Malate synthase from *Streptomyces clavuligerus* NRRL3585: cloning, molecular characterization and its control by acetate

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Malate synthase is a key enzyme of the glyoxylate cycle, which is an anaplerotic pathway essential for growth on acetate as the sole carbon source. The aceB gene, encoding malate synthase from *Streptomyces clavuligerus* NRRL 3585, was cloned using PCR and fully sequenced. The ORF obtained encodes 541 amino acids with a deduced *M* of 60 000, consistent with the observed *M* (62 000–64 000) of most malate synthase enzymes reported so far. The aceB gene has a high G+C content (71.5 mol%), especially in the third codon position. A 50 bp region upstream of the malate synthase ORF was predicted to be a prokaryotic promoter region. The relationship between carbon source, antibiotic (cephalosporin) biosynthesis and malate synthase activity was investigated. Growth of *S. clavuligerus* on acetate as the major carbon source was delayed, compared to that on glycerol. Furthermore, high levels of malate synthase activity were associated with the presence of acetate in the growth medium. Growth on acetate also resulted in lower levels of cephalosporin production, compared to that on glycerol. The cloned *S. clavuligerus* aceB gene was expressed in *Escherichia coli* BL21 (DE3). Transformants exhibited an approximately 71-fold increase in malate synthase activity, compared to the control, thereby demonstrating high-level expression of soluble and enzymically active malate synthase in the heterologous host.

Keywords: *Streptomyces clavuligerus*, acetate metabolism, malate synthase, glyoxylate cycle

**INTRODUCTION**

Malate synthase, which catalyses the reaction

\[
\text{Acetyl-CoA + glyoxylate + H}_2\text{O} \rightarrow \text{malate + CoA}
\]

is a key component of the glyoxylate pathway, leading to the net synthesis of one molecule of malate from two molecules of acetate (Moat & Foster, 1995). This pathway is significant in acetate metabolism, as it allows the replenishment of three-carbon molecules such as phosphoenolpyruvate, which are normally depleted from the TCA cycle for gluconeogenesis (Maloy et al., 1980). Thus, malate synthase activity is detectable in micro-organisms grown in acetate (Hüttner et al., 1997; Reinscheid et al., 1994; Maloy et al., 1980), and bacteria which are defective in malate synthase have been reported to be impaired for growth where acetate is the sole carbon source (Reinscheid et al., 1994).

It is well known that genes encoding primary metabolic enzymes sometimes occur in multiple copies, which are regulated differently to respond to diverse physiological conditions. Consistent with this observation, *Escherichia coli* is known to possess two forms of malate synthase, i.e. A and G, encoded by the aceB and glcB genes, respectively. The A form is predominant in cells growing on acetate, while the presence of the G form accounts for almost the entire malate-synthesizing activity of cells grown on glycolate (Maloy et al., 1980). Similarly, *Saccharomyces cerevisiae* was also found to possess at least two genes encoding malate synthase, namely MLS1 and DAL7. MLS1p participates in carbon metabolism and is susceptible to carbon catabolite repression, while insensitive to nitrogen catabolite repression. Conversely, DAL7p, which participates in catabolism of the nitrogenous compound allantoin via
glyoxylate, is insensitive to carbon catabolite repression, but is highly sensitive to nitrogen catabolite repression (Hartig et al., 1992). However, not all organisms maintain multiple copies of malate synthase genes in their genome. Corynebacterium glutamicum, for example, is thought to possess only one copy of the malate synthase gene, the disruption of which led to impairment of growth on acetate (Reinscheid et al., 1994). Nevertheless, malate synthase is generally an enzyme that has multiple physiological functions in addition to its more obvious role in acetate metabolism.

Due to its physiological significance, genes or cDNAs encoding malate synthase have been cloned and sequenced from bacteria (e.g. E. coli, C. glutamicum and Streptomyces sp), fungi (e.g. S. cerevisiae, Neurospora crassa, Aspergillus nidulans and Candida tropicalis) and plants (e.g. Cucumis sativus and Brassica napus) (Byrne et al., 1988; Molina et al., 1994; Reinscheid et al., 1994; Huttner et al., 1997; Fernandez et al., 1993; Thomas et al., 1988; Sandeman et al., 1991; Hikida et al., 1991; Graham et al., 1989; Comai et al., 1989). While malate synthase has been extensively studied in many organisms, the characterization of a similar glyoxylate cycle has not been reported in Streptomyces spp. However, by chance, a partial DNA sequence encoding Streptomyces clavuligerus malate synthase was identified in the course of another sequencing project. This led to the subsequent cloning of the entire gene, using genome walking techniques based on anchored PCR. Since malate synthase genes are widely distributed among representative groups of prokaryotes as well as eukaryotes, we investigated the diversity of this gene by computational analysis so that the phylogenetic position of the S. clavuligerus malate synthase gene could be determined. The relationship between growth conditions, malate synthase activity and β-lactam antibiotic (cephalosporin) production by S. clavuligerus was also studied via growth experiments, to determine the physiological role of malate synthase. Finally, the gene was expressed with demonstrable enzymic activity in E. coli BL21(DE3), thereby validating the biochemical functionality of the cloned gene.

