Nucleotide Sequence of the Pilin Gene of *Bacteroides nodosus* 340 (Serogroup D) and Implications for the Relatedness of Serogroups

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The gene encoding pilin of *Bacteroides nodosus* 340 has been isolated and the nucleotide sequence determined. The gene is present as a single copy within the *B. nodosus* genome and a protein of M_r 16683 can be predicted from the proposed coding region. A comparison of the predicted amino acid sequence with pilin from other strains of *B. nodosus* indicated that the protein of strain 340 (serogroup D) has a high degree of similarity with pilin of strain 265 (serogroup H). The degree of similarity between pilins from these strains and from other *B. nodosus* serogroups is no greater than that between *B. nodosus* pilins and the homologous proteins of several different bacterial species. These findings suggest that serogroups D and H may form a subset of *B. nodosus* serogroups.

INTRODUCTION

Bacteroides nodosus is the infectious agent of ovine footrot, a contagious disease which has a severe effect on the profitability of sheep production. Although immunity is not a consequence of natural infection, vaccination with killed whole cells of *B. nodosus* elicits a prophylactic response (Egerton & Burrell, 1970; Egerton & Roberts, 1971) and cell-surface appendages referred to as pili (Brinton, 1959), or fimbriae (Duguid *et al.*, 1955), have been identified as the major host-protective immunogen (Every & Skerman, 1982; Stewart, 1978*a, b*; Stewart *et al.*, 1982, 1983*a*).

The pili of *B. nodosus* are flexible filamentous structures 5–6 nm in diameter and up to 10 µm in length (Every, 1979; Stewart, 1973; Walker *et al.*, 1973). They are composed solely of the protein pilin, which has an M_r of 16500 to 19000 (Anderson *et al.*, 1986; Stewart *et al.*, 1986). Many bacterial species are piliated and pilins from *B. nodosus*, *Moraxella bovis*, *M. nonliquefaciens*, *Neisseria gonorrhoeae*, *N. meningitidis* and *Pseudomonas aeruginosa* display extensive N-terminal amino acid sequence homology (Elleman *et al.*, 1986*b*; Marrs *et al.*, 1985; McKern *et al.*, 1983; Olafson *et al.*, 1985). The similarity of these pilins, which have been designated *N*-methylphenylalanyl (N-mPhe) pilins (Elleman *et al.*, 1986*b*; Marrs *et al.*, 1985), has been further demonstrated by the expression of *B. nodosus* pili in *P. aeruginosa* as morphologically correct structures (Elleman *et al.*, 1986*c*; Mattick *et al.*, 1987).

Despite the conservation of sequence at the N-terminus, pilins from different strains of *B. nodosus* are highly variable beyond this region (McKern *et al.*, 1986), giving rise to serological variation. On the basis of cross-reactivity in agglutination reactions involving the pilus, field isolates of the organism have been divided into a number of serogroups, with some groups further divided into subtypes (Claxton *et al.*, 1983). Nine serogroups, designated A through I, are presently recognized (Claxton, 1986) and protection against footrot following vaccination is restricted to the serogroup from which the vaccine was prepared.

An analysis of the differences between the serogroups of *B. nodosus* will require pilin sequence data for at least one representative of each serogroup. The amino acid sequences of pilin from strains 198, 238 and 265, representatives of serogroups A, G and H respectively, have been derived from the nucleotide sequences of the isolated pilin genes (Elleman & Hoyne, 1984; Elleman *et al.*, 1984, 1986*b*; Elleman & von Ahlefeldt, 1987). In addition, the amino acid sequences of pilin from representatives of serogroups B, C and E have been determined (McKern *et al.*, 1986; N. M. McKern, personal communication). In this report, we describe the isolation and characterization of the pilin gene from a representative of the D serogroup, strain 340. The predicted amino acid sequence of strain 340 pilin is of special interest as this additional sequence, together with other data, indicates that there are two subsets of *B. nodosus* serogroups.

METHODS

Preparation of DNA and pili. *B. nodosus* 340 (CSIRO Animal Health Culture Collection) was cultured anaerobically at 37 °C in modified Eugon broth (Claxton *et al.*, 1983). After 2–3 d growth, cells were harvested and DNA and pili were prepared as previously described (Elleman *et al.*, 1984; Stewart *et al.*, 1983*b*).

