Use of bioinformatics and PCR in the search for ABC transporter homology among various bacteria

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ABSTRACT

Bioinformatics databases and search tools are utilised to produce polymerase chain reaction (PCR) primers for the amplification of an ABC transporter gene from the clinically important anaerobe *Finegoldia magna*. On sequencing, a 450 base pair amplicon showed homology with the amino acid transporter of *Enterococcus faecalis*. Little sequence data is available for *F. magna* and the newly isolated DNA could be a useful tool in the identification of this organism in clinical specimens.

KEY WORDS: ABC transporters.
Computational biology.
Finegoldia magna.

Introduction

In 1982, the first ABC transporter, the histidine permease *hisP*, was cloned and sequenced.[1] The ABC transporters (ABC Traffic ATPases[2]) now form part of one of the largest paralogous protein families known. They are highly conserved across species, they derive energy from the hydrolysis of ATP,[3] play a vital role in cell processes, and are characterised by their ability to translocate simple ions as well as complex proteins, even at low concentration, across the cell membrane.

The ABC transporters are used either for efflux or influx (some are involved in both) of solutes against a concentration gradient. However, they are substrate-specific and ABC transporters for amino acids, sugars, inorganic ions, polysaccharides, peptides and even proteins have been characterised. ABC transporters are involved in many basic cell functions such as signal transduction and protein secretion.[4]

A typical ABC transporter is made up of four parts: two membrane-associated domains and two ATP-binding domains. These subunits can be arranged as separate polypeptides or be fused in a variety of configurations. Both ATP-binding domains are required for normal function.

The transmembrane domains are highly hydrophobic[3] and consist of multiple α-helical segments that span the membrane. The ATP-binding domains are characterised in their primary structure by the presence of the Walker A and the Walker B motifs.[5] The Walker A sequence can be represented by GXXGXGKS/T, where 'X' can vary, and the Walker B sequence by hhhhD, where 'h' stands for hydrophobic. The Walker B sequence is preceded by a highly conserved sequence motif, LSGGQ/R/KQR. These conserved regions are shown in Fig. 1. The motif LSGGQ is considered to be a signature sequence for these proteins.[4]

Finegoldia magna (previously *Peptostreptococcus magnus*) is known to be a significant human pathogen,[6] its main habitat being human faeces, the urogenital tract and oral and skin epithelia. It is the most common Gram-positive anaerobic coccus isolated from clinical samples and is an opportunistic bacterium. It has been isolated from various sites of the body such as post-operative wounds, abscesses and ulcers[7]. Usually, *F. magna* is isolated from wounds where other bacteria are present; however, it has been isolated in pure culture in clinical samples, indicating that it has a higher pathogenicity than other species.[8]

In the diagnostic laboratory, this microorganism is classified as fastidious and is mostly overlooked in early cultures. Proper collection of specimens is important, as is its identification. Most strains of *F. magna* take more than 48 hours to grow and cultures for anaerobes take up to 48 hours in most routine microbiology laboratories. Thus, lack of a proper technique results in these anaerobic infections being diagnosed late or not at all.

The aim of this study is to amplify fragments of a gene encoding an ABC transporter in *F. magna* by searching databases for homology in this protein among various bacteria and other organisms. Furthermore, the development of a specific polymerase chain reaction (PCR) method to detect *F. magna*, based on the unconserved regions of the ABC transporter sequences, may be possible.

Materials and methods

The ATP-binding motif is the most highly conserved sequence in all the ABC transporters. GenBank and Swiss-Prot were interrogated for the ATP-binding ABC transporter genes in *Agrobacterium tumefaciens, Bacillus firmus, Encephalitozoon intestinalis, Escherichia coli, Haemophilus influenzae, Campylobacter jejuni, Bacillus subtilis, Staphylococcus aureus, Streptococcus pyogenes, Salmonella choleraesuis, Clostridium acetobutylicum* and also in *Arabidopsis thaliana*. These organisms were chosen on basis of their Gram-stain appearance and on the ATP-binding ABC transporter genes that have been cloned. Using the technique of conserved

