM DTT, and it was loaded onto a MonoQ HR 5/5 column (fast protein liquid chromatography) equilibrated with modified buffer A containing 10 mM potassium phosphate. PchA was eluted by washing the column with standard buffer A at a flow rate of 1 ml/min. PchA-containing fractions (4 ml) were pooled and stored at –80 °C.

**ANALYSIS OF PROTEINS—Protein concentrations were determined by the method of Bradford (27) using a commercial reagent (Bio-Rad) and bovine serum albumin as the standard. The N-terminal sequence of PchA was determined by Dr. P. James (Eidgenössische Technische Hochschule, Zürich, Switzerland) on an Applied Biosystems peptide sequencer model 473A using Edman degradation. The subunit molecular mass of PchA was estimated by SDS-PAGE according to Laemmli and Favre (28) using the low molecular weight calibration kit from Amersham Biosciences as a standard. The molecular mass of native PchA was estimated by gel filtration chromatography on Sephadex G-150 (1.6 x 70 cm, 0.1 ml/min) and Bio-Gel P100 (1.6 x 70 cm, 0.1 ml/min) columns in buffer A with ribonuclease A (13.7 kDa), lysozyme (14.6 kDa), proteinase K (28.8 kDa), pepsin (34.5 kDa), protein A (42 kDa), ovalbumin (43 kDa), and bovine serum albumin (67 kDa) as markers. The elution volumes were plotted against the logarithm of molecular masses for the standards, and the linear regression curve was used to estimate the apparent molecular mass of PchA. The molecular mass of native PchA was also estimated from PAGE in non-denaturing gels of 7.5, 10, 12, 15, and 20% polyacrylamide, with the low molecular weight calibration kit from Amersham Biosciences as a standard, by Ferguson plot analysis (29).**

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**Preparation of Antiserum and Western Immunoblot Procedure—Rabbit polyclonal antibodies were generated by subcutaneous injection of about 410 μg of purified PchA and used in immunoblots as described (21).**

**Coupled ICS Assay—Unless otherwise stated, the incubation mixture contained, in a final volume of 500 μl, 100 mM potassium phosphate buffer, pH 7.0, 10 mM MgCl₂, 10% (v/v) glycerol, 1 mM DTT, 500 μM chorismate (purified by high pressure liquid chromatography), 48 units of purified PchB (corresponding to 3.7 μg) (21), and ≤2 units of PchA. One unit of enzyme activity is defined as the formation of 1 nmol of isochorismate (assayed as salicylate) per minute for ICS and isochorismate pyruvate-lyase. The reaction at 37 °C was initiated by the addition of chorismate to the enzyme solution and terminated after 5 min by the addition of 10 μl of concentrated HCl (10 M), followed by extraction with 3 ml of ethyl acetate. Blanks were obtained from non-incubated complete reaction mixtures. The product of the coupled enzymatic reaction, salicylate, was measured by its fluorescence using an excitation wavelength of 305 nm and an emission wavelength of 440 nm. The amount of salicylate formed was determined from a standard curve obtained with 0.5–8 μM salicylic acid in ethyl acetate. Assays for kinetic studies were performed in triplicate with 0.96 μg of purified PchA and 3.7 μg of PchB in standard incubation buffer with chorismate concentrations varying from 1 to 500 μM. The steady-state kinetic values did not vary by more than ±10%. Initial velocity data were fitted to the equation of Hanes, using Enzpack software (BioSoft).

The influence of Mg²⁺ on the activity of PchA was studied in an incubation mixture containing 100 mM potassium phosphate, pH 7.0, 1 mM DTT, and 10% (v/v) glycerol (21). PchA (3.9 μg) was preincubated at 37 °C for 10 min. EDTA and MgCl₂ were added at concentrations of 1 mM and 10 mM, respectively. The reaction was started by adding PchB (0.54 μg) and 100 μM chorismate and stopped after 5 min.

