Cloning, nucleotide sequence and expression in *Streptomyces lividans* and *Escherichia coli* of *pabB* from *Lactococcus lactis* subsp. *lactis* NCDO 496

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A gene (*pabB*) encoding the aminase activity of *p*-aminobenzoate (PABA) synthase in *Lactococcus lactis* subsp. *lactis* was cloned in pIJ41 and expressed in *Streptomyces lividans* strains defective in PABA biosynthesis. Expression of the gene was associated with a 1.2 kb deletion between the *aph* promoter and the cloning site in pIJ41. Subcloning in pBR322 and expression in *Escherichia coli* AB3295 of the cloned *L. lactis* DNA fragment localized the *pabB*-complementing gene in a 1.9 kb segment. The nucleotide sequence of this segment contained a 1410 bp open reading frame encoding a 470-amino-acid polypeptide of 50937 Da. The deduced amino acid sequence showed substantial similarity to those reported for PabB and TrpE from several organisms. Synonymous codon usage reflected the low G + C content in the genomic DNA of *L. lactis* subsp. *lactis*, and therefore differed markedly from the preferred usage in the *S. lividans* host. The cloned heterologous *pabB* DNA was expressed in amounts that allowed accumulation of excreted PABA in cultures of *S. lividans* transformants.

Introduction

Streptomycetes are filamentous, spore-forming members of the high guanine and cytosine (G + C) subgroup of Gram-positive bacteria (Stackebrandt & Woese, 1981). Interest in their morphological development and in the numerous antibiotics and other secondary metabolites they produce has led to a rapid development of host-vector systems for gene cloning in these organisms (Bibb *et al.*, 1983; Hunter, 1985; Tomich, 1988; Baltz, 1990); progress in defining the molecular requirements for efficient expression of cloned streptomycete genes has increased the versatility of these systems (Hopwood *et al.*, 1986; Strohl, 1992). *Streptomyces lividans* appears to be relatively flexible in its requirements for transcriptional and translational control sequences (Bibb & Cohen, 1982), and has the full complement of tRNAs needed to translate all 61 possible codons (Hopwood *et al.*, 1986). The high (67–74%) G + C content of strepto-

mycete DNA is associated with more than 90% G or C in the third position of codons (Bibb *et al.*, 1985; Seno & Baltz, 1991; Wright & Bibb, 1992). In *Escherichia coli*, genes that are expressed naturally at high levels show a biased codon usage that correlates with the availability of complementary isoaccepting tRNAs (Ikemura, 1981); this may be true in streptomycetes as well, although not yet proven. However, mismatches between tRNA availability and codon usage do not prohibit the expression of foreign genes. This is apparent not only from the expression of several genes from *E. coli* (Schottel *et al.*, 1981; Chater *et al.*, 1982; Kuhstos & Rao, 1983; Shareck *et al.*, 1984), but also of bovine, monkey and human genes (Gray *et al.*, 1984; Muñoz *et al.*, 1985; Pulido *et al.*, 1986; Chang & Chang, 1988; Lichenstein *et al.*, 1988; Koller *et al.*, 1989; Malek *et al.*, 1990). The G + C content of the genes expressed has ranged between 38.3% (human interleukin-2) to 67.6% ((monkey proinsulin).

The versatility of *S. lividans* as a nonrestricting host for cloning foreign DNA (Hopwood *et al.*, 1987) and the availability of mutants deficient in PABA synthesis (Gil & Hopwood, 1983; Paradkar, 1991) suggested that this species might be used to isolate and compare from a variety of organisms the PABA synthase genes associated with primary and secondary metabolism (Aidoo *et al.*, 1990; Criado *et al.*, 1993; Arhin & Vining, 1993). In this paper, we report the expression in *S. lividans* of *pabB* from *Lactococcus lactis* subsp. *lactis*, an organism with a

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Abbreviations: PABA, *p*-aminobenzoic acid.

The nucleotide sequence data reported in this paper have been submitted to GenBank and have been assigned the accession number M64860.

G+C content (37%) about one-half that of streptomycetes. We describe some characteristics of the lactococcal gene.