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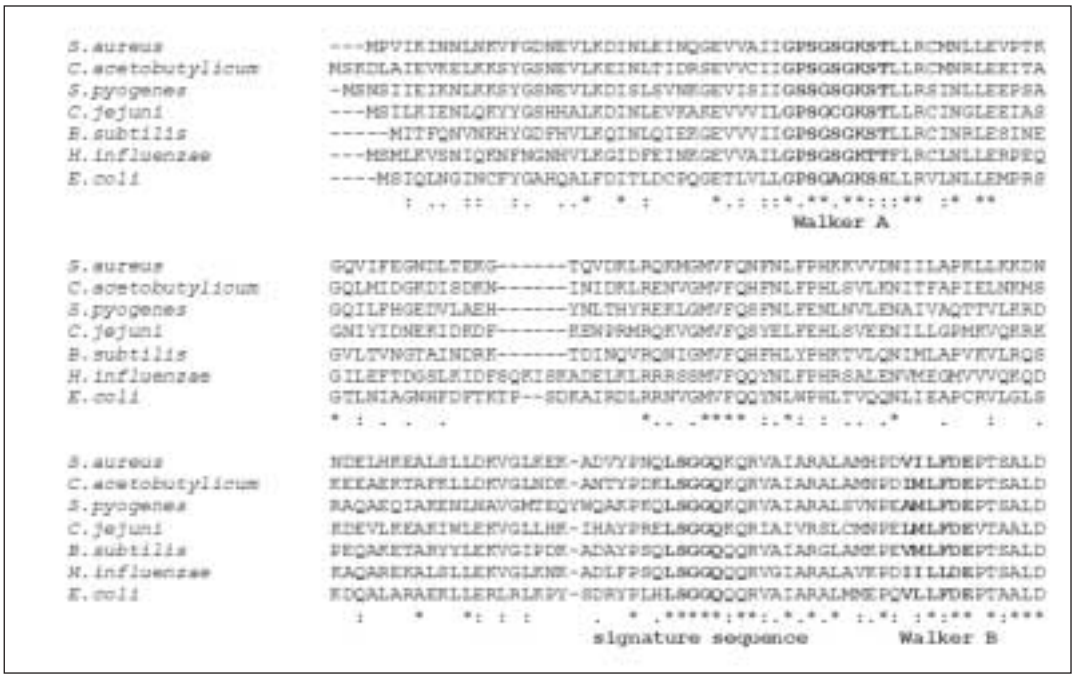

Fig. 1. Sequence alignment of the ATP-binding domains. The conserved sequences (Walker A and Walker B) are shown, as well as the highly conserved motif (signature sequence) that is unique to the members of the ABC transport superfamily (. low homology, : moderate homology, *complete identity).

Table 1. Two PCR products obtained using primers for Walker B.

Sequence 1: ***Finegoldia magna***
CGTCGAGAGGAGTTGCATTTGGGCCTGAAAATTTGGGAGTTCCAAGAGAAGA ACCCCTTNAGNGAGTAGATGAATGTCTTGAACTTGTGGGAATGANCNTTTANA AGNGACATTCACCAGCACCATTATCTGGAGGTCAAAAACAAAGAATTGCAATT GCTGGGATACTAGCAATGAATNCAAAGTGTTTGCTAATGGATGAGCCTACTA.
Sequence 2: ***Enterococcus faecalis***
AGAATATTGTTTTAGCACCAACCGATTTAGGTCGCTTGTCAAAAGACGAAGCCC CNTAAAAAAGCGTTAGAACTTTTAGAACGTGTTGGGTTGGCNCATAAAAAAGAT GCCTATCCAGATTCTTTATCTGGTGGTCAAAAACAACGTGTCGCAATTGCACGT GCGTTAGCGATGAATCCTGATATTATGCTTTTCGATGAGCCTACTAAGCCTACTA

sequence amplification (CSA) by PCR,[9] the genes encoding ATP-binding ABC transporters in various microorganisms were amplified.