Hybridization procedures. The hybridization probe used for the identification of DNA fragments bearing the pilin gene was derived from *B. nodosus* 198 and consisted of the upstream region and 160 nucleotides of the 5' coding region of the pilin gene contained within a *Sau3A*I fragment of DNA cloned in M13mp8 (Elleman & Hoyne, 1984). Following the isolation and sequence determination of the strain 340 gene, a *Hin*PI–*Kpn*I fragment of DNA from this strain was cloned in M13mp18 and used as a probe in further confirmatory hybridization experiments. Probes were prepared using [α -³²P]dATP as described in detail elsewhere (Elleman *et al.*, 1986*b*). Restriction endonuclease fragments bearing the pilin gene were identified with probes following fractionation in submerged agarose gels and transfer to nitrocellulose membrane (Smith & Summers, 1980). Cells transformed by vectors harbouring such fragments of *B. nodosus* DNA were screened by probing colonies grown on nitrocellulose membranes (Maniatis *et al.*, 1982). The highest stringency washes used were done at 65 °C for 1 h with 0.2 × SSC (1 × SSC is 0.15 M-NaCl plus 0.015 M-sodium citrate, pH 7.0) for transferred DNA and 1 × SSC for colony probing, both containing 0.1% (w/v) SDS.

Immunoblotting procedure. Proteins expressed by transformed cells harbouring the pilin gene were extracted by heating cells collected from stationary-phase cultures at 100 °C for 2 min in a solution (1/20 of the culture volume) consisting of 2% (w/v) SDS and 1% (w/v) dithiothreitol. Samples (5 µl) were then subjected to SDS-polyacrylamide gel (15%, w/v) electrophoresis, transferred to nitrocellulose membrane and probed with sheep antiserum against purified pili from *B. nodosus* strain 340 (O'Donnell *et al.*, 1983). Bound antibody was detected with ¹²⁵I-labelled protein A.

Cloning of the pilin gene. *B. nodosus* 340 DNA (8 µg) was digested with *Hind*III and the fragments were separated by submerged agarose gel electrophoresis. A fraction containing fragments of 2.5–3.5 kbp was collected on NA45 membrane (Schleicher & Schuell), extracted and ligated into *Hind*III-digested and phosphatase-treated pBR322 with a threefold molar excess of fragments over vector at a total DNA concentration of approximately 20 µg ml⁻¹. Following the transformation of *Escherichia coli* RR1, cells harbouring the pilin gene were identified by hybridization, and the production of the protein encoded by this gene was confirmed by immunoblotting. Plasmid DNA was prepared from cells lysed with lysozyme/Triton X-100 and purified by CsCl/ethidium bromide equilibrium density-gradient centrifugation as previously described (Elleman *et al.*, 1984).

Nucleotide sequence determination. DNA sequences were determined by the dideoxy chain-termination method (Sanger *et al.*, 1977) using M13-cloned DNA (Messing, 1983) or alkaline-denatured and neutralized double-stranded DNA (Chen & Seeburg, 1985) as templates (Fig. 1). Oligonucleotide primers were synthesized either manually (Elleman & Hoyne, 1984) or automatically on an Applied Biosystems 381A DNA synthesizer and were purified by high-performance liquid chromatography.

RESULTS

Identification of the pilin gene

The probe derived from strain 198 DNA (see Methods) hybridized to a single band of 3.0 kbp in *Hind*III-digested *B. nodosus* 340 DNA, so fragments of about this size were isolated for cloning of the pilin gene from the latter strain. *E. coli* transformants selected with the same probe contained plasmids bearing a single 3 kbp insert at the *Hind*III site (Fig. 1), with the only variation between clones being the orientation of the insert. To verify that strain 340 pilin was encoded by the cloned DNA, protein extracts of these cells were subjected to immunoblotting. A single immunologically reactive protein of *M_r* approximately 17000 was detected in each case

Fig. 1. Characterization of the pilin gene. A 3 kbp fragment of *B. nodosus* 340 DNA cloned in pBR322 is shown, with the pilin-coding sequence boxed. Transcription of the pilin gene occurs from left to right. Nucleotide sequences were derived directly from the plasmid or from subfragments cloned in M13 and are indicated by arrows which define the extent and direction of the determined sequence. The filled circles indicate the use of synthesized primers whose sequences were derived from the strain 340 sequence. *Hind*III and *Kpn*I sites are abbreviated as H and K respectively.

