IDENTIFICATION OF UNKNOWN OCULAR PATHOGENS IN INFECTIOUS UVEITIS USING RIBOSOMAL RNA GENE SEQUENCE ANALYSIS

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Infectious Uveitis

- Characterized by inflammation inside the eye caused by a virus, bacteria, fungus, or a parasite (White, 2007)
- Red eye, pain, light sensitivity, blurred vision, presence of dark spots in vision (Griggs, 2006)
- If left untreated may cause blindness

Diagnosis

- Physical examination, clinical manifestations
- PCR and other molecular techniques

www.calgaryretina.ca/.../SlitLampExamination.jpg

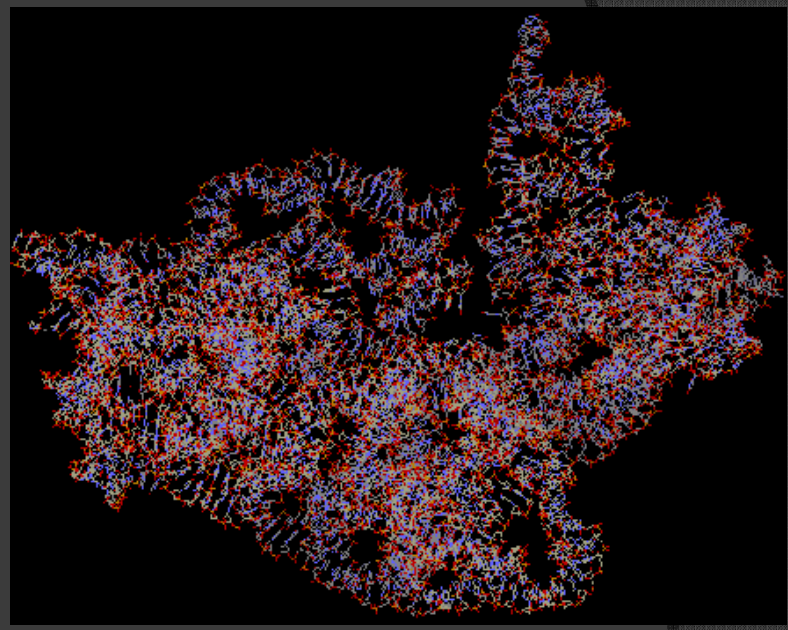
PCR Technology

- PCR requires very small amount of sample
- Conventional Microbiological culture may not be that sensitive (Caroll et al 2000):
  - Small sample size (~100-400ul)
  - Adherence of microorganisms to solid surfaces (intraocular lens, lens remnants, and capsule)
  - Fastidious nature of some microorganisms

www.turbosquid.com

16S rRNA gene

- Highly conserved gene which allows species distinction based on mutation acquired during the course of evolution.
- Universal in bacteria