Several sets of degenerate primers were designed according to the conserved areas of homology in the ATP-binding motif. The conserved motifs targeted for amplification for Walker A were GKST (forward) and DEAT (reverse), and for Walker B the conserved motifs were AMVFQ (forward) and DEPT (reverse). The primers for Walker A were forward 5′ GGHAARWSWACW 3′ and reverse 5′ WGTWGCYTCRTC 3′ and the primers for Walker B were forward 5′ GCWATGGTWTTYCAA 3′ and reverse 5′ WGTWGGYTCRTC 3′. The codon usage for *Micromonas micros* was used as that for *F. magna* was not available at the time the study was undertaken.

The correct combinations of primers used were based on the sequence alignment of *E. coli* published by Higgins in 1992. Only two sets of the degenerate primers produced amplicons. All the primers were tested on the DNA of a variety of aerobes and anaerobes which was extracted by the miniprep bacterial genomic DNA method.[10]

The PCR technique was carried out using standard Mastermix (Promega, 25 units/mL *Thermus aquaticus* [*Taq*] DNA polymerase, 200 µmol each of dATP, dGTP, dCTP and dTTP, and 1.5 mmol/L $MgCl_2$). The PCR reaction was carried out in a 25- µL volume and 1 µL DNA from each anaerobe and aerobe was used. The negative control included water instead of DNA. The PCR programme was run for 30 cycles and the annealing temperature used was 37°C.

American Type Cell Culture (ATCC) strains of *F. magna* (ATCC 29328) and *M. micros* (ATCC 33270) were used. Clinical isolates of organisms obtained from patients at University College London Hospital were also used and included (anaerobes) *F. magna, M. micros (Peptostreptococcus micros), Peptinophilus asaccharolyticus (Peptostreptococcus assacharolyticus), Anaerococcus prevotii (Peptostreptococcus prevotii), Peptostreptococcus anaerobius, Prevotella melaninogenica, Prevotella bivia* and *Bacteroides fragilis*; and (aerobes) *E. coli, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus aureus, Staphylococcus epidermidis*, coagulase-negative staphylococci and group B streptococci.

The PCR amplicons were separated on 1% agarose gel in 1x Tris-boric acid-EDTA (TBE buffer; 0.45 mol/L Tris, 0.44 mol/L boric acid, 0.5 mol/L EDTA [pH 8.3]) by electrophoresis for 1 h at 100 V. In addition, some were sent for further sequencing (Cytomix, Cambridge, UK).

Results

The primers successfully amplified products from the DNA extracted from both aerobes and anaerobes. The PCR products that were obtained when using primers for Walker A were approximately 450 bp in size and are shown in Fig. 2 (anaerobes) and Fig. 3 (aerobes). The PCR products obtained when using primers for Walker B were approximately 360 bp in size and are shown in Fig. 4 (anaerobes) and Fig. 5 (aerobes). As more than one product was amplified, only those reactions that generated a single PCR product were sent for sequencing (Cytomix, Cambridge, UK).

The two PCR products obtained when using primers for Walker B were sequenced successfully and are shown in

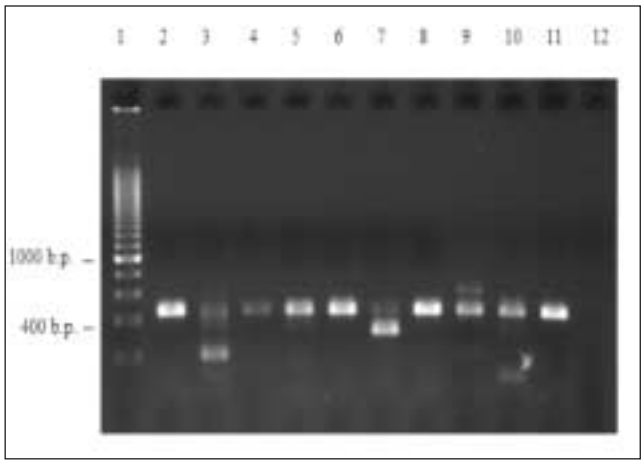

Fig. 2. PCR products of the anaerobes using Walker A primers. Lane 1: 200 bp ladder, lane 2: *F. magna* ATCC, lane 3: *F. magna*, lane 4: *M. micros* ATCC, lane 5: *M. micros*, lane 6: *Peptinophilus asaccharolytica* , lane 7: *Prevotella bivia*, lane 8: *Peptostreptococcus anaerobius*, lane 9: *A. prevotii*, lane 10: *B. fragilis*, lane 11: *Prevotella melaninogenica*, lane 12: negative control.