**RESULTS**

**Purification of the PchA Enzyme—ICS activity was measured in a coupled assay in the presence of an excess of PchB (typically ~30-fold with respect to units of enzyme activity). Thus, the isochorismate formed was converted quantitatively to salicylate during the incubation. Because the pchA gene is expressed only when the pchB gene is present in cis (14), we isolated PchA from *P. aeruginosa* ADD1976 carrying pME3359,
Isochorismate Synthase of \textit{P. aeruginosa}

PchA was purified to apparent homogeneity with 58% yield by three chromatographic steps (Table I). SDS-PAGE of the fraction obtained after the final MonoQ chromatography step indicated a ≈ 98% pure protein of about 50 kDa (data not shown). This subunit molecular mass is in good agreement with that (52.1 kDa) calculated from the deduced sequence of 476 amino acids residues (14). The N-terminal amino acid sequence of the PchA polypeptide (Ser-Arg-Leu-Ala-Pro-Leu-Ser-Gln) obtained by Edman degradation matched that predicted from the PchA gene (30, 31). We tested a range of potential effectors of PchA. However, no effect of any of these compounds on the PchA activity was observed (data not shown). PchA showed hyperbolic saturation kinetics with its substrate, chorismate, with an apparent $K_m$ of 4.5 ± 0.5 μM and a $V_{max}$ of 43.1 ± 4.9 min⁻¹. Optimal activity was observed at pH 7.0 (data not shown). The chorismate-isochorismate interconversion catalyzed by PchA was reversible; incubation of PchA with isochorismate yielded chorismate (Fig. 3). Reversibility has also been observed for both ICSs of \textit{E. coli} (30, 32).

We tested a range of potential effectors of PchA. However, <10% inhibition or activation of ICS activity was found under standard assay conditions after the addition of either the end product pyochelin (100 μM), the pathway intermediates salicylate (10 μM) or dihydroaeruginosinate (Dha in Fig. 1) (100 μM), and the aromatic amino acids tryptophan (100 μM), tyrosine (100 μM) or phenylalanine (100 μM). Furthermore, the addition of Fe²⁺ (100 μM) or cysteine (200 μM) did not significantly alter ICS activity. Thus, there is no evidence that aromatic amino acids or metabolites of the pyochelin pathway in \textit{P. aeruginosa} (Fig. 1) can regulate the activity of the PchA enzyme.

\textbf{PchA Concentration Limits the Rate of Salicylate Formation in Vitro}—To determine the rate-limiting factor in salicylate production, we prepared a crude extract from PAO1 wild type cells grown under iron limitation. A sample of this extract containing ~150 ng of PchA and ~100 ng of PchB, as judged by Western blots (data not shown) per 290 μg of total cellular protein, was incubated in the presence of 100 μM chorismate in 500 μl of incubation buffer. Under these conditions, the formation of salicylate was limited by the PchA concentration in the extract (Fig. 4). This could be seen when an excess (500 ng) of purified PchA was added; thereby, the rate of salicylate synthesis was increased ~3-fold and the transient time, i.e., the lag before steady state conditions were reached in the coupled enzyme reaction, was shortened from 1.5 min to <0.1 min (Fig. 4). This reduction of the transient time illustrates the fact that added PchA enhances the availability of the intermediate isochorismate to the second enzyme in the extract, PchB. By contrast, the addition of 500 ng of purified PchB did not enhance the capacity of the extract to synthesize salicylate (Fig. 4). These results indicate that in a crude \textit{P. aeruginosa} PAO1 extract the activity of the first enzyme of the pathway, i.e., the synthesis of isochorismate, limits the rate of salicylate production.