**METHODS**

**Strains, plasmids and growth conditions.** The bacterial strains and plasmids used in this study are shown in Table 1. E. coli was cultured in LB medium (Sambrook et al., 1989) at 37°C on a rotary shaker at 250 r.p.m. Where necessary, ampicillin or kanamycin was used at a final concentration of 50 μg ml⁻¹. S. clavuligerus seed culture was obtained as described by Aharonowitz & Demain (1978). For the fermentation experiments, the seed culture was inoculated at 2% (v/v) into basal chemically defined medium (Aharonowitz & Demain, 1978) and incubated at 28°C with vigorous shaking (250 r.p.m.). The basal chemically defined medium contained: 0.06% MgSO₄, 0.35% K₂HPO₄ and 1 ml trace salts solution (containing 100 mg each of FeSO₄·7H₂O, MnCl₂·4H₂O, ZnSO₄·H₂O and CaCl₂ per 100 ml water) per litre 0.1 M MOPS buffer. The carbon source used was either 0.5% (w/v) glycerol or 0.5% (w/v) sodium acetate (both from Merck). The nitrogen source used was 0.4% asparagine (Sigma). The initial pH of all media was adjusted to 6.8 with KOH.

**Table 1. Bacterial strains and plasmids used in this study**

<table>
<thead>
<tr>
<th>Strain/plasmid</th>
<th>Description</th>
<th>Source/reference</th>
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<tbody>
<tr>
<td><strong>Strains</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Streptomyces clavuligerus NRRL 3585</td>
<td>Wild-type</td>
<td>ATCC 27064</td>
</tr>
<tr>
<td>Escherichia coli TOP10F'</td>
<td>Cloning host</td>
<td>Clontech</td>
</tr>
<tr>
<td>Escherichia coli BL21(DE3)</td>
<td>Expression host; carries T7 RNA polymerase gene under control of lacUV5 promoter</td>
<td>Novagen</td>
</tr>
<tr>
<td><strong>Plasmids</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pET24a</td>
<td>Kan' Cloning vector provided with TA cloning kit</td>
<td>Novagen</td>
</tr>
<tr>
<td>pAdvantage</td>
<td>Cloning vector</td>
<td>Clontech</td>
</tr>
<tr>
<td>pTZ18U</td>
<td>Cloning vector</td>
<td>BioRad</td>
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<tr>
<td>pSM51</td>
<td>pET24a with S. clavuligerus aceB gene inserted under the control of the phage T7 promoter</td>
<td>This study</td>
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</table>

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**Genome walking, PCR and DNA sequencing.** Genome walking was carried out using the Universal GenomeWalker Kit (Clontech), which is a simple method for cloning unknown genomic sequences adjacent to a DNA fragment of known sequence (Fig. 1). In the first step, pools of uncloned, adapter-ligated genomic DNA 'libraries' from S. clavuligerus were constructed. This was done by complete digestion of separate aliquots of S. clavuligerus genomic DNA with different restriction enzymes. Each batch of digested product was then
Malate synthase of *Streptomyces clavuligerus*

![Diagram of cloning process](image)

Fig. 1. Cloning of the *S. clavuligerus* aceB gene using anchored PCR. Gene-specific primers (GSPs) designed using sequences from the 1865 fragment (containing nt 1–511 of aceB coding region) were used to isolate fragment msGW1, which contained nt 478–968 of the aceB coding region. Subsequently, GSPs designed using sequences from msGW1 were used to isolate msGW2, which contained nt 824–1623 of the aceB coding region. Finally, the DNA sequence of fragments 1865, msGW1 and msGW2 were aligned to obtain the entire aceB gene sequence.

