Small genome of Candidatus Blochmannia, the bacterial endosymbiont of Camponotus, implies irreversible specialization to an intracellular lifestyle

J. J. Wernegreen, Adam B. Lazarus and Patrick H. Degnan

Author for correspondence: J. J. Wernegreen. Tel: +1 508 548 3705 x6650. Fax: +1 508 457 4727. e-mail: jwernegreen@mbl.edu

Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA

INTRODUCTION

Obligate bacterial mutualists are critical to the reproduction and success of over 10% of insect species (Douglas, 1989). In contrast to some facultative bacterial associates, these primary endosymbionts are typically required for host growth and reproduction, occur within specialized host cells called bacteriocytes, and experience stable, maternal transmission through host lineages (Buchner, 1965; Hinde, 1971). Insect endosymbionts are widespread phylogenetically, but several lineages group with the γ-3 subdivision of the Proteobacteria and are closely related to Escherichia coli and other enterobacteria. Molecular evidence shows that γ-3-subdivision endosymbionts include Buchnera aphidicola associated with aphids, Wigglesworthia glossinidia associated with tsetse flies, Carsonella ruddii of psyllids, and Blochmannia of Camponotus, among other insect associates (Charles et al., 2001). Although their functional significance is often unknown, these symbionts are generally thought to play nutritional roles such as providing essential amino acids, vitamins or other nutrients that are lacking in the hosts’ diet (Buchner, 1965; Baumann et al., 1995; Douglas, 1989). For example, Buchnera, the best-characterized insect mutualist, provides essential amino acids that are deficient in the aphid’s diet of plant sap (Douglas, 1998; Baumann et al., 1998).

Like most intracellular pathogens, bacterial endosymbionts of insects have undergone severe genome reduction in the context of their obligate associations with hosts. The small genomes of Buchnera (630–650 kb; Charles & Ishikawa, 1999; Wernegreen et al., 2000) and Wigglesworthia (705–730 kb; Akman & Aksoy, 2001) approach the smallest known bacterial genome size (Mycoplasma genitalium, 580 kb; Fraser et al., 1995) and are very reduced compared to the 4.5–5.5 Mb genome size range for E. coli (Bergthorsson & Ochman, 1995, 1998). Since most bacterial genomes contain primarily coding DNA, genome reduction in endosymbionts must involve the loss of metabolic functions and physiological capacities, with important phenotypic implications (Andersson & Kurland, 1998; Moran & Wernegreen, 2000; Ochman & Moran, 2001). Indeed, full genome sequence data for Buchnera (Shigenobu et al., 2000) and microarray analysis of gene content in Wigglesworthia (Akman & Aksoy, 2001) demonstrate the deletion of several loci for metabolic functions that are not essential within the host cellular environment. The loss of these biochemical capabilities may account for the inability to culture insect endosymbionts without the host.

Keywords: genome reduction, symbiosis, bacteriocytes, asexuality, genetic drift

The GenBank accession number for the sequence reported in this paper is AF495758.
Blochmannia, first discovered in 1887 (Blochmann, 1887) and recently assigned to the new genus ‘Candi-
datus Blochmannia gen. nov.’ (Sauer et al., 2000) is the primary endosymbiont of the ant genus Camponotus, the largest genus in the family Formicidae (ants) that includes 931 species in every biogeographical region (Bolton, 1995). Like most intracellular bacteria, Bloch-
mannia shows accelerated evolutionary rates (unpublished data) and an extremely low G + C content (23 mol%; Dasch, 1975; Dasch et al., 1984). Bloch-
mannia occurs exclusively within ant ovaries and within bacteriocytes, specialized host cells that are intercalated among enterocytes of the ant midgut. Blochmannia is widely distributed across most species of Camponotus, and congruence of host and symbiont phylogenies (Schroder et al., 1996; Sameshima et al., 1999; Sauer et al., 2000) suggests the association is evolutionarily stable and as old as the host genus (20 million years; Wilson, 1985), if not older. In this study, we used PFGE to estimate the genome size of Blochmannia. In the course of this analysis, we further developed methods for isolating genomic DNA of unculturable endosymbionts in sufficient quantity and at sufficient purity for PFGE.