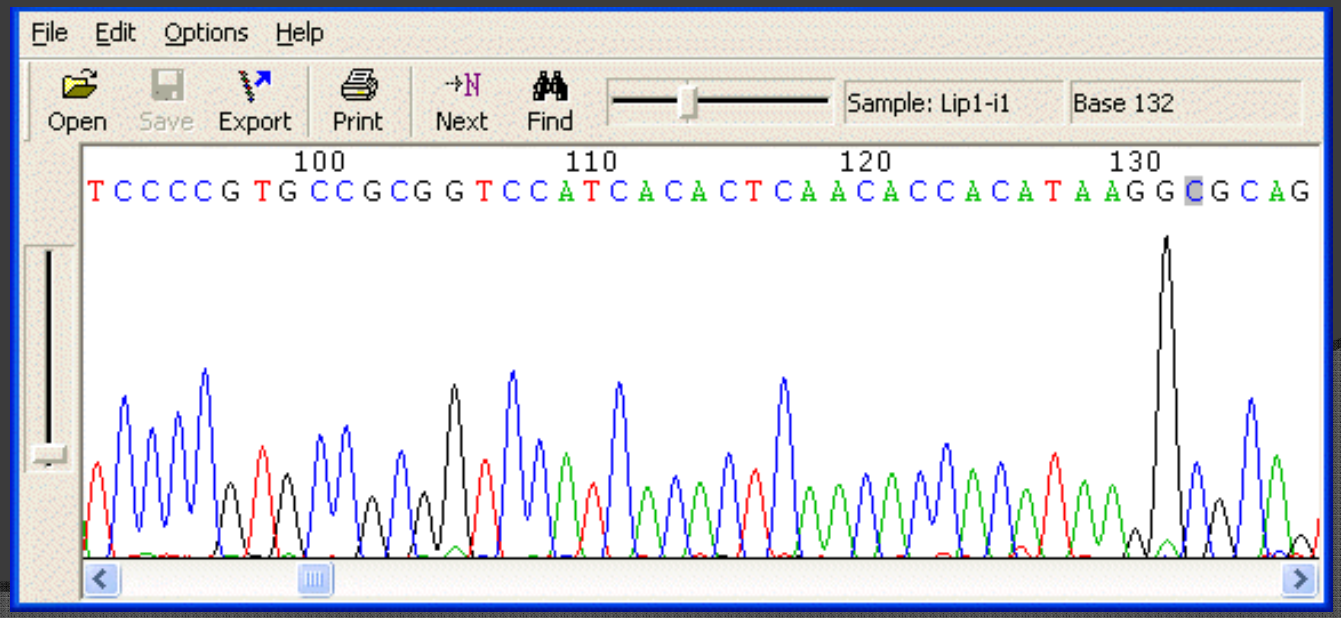


www.biochem.umd.edu/.../ribosome/16SrRNA.html

- Excellent choice in identifying non-culturable bacteria (Clarridge, 2004)

Objective

- Describe the PCR-based detection and DNA sequence-based identification of bacterial pathogens from ocular samples taken from patients diagnosed with infectious uveitis by 16S rRNA gene analysis.