Methods

Bacterial strains and cloning vectors. *Streptomyces lividans* strains 66 (wild-type) and JG10 (*pab*) were provided by Drs D. A. Hopwood, John Innes Institute, Norwich, UK, and J. A. Gil, Universidad de León, Spain, respectively; strain AP3 (*pab*) was obtained in our laboratory by mutagenesis of *S. lividans* TK24 (Paradkar, 1991). The plasmid pIJ41, a low-copy-number vector constructed from the SLP1.2 replicon of *Streptomyces coelicolor* A3(2) (Thompson *et al.*, 1982) was also a gift from Dr Hopwood. *Lactococcus lactis* subsp. *lactis* strain NCDO 496 was obtained from the National Council of Dairy Organisms, Reading, UK; strain AV117 is a plasmid-free derivative of strain NCDO 496 and was obtained in our laboratory (Aidoo, 1985). The sequencing vectors pBluescriptII SK(+) and SK(-), and helper phage VCSM13 were from Stratagene. *Escherichia coli* strains AB3292 (*proA2 his-4 pabA1 ilvC7 argE3 thi-1*) and AB3295 (*his-4 pabB3 ilvC7 argE3 thi-1*) were from the *E. coli* Genetic Stock Centre, Yale University School of Medicine, New Haven, CT, USA.

Chemicals and enzymes. Thiostrepton was a gift from S. J. Lucania of E. R. Squibb and Sons, New Brunswick, NJ, USA. Other biochemical agents, enzymes and culture components were purchased from commercial sources.

Media and culture conditions. *Lactococcus lactis* subsp. *lactis* strains were maintained on M17 agar (Terzaghi & Sandine, 1975). Cells for DNA isolation were grown in M17 medium (500 ml per 2000 ml Erlenmeyer flask) for 24 h at 27 °C without shaking (Terzaghi & Sandine, 1975). *S. lividans* strains were maintained on potato-dextrose agar (BBL) containing 0.5% (w/v) yeast extract. Spores were harvested from such cultures grown at 30 °C and were stored as suspensions in 20% (v/v) glycerol at -20 °C (Hopwood *et al.*, 1985). Cultures for plasmid isolation or vegetative inocula were initiated from spores. Unless stated otherwise, vegetative inocula were grown in liquid media at 30 °C for 48 h on a rotary shaker (220 r.p.m.; 3.7 cm eccentricity), and used in amounts of 1% (v/v) to initiate cultures.

Mycelium used for protoplast formation was from cultures (25 ml per 250 ml Erlenmeyer flask) grown for 36–40 h in YEME medium (Hopwood *et al.*, 1985) supplemented with 34% (w/v) sucrose, 5 mM-MgCl₂ and 0.5% (w/v) glycine. To demonstrate PABA formation, cultures (100 ml per 2000 ml Erlenmeyer flask) were grown for 5 d in glucose-isoleucine medium (Chatterjee *et al.*, 1983). Cultures of *E. coli* used in plasmid isolation or to prepare competent cells were inoculated with a single colony and grown overnight (50 ml per 250 ml Erlenmeyer flask) at 37 °C with shaking in Luria broth (Prival & Magasanik, 1971).

Plasmid and genomic DNA isolation. Plasmid DNA from *S. lividans* or *E. coli* was isolated by the rapid alkaline lysis method of Kieser (1984). For larger amounts the procedure was scaled up 10-fold and was followed by caesium chloride-ethidium bromide density-gradient centrifugation. Genomic DNA from *L. lactis* subsp. *lactis* was isolated and purified by the procedure of Anderson & McKay (1983).

Cloning of the *pabB* gene. Genomic DNA (10 µg) from *L. lactis* subsp. *lactis* NCDO 496 was digested with *Bcl*I and mixed with a *Bcl*I digest of pIJ41 (2 µg). The DNA was precipitated with ethanol, washed twice with 70% ethanol and resuspended in ligation buffer (20 mM-Tris/HCl, pH 7.6, 10 mM-MgCl₂, 10 mM-dithioerythritol, 0.6 mM-ATP) at a concentration of 40 µg ml⁻¹. After incubation with 1 unit of T4 DNA ligase it was used to transform protoplasts of *S. lividans* JG10 by

the procedures of Hopwood *et al.* (1985). Thiostrepton-resistant transformants were replica-plated on minimal medium (Hopwood, 1967) containing 0.25 µg sulphanilamide ml⁻¹ to select clones over-producing PABA.