(Fig. 2), a size comparable to that of pilin from strain 340 and similar to the protein expressed in *E. coli* cells harbouring the pilin gene from other strains of *B. nodosus* (Elleman *et al.*, 1984, 1986b).

Sequence studies indicated a cloned subfragment of strain 340 DNA suitable as a specific hybridization probe for the pilin gene of this strain. The fragment extended from a *Kpn*I site within the pilin coding region to a *Hin*PI site 173 nucleotides downstream from the termination codon (see below). Various restriction enzyme digests of genomic DNA were analysed with this probe to investigate the possibility that multiple pilin genes might be present within the *B. nodosus* genome (Fig. 3). Single bands were detected in *Hind*III, *Hind*III/*Kpn*I and *Hae*II digests with sizes of 3.0, 1.2 and 0.9 kbp respectively, in keeping with the restriction map generated from the pBR322-cloned material (Fig. 1) and the nucleotide sequence (Fig. 4), which revealed that the proposed coding region lay between *Hae*II sites 894 bp apart. These results indicate that the pilin gene occurs as a single copy at a unique locus within the *B. nodosus* genome.

Nucleotide sequence and predicted amino acid sequence

The nucleotide sequence (Fig. 4) contains an open reading frame with features of the pilin-coding sequence identified in other strains of *B. nodosus* (Elleman & Hoyne, 1984; Elleman *et al.*, 1986b). These include a Shine–Dalgarno sequence (Shine & Dalgarno, 1974), ₃₄₆AGGAG, ahead of a possible initiation codon. Although two adjacent ATG triplets are present, translation would most probably be initiated at the second triplet since a minimum of five bases is generally found between the Shine–Dalgarno sequence and the initiation codon (Stormo *et al.*, 1982). With translation initiated at the second ATG triplet, the reading frame would code for a protein of *M*_r 16683, a value in agreement with that estimated for the immunologically reactive protein expressed by the transformed *E. coli* cells. Codon usage within the predicted amino acid sequence reflects trends previously noticed with other pilin structural genes (Elleman & Hoyne, 1984; Elleman *et al.*, 1986b). There is a preferential use of U rather than C in the third base position of quartet codons and a preferential use of A rather than G in the third base position of both quartet and duet codons.

Comparison of sequences within the coding region

The predicted amino acid sequence of the coding region of strain 340 was compared with the sequences of the same region from strains 198 and 265 (Fig. 5). Absolute identity occurs over the

Fig. 2

Fig. 3

Fig. 2. Immunoblot of *E. coli* cells harbouring recombinant plasmid. Lane A, purified *B. nodosus* 340 pili; lanes B and C, *E. coli* proteins from cells harbouring the pilin gene of *B. nodosus* 340 cloned as a *Hind*III fragment in opposite orientations in pBR322. The positions of M_r standards are indicated to the left of the figure.

Fig. 3. Hybridization analysis. *B. nodosus* 340 DNA (0.5 μ g) was digested and fractionated in a submerged agarose gel (1% w/v). After transfer to nitrocellulose membrane, the DNA was probed with a M13mp18-cloned fragment of the pilin gene of *B. nodosus* 340. Lanes A, B and C; *Hind*III, *Hind*III/*Kpn*I, and *Hae*II digests respectively. The positions of size standards (*Eco*RI/*Hind*III-digested phage λ DNA) are indicated to the left of the figure.

first 25 residues of each sequence, a segment which has been suggested as a topogenic signal common to all pilins of the N-mPhe type (Elleman *et al.*, 1986c). Identity of sequence continues for an additional 32 residues between strains 340 and 265, and further extensive similarity throughout the remainder of these sequences results in an overall identity of 70%. In contrast, there is little homology between the strain 198 sequence and that of the other two strains beyond residue 39, and the total identity is not greater than 38% (Fig. 5). This value is within the range of identities found (33–43%) between *B. nodosus* pilins and the N-mPhe-type pilins of other bacterial species (Table 1).

