CURRENT MICROBIOLOGY Vol. 38 (1999), pp. 135–136

**Current Microbiology**
An International Journal


# Detection of Messenger RNA Transcribed from Genes Encoding Enzymes of Amino Acid Biosynthesis in *Buchnera aphidicola* (Endosymbiont of Aphids)

Linda Baumann, Paul Baumann, MyLo Ly Thao

Microbiology Section, University of California, Davis, CA 95616-8665, USA

Received: 10 September 1998 / Accepted: 14 September 1998

**Abstract.** The aphid *Schizaphis graminum* is dependent on *Buchnera aphidicola*, a prokaryotic endosymbiont. One of the functions of the endosymbiont is the synthesis of essential amino acids for the aphid host. Previously we have found that *B. aphidicola* has many of the genes that encode enzymes of amino acid biosynthesis. Using reverse transcriptase and the polymerase chain reaction, we have detected messenger RNA corresponding to genes involved in the synthesis of tryptophan, isoleucine, valine, leucine, and histidine.

*Buchnera aphidicola* is a prokaryotic endosymbiont of the aphid *Schizaphis graminum* [3]. It is found in specialized cells (bacteriocytes) within the body cavity of the aphid. *B. aphidicola* is essential for the survival of the aphid; one of its functions is the synthesis of essential amino acids for the aphid host [3, 8, 10]. Our laboratory has been involved in the study of the genetics of amino acid biosynthesis in *B. aphidicola*. In the endosymbiont of *S. graminum*, we have detected genes that encode enzymes involved in the synthesis of aromatic amino acids (shikimate pathway, tryptophan branch), branched chain amino acids (isoleucine, valine, leucine), histidine, lysine, cysteine, and serine [2–4, 6, 7, 12]. Genes for leucine biosynthesis as well as additional genes for threonine and arginine biosynthesis have also been detected in the endosymbionts of other aphid species closely related to *S. graminum* [5, 11].

To date there is only limited information on the expression of these genes. Endosymbionts have been found to contain tryptophan synthase activity [8], and messenger RNA (mRNA) has been detected corresponding to two genes of threonine and arginine biosynthesis [11]. In this communication we use reverse transcriptase and the polymerase chain reaction (RT-PCR) [9] to detect mRNA corresponding to genes of aromatic amino acid, branched chain amino acid, and histidine biosynthesis.

*Correspondence to:* P. Baumann

Endosymbiont-enriched preparations were obtained as previously described [6]. Total RNA was obtained with the Qiagen RNeasy, midi kit (Qiagen Inc., Valencia, CA, USA), as described by the manufacturer. The RNA preparation (15.5 µg) was treated with 1 U RNase-free DNase I (Ambion, Austin, TX, USA) for 1 h at 37°C. The synthetic oligonucleotides used are described in Table 1. cDNA synthesis was performed in a 20-µl reaction mixture containing 1 µg total RNA, 2 pmole of oligo-B (Table 1), 500 µM dNTPs, 10 mM dithiothreitol, 200 U reverse transcriptase (RT) (Superscript II, Gibco/BRL, Gaithersburg, MD, USA), 250 mM Tris-HCl (pH 8.3), 375 mM KCl, and 15 mM $MgCl_2$. The reaction mixture was incubated at 42°C for 50 min and RT inactivated by heating at 70°C for 15 min. PCR amplification of cDNA was performed in a 50-µl reaction mixture containing 1 µl of the cDNA reaction mixture and 10 pmoles each of oligo-A and oligo-B, 100 mM dNTPs, 2 U AmpliTaq DNA polymerase (Perkin Elmer, Branchburg, NJ, USA), 10 mM Tricine-KOH (pH 8.3 at 72°C), 50 mM KCl, and 2.5 mM $MgCl_2$. The following are the conditions of amplification: [94°C, 5 min (one cycle)]; [94°C, 30 s; 55°C, 30 s; 70°C, 1 min (30 cycles)]; [70°C, 5 min (one cycle)]; [4°C]. In control experiments, 20 ng endosymbiont-enriched DNA was used in the PCR reaction mixture instead of cDNA. Ten microliter of the PCR reaction mixture (cDNA) was electrophoresed in 1% agarose-TPE and stained with ethidium bromide. The amount of the

Table 1. Genes and oligonucleotides used to detect their messenger RNA by RT-PCR

| Gene | Oligo-A | Oligo-B | Size (bp) | GenBank no. |
|---|---|---|---|---|
| *aroH* | 175–203[a] | 505–476 | 331 | U11066 |
| *trpE* | 98–127 | 559–532 | 462 | Z21938 |
| *trpD* | 224–226 | 566–547 | 343 | Z19055 |
| *trpA* | 164–185 | 484–461 | 321 | Z19055 |
| *ilvI* | 70–94 | 469–455 | 400 | AF060492 |
| *ilvD* | 49–74 | 590–560 | 542 | AF008210 |
| *leuA* | 37–62 | 445–419 | 409 | AF041836 |
| *hisG* | 463–494 | 799–767 | 337 | AF067228 |

[a] Nucleotide numbers of the corresponding gene.

control reaction mixture containing endosymbiont-enriched DNA as template was adjusted to give bands approximately equivalent to that in the cDNA PCR reaction mixture. Additional standard molecular biology methods used are described in [1, 9].