DNA hybridization. Genomic DNA fragments from restriction enzyme digests were separated by electrophoresis, transferred to a nylon membrane and hybridized with the [α -³²P]dCTP-labelled 6 kb *Bgl*II DNA fragment from pDQ250 in a solution at 65 °C containing 5 × SSPE (1 × SSPE is 0.18 M-NaCl, 0.01 M-sodium phosphate and 1 mM-EDTA, pH 7.7), 5 × Denhardt's solution (Denhardt, 1966), 0.5% (w/v) SDS and sheared herring sperm DNA (100 µg ml⁻¹). The membrane was washed twice at 70 °C for 15 min with the following solutions in sequence: 5 × SSPE containing 0.1% (w/v) SDS, 2 × SSPE containing 0.1% (w/v) SDS, and 0.1 × SSPE containing 0.1% (w/v) SDS. The stringency of the final wash should have removed DNA not possessing complete nucleotide sequence identity with the probe.

Analyses. Total aromatic amino compounds in culture broths were determined colorimetrically as described by Levine & Fischbach (1951) but without dithionite reduction. Samples for thin-layer chromatography (TLC) were extracted from filtered broth at pH 5.0 with ethyl acetate. Chromatograms (silica gel Sil60 F₂₅₄; E. Merck) were developed with benzene/acetic acid/water (42:24:1, by vol. and n-butanol/acetic acid/water (12:3:5, by vol.). PABA was detected at *R_F* 0.50 and 0.75, respectively, as a quenching zone under ultraviolet light (254 nm) and as a yellow zone when the chromatogram was sprayed with 1% (w/v) *p*-dimethylaminobenzaldehyde in ethanol/conc. HCl (9:1, v/v).

Nucleotide sequencing. Restriction fragments were subcloned in pBluescriptII SK(+) and pBluescriptII SK(-), and ordered deletion clones were prepared; single-strand DNA was obtained by the procedure of Karger & Jessee (1990). The nucleotide sequence of both strands of the cloned DNA was determined by the dideoxy chain-termination method of Sanger *et al.* (1977) using [α -³⁵S]dATP and a modified T7 DNA polymerase (Sequenase, United States Biochemical). Database searches in GenBank used the BLASTP program (Altschul *et al.*, 1990).

Results and Discussion

Cloning of the *pabB* gene

Streptomyces lividans JG10 contains a mutation within the genes for PABA synthase; it exhibits an unambiguous requirement for PABA when grown in media supplemented at low concentration with a sulphonamide, which competitively inhibits the incorporation of PABA into folic acid (Gil & Hopwood, 1983). When *Bcl*I-digested genomic DNA from *L. lactis* subsp. *lactis* NCDO 496 was shotgun-cloned in pIJ41 and introduced into *S. lividans* JG10 (*pab*), one of 15000 thiostrepton-resistance transformants was able to grow after transfer to a medium containing 0.25 µg sulphanilamide (an unsubstituted sulphonamide) ml⁻¹. The transformant contained a 32 kb recombinant plasmid (pDQ250) which also transformed *S. lividans* AP3 (*pab*) to PABA independence in cultures containing sulphanilamide. Restriction enzyme analysis of pDQ250 indicated that an 18.3 kb DNA fragment had been inserted into the *Bcl*II site of the vector, and that a 1.2 kb vector segment

Fig. 1. Origin of pDQ250, pDQ251 and pDQ253. Shotgun-cloning of genomic DNA from *S. lactis* subsp. *lactis* NCDO496 in pIJ41 and selection in *S. lividans* for thiostrepton and sulphanilamide resistance yielded pDQ250. A 6.0 kb *Bgl*II fragment from pDQ250 was cloned in pBR322; an ampicillin-resistant prototrophic transformant, selected in *E. coli* AB3295, yielded pDQ251. The 6.0 kb *Bgl*II fragment from pDQ250 was partially digested with *Bam*HI and ligated to pBR322 linearized with *Bam*HI; an ampicillin-resistant, prototrophic transformant of *E. coli* AB3295 yielded pDQ253. Vector sequences (pIJ41 or pBR322) are represented by single thin lines. DNA cloned from *L. lactis* is shown as a double line either cross-hatched (minimum *pabB*-complementing region subcloned in pDQ253), stippled (remaining region subcloned in pDQ251) or open (remaining region initially cloned in pIJ41). Resistance genes are indicated by arrows showing the direction of transcription. Recognition sites for restriction enzymes on pIJ41 are redrawn from Thompson *et al.* (1982). The approximate location of the 1.2 kb deletion is indicated in pDQ250. The numbers in parenthesis are the positions (in kb) of restriction enzyme sites relative to the *Eco*RI site (0.0).

