Papers relating to Pseudomonas fluorescens strain LP6a = UAMH 11620 or other aspects of degradation of hydrocarbons by bacteria.

Report

Foght, J.M. 1999. Identification of the bacterial isolates comprising the Environment Canada freshwater and cold marine standard inocula, using 16sRNA gene sequencing and analysis. Report EE-164, Environment Protection Service, Environment Canada

Publications

1. Adebusuyi AA, Foght JM, An alternative physiological role for the EmhABC efflux pump in Pseudomonas fluorescens cLP6a. BMC Microbiol. 2011 Nov 15;11:252.
ABSTRACT: BACKGROUND: Efflux pumps belonging to the resistance-nodulation-division (RND) superfamily in bacteria are involved in antibiotic resistance and solvent tolerance but have an unknown physiological role. EmhABC, a RND-type efflux pump in Pseudomonas fluorescens strain cLP6a, extrudes hydrophobic antibiotics, dyes and polycyclic aromatic hydrocarbons including phenanthrene. The effects of physico-chemical factors such as temperature or antibiotics on the activity and expression of EmhABC were determined in order to deduce its physiological role(s) in strain cLP6a in comparison to the emhB disruptant strain, cLP6a-1. RESULTS: Efflux assays conducted with 14C-phenanthrene showed that EmhABC activity is affected by incubation temperature. Increased phenanthrene efflux was measured in cLP6a cells grown at 10°C and decreased efflux was observed at 35°C compared with cells grown at the optimum temperature of 28°C. Membrane fatty acids in cLP6a cells were substantially altered by changes in growth temperature and in the presence of tetracycline. Changed membrane fatty acids and increased membrane permeability were associated with ~30-fold increased expression of emhABC in cLP6a cells grown at 35°C, and with increased extracellular free fatty acids. Growth of P. fluorescens cLP6a at supra-optimal temperature was enhanced by the presence of EmhABC compared to strain cLP6a-1. CONCLUSIONS: Combined, these observations suggest that the EmhABC efflux pump may be involved in the management of membrane stress effects such as those due to unfavourable incubation temperatures. Efflux of fatty acids replaced as a result of membrane damage or phospholipid turnover may be the primary physiological role of the EmhABC efflux pump in P. fluorescens cLP6a.

2. Abbasnezhad H, Gray M, Foght JM. Influence of adhesion on aerobic biodegradation and bioremediation of liquid hydrocarbons. Appl Microbiol Biotechnol. 2011 Nov;92(4):653-75.
Biodegradation of poorly water-soluble liquid hydrocarbons is often limited by low availability of the substrate to microbes. Adhesion of microorganisms to an oil-water interface can enhance this availability, whereas detaching cells from the interface can reduce the rate of biodegradation. The capability of microbes to adhere to the interface is not limited to hydrocarbon degraders, nor is it the only mechanism to enable rapid uptake of hydrocarbons, but it represents a common strategy. This review of the literature indicates that microbial adhesion can benefit growth on and biodegradation of very poorly water-soluble hydrocarbons such as n-alkanes and large polycyclic aromatic hydrocarbons dissolved in a non-aqueous phase. Adhesion is particularly important when the hydrocarbons are not emulsified, giving limited interfacial area between the two liquid phases. When mixed communities are involved in biodegradation, the ability of cells to adhere to the interface can enable selective growth and enhance bioremediation with time. The critical challenge in understanding the relationship between growth rate and biodegradation rate for adherent bacteria is to accurately measure and observe the population that resides at the interface of the hydrocarbon phase.

3. Gieg LM, Jack TR, Foght JM. Biological souring and mitigation in oil reservoirs. Appl Microbiol Biotechnol. 2011 Oct;92(2):263-82.
Souring in oil field systems is most commonly due to the action of sulfate-reducing prokaryotes, a diverse group of anaerobic microorganisms that respire sulfate and produce sulfide (the key souring agent) while oxidizing diverse electron donors. Such biological sulfide production is a detrimental, widespread phenomenon in the petroleum industry, occurring within oil reservoirs or in topside processing facilities, under low- and high-temperature conditions, and in onshore or offshore operations. Sulfate reducers can exist either indigenously in deep subsurface reservoirs or can be "inoculated" into a reservoir system during oil field development (e.g., via drilling operations) or during the oil production phase. In the latter, souring most commonly occurs during

water flooding, a secondary recovery strategy wherein water is injected to re-pressurize the reservoir and sweep the oil towards production wells to extend the production life of an oil field. The water source and type of production operation can provide multiple components such as sulfate, labile carbon sources, and sulfate-reducing communities that influence whether oil field souring occurs. Souring can be controlled by biocides, which can non-specifically suppress microbial populations, and by the addition of nitrate (and/or nitrite) that directly impacts the sulfate-reducing population by numerous competitive or inhibitory mechanisms. In this review, we report on the diversity of sulfate reducers associated with oil reservoirs, approaches for determining their presence and effects, the factors that control souring, and the approaches (along with the current understanding of their underlying mechanisms) that may be used to successfully mitigate souring in low-temperature and high-temperature oil field operations.