www.technelysium.com.au/chromas_lite.html

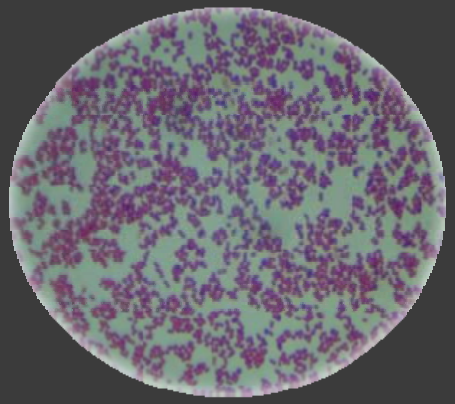
Methods

Vitreous aspirate, Anterior chamber tap, other eye specimens

Gram stain

16S rRNA gene analysis

MacConkey agar, Tryptic soy agar, Blood agar



Sphingomonas sp.
1000X

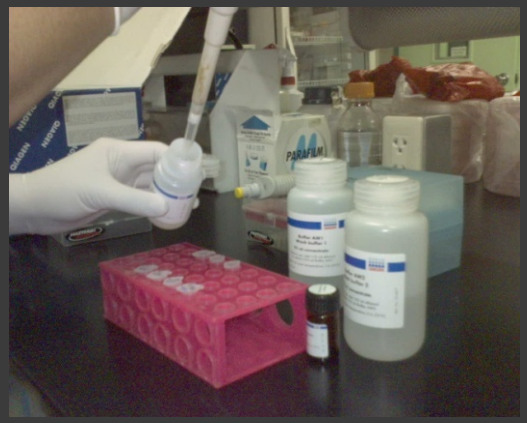
Isolation & Purification

Gram stain

BD BBL Crystal ID System

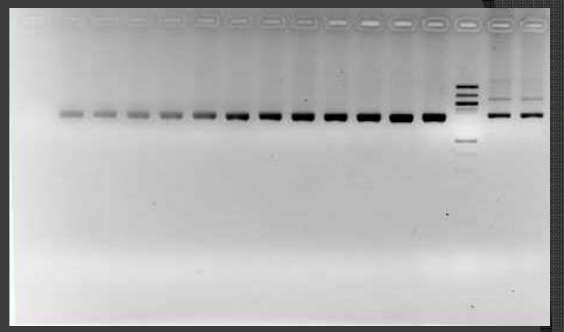
16S rRNA gene Analysis

DNA Extraction



PCR

Agarose Gel Electrophoresis



PCR Product Purification

www.biokitscom

DNA Sequencing

MACROGEN
Advancing through Genomics

www.macrogen.com

Sequence Analysis & Identification

Analysis of sequence data

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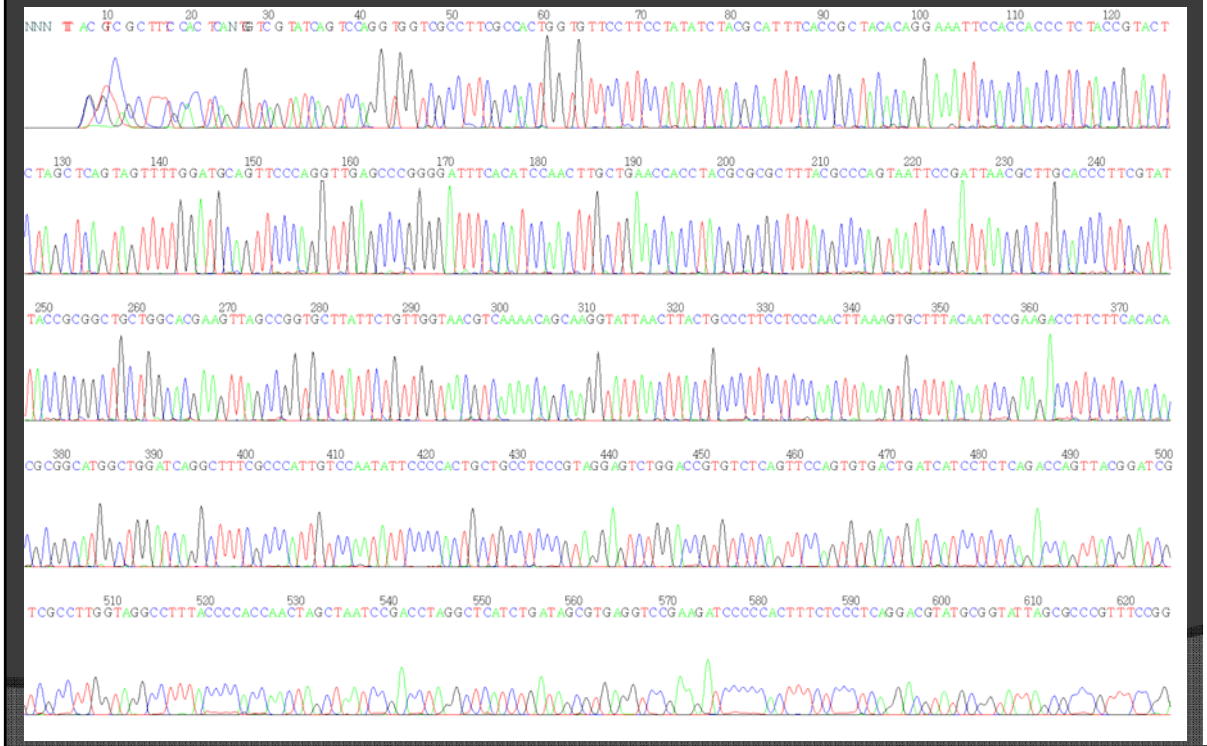
Comparative sequence analysis



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>  gb|FJ190080.1|  Pseudomonas aeruginosa strain Am7 16S ribosomal RNA gene,
partial
sequence
Length=1393

 Score = 1343 bits (727),  Expect = 0.0
 Identities = 738/743 (99%), Gaps = 4/743 (0%)
 Strand=Plus/Minus

Query  12   CGCTTTC-CA-CTCANTGGTC-GTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCC  68
            ||||||| || |||| | ||| ||||||||||||||||||||||||||||||||||||||
Sbjct  743  CGCTTTCGCACCTCAGT-GTCAGTATCAGTCCAGGTGGTCGCCTTCGCCACTGGTGTTCC  685

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            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Results

- Ninety-two samples from infected eyes of 56 patients and 4 control samples were tested.

	Gram Stain	Culture	Control samples
Positive	3 (3.3%)	7 (7.6%)	0
Negative	89	85	4

Table 1. Identification of bacterial pathogens of the eye based on biochemical test profiles and 16S rRNA gene sequence analysis.