containing the carboxy-terminus of the *aph* gene had been deleted (Fig. 1). Consistent with such a deletion, transformants containing pDQ250 differed from those

containing pIJ41 in their failure to grow on media supplemented with neomycin. When the 18.3 kb insert was excised from pDQ250 by digestion with *Bcl*I and introduced into the *Bcl*I site of pIJ41, the recombinant plasmid did not complement the *pab* mutations in *S. lividans* strains JG10 and AP3. This suggested that the insert lacked promoter activity in *S. lividans* and that the deletion in pDQ250 allowed *pab* expression from the *aph* promoter. Similar vector deletions allowing expression of *E. coli* genes in *Streptomyces* from *Streptomyces* vector promoters have been reported (Schottel *et al.*, 1981; Shareck *et al.*, 1984).

Subcloning in *E. coli*

A 6.0 kb *Bgl*II restriction fragment internal to the 18.3 kb *Bcl*I insert was cloned in both orientations in the *Bam*HI site of pBR322. The orientation in pDQ251 (see Fig. 1) complemented the *pabB* mutation in *E. coli* AB3295 but not the *pabA* mutation in *E. coli* AB3292 (The *pabB* and *pabA* gene products form the aminase and glutamine amidotransferase components, respectively, of PABA synthase. A mutation in either gene can prevent PABA synthesis.); the plasmid with the 6.0 kb fragment cloned in the opposite orientation did not complement either mutation. Complementation only when the fragment was cloned in a particular orientation suggested that *pabB* was again expressed from a vector promoter, presumably the *tet* promoter in pBR322. In support of this conclusion, a mixture of sulphanilamide-resistant and -sensitive transformants was obtained when the 6.0 kb *Bgl*II fragment was ligated to the 13.7 kb vector fragment obtained by digestion of pDQ250 with *Bcl*I, and the ligation mixture was used to transform *S. lividans* JG10 or AP3 protoplasts. Sulphanilamide resistance in the transformants correlated with the presence of a 19.7 kb plasmid (pDQ255) carrying the 6.0 kb insert oriented away from the *aph* promoter site. The *pabB*-complementing sequence within the 6.0 kb *Bgl*II restriction fragment was localized by excision of the DNA from a *Bgl*II digest of pDQ250, partial digestion with *Bam*HI and ligation of the fragments into pBR322 linearized with *Bam*HI. Transformation of *E. coli* AB3295 with the ligation mixture yielded a prototrophic strain containing a recombinant plasmid with a 1.87 kb *Bgl*II–*Bam*HI insert (see Fig. 1). The insert DNA complemented the *pabB* mutation when present in the *Bam*HI site of pBR322 in the same orientation as in pDQ251; the plasmid was designated pDQ253.

A 32 P-labelled sample of the 6.0 kb *Bgl*II fragment of *L. lactis* DNA excised from the *Bcl*I insert in pDQ250 hybridized with single 6.0 kb fragments in *Bgl*II digests of genomic DNA from *L. lactis* subsp. *lactis* strains AV117 and NCDO 496. When *Bcl*I digests of the

genomic DNA samples were used, the probe hybridized with single fragments of about 20 kb. The evidence that the cloned DNA was present in the plasmid-free strain AV117 as well as in the wild-type strain NCDO 496 indicated that the fragment originated from the chromosome of *L. lactis*. The probe also hybridized with pDQ253, but not with the genomic DNA of streptomycetes. The results were consistent with the shotgun-cloning in pDQ250 of an 18.3 kb *Bcl*I fragment from the genomic DNA of *L. lactis* subsp. *lactis* NCDO 496, and with the subcloning in pDQ251 of 6.0 kb *Bgl*II and 1.87 kb *Bgl*II–*Bam*HI segments of this DNA, each of the recombinant plasmids conferring resistance to sulphanilamide.

Production of PABA by *S. lividans* strains

When grown as adjacent patches on minimal agar medium containing 0.25 µg sulphanilamide ml⁻¹, strains containing pDQ250 cross-fed the *S. lividans* *pab* mutants. Cultures of *S. lividans* JG10 (*pab*) and AP3 (*pab*) as well as their transformed derivatives containing pIJ41 or pDQ250 were grown in a defined medium, and clarified broths were examined for aromatic amines. Positive colorimetric assays were obtained only for the transformants containing pDQ250; the concentration of PABA in their cultures was 13–15 µg ml⁻¹. When the broths of these strains were examined by TLC, zones were observed at the *R_F* values and with the characteristics of authentic PABA. The results established that the *S. lividans* transformants with enhanced resistance to sulphanilamide overproduced PABA, and indicated that the cloned *L. lactis* subsp. *lactis* fragment was involved in PABA production.