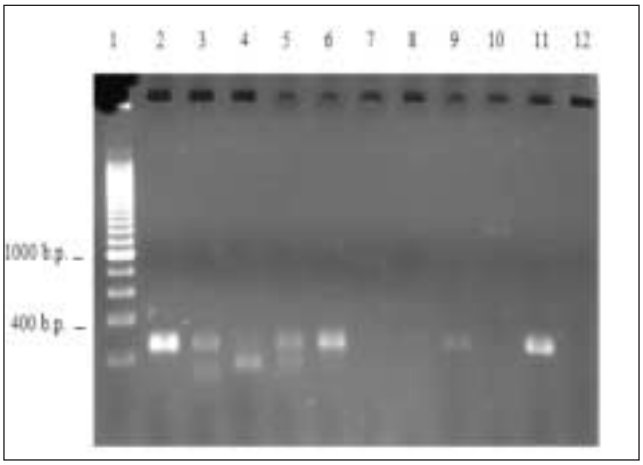

Fig. 4. PCR products of the anaerobes using Walker B primers. Lane 1200 bp ladder, lane 2: *F. magna* ATCC, lane 3: *F. magna*, lane 4: *M. micros* ATCC, lane 5: *M. micros*, lane 6: *Peptinophilus asaccharolyticus*, lane 7: *Prevotella bivia*, lane 8: *Peptostreptococcus anaerobius*, lane 9: *A. prevotii*, lane 10: *B. fragilis*, lane 11: *Prevotella melaninogenica*, lane 12: negative control.

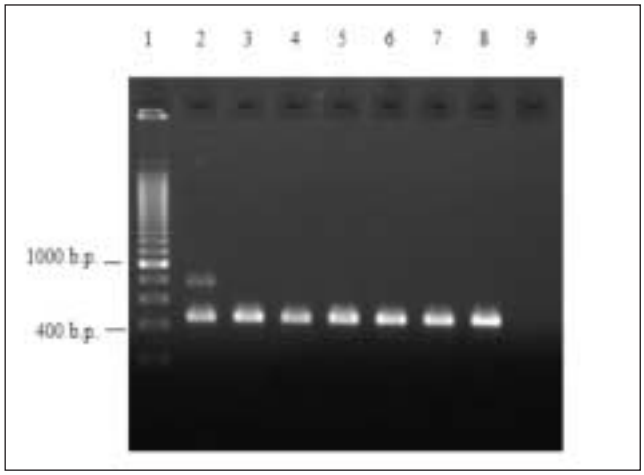

Fig. 3. PCR products of the aerobes using Walker A primers. Lane 1: 200 bp ladder, lane 2: *E. coli*, lane 3: group B streptococcus, lane 4: *S. aureus*, lane 5: *S. epidermidis*, lane 6: coagulase-negative staphylococcus, lane 7: *E. faecalis*, lane 8: *Pseudomonas aeruginosa*, lane 9: negative control.

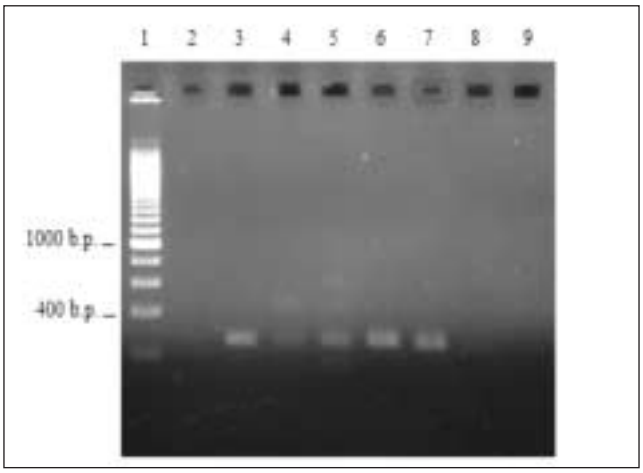

Fig. 5. PCR products of the aerobes using Walker B primers. Lane 1: 200 bp ladder, lane 2: *E. coli*, lane 3: group b streptococcus, lane 4: *S. aureus*, lane 5: *S. epidermidis*, lane 6: coagulase-negative staphylococcus, lane 7: *E. faecalis*, lane 8: *Pseudomonas aeruginosa*, lane 9: negative control.