Diagonal matrices (Staden, 1982) were used to compare the nucleotide sequences of the pilin-

5'GCGCACGGTAAAAAGCCGCGGATTTCGGTTTGTCCTTCTGCTAACGCTGCTA
50
ATAATAAGGCGCGATGCGACATTGATTATCGCCGCATATCGTTATTTTCGCCGAAAGCGCTGAGACGGGCGCGAT
100
GTGCCATATATTCATCATTATTCCTTTTGTGAAACGGGCGTTATTGTGCCGAAAAATAGTAGAAAAAATGCAAA
150 200
AAAAGCGCGTGTGCCAGAAAAATAATTTTTTAAATTATTGTTTTTAAATATAAAAAATAATGTTGGCATTGATGACG
250
CATAATGAAAGGCGTCAGGCAACTGACTCTAAACAAGATGATATTTAAATGTTACATTCTTAATAGGAGAATATG
300 350
Met Lys Ser Leu Gln Lys Gly Phe Thr Leu Ile Glu Leu Met Ile Val Val Ala Ile
ATG AAA AGT TTA CAA AAA GGT TTT ACC TTA ATC GAA CTC ATG ATT GTA GTT GCA ATT
400
20
Ile Gly Ile Leu Ala Ala Ile Ala Ile Pro Gln Tyr Gln Asn Tyr Ile Ala Arg Ser
ATC GGT ATC TTA GCT GCA ATC GCT ATT CCA CAA TAC CAA AAC TAC ATC GCT CGT TCA
450
40
Gln Val Ser Arg Val Met Ser Glu Thr Gly Gln Met Arg Thr Ala Ile Glu Thr Cys
CAA GTT AGC CGC GTT ATG TCA GAA ACT GGA CAA ATG CGC ACC GCC ATC GAA ACT TGC
500
60
Val Leu Asp Gly Lys Glu Ala Asp Lys Cys Phe Ile Gly Trp Thr Gly Ser Asn Leu
GTT TTG GAT GGT AAA GAA GCC GAT AAA TGC TTC ATT GGT TGG ACT GGT AGT AAT TTG
550
80
Leu Asp Gly Glu Phe Thr Ala Gly Thr Glu Ser Thr Ala Ala Ala Thr Gly Gln Thr
TTG GAC GGT GAA TTT ACT GCT GGT ACC GAA TCT ACA GCA GCA GCA ACA GGT CAA ACA
600
100
Gly Ile Thr Ile Lys Tyr Pro Val Ala Ala Asp Asp Glu Gly Asn Ile Val Ala Thr
GGA ATC ACA ATT AAG TAC CCG GTT GCA GCC GAC GAT GAG GGA AAT ATT GTT GCA ACA
650
120
Phe Gly Arg Asn Ala Ala Ala Ala Ile Lys Pro Gln Thr Leu Thr Trp Ser Arg Ser
TTT GGT CGA AAT GCT GCA GCG GCT ATT AAA CCT CAA ACA TTG ACA TGG TCA CGT AGC
700 750
140
Lys Glu Gly Thr Trp Thr Cys Ala Thr Thr Val Glu Ala Lys Phe Gln Pro Thr Gly
AAA GAA GGT ACT TGG ACA TGT GCA ACA ACT GTT GAA GCC AAA TTC CAA CCA ACT GGT
800
Cys Lys Asp Gly Lys
TGT AAA GAT GGT AAA TAGTTTTCTCTCGCAGAAACAATCAAAGGTGTACTTCGGTACACCTTTTTTAT
850
AATTGTAGCATTTTACCATGTTAAAAAATATTGATTTTTCCGCTTTTCAATAAAAGTGATTGCCATCATTGCGAT
900
GAGCCTCGATCATTCGCTTTTGCTTTTTCTTTCTGATGGTAGCTGGCGC 3'
1000

Fig. 4. Nucleotide sequence containing the pilin gene of *B. nodosus* 340. The predicted amino acid sequence is presented above the nucleotide sequence.

coding regions of *B. nodosus* (data not shown) and revealed homologies corresponding to those seen in the amino acid sequences. Nucleotide sequence similarity was apparent throughout the comparison of strains 340 and 265 while similarity between these two strains and strain 198 was limited to the sequences encoding the N-terminal region (residues 1 to 40) and the last 14 amino acids. Within the region encoding the invariant amino acids (residues 1 to 25), only three silent base changes have occurred between the strains, remarkably few considering the divergence of strains 340 and 265 relative to strain 198.