Nucleotide sequence analysis

The nucleotide sequence of the 1.87 kb *Bgl*II–*Bam*HI fragment showed one large open reading frame (ORF) beginning with ATG at nucleotides 147–149 and terminating with TAA at nucleotides 1557–1559 (Fig. 2). A putative ribosome-binding site (RBS) with a sequence GAAAGGA complementary to a region near the 3' end of 16S rRNA of *L. lactis* subsp. *lactis* (Ludwig *et al.*, 1985) was present upstream of and separated by five bases from the ATG start codon. The sequence and its separation from the translational start site fall within the range observed in streptomycetes (Strohl, 1992) and *E. coli* (Rosenberg & Court, 1979), and are similar to RBSs recognized in lactococci (De Vos, 1987). The region upstream of the ORF contained no appropriately spaced hexamers corresponding to the consensus –10 and –35 promoter sequences recognized by the common sigma

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1  AGATCTCTTATAGAATAGCAATGAAATCTTGGGCGTATACGTAATATACTGAATATATCA
61  ATCGTATTGAGCATGGTATATCATCTGTGCAAAAGCATACGGACTGACCTGAACAGTAGCGT
121  GGCATAGTCGATCGAAAGGAATTAGATGTTTCACAAATCAGTGGAGTAGTATTTGATACAA
1  M F T I S G V V L I T R
181  GACCAGTATACGATGAAGGAAGTTTGAATTACTGTCTCAGAGTGGAGCAATGAACAAACGGAA
13  P V Y D E G S L N Y C Q S G A M N N I
241  TCTTGTGGAAAGTGTAGAAGGAACCAAGCTCGATACAGATTCGGAGGAGCAGAACCTA
33  L E S V E G N K P R Y S I G G A E P I
301  TCGGAATATCAACGCAACGACGATTTGACTGCAGCAACTTACGCAGAAAGATGTAAGT
53  G T I N A N A V L T A T Y A E D V K F
361  TCACCTGATGAGATCCCTTGAACGGAACATAGAGTAGCAATCTGTAACGGAGAGATACTC
73  T D A D P L N G T R V A I C N G E D T Q
421  AACAAAGAAATGGGATCCAGGAGGAGCATTGGGATCTTCCGATACGATGATAGGAA
93  Q E E M G F Q G G A L G Y F A Y D V G R
481  GAAGATTGGAAGGATACACGATTTGGGAATCGAAGATTGGGCAATCCGAGATTGGCAG
113  R L E G Y N D L G I E D W A I P D L A G
541  GAAGTAGTTACGAAATCGGAGTAAGTGCAGATCATCAAAACGATGTAATCGTATTGATCG
133  S Y E I G V S A D Q N D V I V L I A
601  CACATGCAAGTGCAGATGGAAGCATGATGATTCATCAAGATAGTAGAATTTGAGTAGTG
153  H A S A D G N D V F I T S S R Q L S M V
661  TAGCAGGACCAACATGTTGTGCAAGTGGAGATGTAGAATCTTGAAGAACAGATTGTCATT
173  A G P T C C A S G D V E I L R N K L H Y
721  ACTACGAGTAAATCCCATTCAGTCAAGATGATTGTGGATTCACAGATTGAAAGATTACT
193  Y G V I P F S Q D D C G F N R L K D Y L
781  TGGGAAGTGGAGATGATGATACCAATTAATTTGGGAACAGAAACGATGAGCAATCGTAA
213  G S G D M Y Q V N L G N R N V G A I V M
841  TGACATTGTTCCAAAGGATACCAACATTTGAGATTGATGAACCGGAGCATATGGTAT
233  T L F Q G Y N Q L R L M N P G P Y M V F
901  TCTTGGATGAAGCAACATCATCGCAAGTCCAGAAATCGTATTGGCAGATGACGACAA
253  L D E A N I I M A S P E I V L A D E A N
961  ACGATTGTAACACAAGCAATCGCAGGAACATTGATGAGATTGAACGAGCAAGTAGAAG
273  D L N T R P I A G T L M R L N E Q D E D
1021  ATGAGTAAACGAGCATGCTGGGCAACATCATAGGATAGAGCAGAAATGATGATGA
293  G V N A A C L G Q H H K D R A E H M I
1081  TCGTAGATTGGTAAGAACGATTTGGGAAGAGTGGGAAGATTCCGAAGTGAACGTAC
313  V D L V R N D L G R V G R F G S V N V Q
1141  AAGAAATCGTAGGAGCTGAAACATACAGTGTAGTATGATCATCGTAAGTAGATTAACAG
333  E I V G A E N Y S V V M H I V S R V T G
1201  GATCCTTGAACGAAGCATTCGAAGCAATGGAATCATCAGAGCAGGATTCCCGAGGAGAA
353  S L N E A F E A M E I I R A G F P G G S
1261  GTATCAGGAGCACCAGGTAAGAGCAATGGAATCATCGAAGATTGGAACCCACAA
373  I T G A P K V R A M E I I E E L E P Q R
1321  GACGAGATGGATGGGAGGAAGTATCGGATACATCGCATCAGAGGAACATCGGTTACA
393  R D G W G S I G Y I A Y R G N I G Y R
1381  GAATCGCAATCAGAACATTTGCTGCTGTGAACGGAATTTGTCGAAGTAGTGGAGCAG
413  I A I R T L F A C N G Q L F A S S G A G
1441  GATTGGTAGGATAGTATGGAAGACGGAGATACAAACCAATTCGAAAGATGAGAG
433  L V G D S M E D G E Y N E T F E K M R A
1501  CATTGAGAAGTTCTCTGTGCGAGGTACATATGGGAAGACACCATCTACTTGATTAAT
453  L R S F F C A A V H M G K T P Y L S *
1561  TACGATCCGTAATATGTACTTATAGCGTTCGAATATGGTAAAGTAGTACTTAAATACGT
1621  AATGTTTATTCAATAGTAATAGATCATGATAGATATCGAATGTAAATAGATCTGATG
1681  TAATTAATTCAGTATAATCTGATCATGACAAAATGCAATGTAATCGTAACTATATA
1741  ATGCTCAGTATGATGATCGATCGATCGCTGCGTATGAGGATTAACTAAAGCTAGCATGATA
1801  AATAGATAGATACTGATACAAATCATGATCATACATTGACTATAGTACGCGGTACGT
1861  ATAGATTCTGTAATCCGTACGTAGATCC

