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Got black swimming dots in your cell culture? Identification of *Achromobacter* as a novel cell culture contaminant

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ARTICLE SUMMARY

Cell culture model systems are utilized for their ease of use, relative inexpensiveness, and potentially limitless sample size. Reliable results cannot be obtained, however, when cultures contain contamination. This report discusses the observation and identification of mobile black specks observed in multiple cell lines. Cultures of the contamination were grown, and DNA was purified from isolated colonies. The 16S rDNA gene was PCR amplified using primers that will amplify the gene from many genera, and then sequenced. Sequencing results matched the members of the genus *Achromobacter*, bacteria common in the environment. *Achromobacter* species have been shown to be resistant to multiple antibiotics. Attempts to decontaminate the eukaryotic cell culture used multiple antibiotics at different concentrations. The contaminating *Achromobacter* was eventually eliminated, without permanently harming the eukaryotic cells, using a combination of the antibiotics ciprofloxacin and piperacillin.

Keywords

achromobacter; bacteria; cell culture; contamination

INTRODUCTION

Cell culture contamination is a universal challenge facing most researchers at one time or another in their career. An obvious consequence of contamination is loss of experiments, time, and money. Contamination can be difficult to identify, particularly with a transient workforce as often the case in research and, especially, academic, settings. However, if cell culture contamination remains unidentified, it can result in erroneous results not actually due to treatments but rather the cellular reaction to the contaminate itself.

Researchers take multiple steps, including aseptic culture handling, filter sterilization, purchasing of liquid media and solutions, and inclusion of antibiotics in the growth media, to keep the cultures free of unwanted organisms. An additional complication arises when

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researchers use cell lines that are not commercially available and are often irreplaceable, difficult to obtain, or need rederivation from primary cells.

In the case of non-commercially available lines, all attempts are usually taken to clean up the culture by selectively killing the contamination without harming the cell line. Often the culprit of culture contamination is mycoplasma, intracellular bacteria that can be almost undetectable in culture. Approximately 28% of 460 human cell lines surveyed contained mycoplasma [1]. Numerous commercial kits are available to identify mycoplasma contamination [2,3] and antibiotic solutions are commercially available to rid cultures of them. Additional culture contamination includes other bacteria, mold, and fungi [4].

Unknown bacterial contamination can be transient. Contamination is common with poor aseptic technique and can be devastating in a research setting [3]. While many undesired organisms may steal nutrients from cell lines in culture, they may also prey on the cells themselves. Predatory bacteria have been shown to feed on other bacteria [5,6], particularly in a limited nutrient environment [7]. Experimental results may also be altered due to unwanted activation of cells. Different cellular functions, including those triggered by Toll-like receptors, can be activated by a variety of bacterial components [8–10].

Several online science blogs discuss a cell culture contaminate that looks like black specks in the cell culture (<http://www.cellculturechat.com/i.html/T3470/H3448.html>; <http://www.protocol-online.org/forums/index.php?s=bc4fec655ee6898191ab6ed43f5a5a7&showtopic=9373&pid=30730&st=0&#entry30730>). The researchers on these blogs describe black dots that fidget or swim and look more like rods than dots. Our laboratory has experienced sporadic cell culture contamination fitting this description. Here we report the identification of a bacterial cell culture contaminate, a member of the *Achromobacter* genus, matching anecdotal descriptions of cell culture contaminants of multiple eukaryotic cell lines. In addition, we provide information regarding antibiotic resistance and treatment effectiveness.

MATERIALS AND METHODS

Chemicals

All chemicals were purchased from Sigma (St. Louis, MO, USA) unless otherwise noted. Growth media, insulin/transferrin/selenium, and murine interferon (IFN- γ) and were purchased from Life Technologies (Rockville, MD, USA). Neonatal calf serum was purchased from Gemini Bio-Products (Woodland, CA, USA).