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198   MKSLQKGFTLIELMIVVAIIGILAAFAIPAYNDVIARSQAAEGLTLADGLKVRISDHLES
340   MKSLQKGFTLIELMIVVAIIGILAAIAIPQYQNYIARSQVSRVMSETGQMRTAIETCVLD
265   MKSLQKGFTLIELMIVVAIIGILAAIAIPQYQNYIARSQVSRVMSETGQMRTAIETCLLD

198   G-ECKGDANPASGSLGNDDKGKY-----ALATIDGDYNKDAKTADEKNGCKVVITYGQG
340   GKEADKCFIGWTGSNLLDGEFTAGTE-STAAATGQTGITIKYPVAADEG-NIVATFGRN
265   GKEGKDCFIGWTTSNLLAAAGGSTTNNATAADFGQGGLNITYALESTAEN-KIEATFGQN

198   TAGEKISKLIVGKKLVLDQFVNGSYKYNEGETDLELKFIPNAVKN
340   AAA-----AIKPQTLTWSRSKEGTW---TCATTVEAKFQTGCKDGK
265   AAA-----TLHGKKLTWTRSPEATW---SCSTDVDEKFKPTGCKK

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Fig. 5. Comparison of predicted amino acid sequences of pilin from *B. nodosus* 198, 340 and 265. Sequences were aligned with the aid of the ALIGN program (Dayhoff, 1976); residues in one sequence differing from the other two sequences at corresponding positions are shaded.

Table 1. Percentage identity between predicted amino acid sequences of bacterial pilins

Additional sequences were obtained from the Genbank or NBRF databases. Sequences were aligned using the Needleman-Wunsch algorithm (Needleman & Wunsch, 1970) with a unitary matrix and a gap penalty of 2. Percentage identity is expressed as the number of matches relative to the total number of possible matches.

	<i>B. nodosus</i> 198	<i>M. bovis</i> EPP63 β	<i>N. gonorrhoeae</i> MS11	<i>P. aeruginosa</i> PAK
<i>B. nodosus</i> 340	36	43	36	41
<i>B. nodosus</i> 198		36	39	37
<i>M. bovis</i> EPP63 β			37	37
<i>N. gonorrhoeae</i> MS11				33

Comparison of nucleotide sequences flanking the coding region

In an alignment of nucleotide sequences 3' downstream of the proposed coding regions of the pilin gene from strains 198, 265 and 340, there is substantially greater similarity between strains 340 and 265 than between either of these two strains and strain 198 (Fig. 6). While strains 265 and 340 show 84% identity over the sequences compared, strain 198 displays less than half the number of identities. Within the downstream sequence of each strain, a region of hyphenated dyad symmetry followed by a string of thymidine residues is present which might constitute a transcription terminator for RNA polymerase (Platt, 1986). The potential stem and loop structures of the terminators from strains 340 and 265 are identical, with free energies ($\Delta G_{25^\circ\text{C}}$) of -64 kJ mol^{-1} compared with -77 kJ mol^{-1} for the strain 198 sequence (Elleman *et al.*, 1986*b*).

Sequences upstream of the pilin-coding regions of strains 198, 265 and 340 (Fig. 7) show more extensive similarities (>95% identity) than the downstream regions. However, contrary to the relatively greater similarity of strains 265 and 340 in both pilin-coding regions and downstream sequences, the most extensive similarity (99% identity) in the upstream region is between strains 198 and 265. Very few base changes have occurred in the sequence immediately adjacent to the pilin-coding region and a potential promoter of the pilin gene has been identified in this area (Johnson *et al.*, 1986). Further upstream, an open reading frame is present in the opposite

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198      TAATAG-CTAGCTCTTAATGCG-----AAAGCCTCTCTCTTGAGAGGCCTTTT
340      TAGTTTTTCTCTCGCAGAAACAATCAAAGGTGTACTTCGGTACACCTTTT
265      TAGAGACTAATAGTCTTTTGCTTACAGGCGCAATCAAAGGTGTACTTCGGTACACCTTTT