4. Siddique T, Penner T, Semple K, Foght JM. Anaerobic biodegradation of longer-chain n-alkanes coupled to methane production in oil sands tailings. Environ Sci Technol. 2011 Jul 1;45(13):5892-9.
Extraction of bitumen from mined oil sands ores produces enormous volumes of tailings that are stored in settling basins (current inventory ≥ 840 million m(3)). Our previous studies revealed that certain hydrocarbons (short-chain n-alkanes [C(6)-C(10)] and monoaromatics [toluene, o-xylene, m-xylene]) in residual naphtha entrained in the tailings are biodegraded to CH(4) by a consortium of microorganisms. Here we show that higher molecular weight n-alkanes (C(14), C(16), and C(18)) are also degraded under methanogenic conditions in oil sands tailings, albeit after a lengthy lag (~180 d) before the onset of methanogenesis. Gas chromatographic analyses showed that the longer-chain n-alkanes each added at ~400 mg L(-1) were completely degraded by the resident microorganisms within ~440 d at ~20 °C. 16S rRNA gene sequence analysis of clone libraries implied that the predominant pathway of longer-chain n-alkane metabolism in tailings is through syntrophic oxidation of n-alkanes coupled with CO(2) reduction to CH(4). These studies demonstrating methanogenic biodegradation of longer-chain n-alkanes by microbes native to oil sands tailings may be important for effective management of tailings and greenhouse gas emissions from tailings ponds.

5. Kumaraswamy R, Ebert S, Gray MR, Fedorak PM, Foght JM. Molecular- and cultivation-based analyses of microbial communities in oil field water and in microcosms amended with nitrate to control H2S production. Appl Microbiol Biotechnol. 2011 Mar;89(6):2027-38.
Nitrate injection into oil fields is an alternative to biocide addition for controlling sulfide production ('souring') caused by sulfate-reducing bacteria (SRB). This study examined the suitability of several cultivation-dependent and cultivation-independent methods to assess potential microbial activities (sulfidogenesis and nitrate reduction) and the impact of nitrate amendment on oil field microbiota. Microcosms containing produced waters from two Western Canadian oil fields exhibited sulfidogenesis that was inhibited by nitrate amendment. Most probable number (MPN) and fluorescent in situ hybridization (FISH) analyses of uncultivated produced waters showed low cell numbers (≤10(3) MPN/ml) dominated by SRB (>95% relative abundance). MPN analysis also detected nitrate-reducing sulfide-oxidizing bacteria (NRSOB) and heterotrophic nitrate-reducing bacteria (HNRB) at numbers too low to be detected by FISH or denaturing gradient gel electrophoresis (DGGE). In microcosms containing produced water fortified with sulfate, near-stoichiometric concentrations of sulfide were produced. FISH analyses of the microcosms after 55 days of incubation revealed that Gammaproteobacteria increased from undetectable levels to 5-20% abundance, resulting in a decreased proportion of Deltaproteobacteria (50-60% abundance). DGGE analysis confirmed the presence of Delta- and Gammaproteobacteria and also detected Bacteroidetes. When sulfate-fortified produced waters were amended with nitrate, sulfidogenesis was inhibited and Deltaproteobacteria decreased to levels undetectable by FISH, with a concomitant increase in Gammaproteobacteria from below detection to 50-60% abundance. DGGE analysis of these microcosms yielded sequences of Gamma- and Epsilonproteobacteria related to presumptive HNRB and NRSOB (Halomonas, Marinobacterium, Marinobacter, Pseudomonas and Arcobacter), thus supporting chemical data indicating that nitrate-reducing bacteria out-compete SRB when nitrate is added.