Table 1. They were first translated into amino acid sequences and then analysed using the EMBOSS transeq software (www.ebi.ac.uk/emboss/transeq/). The results of the translations included the ABC transporter signature sequence LSGGQ (data not shown).

Discussion

In this study, the ABC transporter genes were amplified correctly using primer designs based on homology and conserved sequence amplification. Previously, this technique was used successfully in the cloning of the glycosidase genes from the protozoan *Tritrichomonas foetus*.[9]

Although the ABC transporter gene sequence in *F. magna* should be similar to ABC transporter genes in other microorganisms, it would be expected to show differences because the four domains of the ABC transporters are encoded by different polypeptides. Based on this difference in sequence, a specific PCR method to detect only the ABC transporter gene in *F. magna* can be designed and used as a diagnostic tool for the rapid identification of *F. magna* in clinical samples.

However, the short ATP-binding motifs (Walker motifs) are known to occur on other types of transporters (eg F-type ATPases, P-type ATPases and the Ars arsenite/arsenate export system).[11] Interestingly, when the sequences obtained in this study were translated into amino acid sequences, the consensus and signature sequence LSGGQ was present. This special sequence identifies members of the ABC superfamily.[11]

Data generated from this study are new, as no information on ABC transporters for *F. magna* is currently available in any bioinformatics database. Interestingly, the *F. magna* sequence data showed 63% homology with the ABC transporter in *Clostridium perfringens*. The latter is also an anaerobe, which

suggests that the sequence obtained is genomic information for *F. magna*. More importantly, the sequence data showed 59% homology with the ATPase component of the anaerobes *C. acetobutylicum* and *Thermoanaerobacter tengcongensis*. The *F. magna* sequence obtained showed 54% homology with the ABC transporter, more specifically the ATP-binding domain, of *Streptococcus pneumoniae* and *S. pyogenes*. This reflects the specificity of the primers used in this study.

The sequence obtained from the clinical isolate of *F. magna* showed 96% homology with the multidrug-resistance protein (cel:C54D1.1). Not surprisingly, this clinical isolate was obtained from a wound infection.

ABC transporters have been associated with antibiotic resistance, and multidrug resistance pumps have also been found in other pathogens.[12] ABC transporters actively pump antibiotics out of the cell, and some that are found in microorganisms of medical importance have been identified. One such example is the ABC protein MrsA, which has been postulated to cause erythromycin resistance in staphylococci.[13] However, not all antibiotic resistance by export involves ABC proteins.

The sequence data obtained for the amplicon from *Enterococcus faecalis* showed 90% homology with the *abc* gene coding for an amino acid ABC transporter in an *E. faecalis* vancomycin-resistant V583 clinical isolate. Thus, *E. faecalis* was identified to the species level and the amplicon obtained was most probably that of the amino acid ABC transporter.

Genome research that involves comparative analysis of ABC transporters in completed microbial genomes will provide greater insight into their roles and functions. One such study was carried out by Tomii and Kaneshisa.[14] They analysed various completed genomes and studied the number of ABC-type ATP-binding proteins that may be present in Gram-negative bacteria, Gram-positive bacteria, cyanobacteria and archaea. They also studied the functional predictions of the ATP-binding proteins. In this way, the different types and numbers of ABC transporters can be lodged in a database so that ABC transporters can be identified in other microorganisms using bioinformatics and homology.

Currently, antibiotics are prescribed prophylactically to the elderly when they undergo dental procedures because the transfer of *F. magna* infections to hip prostheses have occurred in such cases.[15] Use of antibiotics in this way will lead to increasing resistance among the different *F. magna* isolates.

Clearly, in the face of growing antibiotic resistance of microorganisms, the study of microbial ABC transporters is of the utmost importance. In this respect, the use of rapid DNA-based diagnostic tests[16] will prove extremely useful. □

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