\textbf{PchA Concentration Limits the Production of Salicylate and Pyochelin in Vivo}—To test the role of the first enzyme in salicylate formation and to see how salicylate availability influences the productivity of the pyochelin pathway, we constructed the pchBA overexpression plasmid pME3395 (Fig. 2) in which the pchBA genes were fused to the inducible tac promoter ($P_{tac}$), and the pchB function was inactivated by an in-frame deletion, removing notably the codon for the essential Ile-88 residue of isochorismate pyruvate-lyase (21). We verified that in \textit{vivo} the internally truncated PchB protein was totally devoid of isochorismate pyruvate-lyase activity. The mutated protein also lacked chorismate mutase activity (data not shown), the second PchB function (21). Using this somewhat unconventional construct, we overcame the problem that the pchA open reading frame cannot be expressed alone, even when it is equipped with a strong promoter and a good ribosome binding site (14).

Salicylate, Dha, and pyochelin were measured in cultures of the wild type PAO1, with or without the $P_{tac}$ pchA overexpression construct pME3395. The growth medium used (GGP) contains glycerol and proteose peptone and favors pyochelin production because of limited iron availability (16, 33). Proteose peptone, a milk fraction containing mostly casein cleavage products, is a rich source of amino acids with the notable exception of cysteine, which is underrepresented (34). We therefore also conducted a series of experiments using GGP medium amended with 2 mM L-cysteine. In both media, pchA overexpression driven by the addition of the inducer IPTG caused strong salicylate accumulation and pyochelin overproduction during stationary phase, concomitant with the increased accumulation of Dha (Table II). During late exponential phase, the addition of 2 mM L-cysteine significantly enhanced the conversion of salicylate to pyochelin; however, irrespective of cysteine addition, pyochelin concentrations were consistently increased by pchA overexpression in comparison.
Isochorismate Synthase of P. aeruginosa

Table I

| Purification step | Protein | Activity | Specific activity | Yield | Purification |
|-------------------|---------|----------|-------------------|-------|--------------|
| Crude cell extract| 133     | 10,369   | 78                | 100   | 1            |
| DEAE-Sephrose CL-6B| 31.6    | 8,814    | 279               | 85    | 3.6          |
| Phenyl-Sepharose CL-4B| 8.0     | 7,105    | 890               | 69    | 11.4         |
| MonoQ HR 5/5      | 3.6     | 6,015    | 1,671             | 58    | 21.4         |

Data shown are taken from a typical preparation. In three independent experiments, the reproducibility of the purification factors was ±10%.

FIG. 3. Reversibility of the reaction catalyzed by PchA. Purified PchA (1 μg) from P. aeruginosa was incubated with 200 μM isochorismate in 100 mM potassium phosphate, pH 7.0, containing 10 mM MgCl₂, 10% (v/v) glycerol, and 1 mM DTT. The reaction was stopped after 10 min (——) by the addition of 10 μl concentrated HCl, followed by extraction with ethyl acetate. In the control (----), the enzyme was omitted. After evaporation of the organic phase, the dry residue was dissolved in 100 μl 50% (v/v) acetonitrile and 0.43% (w/v) H₃PO₄. A 20-μl aliquot was injected into a Hewlett-Packard 1050 series LC system. Elution of the Nucleosil C-18 column (4 × 250 mm) at 1 ml/min was carried out with a linear gradient consisting of solvent A (0.43% (w/v) H₃PO₄) and solvent B (95% (v/v) acetonitrile in 0.43% (w/v) H₃PO₄), whereby solvent B increased from 7% (v/v) at 0 min to 54% (v/v) at 15 min. Compounds were identified by their retention times (established with synthetic compounds) and UV spectra. Isochorismate and chorismate were eluted at 8.7 and 11.2 min, respectively.