Sample #	Specimen	Identity of bacterial isolate	
		Biochemical test profiling (BD BBL Crystal ID)	16S rRNA gene sequence (% identity)
03a-2004	Vitreous tap	Burkholderia cepacia	Ralstonia mannitolilytica (99%) (778/780)
03c-2004	Vitreous aspirate	Burkholderia cepacia	Ralstonia mannitolilytica (97%); (748/749)
04a-2004	Anterior chamber tap	Streptococcus sanguis	Uncultured bacteria (99%) Streptococcus oralis (98%); (754/765) Streptococcus sp. (98%); (765/778)
04b-004	Vitreous tap	Streptococcus constellatus	(Sequences of poor quality)
05b-2004	Vitreous tap	Hafnia alvei Enterobacter cloacae	Hafnia alvei (98.5%); (605/611) Escherichia coli (98.5%) Salmonella enterica (98.5%)
53a-2007	Vitreous tap	Pseudomonas aeruginosa	Pseudomonas aeruginosa (99%) (738/743)
53b-2007	Corneal scraping	Pseudomonas aeruginosa	Pseudomonas aeruginosa (99%) (747/748)

	16S rRNA gene detection
Positive	23 (25%)
Negative	69

- Out of the 23:
  - 16 did not grow in the 3 media used.
  - 18 products were sequenced.
  - 16 out of the 18 products produced sequences of good quality.

Table 2. Identification of unculturable bacaterial pathogens of the eye by 16S rRNA gene sequence analysis

Sample #	Specimen	Identity of bacterial isolate	
		Biochemical test profiling	16S rRNA gene sequence (% identity)
12a-2004	Anterior chamber tap	No growth after 72 hours	Sphingomonas sp. (97%); (633/641)
12b-2004	Vitreous tap	No growth after 72 hours	Sphingomonas sp. (98.1-97.1); (624/695)
13b-2004	Vitreous tap	No growth after 72 hours	Xanthomonas sp. (98%); (759/774)
22b-2005	Anterior chamber tap	No growth after 72 hours	Haemophilus influenzae (99%); (610/615)
24-2005	Eyeball	No growth after 72 hours	Klebsiella pneumoniae (99%); (733/737)
35a-2005	Anterior chamber tap	No growth after 72 hours	Staphylococcus haemolyticus (99%); (723/725)
35b-2005	Vitreous tap	No growth after 72 hours	Staphylococcus haemolyticus (99%); (723/724)
38b-2005	Vitreous tap	No growth after 72 hours	Mycobacterium sp. (96%); (658/679)
44a-2006	Anterior chamber tap	No growth after 72 hours	Klebsiella pneumoniae (96%); (432/449)
46a-2006	Anterior chamber tap	No growth after 72 hours	Morganella morganii (96%); (580/604)
47b-2006	Vitreous tap	No growth after 72 hours	Morganella morganii (94%); (561/594)

- Sequence-based identification produced different results obtained by culture-based methods in one sample – *Ralstonia mannitolilytica* vs. *Burkholderia cepacia* (De Baere et al. 2001)

- Identification of bacterial pathogens rarely seen in infectious uveitis (*Ralstonia mannitolilytica, Xanthomonas sp.*, and *Sphingomonas sp.*)

Discussion

- A few of the identified bacteria are associated with nosocomial infections:
  - *Pseudomonas aeruginosa* (*Froggat, 1989)*
  - *Sphingomonas sp.* (Kelley, 2004)

- Some have been reported to cause eye infections:
  - *Morganella morganii* (Miller, 2008)
  - *Klebsiella pneumoniae* (Lindstorm, 1997)
  - *Streptococcus sp.* (Narayanan, 2006; Okharvi, 2000)
  - *Mycobacterium sp.* (Freitas, 2003)

Discussion

- Other bacteria were found to be opportunistic pathogens especially in immunocompromised patients:
  - *Staphylococcus haemolyticus* (Falcone, 2007)

Discussion

- An advantage of this technique lies with its capability to identify bacteria that may be difficult to culture (*Haemophilus influenzae*, *Mycobacterium sp*.) due to:
  - Small sample amount (~100-400 ul)
  - Low bacterial load in the sample
  - Fastidiousness of the pathogen

Limitations

- Presence of inhibiting substances in the sample submitted that may lead to false negative results.
- Presence of contaminating agents that may lead to false positive results
- Poor sequence quality
- Length of the product being sequenced

Conclusion

- 16S rRNA gene sequence analysis has successfully identified both common and unusual bacteria associated with infectious uveitis.

- May also lead to identification of novel pathogens.

Conclusion

- 16S rRNA gene sequence analysis can be an adjunct to conventional culture method for the identification of bacteria in very minute amounts of sample and that are difficult to culture.

Significance

- The data gathered from this study may be used to construct a database of pathogens of the eye
- Epidemiological studies and further scientific research

Thank you and good day to everyone!

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