ligated separately to the GenomeWalker Adaptor. After this, a primary PCR amplification was carried out using an outer adaptor primer (AP1) provided in the kit and an outer, user-defined gene-specific primer (GSP1), which was designed according to known DNA sequences near the unknown target region. The resultant PCR mixture was then diluted and used as a template for a secondary, ‘nested’ PCR with the nested adaptor primer (AP2) and a nested gene-specific primer (GSP2). These primers were located downstream of AP1 and GSP1, respectively, closer to the unknown region to be amplified. This secondary amplification step serves to confirm the identity of the positive band and also to intensify the band to facilitate cloning. After amplification, the resultant mixture was electrophoresed through an agarose gel. The major PCR product was isolated using the Geneclean kit (Bio101) and cloned into pAdvantage vectors using the TA cloning kit (Clontech) for sequencing. All PCR reactions were carried out using the Advantage *Tth* Polymerase Mix (Clontech) and the amplification cycle stipulated in the instruction manual for the GenomeWalker Kit.

DNA sequencing was performed using the ABI Prism Dye Terminator Cycle Sequencing Ready Reaction Kit. Electrophoretic separation of the completed sequencing reaction was performed on an ABI 373 automated DNA sequencer.

**Computer analysis and phylogenetic tree construction.** The sequences used for multiple alignment of malate synthase amino acid sequences were downloaded from the GenBank, GenBank Updates and SWISS-PROT Databases. Promoter prediction was carried out using the Prokaryotic Promoter Prediction by Neural Network software distributed by the Baylor College of Medicine, Houston, USA. *M* was deduced using computational programs at the ExPASy molecular biology server of the Geneva University Hospital and the University of Geneva. Multiple-alignment and neighbour-joining trees (Saitou & Nei, 1987) were constructed using the
CLUSTAL W program (Thompson et al., 1994). Parsimony analysis, bootstrap calculations and consensus tree building were carried out using the PROTPARS, SEQBOOT and CONSENSE programs, respectively, from the PHYLIP phylogenetic analysis package (Felsenstein, 1993). Trees were visualized using the TreeView 3.2 program, distributed by the University of Glasgow, UK.

Preparation and analysis of cell-free extracts. S. clavuligerus cells were harvested by centrifugation, washed, resuspended in TDE buffer (Jensen et al., 1986) and lysed by sonication using the MSE Soniprep with a 1/8 inch (3 mm) probe. Cell debris was removed by centrifugation and the resulting cell-free extract was used for the malate synthase assay and protein determination. The malate synthase assay was performed as described by Ornston & Ornston (1969). Protein concentration was determined using the Protein Assay Reagent (Bio-Rad) according to the manufacturer’s instructions. The bioassay for cephalosporin production was carried out by the indicator strain and cephalosporin C (Sigma) as the standard, as previously described (Brana et al., 1985). For determination of dry cell weight, mycelia were filtered with a Gelman 0.45 μm filter, washed and dried in a 60 °C oven overnight.

Heterologous expression of aceB in E. coli. BL21(DE3). For heterologous expression of S. clavuligerus aceB gene, the pET system (Novagen) was employed, using E. coli BL21(DE3) as the expression host. Primers annealing to the 5' and 3' ends of the coding regions of aceB were synthesized, to allow in-frame insertion of the aceB gene into the Ndel BamHI cloning sites of the pET24a vector. This placed the aceB gene under the control of strong bacteriophage T7 transcription and translation signals, in-frame with an ATG start site located in the vector. The inserted gene was fully sequenced to confirm its identity and frame of insertion. The resulting plasmid, designated pSMS1, was transformed into E. coli BL21(DE3). As the E. coli BL21(DE3) host contains a chromosomal copy of the T7 RNA polymerase gene under lacUV5 control, expression was induced by addition of IPTG (Clontech) at a final concentration of 1 mM. The induced cells were harvested after incubation at ambient temperature (22–25 °C) with shaking at 250 r.p.m. for 15 h and tested for malate synthase activity using the same procedure as for S. clavuligerus.

Reproducibility. To ensure accurate sequence data, clones obtained from the genome walking experiments were sequenced on both strands, with each sequencing reaction performed twice to ensure reproducibility. For the fermentation experiments, representative results from single experiments are shown, all of which were performed at least twice.