YAMC and IMCE Cell Culture Conditions

The young adult murine colon epithelial (YAMC) cells were developed from the transgenic SV40 large T antigen mouse [11]. The immorto-min mouse colon epithelial (IMCE (*Apc*^{Min/+})) cells were derived from an F1 hybrid between the Simian vacuolating virus 40 (SV40) large T antigen transgenic mouse and the *Apc*^{Min/+} mouse [12]. Both of these cell lines are non-tumorigenic in nude mice, do not grow in soft agar and survive in culture only on extracellular matrix proteins such as collagen I [12]. Both YAMC (*Apc*^{+/+}) and IMCE (*Apc*^{Min/+}) cells express the heat-labile SV40 large T antigen under the control of an interferon (IFN)- γ -inducible promoter. At 33°C, the temperature-sensitive SV40 large T antigen is active and drives cell proliferation. At 39°C, the temperature-sensitive mutation yields an inactive protein, and cells behave as non-proliferating, differentiated colon epithelial cells [13]. Cells were cultured as previously described [14]. Cells were maintained at 33°C for all antibiotic treatments.

MC38 Cell Culture Conditions

The murine carcinoma-38 (MC38) colon cancer cell line was derived from a murine colon tumor, grade III carcinoma, which was chemically induced in the C57Bl/6 female mouse [15]. This cell line was kindly gifted by Dr. Steve Hursting (University of Texas; Austin, TX, USA) and cultured in DMEM supplemented with 10% (v/v) fetal bovine serum (FBS) and 1% (v/v) penicillin/streptomycin (Gibco; Rockville, MD, USA) in a 37°C incubator with 5% CO₂ [16].

RAW 264.7 and L929 Cell Culture Conditions

RAW 264.7 murine macrophage cells and L929 murine fibroblasts (kindly provided by Dr. James Pestka, Michigan State University; East Lansing, MI, USA) were cultured in Dulbecco's Modified Eagle's Medium supplemented with 10% (v/v) heat-inactivated FBS and 1% (v/v) penicillin/streptomycin in a 37°C incubator with 5% CO₂.

Bacterial Cell Lysis and DNA Isolation

Contaminated supernatant from the IMCE cells was added to cultures of L929 murine fibroblasts. The L929 cells were susceptible to the contamination and used to determine if cells, media, and other items in the laboratory, such as tap and distilled water sources, FBS and newborn calf serum (NCS) from various sources, and water from the water bath were contaminated with the unknown organism. The contamination was then initially plated on MRS agar for growth in a 37°C incubator with 10% CO₂.

Colony material was scraped off the MRS agar and cultured in MRS broth. Aliquots of the liquid cultures and colony material from the MRS agar was plated on blood agar plates and incubated in a 34°C incubator with 10% CO₂. After bacterial growth was visible, the cultures were streaked on blood agar plates for isolation. Two days later, isolated colonies, from four different cultures, were spread on blood agar. Once the lawn grew on the plates, colony material was scraped off and resuspended in 50 mM EDTA for genomic DNA isolation using the Wizard Genomic DNA Isolation Kit (Promega; Madison, WI, USA) following the manufacturer's instructions. Although a Gram stain identified the bacteria as Gram negative, due to prior difficulty in lysing the unknown bacteria, the protocol for Gram positive bacteria was followed in order to ensure lysis. The optional lysis step was carried out with lysozyme (50 mg/ml, Roche; Indianapolis, IN, USA) and was incubated for 30 min. The DNA pellet was resuspended in the provided DNA Rehydration Solution (10mM Tris-HCl/1mM EDTA, pH 7.4) overnight at 4°C.

Sequencing and Identification

After quantitation with a NanoDrop 1000 (NanoDrop, Wilmington, DE, USA), 50 ng of the DNA was added to a PCR reaction consisting of GoTaq® Colorless Master Mix (Promega; Madison, WI, USA), the primers 8F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') [17], and RNase/DNase free water, to 50 µl. The reactions were run using the following cycling parameters: 95°C for 3 min, 30 cycles of 30 sec at 95°C, 30 sec at 55°C, and 45 sec at 72°C, with a final elongation step of 10 min at 72°C, before a 4°C hold.