METHODS

Collection and maintenance of Camponotus host species. We selected Blochmannia associated with Camponotus pennsyl-
vanicus because of the local abundance of this carpenter ant. C. pennsylvanicus colonies were collected from two sites in Falmouth, MA, USA, less than 1 mile (~2.4 km) apart. Colonies were maintained for 4–6 months in the laboratory on a modified Bhatkar diet and live or frozen insects (Ho and Ishikawa, 1999). DNA sequence data support a low genomic restriction buffer (30 min per wash). Digestions were per-
which were omitted, to reduce the activity of nucleases. The
limiting factor was 25 °C. Beta-Agarase was added to the digestion buffer to ensure complete digestion. Digestion of DNA within plugs using G
Ishikawa, 1999) suggests the association is evolutionarily stable and as old as the host genus (20 million years; Wilson, 1985), if not older. In this study, we used PFGE to estimate the genome size of Blochmannia. In the course of this analysis, we further developed methods for isolating genomic DNA of unculturable endosymbionts in sufficient quantity and at sufficient purity for PFGE.

RESULTS AND DISCUSSION

One hallmark of bacterial genomes is their potential to undergo radical changes in size and organization through gene duplication, gene loss, and rearrangements of large portions of the chromosome, as well as to acquire genes via viruses, plasmids and transposable elements. Since most bacterial genomes contain over 85% coding DNA (Bergthorsson & Ochman, 1998) the acquisition or loss of a large portion of DNA may define the biochemical capabilities and range of environments available to particular bacterial lineages. Across bacteria, the most rapid and severe changes in genome sizes are coupled with transitions to an intracellular lifestyle (Moran & Wernegreen, 2000). Obligate endosymbionts that are sequestered within specialized host cells may lack opportunities for recombination with genetically distinct strains (e.g. Funk et al., 2000). Therefore, severe gene loss in endosymbionts may represent an irreversible evolutionary trajectory that constrains existence outside a eukaryotic cell, and limits transitions in symbiotic lifestyles (e.g. parasitic versus mutualistic associations with hosts) (Moran & Wernegreen, 2000; Tamas et al., 2001).

In this study, we demonstrate that the Blochmannia genome is severely reduced compared to closely related free-living bacteria such as E. coli. We estimated the size of the Blochmannia genome by summing the sizes of
Severe genome reduction in an endosymbiont of ants

**Fig. 1.** PFGE of endonuclease-digested DNA of *Blochmannia* associated with *C. pennsylvanicus*. Sizes of standard ladders are marked in kb. *Blochmannia* DNA samples are labelled with the restriction enzyme used. Estimated sizes of *Blochmannia* DNA fragments were generally consistent across reactions and across gels (see Table 1). General running conditions are described in the text. Specific run conditions were programmed as follows: (a) 300–950 kb [initial switch time (IST) 35–43 s, final switch time (FST) 1 min 28.73 s, run time (RT) 34 h 8 min], 1% gel; (b) 200–1000 kb (IST 24.03 s, FST 1 min 33.69 s, RT 29 h 57 min), 1% gel; (c) 30–500 kb (IST 2.17 s, FST 44.76 s, RT 26 h 40 min), 1% gel; (d) 60–650 kb (IST 7.33 s, FST 59.04 s, RT 27 h 47 min); (e) 5–100 kb (IST 0.23 s, FST 8.55 s, RT 15 h 16 min), 1% gel.
Table 1. Fragment sizes resulting from digestion of *Blochmannia* DNA with four endonucleases

Fragment sizes were estimated using GelPro 2.0, as described in the text. Restriction fragment sizes were summed for each enzyme to estimate genome size. Fragment sizes are labelled with the gel (Fig. 1, a–e) from which that estimate was derived. Sizes in bold are considered the most reliable, due to their close proximity to a standard size ladder and/or their location within a highly resolved region of the gel.