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Fig. 2. The nucleotide and derived amino acid sequences of the *pabB*-containing DNA fragment cloned from *L. lactis* subsp. *lactis*. The nucleotide/amino acid positions are numbered above the sequences. The putative RBS (nucleotides 135–141) as well as the start and stop codons are underlined.

factors of *L. lactis*, *S. lividans* and *E. coli* (McClure, 1985; De Vos, 1987; Strohl, 1992). This may indicate that the gene is transcribed from an upstream promoter that was not cloned with the fragment. However, the presence of a promoter region that deviates from the consensus, and possibly also requires for its function ancillary proteins that may be absent in *S. lividans* and *E. coli* (Irani *et al.*, 1989), cannot be excluded. Either of these alternatives could explain why expression of the *L. lactis* gene in both *S. lividans* and *E. coli* required a vector promoter. A similar apparent requirement for vector promoters was observed when a citrate permease gene from *L. lactis* was cloned and expressed in *E. coli*; the cloned sequence did not contain any regions resembling *E. coli* or *L. lactis* consensus promoter sequences for transcription initiation (David *et al.*, 1990). Lack of expression due to absence of appropriate promoter sequences in cloned DNA from *L. lactis* subspecies *lactis* containing genes

Table 1. Comparison of deduced amino acid sequences

The sequence deduced from the ORF in cloned *L. lactis* subsp. *lactis* DNA was compared with sequences deduced for related genes of other bacteria using the BLASTP programme from Experimental GENINFO(R) BLAST Network Service, National Center for Biotechnology Information (National Library of Medicine, Bethesda, MD, USA).

Gene	Identity (%)	Overlap (number of amino acids)	Reference
<i>Streptomyces lividans pabB</i>	63	169	Arhin & Vining (1993)
<i>Bacillus subtilis pabB</i>	54	192	Slock <i>et al.</i> (1990)
<i>Escherichia coli pabB</i>	53	198	Goncharoff & Nichols (1984)
<i>Salmonella typhimurium pabB</i>	52	198	Goncharoff & Nichols (1988)
<i>Klebsiella aerogenes pabB</i>	52	198	Goncharoff & Nichols (1988)
<i>Pseudomonas aeruginosa trpE</i>	46	197	Essar <i>et al.</i> (1990)
<i>Bacillus subtilis trpE</i>	43	197	Henner <i>et al.</i> (1984)
<i>Lactococcus lactis trpE</i>	41	205	Bardowski <i>et al.</i> (1992)

for histidine or branched-chain amino acid biosynthesis may also have accounted for inconsistent complementation of *his* and *ilv* auxotrophy in *Bacillus subtilis* and *E. coli* (Delorme *et al.*, 1992; Godon *et al.*, 1992). However, an operon containing all of the genes required for tryptophan biosynthesis was fully expressed in *E. coli* (Bardowski *et al.*, 1992).