**RESULTS**

**PCR cloning of aceB from S. clavuligerus**

A 1.3 kb DNA fragment, 186S, containing a partial ORF carrying nt 1–511 of the coding region of a gene from S. clavuligerus which bears high homology to known malate synthase genes, was obtained during a sequencing project (Fig. 2). Primers designed from the 3' end of the partial reading frame were used in a genome-walking experiment to probe the adjoining gene sequence downstream using anchored PCR. The sequences of the GSPl and GSP2 primers used for this round of genome walking were 5' AGTCCTAGCTCTACGCTGGAGGGAATTG and 5' GATGCCGATGGCATCCTCGG, respectively. The GSPl and GSP2 primers were designed for this round of amplification, the GSPl and GSP2 primers were designed for this round of genome walking msGW1 fragment showing that it still did not carry the 3' end of the aceB gene. Hence, a second genome walking experiment was carried out to obtain the remaining downstream region of aceB. For this round of amplification, the GSP1 and GSP2 primers were designed based on the 3' end of msGW1; their sequences were 5' ATTCGTACGAGCTGCCACCGGACCACCACC and 5' GCTGACCAGGGGATCGGGTGGTGGTGGG, respectively. A third fragment, msGW2, containing the remaining 3' aceB sequence including the stop codon, was cloned and sequenced. The 3' end of msGW1 overlaps with 144 bp at the 5' end of msGW2, confirming that the cloned fragment was correct. Finally, the complete aceB gene sequence was obtained by alignment of the 186S, msGW1 and msGW2 sequences.

**Sequence analysis and phylogenetic analysis**

The results of successive cloning and sequencing experiments showed that the aceB coding region is 1623 bp long and encodes 541 amino acids with a predicted Mr of about 60000. The predicted Mr is consistent with the observed Mr (62000–64000) of most malate synthase enzymes (Molina et al., 1994). The coding region has a G+C content of 71.5 mol% and is extremely biased.
Malate synthase of *Streptomyces clavuligerus*

Fig. 3. Nucleotide and amino acid sequences of part of the 5' region of the *S. clavuligerus* aceB coding region, and its upstream nucleotide sequence. The underlined sequence indicates a putative promoter region, described in the text. The putative ribosome-binding site is boxed.

**Table 2.** Comparison between an *E. coli*-like streptomycete promoter consensus sequence and the putative aceB promoter.

<table>
<thead>
<tr>
<th>-35 region</th>
<th>No. of bp in spacer</th>
<th>-10 region</th>
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<tbody>
<tr>
<td>Consensus</td>
<td>T T G A C Pu</td>
<td>T A g Pu Pu T</td>
</tr>
<tr>
<td>Percentage</td>
<td>86 90 100 69 66 62</td>
<td>100 59 86 41 69 72 100</td>
</tr>
<tr>
<td>Observed putative promoter</td>
<td>T T G T T A T</td>
<td></td>
</tr>
<tr>
<td>Agreement with consensus</td>
<td>yes yes yes yes yes yes yes yes</td>
<td></td>
</tr>
</tbody>
</table>

Towards codons containing G or C in the third position (not shown). This position-specific preference for G and C nucleotides is characteristic of *Streptomyces* coding regions and is thought to reflect the adaptations to tRNA pools for the purpose of regulation of gene expression (Bibb et al., 1984). Using promoter-prediction software, a 50 bp region upstream of the cloned aceB gene was predicted to be a prokaryotic promoter region (Fig. 3). Interestingly, this region has a distinctly lower G+C content (48 mol %) than the aceB coding region (71.5 mol %). This suggests the presence of an *E. coli*-like streptomycete promoter region, which are known to have a characteristically low G+C content (57–62 mol %), compared to the coding regions (69–78 mol %) (Strohl, 1992). Putative -10 and -35 sequences were identified (Table 2) within the 50 bp upstream region, which bears strong homology to an *E. coli*-like streptomycete consensus promoter sequence compiled by Strohl (1992).

As malate synthase has been reported to be present in both eukaryotes and prokaryotes, it was interesting to investigate its ancestral diversification by sequence alignment and phylogenetic tree construction. Pairwise percentage identity between known malate synthase sequences indicated the presence of two distinct classes of enzymes: class A, formed by all malate synthase genes except *E. coli glcB* and *C. glutamicum aceB*; and class G, formed by the latter two bacterial genes alone (Fig. 4). Within class A, the homology ranges from 39–98%, while the two genes belonging to class G show 59% homology. In contrast, the homology between these two
classes of sequences is very low, in the range 13–18%. Phylogenetic trees were drawn using known sequences from sequence databases. As mentioned above, E. coli glcB and C. glutamicum aceB are very diverged from the rest of the malate synthase genes. Hence, these sequences were excluded for construction of the trees presented in Fig. 5 because their inclusion may lead to unreliable prediction due to stochastic errors as a result of random substitutions and compositional biases (Hashimoto et al., 1995). Otherwise, the data sets used for Figs 4 and 5 were identical. Phylogenetic trees plotted using two independent algorithms, i.e. maximum parsimony and neighbour joining, were found to give largely the same results. Sequences from plants and fungi formed clear monophyletic groups in trees constructed with both methods. On the other hand, bacterial sequences seemed to be more diversified and were not organized in a monophyletic group.