Figure 1 presents the results. In all cases, in the presence of total *B. aphidicola* RNA and the absence of RT, no bands were detected, indicating that the RNA preparation was not contaminated with DNA. In the presence of *B. aphidicola* RNA and RT, a band was detected that corresponded to that obtained when *B. aphidicola* DNA was used as the template. These results indicate that in *B. aphidicola* the tested genes encoding enzymes of amino acid biosynthesis were transcribed into mRNA. From past studies it is known that in *B. aphidicola trpE* and *leuA* are found on plasmids [3, 4], whereas the remaining genes are chromosomal [3, 4]. There is evidence that *trpD* and *trpA* are part of a single transcription unit [*trpDC(F)BA*], while all of the remaining genes are located on different transcription units [6, 7, 12].

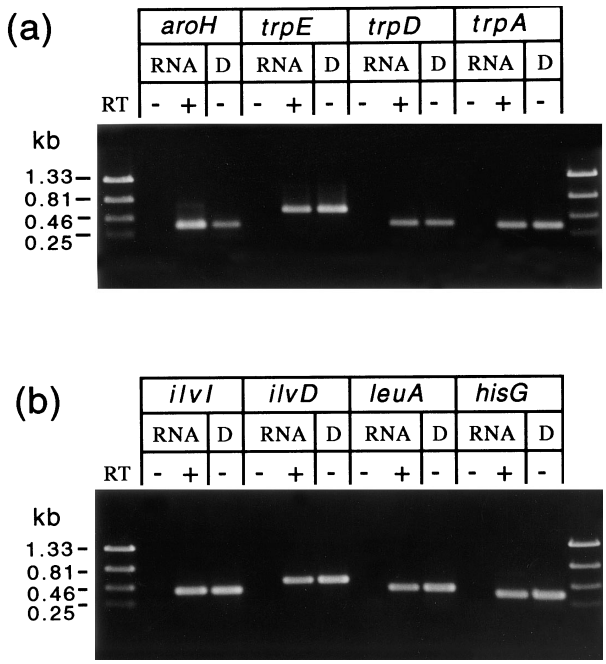


Fig. 1. Detection by RT-PCR of mRNA transcribed from genes encoding enzymes of amino acid biosynthesis in *B. aphidicola*. (a) Genes of the shikimate pathway (*aroH*) and the tryptophan branch (*trpE, trpD, trpA*). (b) Genes of branched chain amino acid (*ilvI, ilvD, leuA*) and histidine (*hisG*) biosynthesis. RNA, total RNA preparation from *B. aphidicola*; D, DNA from *B. aphidicola*; RT, reverse transcriptase.

**ACKNOWLEDGMENTS**

This material is based on work supported by National Science Foundation Award MCB-9807145 and the University of California Experiment Station.

## Literature Cited

1. Ausubel FM, Brent R, Kingston RE, Moore DD, Seidman JG, Smith JA, Struhl K (eds) (1998) Current protocols in molecular biology. New York: Wiley
2. Baumann L, Baumann P, Moran NA, Sandström J, Thao ML (1998) Genetic characterization of plasmids containing genes encoding enzymes of leucine biosynthesis in endosymbionts (*Buchnera*) of aphids. J Mol Evol, in press
3. Baumann P, Baumann L, Lai C-Y, Rouhbakhsh D, Moran NA, Clark MA (1995) Genetics, physiology, and evolutionary relationships of the genus *Buchnera*: intracellular symbionts of aphids. Annu Rev Microbiol 49:55–94
4. Baumann P, Baumann L, Clark MA, Thao ML (1998) *Buchnera aphidicola*: the endosymbiont of aphids. ASM News 64:203–209
5. Bracho AM, Martínez-Torres D, Moya A, Latorre A (1995) Discovery and molecular characterization of a plasmid localized in *Buchnera* sp., a bacterial endosymbiont of the aphid *Rhopalosiphum padi*. J Mol Evol 41:67–73
6. Clark MA, Baumann P (1998) Sequence analysis of a 34.7-kb DNA segment from the genome of *Buchnera aphidicola* (endosymbiont of aphids) containing *groEL, dnaA*, the *atp* operon, *gidA*, and *rho*. Curr Microbiol 36:158–163
7. Clark MA, Baumann L, Baumann P (1999) *Buchnera aphidicola* (endosymbiont of aphids) contains genes encoding enzymes of histidine biosynthesis. Curr Microbiol, in press
8. Douglas AE (1998) Nutritional interactions of insect-microbial symbioses: aphids and their symbiotic bacteria. Annu Rev Entomol 43:17–37
9. Gause WC, Adamovicz J (1995) Use of PCR to quantitate relative differences in gene expression. In: Dieffenbach CW, Dveksler GS (eds) PCR primer; a laboratory manual. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press, pp 293–311
10. Liadouze I, Febvay G, Guillaud J, Bonnot G (1996) Metabolic fate of energetic amino acids in the aposymbiotic pea aphid *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae). Symbiosis 21:115–127
11. Nakabachi A, Ishikawa H (1997) Differential display of mRNAs related to amino acid metabolism in endosymbiotic systems of aphids. Insect Biochem Mol Biol 27:1057–1062
12. Thao ML, Baumann P (1998) Sequence analysis of a DNA fragment from *Buchnera aphidicola* (aphid endosymbiont) containing the genes *dapD–htrA–ilvI–ilvH–ftsL–ftsI–murE*. Curr Microbiol 37:214–216