Amino acid sequence analysis

The ORF encodes a polypeptide of 470 amino acids (see Fig. 2) that is similar in size (50937 Da) to the deduced *pabB* gene products from *E. coli* (53400 Da; Goncharoff & Nichols, 1984) and *B. subtilis* (approximately 50000 Da; Slock *et al.*, 1990); its content of hydrophobic (45.3%), hydrophilic (36.5%), basic (8.9%) and acidic (8.8%) amino acids is similar to that reported for *E. coli* PabB (Goncharoff & Nichols, 1984). The product of the *L. lactis* ORF shares substantial sequence identity with PabB of other organisms and also with the TrpE polypeptide of several bacteria, including that of *L. lactis* subspecies *lactis* (Table 1). The closest identity was with PabB of *S. lividans*. The presence of a single ORF encoding a polypeptide comparable to PabB in *E. coli* explains the complementation of *pabB* but not *pabA* mutations in that organism. The similarity of the *L. lactis* PabB to TrpE as well as to PabB polypeptides from various bacteria (Fig. 3) is consistent with the evidence from enteric bacteria that PabB and TrpE are very similar (Goncharoff & Nichols, 1988), and with the suggestion by these and other workers (Crawford, 1989) that the *pab* and *trp* genes coding for the polypeptides evolved from a common ancestor. As observed by Crawford (1989) for other PabB and TrpE sequences, alignment of identical and similar amino acids was markedly better in the carboxy-terminal than in the

amino-terminal halves of the polypeptides. It is noteworthy that the deduced amino acid sequence for *pabB* of *L. lactis* showed a similar degree of identity to the sequences deduced for *trpE* from the same organism (41%), from *B. subtilis* (43%) and from *Pseudomonas aeruginosa* (46%); moreover, this identity was consistently lower than with sequences deduced for *pabB* from various bacteria.

No additional ORFs were detected in the 1.87 kb fragment of *L. lactis* DNA; therefore, the organization of PABA synthase genes differs from that in *B. subtilis* where *pabB*, an amphibolic *pabA/trpG* and *pabC* are translationally coupled as part of a presumptive operon directing folic acid biosynthesis (Slock *et al.*, 1990). It also differs from that in *S. lividans* where *pabB* and *pabA* are closely linked but separate from *pabC* (Arhin & Vining, 1993). If *pabA* and *pabC* have separate locations, the distribution of PABA synthase genes in *L. lactis* subsp. *lactis* would resemble that in enteric bacteria (Bachmann, 1990). The apparent absence in *L. lactis* of a cluster containing all PABA synthetase genes contrasts with the adjacent arrangement in that organism of the genes for tryptophan biosynthesis, an arrangement which, in several respects, also resembles that in the *Enterobacteriaceae* (Bardowski *et al.*, 1992).

Codon usage in the ORF

The 41.4% G+C content of the coding region in the cloned *L. lactis* DNA is somewhat higher than the 37% overall G+C content in the DNA of the genome (Schleifer *et al.*, 1985). Table 2 shows the expected biased use of synonymous codons rich in A and T nucleotides. Consistent with this preference, the first position in the codons averages 49.3% G+C, the second position 39.7% and the degenerate third position only 34.9%. Use

Table 2. Codon usage in *L. lactis* pabB and *S. lividans* genes

Frequencies in 15 sequenced genes from *S. lividans* JI1326 were calculated using the CODONFREQUENCY program in software from Genetics Computer Group Inc., Madison, WI, USA. The genes (and accession numbers) were: *celA* (M82807), *cmlG* (X59968), *irm/mgt* (M74717), *pabA/pabB* (M64859), *P49/XP55* (Y00142), *snpA/snpR* (M81703), *tet* (M74079), *tipA* (M24524), *xlnA* (M64551), *xlnB* (M64552) and *xlnC* (M64553).