198      TT-ATCGTTTATTGTTTCTATCATTTAAACAAAAGCAAAATTAACTCATATCATCTACTC
340      TTTATAATTGTAGCATTTTACCATGTTAAAAAAATATTGATTTTTCCGCTTTTCAATTAAA
265      TTTATAATG-----TTTTATCATGTCAAGAAAATATCGATTTTTCCGCTTTTCAATTAAA

198      TATATCTTGTCTAAGTAGG
340      AGTGATTGCCATCATTGCG
265      AATGATTGCCATCATGGCG
  
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Fig. 6. Comparison of nucleotide sequences 3' downstream of the coding region of the pilin gene from *B. nodosus* 198, 340 and 265. Bases in one sequence differing from the other two sequences at corresponding positions are shaded. The ALIGN program (Dayhoff, 1976) was used to maximize matches between the sequences. Pilin termination codons are underlined and the arrows denote a region of hyphenated dyad symmetry in each sequence, the presumed transcription terminator.

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198      C
340      GCGCACGGTAAAAAGCCGCGGATTTCGGTTTGCTCTTCTGCTAACGCTGCTAATAATAAG
265      C

198
340      GCGCGATGCGACATTGATTTATCGCCGCATATCGTTATTTGCGCGGAAAGCGCTGAGACG
265      A

198      G      CGT      C T C      A A      CAA
340      GGCGCGATGTGCCATATATT---CATCATTATTCCTTTTGTGAAACGGGCGTTATTGTG
265      CGT      C T C      A A      CAA

198
340      CCGGAAAAATAGTAGAAAAAATGCAAAAAAGCGCGTGTGCCAGAAAAATAATTTTTTAA
265      G G

198      C C
340      ATTATTGTTTTTAAATATAAAAATAATGTTGGCATTGATGACGCATAATGAAAGGCGTCA
265      C

198
340      GGCAACTGACTCTAAACAAGATGATATTTAAATGTTACATTCTTAATAGGAGAAATATG
265
  
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Fig. 7. Comparison of nucleotide sequences from the 5' upstream region of the pilin gene from *B. nodosus* 198, 340 and 265. The comparison begins at nucleotide 1 of Fig. 4 and ends at the nucleotide (356) immediately preceding the pilin-coding region. For the strain 198 and 265 sequences, only those bases that differ from the strain 340 sequence are shown. Regions of hyphenated dyad symmetry are indicated by arrows.

orientation to that of the pilin coding sequence (Fig. 7) and this extends considerably beyond the presented data (T. A. A. Dopheide, personal communication). Within the open reading frame, an ATG triplet (reverse complement of $_{141}$ CAT) is well situated with respect to a potential Shine-Dalgarno sequence, AAGGA (reverse complement of $_{150}$ TCCTT) (Stormo *et al.*, 1982). Many of the differences between strain 340 and strains 198 and 265 are concentrated in the extended ribosome-binding site (Stormo *et al.*, 1982), including the first amino acid residues of this potential coding region. A sequence motif possessing hyphenated dyad symmetry is present mid-way between the initiation codons of the potential upstream coding region and the pilin-coding region (Fig. 7).

DISCUSSION

Despite an absence of protein sequence data for pilin of *B. nodosus* 340, the hybridization and immunoblotting results demonstrate that the DNA fragment isolated from this strain contains the pilin structural gene. In addition, the amino acid sequence predicted from the coding region of this gene has features characteristic of *B. nodosus* pilins and is especially similar to the protein from strain 265. This similarity permits several predictions to be made with regard to the mature pilin of strain 340. Previous comparisons of nucleotide and amino acid sequences from *B. nodosus* 198 or 265 (Elleman *et al.*, 1986*b*; McKern *et al.*, 1983) have indicated that pilin is encoded as a preprotein from which the first seven residues are cleaved post-translationally in *B. nodosus* (Elleman & Hoyne, 1984; Elleman *et al.*, 1986*a,b*). Since the encoded sequences of the pilin genes of strains 198, 265 and 340 are identical in the N-terminal region (Fig. 5), it is reasonable to assume that the strain 340 gene also encodes a preprotein which is subsequently cleaved in the same position as the preprotein of strains 198 and 265. The relative mobilities of *B. nodosus* pilin and the immunologically reactive protein produced by *E. coli* cells harbouring the pilin gene (Fig. 2) support this assumption since the former has a slightly greater mobility than the *E. coli* protein from which the leader sequence is not removed (Elleman & Hoyne, 1984).