**Dependence of S. clavuligerus malate synthase activity on culture conditions**

Since the isolation of a gene encoding malate synthase suggests the operation of a glyoxylate cycle, it was of interest to examine malate synthase activity in S. clavuligerus and to determine the dependence of malate synthase activity on culture conditions. Studies on carbon-source requirements of S. clavuligerus have previously been conducted (Aharonowitz & Demain, 1978). However, we could not ascertain the utilization of acetate from those data, as growth was monitored up to only 96 h, and biomass increase seemed to be minimal. Hence, we grew S. clavuligerus cells in 0.5% (w/v) acetate or 0.5% (w/v) glycerol, and monitored growth for up to 16 d. Interestingly, growth in acetate was much delayed, compared to that in glycerol. Similar levels of maximal biomass could be attained using both substrates (Fig. 6). However, unlike growth in glycerol, growth in acetate was accompanied by a marked alkalination of the culture medium, an observation that is consistent with previous findings on the growth of S. clavuligerus on organic acids (Aharonowitz & Demain, 1978).

Malate synthase activity was detected at high levels in S. clavuligerus when cells were grown in acetate medium. The increase in malate synthase activity was not associated with accumulation of biomass. Rather, the malate synthase activity increased sharply after growth in acetate.

Secondary metabolism is often connected to, and influenced by, pathways of primary metabolism, it therefore became of interest to observe whether the high-level, but late, expression of malate synthase might affect cephalosporin production. The results showed that growth in acetate was not accompanied by the usual optimal level of cephalosporin production when glycerol was provided.
Heterologous expression of *S. clavuligerus* aceB in *E. coli* BL21(DE3)

To date, the only malate synthase gene to be cloned and expressed in *E. coli* is aceB of *C. glutamicum* (Reinscheid et al., 1994). To investigate whether a similar gene from *S. clavuligerus* can also be expressed in *E. coli*, the aceB gene had to be cloned in its entirety. We therefore constructed pSMS1, in which the aceB gene was placed under the control of a strong IPTG-inducible bacteriophage T7 promoter (see Fig. 2). The clones transformed with pSMS1 exhibited an approximately 71-fold increase in malate synthase specific activity (781 nmol min⁻¹ mg⁻¹) compared to cells transformed with pET24a, which showed a very low level of activity (11 nmol min⁻¹ mg⁻¹). Moreover, when the assay was repeated with the omission of either acetyl-CoA or glyoxylate, no activity was detectable in the cell-free extracts of the clones transformed with pSMS1. This shows that the cloned aceB gene indeed encodes a functional malate synthase which is enzymically active when inducibly expressed in *E. coli* BL21(DE3).

**DISCUSSION**

**Diversification of malate synthase genes**

Many organisms possess multiple copies of malate synthase genes. Gene duplication followed by divergent evolution is sometimes invoked to explain the existence of such divergent classes of isoenzymes. It was thought that this would enable enzymes catalysing the same reaction to respond to different regulatory signals. Since the streptomyces are ubiquitous soil organisms, it would be interesting to explore the existence of malate synthase genes present in various species.

The wide distribution of malate synthase genes among representative groups of different organisms (plants, fungi, yeasts, Gram-positive and Gram-negative bacteria), has prompted the search for the ancestral hierarchy of this gene. Interestingly, two distinct classes of malate synthase genes have been identified, based on multiple alignment and homology of protein sequences. It was also noted that in *E. coli*, genes encoding the two classes, A and G, of malate synthase are regulated differently. Incidentally, the majority of malate synthase genes found belong to class A, while class G contained only two members. However, in *C. glutamicum*, there is only one malate synthase gene, which belongs to class G, and is mainly related to acetate metabolism, similar to some of the genes that form class A. Moreover, although the two malate synthase genes of *S. cerevisiae* are differentially regulated, their coding regions are basically similar and they belong to class A. Hence, there are no conclusive data to explain the distribution of the two classes of malate synthase genes. Analysis of the phylogenetic trees revealed a close relationship between the prokaryotic and the eukaryotic malate synthase...
genes, where both groups of genes were identified as paraphyletic. Moreover, this observation remains valid even if independent methods (i.e. distance and parsimony analyses) are employed for phylogenetic tree construction. In contrast, phylogenetic analyses of rRNA and recA genes (Woese, 1987; Brendel et al., 1997; Rijik et al., 1995), which are thought to reflect the evolutionary history of organisms, suggest that both prokaryotes and eukaryotes may have monophyletic origins. Hence, based on current information, the diversification pattern of the malate synthase gene is apparently different from that of the rRNA and recA genes. This suggests that the lineage of malate synthase genes in bacteria may be polyphylectic, and thus may have more than one evolutionary origin. It is also clear that the S. clavuligerus aceB gene we isolated is most closely related to that of S. arenaceus, followed by those of fungi and then by those of the other bacteria reported.