FIG. 4. Salicylate formation in cell extract from strain PAO1. Crude extract was prepared by sonication from strain PAO1 grown under iron limitation in GGP medium (33) to 2.1 × 10⁹ cells/ml. The incubation mixture (500 μl) contained 290 μg of protein from this extract in 100 mM potassium phosphate, pH 7.0, containing 10 mM MgCl₂, 10% (v/v) glycerol, 1 mM DTT, and 100 μM chorismate. +PchA, addition of 0.5 μg purified PchA; +PchB, addition of 0.5 μg purified PchB. The reaction was started by addition of the substrate and carried out at room temperature. Salicylate formation was monitored continuously in a luminescence spectrometer at 440 nm using an excitation wavelength of 305 nm.

whether or not the medium contains an extra supply of the cosubstrate L-cysteine. (iii) Excess iron brings the expression of PchA (and the other enzymes of the pathway as well) to a halt by Fur-mediated repression of the pchDCBA operon (15, 16).

DISCUSSION

Using the siderophore pyochelin of P. aeruginosa as an example, we have addressed the question of where the bottleneck lies in a bacterial biosynthetic pathway leading to extracellular products. Clearly, the classical pattern, i.e. feedback inhibition and repression by the end product, is not observed here. Instead, we propose that the concentration of the first, nonallosteric enzyme, ICS, is one key determinant controlling the productivity of the pyochelin pathway (Table II). A similar observation has been made in filamentous fungi where penicillin production is critically dependent on the amount of the first enzyme, δ-(L-aminoadipyl)-L-cysteinyl-D-valine synthetase (35). Furthermore, in Streptomyces clavuligerus the reaction catalyzed by this enzyme is a rate-limiting step in cephalosporin biosynthesis (36). However, in other bacterial pathways producing extracellular compounds, the rate-limiting steps have rarely (if ever) been investigated.

In its native context, the pchA gene is placed last in the pchDCBA operon, which encodes, in this order, a salicylate-activating enzyme (22), a thioesterase of unknown function (15), isochorismate pyruvate-lyase (21), and ICS (Ref. 14, and this study). The promoter of this operon is positively controlled by the PchR protein in the presence of pyochelin (16) and negatively by the Fur repressor in the presence of iron (15). Within the operon, the expression of the pchDC and pchBA genes is tightly coordinated at a post-transcriptional level (14).
Thus, iron availability and pyochelin acting as an autoinducer are two major signals that determine the productivity of the pathway by regulating pchA expression.

The high affinity of PchA for chorismate \( (K_m = 4.5 \mu M) \) enables this enzyme to draw effectively on the chorismate pool in competition with the other enzymes of aromatic metabolism in \( P.\ aeruginosa \) (Fig. 1). In strain PAO1/pME3395, maximizing PchA expression by IPTG addition caused no measurable reduction in exponential growth rate and growth yield (data not shown), suggesting that maximal pyochelin synthesis does not seriously deplete the chorismate resources.

The data of Table II also show that cysteine availability in the growth medium improves the conversion of salicylate to pyochelin during growth and enhances the yield of pyochelin, especially in the stationary phase. Similar observations have recently been reported for another strain of \( P.\ aeruginosa \) (37). It is not known, however, whether there are regulatory links between pyochelin and cysteine synthesis in \( P.\ aeruginosa \).

A multiple sequence alignment (Fig. 6) (38) places PchA of \( P.\ aeruginosa \) in a family of a dozen microbial ICSs that are currently known. As noted previously (14), the TrpE component of anthranilate synthase and the PabB component of the aminobenzoate synthase of various microorganisms show significant sequence identities with PchA, essentially because of a shared chorismate binding domain (39, 40). However, the members of the ICS family do not intermingle with the other enzymes of aromatic metabolism and the aminodeoxychorismate synthase of various microorganisms. Therefore, the TrpE component of anthranilate synthase and the PabB component of the aminobenzoate synthase of various microorganisms are represented. Accession number (protein identification number given by the NCBI) and strain are given in parenthesis.