6. Abbasnezhad H, Foght JM, Gray MR. Adhesion to the hydrocarbon phase increases phenanthrene degradation by Pseudomonas fluorescens LP6a. Biodegradation. 2011 Jun;22(3):485-96.
Microbial adhesion is an important factor that can influence biodegradation of poorly water soluble hydrocarbons such as phenanthrene. This study examined how adhesion to an oil-water interface, as mediated by 1-dodecanol, enhanced phenanthrene biodegradation by Pseudomonas fluorescens LP6a. Phenanthrene was dissolved in heptamethylnonane and added to the aerobic aqueous growth medium to form a two phase mixture.

1-Dodecanol was non-toxic and furthermore could be biodegraded slowly by this strain. The alcohol promoted adhesion of the bacterial cells to the oil-water interface without significantly changing the interfacial or surface tension. Introducing 1-dodecanol at concentrations from 217 to 4,100 mg l(-1) increased phenanthrene biodegradation by about 30% after 120 h incubation. After 100 h incubation, cultures initially containing 120 or 160 mg l(-1) 1-dodecanol had mineralized >10% of the phenanthrene whereas those incubated without 1-dodecanol had mineralized only 4.5%. The production and accumulation of putative phenanthrene metabolites in the aqueous phase of cultures likewise increased in response to the addition of 1-dodecanol. The results suggest that enhanced adhesion of bacterial cells to the oil-water interface was the main factor responsible for enhanced biodegradation of phenanthrene to presumed polar metabolites and to CO(2).

7. Penner TJ, Foght JM. Mature fine tailings from oil sands processing harbour diverse methanogenic communities. Can J Microbiol. 2010 56(6):459-70.
Processing oil sands to extract bitumen produces large volumes of a tailings slurry comprising water, silt, clays, unrecovered bitumen, and residual solvent used in the extraction process. Tailings are deposited into large settling basins, where the solids settle by gravity to become denser mature fine tailings (MFT). A substantial flux of methane, currently estimated at ~40 million L/day, is being emitted from the Mildred Lake Settling Basin. To better understand the biogenesis of this greenhouse gas, the methanogenic consortia in MFT samples from depth profiles in 2 tailings deposits (Mildred Lake Settling Basin and West In-Pit) were analyzed by constructing clone libraries of amplified archaeal and bacterial 16S rRNA genes. The archaeal sequences, whose closest matches were almost exclusively cultivated methanogens, were comparable within and between basins and were predominantly (87% of clones) affiliated with acetoclastic Methanosaeta spp. In contrast, bacterial clone libraries were unexpectedly diverse, with the majority (~55%) of sequences related to Proteobacteria, including some presumptive nitrate-, iron-, or sulfate-reducing, hydrocarbon-degrading genera (e.g., Thauera, Rhodoferax, and Desulfatibacillum). Thus, MFT harbour a diverse community of prokaryotes presumptively responsible for producing methane from substrates indigenous to the MFT. These findings contribute to our understanding of biogenic methane production and densification of MFT in oil sands tailings deposits.

8. Abbasnezhad H, Gray MR, Foght JM. Two different mechanisms for adhesion of Gram-negative bacterium, Pseudomonas fluorescens LP6a, to an oil-water interface. Colloids Surf B Biointerfaces. 2008 Mar 15;62(1):36-41. Epub 2007 Oct 1.
Microbial adhesion to the oil-water interface is an important parameter in biodegradation of hydrocarbons to enhance uptake and metabolism of compounds with very low aqueous solubility, but the mechanisms of adhesion are not well understood. Our approach was to study a range of compounds and mechanisms to promote the adhesion of a hydrophilic bacterium, Pseudomonas fluorescens strain LP6a, to an oil-water interface. The cationic surfactants cetylpyridinium chloride (CPC), poly-l-lysine and chlorhexidine gluconate (CHX) and the long chain alcohols 1-dodecanol and farnesol increased the adhesion of P. fluorescens LP6a to n-hexadecane from ca. 30 to 90% of suspended cells adhering. In contrast, adjusting the ionic strength of the suspending medium only increased the adhesion from about 8 to 30%. The alcohols, 1-dodecanol and farnesol, also caused a dramatic change in the oil-water contact angle of the cell surface, increasing it from 24 degrees to 104 degrees , whereas the cationic compounds had little effect. In contrast, cationic compounds changed the electrophoretic mobility of the bacteria, reducing the mean zeta potential from -23 to -7 mV in 0.01 M potassium phosphate buffer, but the alcohols, 1-dodecanol and farnesol, had no effect on zeta potential. Even though both types of compounds promoted cell adhesion to the n-hexadecane interface, the mechanisms were different. Alcohols acted through altering the cell surface hydrophobicity, whereas cationic surfactants changed the surface charge density.