Physiological role of malate synthase

In the growth experiments, it was observed that S. clavuligerus was able to grow well on acetate, resulting in a growth yield of about 25 g dry cell materials per mole of acetate. This is comparable to the observed growth yield in glycerol, which was about 28 g of dry cell materials per mole of glycerol. The molar growth yield comparison between glycerol and acetate is remarkable, as acetate contains 50% less carbon per mole. This finding may be of considerable industrial interest in terms of biomass production, as glycerol was thought to be the most favourable carbon source for growth of S. clavuligerus (Aharonowitz & Demain, 1978). However, growth on acetate was delayed, compared to that on glycerol, and maximal growth was attained after about 10 d fermentation. This suggests that previous studies using short fermentation periods (such as 96 h) may not be sufficient for studying growth of S. clavuligerus on certain carbon sources, and that the possibility of delayed growth should be investigated by prolonged cultivation of cells.

Furthermore, it was found (data not shown) that malate synthase activity was induced to even higher levels when late-stationary-phase cells of S. clavuligerus, pre-grown in glycerol medium, were washed and transferred to fresh medium containing 1% acetate but without glycerol. These observations suggest that high levels of malate synthase activity are associated with the presence of acetate in the growth medium. The increase in malate synthase activity was not associated with accumulation of biomass. This may suggest a rather late role for malate synthase in the life cycle of S. clavuligerus, under the growth condition used in this study. Due to its central role in primary metabolism, the glyoxylate cycle may have several other important physiological implications, as observed in other organisms. For example, biodegradation of high-M, polyethylene glycols by a Pseudomonas stutzeri isolate was thought to involve the glyoxylate cycle when malate synthase was detected in the PEG-grown cells (Obrandors & Aguilar, 1991).

Additionally, lipid reserves in fungal spores are metabolized via the glyoxylate pathway for the biosynthesis of amino acids and polysaccharides during spore germination (Jennings & Lysek, 1996). Hence, it is worthwhile investigating whether the glyoxylate cycle in S. clavuligerus is related to sporulation, lipid metabolism or degradation of PEGs.

Heterologous expression of the aceB gene from S. clavuligerus

The aceB gene has recently been cloned from S. arenaceus. It shows strong homology (70% identity) with the S. clavuligerus aceB gene. However, the functionality of the cloned S. arenaceus aceB gene, and its relationship to the malate synthase protein purified from S. arenaceus cells, was not clearly evident from that study (Hüttner et al., 1997). In this study, the expression of the cloned S. clavuligerus aceB gene in E. coli and its catalytic activities in in vitro experiments clearly validate its functionality. In addition, the high levels of production of S. clavuligerus malate synthase in E. coli in its soluble form should facilitate future studies on the structure-function relationship of the S. clavuligerus malate synthase.

Concluding remarks

This study attempts to present an insight, albeit limited, into the primary metabolism of S. clavuligerus. We have investigated, via cloning and physiological studies, the operation of the malate synthase component of the glyoxylate cycle and its relationship to growth in acetate. Due to the commercial and medical importance of antibiotics produced by Streptomyces spp., much of the research in S. clavuligerus has so far focused on secondary metabolism, and very few primary metabolic genes have been cloned. Although our knowledge of primary metabolic pathways is frequently based on those of E. coli, recent investigations have revealed that primary metabolism in actinomycetes may differ from that in E. coli (Alves et al., 1994). Information on primary metabolism is crucial to the understanding of antibiotic production, as secondary metabolites are derived from the intermediates of primary metabolism. Hence, data on the primary metabolism, such as those obtained in this study, may be important for the development of fermentation processes for secondary metabolite production.

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