![Fig. 5. Immunoblot analysis of PchA. A, samples were taken from cultures of PAO1, PAO1/pME3395, and PAO1/pME3395 (induced with IPTG) grown as described in Table II. 10^6 cells were lysed in 50 μl of 10 mM Tris-HCl, pH 8.0, containing 1 mM EDTA, 2.5% (v/v) SDS, and 5% (v/v) β-mercaptoethanol, and one-twentieth of this lysate was separated by SDS-PAGE on a 12.5% gel. Western blot analysis using a polyclonal anti-PchA antiserum was carried out as described (25). PchA, 50 ng of purified PchA. B, total cellular protein (12 μg) from strain PAO1 grown in GGP medium (33) without or with 100 μM FeCl3, was electrophoresed and subjected to immunoblotting as above. PchA, 100 ng of purified PchA.

![Fig. 6. Sequence comparison of ICS, anthranilate synthase (TrpE), and p-aminobenzoate synthase (PabB). Sequences were aligned using ClustalW (38). All currently known ICSs of bacteria are shown, whereas only selected examples of TrpE and PabB sequences are represented. Accession number (protein identification number given by the NCBI) and strain are given in parenthesis. PchA (S58229, \( P.\ aeruginosa \)); VibC (NP_230422, \( V.\ cholerae \)); Dhbc (NP_391079, \( B.\ subtilis \)); PmsC (CAA70528, \( Pseudomonas\ fluorescens \)); EntC (AAB40793, \( E.\ coli \)); AnaA (P23390, \( Aeromonas\ hydrophila \)); MenF (NP_390961, \( B.\ subtilis \)); MenF (NP_231610, \( V.\ cholerae \)); MenF (P38051, \( E.\ coli \)); MbtI (NP_216902, \( M.\ tuberculosis \)); IpP9 (CAE46570, \( Y.\ enterococci \)); YbtS (NP_405477, \( Y.\ pestis \)); Trpe (NP_230819, \( V.\ cholerae \)); Trpe (NP_415780, \( E.\ coli \)); Trpe (NP_405749, \( Y.\ pestis \)); Trpe (NP_230149, \( B.\ subtilis \)); Trpe (NP_249300, \( P.\ aeruginosa \)); Trpe (NP_206125, \( M.\ tuberculosis \)); PabB (NP_387965, \( B.\ subtilis \)); PabB (NP_405340, \( Y.\ pestis \)); PabB (NP_416326, \( E.\ coli \)); PabB (NP_215521, \( M.\ tuberculosis \)).]
the PabB and TrpE families, contrary to the intrusion of the PabB of Bacillus subtilis into the TrpE cluster (Fig. 6, left side). The ICS tree constructed by sequence alignment (Fig. 6, right side) has no resemblance with phylogenetic trees based on a sequence comparison of 16 S RNAs or housekeeping proteins (41), strictly that the ICS genes have traveled widely in the microbial world. This idea is supported by the finding that the ird-E and ybtS genes, which are required for yersiniabactin biosynthesis, are part of mobile pathogenicity islands (42). Other ICS genes might also be part of pathogenicity islands and, as such, are transmissible between different bacteria. It has been speculated that MbtI of Mycobacterium tuberculosis, YbtS of Yersinia pestis, and Irp-9 of Yersinia enterocolitica, which are peripheral ICS family members (Fig. 6) and interacts with the vicinity of the ICS genes (43, 44). We have examined the purified P. aeruginosa ICS for its ability to produce salicylate, whereas the same strain carrying PabB homolog has been found to produce salicylate, whereas the same strain carrying pme3368 (pchAB−) did (14). If the MbtI, YbtS, and Irp-9 proteins catalyzed just the chorismate-to-isochorismate conversion, another explanation should be sought. We found that the PchB enzyme of P. aeruginosa is structurally and perhaps also functionally related to chorismate mutase (21). It is therefore conceivable that in Yersinia and Mycobacterium spp. the isochorismate pyruvate-lyase reaction might be executed by a chorismate mutase. A similar situation may possibly also occur in Arabidopsis thaliana, where a PchA-like enzyme but no PchB homolog has been found (40).

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