PCR reactions (5 µl) were visualized on a 1% tris-borate-EDTA (TBE) agarose gel. A single product was observed, at approximately 1400 base pairs. The PCR products were isolated by ethanol precipitation. Briefly, 2.5 µl of 3 M sodium acetate (pH 4.6) and 50 µl of 95% ethanol was added to 25 µl of the PCR reaction, and incubated at -20°C for 1 hr. The tubes were centrifuged, supernatant removed, and the pellet washed with 70% ethanol. The pellet was dried at room temperature and resuspended in water.

After quantitation of the PCR product, with a NanoDrop, DNA (all four sample amounts were between 27.5 and 50 ng) was submitted for sequencing to Michigan State University's Research Technology and Support Facility, along with 30 pmoles of primer 8F or 1492R. The reverse complement of the sequences originating from the 1492R primers was obtained from http://www.bioinformatics.org/sms/rev_comp.html. The two sequences, one originating from the 8F primer and the other from the 1492R primer were aligned using NCBI's BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>; accessed on August 7, 2009). A sequence of over 1200 base pairs was obtained for each of the four samples. All four sequences, isolated from four different colonies, were queried using NCBI's BLAST and Ribosomal Database Project (<http://rdp.cme.msu.edu/index.jsp?jsessionid=99951FE2A428D273BA5B9808031BEB99>; accessed on August 7, 2009).

Microscopy

Cultures of the contaminant, in MRS broth, were mixed with an equal volume of agarose, resulting in 0.25 to 1% agarose. Approximately 25 μ l of this mix was placed on a slide and a cover slip was sealed in place. Slides were taken to Michigan State University's Center for Advanced Microscopy and examined on a Confocal Laser Scanning Microscope.

Antibiotic Treatment

Numerous antibiotics were tested at various concentrations. These include gentamicin, amikacin, tobramycin, imipenem, erythromycin, ciprofloxacin, chloramphenicol, piperacillin, and Plasmocin (InvivoGen; San Diego, CA, USA). Doses are shown in Table 1. Antibiotics and doses were chosen based on treatments used in the literature [18,19]. Antibiotics were added to the complete media which was replaced every 3–5 days with fresh media and antibiotics.

RESULTS AND DISCUSSION

Achromobacter can survive and multiply in water and moist soil. Sources have been identified as untreated well water, swimming pools, dialysis fluids, distilled water, deionized water, tap water, disinfectants, water systems, ventilators, humidifiers, IV fluids, and infant formula [20,21]. Four of our cell lines, IMCE, YAMC, MC38 and 3T3-L1, were susceptible to the contamination. Other cell lines in the lab, RAW 264.7 murine macrophages and U937 human monocytes appeared to spontaneously clear the contaminate. After initial difficulty with culturing the contamination, the bacterial contaminant was isolated from the IMCE cultures. Cell cultures with contamination had qualitative growth differences, such as slow growth and reduced cell viability (data not shown).

Initial attempts to lyse the culture contamination were done in the eukaryotic cell culture supernatant. Boiling (5–10 min at 95°C) and sonicating (15–30 sec at output power 4) did not result in available DNA for PCR. It is possible that the large amount of protein present in eukaryotic culture media, primarily from the FBS, could have protected the bacterial cells. DNA was finally isolated using a commercial kit from Promega from isolated colonies grown on blood agar.

The PCR products, about 1400 base pairs in size, were isolated by ethanol precipitation and sent for sequencing. After the sequencing, the resulting sequences were checked for similarity to other known sequences using NCBI's BLAST and the Ribosomal Database Project (RDP). Sequence 1 shared 99% sequence identity with *Alcaligenes* and *Achromobacter* 16S rDNA gene sequences, as listed in Table 2. Members of the *Achromobacter* and *Alcaligenes* genera have reclassified to and from these two genera, including *Achromobacter* (*Alcaligenes*) *xylosoxidans* and *Achromobacter* (*Alcaligenes*) *denitrificans* [22,23]. It is unclear whether the

similarity to both members of the *Achromobacter* and *Alcaligenes* genera is due to uncertainty in the nomenclature of previously submitted sequences or due to the actual sequence of the contaminating bacteria. RDP, a web-based program containing only 16S rDNA sequences, was utilized to confirm the contaminating bacteria's genus. Results using SeqMatch from the RDP identified the sequences as belonging to the genus *Achromobacter*. Phylogenetic trees based on the BLAST and RDP results grouped the bacteria with predominantly *Achromobacter* 16S rDNA sequences (data not shown). Sequences 2, 3, and 4 share 99% sequence identity with sequence 1 (Table 3).