The similarity throughout the amino acid sequences of pilin from strains 265 and 340 also allows a prediction to be made with regard to the disposition of disulphide linkages within the strain 340 protein. Cysteine residues are present at positions 57, 67, 140 and 153 of the predicted strain 340 sequence, and by analogy with pilin of strain 265, in which cysteine residues are similarly located and of known linkage, disulphide linkages may exist between residues 57 and 67 and residues 140 and 153 of strain 340 pilin (Fig. 4). A disulphide loop near the C-terminus has been recognized as a homologous feature of pilins from several bacterial species (Elleman *et al.*, 1986*b*). Although this feature is present in strains 265 and 340, representatives of serogroups H and D respectively, it is absent from pilins of other *B. nodosus* serogroups, which contain a single, larger and more centrally located disulphide loop (McKern *et al.*, 1983; N. M. McKern, personal communication).

Despite the extensive similarity between pilin of strains 340 (serogroup D) and 265 (serogroup H), further processing by a central cleavage within the polypeptide chain, as found in pilins of serogroup H, does not occur in pilins of serogroup D. Pilin of strain 265, although encoded as a single-chain protein of M_r 16637 (Elleman *et al.*, 1968*b*), exists in pili largely as two subunits of M_r about 8000 (Anderson *et al.*, 1986; Elleman *et al.*, 1986*b*; Stewart *et al.*, 1986), a presumed proteolytic cleavage having occurred at an Ala-Ala bond between residues 79 and 80 (Fig. 5). Sequence differences in the corresponding region of strain 340 pilin might account for the absence of cleavage if the appropriate protease is present in this strain also. All other serogroups of *B. nodosus* are like serogroup D in that pilin exists in pili as a single polypeptide chain (Anderson *et al.*, 1986; Stewart *et al.*, 1986).

Comparison of pilin amino acid sequences from representatives of different *B. nodosus* serogroups illustrates relationships beyond those recognized on the basis of serology (Claxton *et al.*, 1983). As noted above, 70% identity exists between strains 265 (serogroup H) and 340 (serogroup D). However, no greater than 38% identity is shown between the protein from either of these strains and strain 198 pilin (serogroup A). In contrast, approximately 70% identity of sequence exists between pilins from representatives of serogroups A, B, C and E (Elleman *et al.*,

1986b). An identity of about 70% is also found when the amino acid sequences of pilins from representatives of serogroups F (Dalrymple & Mattick, 1987) and G (Elleman & von Ahlefeldt, 1987) are compared with strain 198 pilin. There are thus two different types of *B. nodosus* pilins, those of serogroups D and H forming one subset and those of serogroups A, B, C, E, F and G another. This division of serogroups into subsets is further supported by comparison of the nucleotide sequences downstream of the coding region (Fig. 6): a considerably higher degree of similarity exists between strains 265 and 340 than between these sequences and that of strain 198. Conversely, the downstream sequence of strain 198 is virtually identical to strain 238 of serogroup G in this region (Elleman & von Ahlefeldt, 1987).

The comparison of pilins from *B. nodosus* with N-mPhe-type pilins of other bacterial species shows that the similarity between different species is about the same as between the subsets of *B. nodosus* (Table 1). Clearly, generation of the subsets has occurred through more extensive nucleotide rearrangement than the changes responsible for the diversity of serogroups within a subset. A single pilin gene is present in the genome of *B. nodosus*, so the opportunity for homologous recombination does not exist as it does in species carrying multiple pilin gene sequences such as *N. gonorrhoeae* (Hagblom *et al.*, 1985). However, recombination between non-homologous sequences or foreign DNA may have given rise to sequence variation in the ancestral pilin genes of *B. nodosus* which resulted in formation of the subsets.

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