9. Hearn EM, Gray MR, Foght JM. Mutations in the central cavity and periplasmic domain affect efflux activity of the resistance-nodulation-division pump EmhB from Pseudomonas fluorescens cLP6a. J Bacteriol. 2006 Jan;188(1):115-23.
The EmhABC efflux system in Pseudomonas fluorescens cLP6a is homologous to the multidrug and solvent efflux systems belonging to the resistance-nodulation-division (RND) family and is responsible for polycyclic aromatic hydrocarbon transport, antibiotic resistance, and toluene efflux. To gain a better understanding of substrate transport in RND efflux pumps, the EmhB pump was subjected to mutational analysis. Mutagenesis of amino acids within the central cavity of the predicted three-dimensional structure of EmhB showed selective

activity towards antibiotic substrates. An A384P/A385Y double mutant showed increased susceptibility toward rhodamine 6G compared to the wild type, and F386A and N99A single mutants showed increased susceptibility to dequalinium compared to the wild type. As well, the carboxylic acid side chain of D101, located in the central cavity region, was found to be essential for polycyclic aromatic hydrocarbon transport and resistance to all antibiotic substrates of EmhB. Phenylalanine residues located within the periplasmic pore domain were also targeted for mutagenesis, and the F325A and F281A mutations significantly impaired efflux activity for all EmhB substrates. One mutation (A206S) in the outer membrane protein docking domain increased antibiotic resistance and toluene tolerance, demonstrating the important role of this domain in transport activity. These data demonstrate the roles of the central cavity and periplasmic domains in the function of the RND efflux pump EmhB.

10. Kirkwood KM, Ebert S, Foght JM, Fedorak PM, Gray MR. Bacterial biodegradation of aliphatic sulfides under aerobic carbon- or sulfur-limited growth conditions. J Appl Microbiol. 2005;99(6):1444-54.
AIMS: To isolate bacteria capable of cleaving aliphatic carbon-sulfur bonds as potential biological upgrading catalysts for the reduction of molecular weight and viscosity in heavy crude oil. METHODS AND RESULTS: Thirty-one bacterial strains isolated from enrichment cultures were able to biotransform model compounds representing the aliphatic sulfide bridges found in asphaltenes. Using gas chromatography and mass spectrometry, three types of attack were identified: alkyl chain degradation, allowing use as a carbon source; nonspecific sulfur oxidation; and sulfur-specific oxidation and carbon-sulfur bond cleavage, allowing use as a sulfur source. Di-n-octyl sulfide degradation produced octylthio- and octylsulfonyl-alkanoic acids, consistent with terminal oxidation followed by beta-oxidation reactions. Utilization of dibenzyl sulfide or 1,4-dithiane as a sulfur source was regulated by sulfate, indicating a sulfur-specific activity rather than nonspecific oxidation. Finally, several isolates were also able to use dibenzothiophene as a sulfur source, and this was the preferred organic sulfur substrate for one isolate. CONCLUSIONS: The use of commercially available alkyl sulfides in enrichment cultures gave isolates that followed a range of metabolic pathways, not just sulfur-specific attack. SIGNIFICANCE AND IMPACT OF THE STUDY: These results give new insight into biodegradation of organosulfur compounds from petroleum and for biotreatment of such compounds in chemical munitions.

11. Dorobantu LS, Yeung AK, Foght JM, Gray MR. Stabilization of oil-water emulsions by hydrophobic bacteria. Appl Environ Microbiol. 2004 Oct;70(10):6333-6.
Formation of oil-water emulsions during bacterial growth on hydrocarbons is often attributed to biosurfactants. Here we report the ability of certain intact bacterial cells to stabilize oil-in-water and water-in-oil emulsions without changing the interfacial tension, by inhibition of droplet coalescence as observed in emulsion stabilization by solid particles like silica.