Codon			S.			Codon			S.		
			<i>L. lactis</i>	<i>lividans</i>					<i>L. lactis</i>	<i>lividans</i>	
Phe	UUU	0	0.01		Tyr	UAU	0	0.02			
	UUC	1.00	0.99			UAC	1.00	0.98			
Leu	UUA	0	0		ter	UAA	1.0	0			
	UUG	0.97	0.3			UAG	0	0.07			
Leu	CUU	0	0.02		His	CAU	1.00	0.03			
	CUC	0	0.36			CAC	0	0.97			
	CUA	0	0.01		Gln	CAA	0.93	0.05			
	CUG	0.03	0.59			CAG	0.07	0.95			
Ile	AUU	0	0.02		Asn	AAU	0.03	0.01			
	AUC	0.97	0.95			AAC	0.97	0.99			
Met	AUA	0.03	0.03		Lys	AAA	0	0.04			
	AUG	1.00	1.00			AAG	1.00	0.96			
Val	GUU	0	0.01		Asp	GAU	0.97	0.03			
	GUC	0	0.60			GAC	0.03	0.97			
	GUA	0.97	0.03		Glu	GAA	0.97	0.14			
	GUG	0.03	0.36			GAG	0.03	0.86			
Ser	UCU	0	0.01		Cys	UGU	1.00	0.07			
	UCC	0.04	0.34			UGC	0	0.93			
	UCA	0	0.01		ter	UGA	0	0.93			
	UCG	0	0.26		Trp	UGG	1.00	1.00			
Pro	CCU	0.20	0.02		Arg	CGU	0	0.06			
	CCC	0	0.40			CGC	0	0.47			
	CCA	0.73	0.03			CGA	0.08	0.04			
	CCG	0.07	0.54			CGG	0	0.36			
Thr	ACU	0.33	0.02		Ser	AGU	0.96	0.05			
	ACC	0	0.64			AGC	0	0.34			
	ACA	0.67	0.03		Arg	AGA	0.92	0.01			
	ACG	0	0.31			AGG	0	0.06			
Ala	GCU	0.05	0.01		Gly	GGU	0.02	0.07			
	GCC	0	0.56			GGC	0	0.71			
	GCA	0.93	0.04			GGA	0.98	0.08			
	GCG	0.02	0.38			GGG	0	0.13			

of the termination codon TAA instead of TGA or TAG follows the trend towards codons with low G+C content.

A comparison of codon usage in the *L. lactis* pabB ORF with the average usage in 15 genes cloned from *S. lividans* shows sharp differences between the two bacteria (Table 2). Since the genes included are unlikely to be

highly expressed, the distribution of isoaccepting tRNA species in *S. lividans* may be even more polarized than is suggested by the synonymous codon usage data (Wright & Bibb, 1992). The potential imbalance between specific tRNA availability and codon preference did not prevent expression of the cloned *L. lactis* pabB in *S. lividans* at a level that reversed sulphanilamide inhibition through overproduction of PABA. This result extends the variety of examples where streptomycetes, and notably *S. lividans*, has been used as the host for expressing genes cloned in recombinant vectors. It confirms earlier evidence (Pulido *et al.*, 1986) that the codon usage bias in *S. lividans* does not preclude expression of genes from organisms with markedly different codon preferences. Andersson & Kurland (1990) have pointed out that the overall frequency with which an mRNA is translated depends more upon its ability to initiate protein synthesis by sequestering ribosomal components than on the rate at which the message is subsequently translated; codon preference could potentially limit the rate at which a gene is expressed, but this too might be offset by mechanisms regulating the expression of individual isoaccepting tRNAs. At present there is no evidence that the degree to which codon preferences match the distribution of isoaccepting tRNAs controls gene expression.

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Fig. 3 (facing page). Alignment of PabB and TrpE proteins from *Bacillus subtilis* (BA), *Streptomyces lividans* (ST), *Lactococcus lactis* subsp. *lactis* (LAC), *Escherichia coli* (EC), *Salmonella typhimurium* (SA), *Klebsiella aerogenes* (KL) and *Pseudomonas aeruginosa* (PS). Sequences were analysed by the MULTALIN programme (Corpet, 1988). Amino acids identical in a large majority of the sequences are printed in the consensus line; similar residues are identified as ! (I or V), \$ (L or M), % (F or Y) and + (B, D, E, N, Q, or Z). Dots indicate gaps introduced to increase similarity (gap penalty 8).

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