Achromobacter is a genus containing members that are Gram negative, oxidase positive and are mainly non-fermenters with members from a range of environments, including soil, water, and samples from humans. They are rod shaped, have flagella, and grow well at 37–42°C with a pH of 6.5–8.5 [24]. In our laboratory, we found that it would also grow at room temperature, 33°C, and 39°C. This contaminant was cultured from FBS and NCS, tap water, distilled water, the water bath (in the water and colony material on the metal), phospho-buffered saline, and tris-buffered saline. FBS and NCS were suspected as the primary, but not only, sources of contamination for several reasons. First, cells thawed from cryostorage (stored in 95% FBS or NCS and 5% dimethyl sulfoxide), which had been previously uncontaminated, contained the bacteria once thawed. Second, multiple sources of FBS and NCS appeared to be contaminated with the moving rod-like motile structures, presumed to be bacteria, when samples were examined under a microscope. Third, this contaminant morphology and motility has been observed in cells obtained from other labs using NCS from the same source.

Cultures of *Achromobacter* were visualized using microscopy. Cultures were mixed with an equal volume of agarose and placed on a slide. Figure 1A shows many bacterial cells while 1B shows individual cells. Cells were approximately 4–5 µm and were rod-shaped.

One member of the genus, *Achromobacter xylosoxidans* has been found to be the causative agent in multiple cases of septicemia, mainly in immunocompromised individuals, and sometimes due to contaminated medical supplies [25–30]. *Achromobacter* is becoming a serious threat to the health of individuals with cystic fibrosis [31]. Other infections linked to the presence of *A. xylosoxidans* are meningitis, pneumonia, urinary tract infections, and pharyngitis [32].

A. xylosoxidans has been shown to be resistant to multiple antibiotics and one strain even produces a thermostable penicillin G acylase with a temperature optima of around 60°C [33]. Additionally, different members of the *Achromobacter* genus are resistant to multiple antibiotics. *Achromobacter* contamination in the laboratory was resistant to most types and doses of antibiotics tested (see Table 1 for antibiotics and doses tested). Eukaryotic cells were killed at various concentrations of antibiotics, depending on cell type. For example, a ciprofloxacin concentration greater than 60 µg/ml killed both MC-38 and YAMC cells. A combination of two antibiotics, piperacillin and ciprofloxacin, at a concentration of 10 µg/ml each, was best at selectively killing the *Achromobacter* contaminant without appearing to harm the eukaryotic cells. *Achromobacter* have been previously shown to be susceptible to antibiotics administered together. *A. xylosoxidans*, one species of *Achromobacter* is resistant to ciprofloxacin, but susceptible to a combination of piperacillin and tazobactam [34,35].

In this study, isolation of an unknown contaminant was revealed as member of the genus *Achromobacter*. The bacteria in this genus can survive in a wide range of environments including tap water, the waterbath, FBS, NCS, and many common laboratory solutions. In addition, commercial cells were found to contain this contaminant. It is important that researchers utilizing cell culture for experiments become aware of this multi-drug resistant bacterial contaminant. Selective killing of these bacteria in eukaryotic cell culture was

successful only with a combination of two antibiotics, piperacillin and ciprofloxacin, both at a concentration of 10 µg/ml each. This report presents the novel identification of this cell culture contaminant and a possible way to remove the swimming black dots from cell culture.