<table>
<thead>
<tr>
<th>Restriction enzyme</th>
<th>Enzyme fragment sizes (kb)</th>
<th>Sum of fragments (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AscI</td>
<td>809 (a) 810 (b)</td>
<td>809</td>
</tr>
<tr>
<td>NotI</td>
<td>332 (c) 334 (d) 325 (c) 151 (c) 153 (d) 147.5 (e)</td>
<td>808</td>
</tr>
<tr>
<td>Smal</td>
<td>267 (c) 264 (d) 178 (c) 175 (d) 101 (c) 96 (c) 84 (c) 81 (c) 78 (c) 80 (c) 44 (c) 40 (c) 38 (c) 34 (c)</td>
<td>790*</td>
</tr>
<tr>
<td>ApaI</td>
<td>234 (c) 227 (d) 156 (c) 153 (d) 132 (c) 128 (c) 77 (c) 80 (e) 64 (c) 63 (e) 61 (c) 61 (e) 53 (c) 54 (e) 36 (c) 32 (c)</td>
<td>813</td>
</tr>
</tbody>
</table>

*The slightly lower genome size estimate based on Smal digestions may result from the presence of faint, low-molecular-mass restriction fragments that were not scored (see text).
clearly demonstrate that the Blochmannia genome is severely reduced, and totals less than one-fifth the genome size of E. coli.

Genome reduction in Blochmannia and other obligately intracellular bacteria is consistent with a shift in the selection–mutation balance in these species. The persistence of a gene within a bacterial lineage depends on the selection coefficient for its maintenance, the efficacy of this selection, as determined by effective population size (Ne), and mutation rates (Lawrence, 1999). Each of these three factors may shift in obligate endosymbionts. First, selective coefficients for the maintenance of genes for metabolic diversity may be reduced in an intracellular environment, resulting in the loss of genes for the biosynthesis of compounds they can acquire from the host cytoplasm. For example, the full genome sequence of Buchnera sp. APS (Shigenobu et al., 2000) demonstrates that the exchange of gene products between the symbiont and host is complementary and mutually dependent. *Buchnera* provides the host with essential amino acids and, as expected, possesses a complete set of genes for the biosynthesis of essential amino acids. However, *Buchnera* lacks genes for the biosynthesis of non-essential amino acids that it must import from the host cytoplasm. In addition, the pantothenate → pyruvate → CoA pathway illustrates the mutual dependency between host and symbiont genomes. *Buchnera* performs the pantothenate → pyruvate reaction while the host converts pyruvate to CoA.

Second, endosymbionts may have small Ne due to severe bottlenecks upon transmission to host offspring, and few, if any, opportunities for recombination with genetically distinct strains. According to the nearly neutral theory (Ohta, 1973), reduced Ne is expected to increase the effect of genetic drift and to reduce the efficacy of selection. Previous studies show elevated rates of fixation of slightly deleterious mutations at particular genes of endosymbionts, consistent with a strong effect of genetic drift (Moran, 1996). On a genome-wide level, genetic drift may have a cumulative effect of reducing genome size if it increases the selective coefficient required to maintain a given gene (Lawrence, 1999; Lawrence & Roth, 1999).

Third, small genomes of intracellular bacteria may reflect stronger effects of mutational biases due to the loss of several DNA-repair genes (Moran & Wernegreen, 2000). Mutational bias toward deletions may account for shorter lengths of endosymbiont genes (Charles et al., 1999), and recent work suggests that deletion biases in bacteria may have greater effects on endosymbiont genomes, in which more sites are effectively neutral (Mira et al., 2001).

Further genomic studies of Blochmannia may inform our understanding of the functional significance of this endosymbiont, which is currently unknown. The proximity of Blochmannia to the ant midgut, and the ability to cure Camponotus of Blochmannia if the host is fed a very complex diet (Boursaux-Eude & Gross, 2000) both suggest that Blochmannia may provide the host with essential nutrients (Dasch et al., 1984). However, the insect and plant diet of Camponotus (Hölldobler & Wilson, 1990) is more complex than the unbalanced diets of other insects with bacteriocyte-associated symbionts (e.g. the phloem diet of aphids and other sap-sucking insects, and the blood diet of tsetse flies). Given the relative complexity of the Camponotus diet, it is quite possible that Blochmannia provides alternative, non-nutritional functions for the host. Notably, we found that the Blochmannia genome is slightly larger than those of the closely related insect endosymbionts Buchnera and Wigglesworthia. Genes that are present in Blochmannia but absent from related symbionts will provide promising candidates for loci with functional significance for this particular host association.

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