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REFERENCES

1. Uphoff CC, Drexler HG. Prevention of mycoplasma contamination in leukemia-lymphoma cell lines. *Hum Cell* 2001;14(3):244–247. [PubMed: 11774744]
2. Garner CM, Hubbard LM, Chakraborti PR. Mycoplasma detection in cell cultures: a comparison of four methods. *Br J Biomed Sci* 2000;57(4):295–301. [PubMed: 11204859]
3. Hay RJ. Operator-induced contamination in cell culture systems. *Dev Biol Stand* 1991;75:193–204. [PubMed: 1794620]
4. Mirjalili A, et al. Microbial contamination of cell cultures: a 2 years study. *Biologicals* 2005;33(2): 81–85. [PubMed: 15939285]
5. Hillesland KL, Lenski RE, Velicer GJ. Ecological variables affecting predatory success in *Myxococcus xanthus*. *Microb Ecol* 2007;53(4):571–578. [PubMed: 17410395]
6. Strauch E, Schwudke D, Linscheid M. Predatory mechanisms of *Bdellovibrio* and like organisms. *Future Microbiol* 2007;2:63–73. [PubMed: 17661676]
7. Nandy SK, Bapat PM, Venkatesh KV. Sporulating bacteria prefers predation to cannibalism in mixed cultures. *FEBS Lett* 2007;581(1):151–156. [PubMed: 17184776]
8. Blazkova H, et al. Bacterial Intoxication Evokes Cellular Senescence with Persistent DNA Damage and Cytokine Signaling. *J Cell Mol Med*. 2009
9. Testro AG, Visvanathan K. Toll-like receptors and their role in gastrointestinal disease. *J Gastroenterol Hepatol* 2009;24(6):943–954. [PubMed: 19638078]
10. Zenk SF, Jantsch J, Hensel M. Role of *Salmonella enterica* lipopolysaccharide in activation of dendritic cell functions and bacterial containment. *J Immunol* 2009;183(4):2697–2707. [PubMed: 19625639]
11. Whitehead RH, et al. Establishment of conditionally immortalized epithelial cell lines from both colon and small intestine of adult H-2Kb-tsA58 transgenic mice. *Proc Natl Acad Sci U S A* 1993;90(2): 587–591. [PubMed: 7678459]
12. Whitehead RH, Joseph JL. Derivation of conditionally immortalized cell lines containing the Min mutation from the normal colonic mucosa and other tissues of an "Immortomouse"/Min hybrid. *Epithelial Cell Biol* 1994;3(3):119–125. [PubMed: 7894569]
13. D'Abaco GM, Whitehead RH, Burgess AW. Synergy between *Apc min* and an activated *ras* mutation is sufficient to induce colon carcinomas. *Mol Cell Biol* 1996;16(3):884–891. [PubMed: 8622690]
14. Fenton JJ, et al. Membrane-type matrix metalloproteinases mediate curcumin-induced cell migration in non-tumorigenic colon epithelial cells differing in *Apc* genotype. *Carcinogenesis* 2002;23(6): 1065–1070. [PubMed: 12082030]
15. Corbett TH, et al. Tumor induction relationships in development of transplantable cancers of the colon in mice for chemotherapy assays, with a note on carcinogen structure. *Cancer Res* 1975;35(9):2434–2439. [PubMed: 1149045]
16. Pajtasz-Piasecka E, et al. Loss of tumorigenicity of murine colon carcinoma MC38/0 cell line after transduction with a retroviral vector carrying murine IL-12 genes. *Folia Biol (Praha)* 2004;50(1):7–14. [PubMed: 15055737]
17. Rodrigues JL, et al. Phylogenetic characterization and prevalence of "*Spirobacillus cienkowskii*," a red-pigmented, spiral-shaped bacterial pathogen of freshwater *Daphnia* species. *Appl Environ Microbiol* 2008;74(5):1575–1582. [PubMed: 18192404]
18. Tsay RW, et al. *Alcaligenes xylosoxidans* bacteremia: clinical features and microbiological characteristics of isolates. *J Microbiol Immunol Infect* 2005;38(3):194–199. [PubMed: 15986070]

19. Kish MA, Buggy BP, Forbes BA. Bacteremia caused by *Achromobacter* species in an immunocompromised host. *J Clin Microbiol* 1984;19(6):947–948. [PubMed: 6332118]
20. Spear JB, Fuhrer J, Kirby BD. *Achromobacter xylosoxidans* (*Alcaligenes xylosoxidans* subsp. *xylosoxidans*) bacteremia associated with a well-water source: case report and review of the literature. *J Clin Microbiol* 1988;26(3):598–599. [PubMed: 3281982]
21. Weitkamp JH, et al. Recurrent *Achromobacter xylosoxidans* bacteremia associated with persistent lymph node infection in a patient with hyper-immunoglobulin M syndrome. *Clin Infect Dis* 2000;31(5):1183–1187. [PubMed: 11073750]
22. Clermont D, Harmant C, Bizet C. Identification of strains of *Alcaligenes* and *Agrobacterium* by a polyphasic approach. *J Clin Microbiol* 2001;39(9):3104–3109. [PubMed: 11526136]
23. Coenye T, et al. *Kerstersia gyiorum* gen. nov., sp. nov., a novel *Alcaligenes faecalis*-like organism isolated from human clinical samples, and reclassification of *Alcaligenes denitrificans* Ruger and Tan 1983 as *Achromobacter denitrificans* comb. nov. *Int J Syst Evol Microbiol* 2003;53(Pt 6):1825–1831. [PubMed: 14657111]
24. Plhachova K, et al. Isolation and characterization of a new strain of *Achromobacter* sp. with beta-lactam antibiotic acylase activity. *Appl Microbiol Biotechnol* 2003;62(5–6):507–516. [PubMed: 12827318]
25. Asano K, et al. A novel bacterium *Achromobacter xylosoxidans* as a cause of liver abscess: three case reports. *J Hepatol* 2005;43(2):362–365. [PubMed: 15978693]
26. Gomez-Cerezo J, et al. *Achromobacter xylosoxidans* bacteremia: a 10-year analysis of 54 cases. *Eur J Clin Microbiol Infect Dis* 2003;22(6):360–363. [PubMed: 12750959]
27. Kanellopoulou M, et al. Persistent colonization of nine cystic fibrosis patients with an *Achromobacter* (*Alcaligenes*) *xylosoxidans* clone. *Eur J Clin Microbiol Infect Dis* 2004;23(4):336–339. [PubMed: 15024624]
28. Molina-Cabrillana J, et al. Outbreak of *Achromobacter xylosoxidans* pseudobacteremia in a neonatal care unit related to contaminated chlorhexidine solution. *Eur J Clin Microbiol Infect Dis* 2007;26(6):435–437. [PubMed: 17516103]
29. Ramos JM, et al. [Bacteremia caused by *Alcaligenes* (*Achromobacter*) *xylosoxidans*. Description of 3 cases and review of the literature]. *Enferm Infecc Microbiol Clin* 1996;14(7):436–440. [PubMed: 8991439]
30. Vu-Thien H, et al. Investigation of an outbreak of wound infections due to *Alcaligenes xylosoxidans* transmitted by chlorhexidine in a burns unit. *Eur J Clin Microbiol Infect Dis* 1998;17(10):724–726. [PubMed: 9865987]
31. Davies JC, Rubin BK. Emerging and unusual gram-negative infections in cystic fibrosis. *Semin Respir Crit Care Med* 2007;28(3):312–321. [PubMed: 17562501]
32. Reverdy ME, et al. Nosocomial colonization and infection by *Achromobacter xylosoxidans*. *J Clin Microbiol* 1984;19(2):140–143. [PubMed: 6699141]
33. Houghton J, et al. Gastric cancer originating from bone marrow-derived cells. *Science* 2004;306(5701):1568–1571. [PubMed: 15567866]
34. Tena D, et al. Urinary tract infection due to *Achromobacter xylosoxidans*: report of 9 cases. *Scand J Infect Dis* 2008;40(2):84–87. [PubMed: 17852927]
35. Magni A, et al. Emerging cystic fibrosis pathogens: incidence and antimicrobial resistance. *New Microbiol* 2007;30(1):59–62. [PubMed: 17319602]

A

B

Figure 1.

A) Phase contrast microscopy of *Achromobacter* contaminant in 0.5% agarose (400 $\times$) as it might look in culture under a typical microscope. B) Magnification (3200 $\times$) of *Achromobacter* contaminant in 0.5% agarose.

Table 1Antibiotics and doses tested to treat *Achromobacter* contaminated cells.

Antibiotic	Dose (µg/ml)
Gentamicin	500–2000
Amikacin	50–2500
Tobramycin	50–600
Imipenem	2–64
Erythromycin	25–500
Piperacillin	0.5–1000
Choramphenicol	50–500
Ciprofloxacin	10–750
Plasmocin	25–62.5

Table 2

16S rDNA sequences from the cell culture contaminant and similar sequences identified through NCBI's BLAST.

Sequence 1 (GenBank accession number: GQ896352)	Sequence homology and percent sequence identity
cgagtggcgaacgggtgagtaattgtatcggaacgtgccagtagcg ggggataactacgcgaagcgtagctaataccgcatacgcctacgg gggaaagcagggatcgtgaagaccttgactattggagcgccgata tcggattagctagttgggtgggtaacggctaccaaggcgacgatcc gtagctggtttgagaggacgaccagccacactgggactgagacacg gccagactctacgggagggcagcagtggggaatttggacaatggg ggaaacccgtatccagccatcccgtgtgctgaggaaggcctcggg ttgtaaacactttggcaggaaagaaacgtcatgggttaatacccg gaaactgacgggtacgtcagaataagcaccggctaactacgtgccag cagcccggtataactgtaggggtcaagcgttaactggaattactgggc gtaaacgtgctgcagcggtgtcggaaagaaatgtgaaatcccag agcttaactttggaactgcatttttaactaccgggctagagtgtgcaga gggaggtggaattccgcgtgtagcagtgaatgcgtagatatcgga ggaacaccgatggcgaaggcagcctcctgggataacactgacgtc atgcacgaaagcgtggggagcaaacaggattagataccctggtagtc cagccctaacgatgtcaactagctgttggggccttcgggccttggtg gcgcagctaaccgtgaagttagaccgctggggagtagggtcgaa gattaaaactcaaggaattgacggggacccgcacaagcgggtgatg atgtggattaattcgaacgcgaaaaaccttacacctgacat gtctggaatcctgaagagatttaggagtgctcgcaagagaaccggaa cacaggtgctgcatggctgtcgtcagctcgtgtcgtgagatgttgggt aagtcgccgaacgagcgaacccttgtcattagttgctacgaaagggc actctaagagactgccggtagacaacccggagggaagggtgggatga cgtcaagtcctcatggcccttatgggtagggttcacacgtcatacaat ggctgggacagagggtcgcaaccgcgagggggagccaatcca gaaacccgatcgtatcgatcgatcgtcgaactcgactgcgtgaa gtcggaatcgctagtaatcgcgatcagcatgtcgggtgaatacgtt cccgggtctgtacacacggcccgctacaccatgggagtggttttac cagaagtagtagc (1339 bases)	Alcaligenes sp. CCBAU 10750 16S ribosomal RNA gene, partial sequence (EF377320.1) 99% identical
	Achromobacter sp. MGT3 16S ribosomal RNA gene, partial sequence (EU643799.1) 99% identical
	Alcaligenes denitrificans 16S rRNA gene (Y14907.1) 99% identical

Table 3

Percent identity of Sequence 1 and the other three sequences.

Sequence (GenBank accession number)	Number of gaps in the sequence/number of bases in common	Percent identical to sequence 1
1 (comparison sequence; GQ896352)	0/1339	100%
2 (GQ896351)	5/1338	99%
3 (GQ896350)	3/1341	99%
4 (GQ896349)	2/1271	99%