12. Hearn EM, Dennis JJ, Gray MR, Foght JM. Identification and characterization of the emhABC efflux system for polycyclic aromatic hydrocarbons in Pseudomonas fluorescens cLP6a. J Bacteriol. 2003 Nov;185(21):6233-40.
The hydrocarbon-degrading environmental isolate Pseudomonas fluorescens LP6a possesses an active efflux mechanism for the polycyclic aromatic hydrocarbons phenanthrene, anthracene, and fluoranthene but not for naphthalene or toluene. PCR was used to detect efflux pump genes belonging to the resistance-nodulation-cell division (RND) superfamily in a plasmid-cured derivative, P. fluorescens cLP6a, which is unable to metabolize hydrocarbons. One RND pump, whose gene was identified in P. fluorescens cLP6a and was designated emhB, showed homology to the multidrug and solvent efflux pumps in Pseudomonas aeruginosa and Pseudomonas putida. The emhB gene is located in a gene cluster with the emhA and emhC genes, which encode the membrane fusion protein and outer membrane protein components of the efflux system, respectively. Disruption of emhB by insertion of an antibiotic resistance cassette demonstrated that the corresponding gene product was responsible for the efflux of polycyclic aromatic hydrocarbons. The emhB gene disruption did not affect the resistance of P. fluorescens cLP6a to tetracycline, erythromycin, trimethoprim, or streptomycin, but it did decrease resistance to chloramphenicol and nalidixic acid, indicating that the EmhABC system also functions in the efflux of these compounds and has an unusual selectivity. Phenanthrene efflux was observed in P. aeruginosa, P. putida, and Burkholderia cepacia but not in Azotobacter vinelandii. Polycyclic aromatic hydrocarbons represent a new class of nontoxic, highly hydrophobic compounds that are substrates of RND

efflux systems, and the EmhABC system in P. fluorescens cLP6a has a narrow substrate range for these hydrocarbons and certain antibiotics.

13. Bugg T, Foght JM, Pickard MA, Gray MR. Uptake and active efflux of polycyclic aromatic hydrocarbons by Pseudomonas fluorescens LP6a. Appl Environ Microbiol. 2000 Dec;66(12):5387-92.
The mechanism of transport of polycyclic aromatic hydrocarbons (PAHs) by Pseudomonas fluorescens LP6a, a PAH-degrading bacterium, was studied by inhibiting membrane transport and measuring the resulting change in cellular uptake. Three cultures were used: wild-type LP6a which carried a plasmid for PAH degradation, a transposon mutant lacking the first enzyme in the pathway for PAH degradation, and a cured strain without the plasmid. Washed cells were mixed with aqueous solutions of radiolabelled PAH; then the cells were removed by centrifugation, and the concentrations of PAH in the supernatant and the cell pellet were measured. The change in the pellet and supernatant concentrations after inhibitors of membrane transport (azide, cyanide, or carbonyl cyanide m-chlorophenyl hydrazone) were added indicated the role of active transport. The data were consistent with the presence of two conflicting transport mechanisms: uptake by passive diffusion and an energy-driven efflux system to transport PAHs out of the cell. The efflux mechanism was chromosomally encoded. Under the test conditions used, neither uptake nor efflux of phenanthrene by P. fluorescens LP6a was saturated. The efflux mechanism showed selectivity since phenanthrene, anthracene, and fluoranthene were transported out of the cell but naphthalene was not.

14. Foght, J., K. Semple, D.W.S. Westlake, S. Blenkinsopp, G. Sergy, Z. Wang and M. Fingas. 1998. Development of a standard bacterial consortium for laboratory efficacy testing of commercial freshwater oil spill bioremediation agents. *J. Ind. Microbiol. Biotechnol.* 21:322-330. [abstract not available]

15. Telang AJ, Ebert S, Foght JM, Westlake D, Jenneman GE, Gevertz D, Voordouw G. Effect of nitrate injection on the microbial community in an oil field as monitored by reverse sample genome probing. Appl Environ Microbiol. 1997 May;63(5):1785-93.
The reverse sample genome probe (RSGP) method, developed for monitoring the microbial community in oil fields with a moderate subsurface temperature, has been improved by (i) isolation of a variety of heterotrophic bacteria and inclusion of their genomes on the oil field master filter and (ii) use of phosphorimaging technology for the rapid quantitation of hybridization signals. The new master filter contains the genomes of 30 sulfate-reducing, 1 sulfide-oxidizing, and 16 heterotrophic bacteria. Most have been identified by partial 16S rRNA sequencing. Use of improved RSGP in monitoring the effect of nitrate injection in an oil field indicated that the sulfide-oxidizing, nitrate-reducing isolate CVO (a Campylobacter sp.) becomes the dominant community component immediately after injection. No significant enhancement of other community members, including the sulfate-reducing bacteria, was observed. The elevated level of CVO decayed at most sampling sites within 30 days after nitrate injection was terminated. Chemical analyses indicated a corresponding decrease and subsequent increase in sulfide concentrations. Thus, transient injection of a higher potential electron acceptor into an anaerobic subsurface system can have desirable effects (i.e., reduction of sulfide levels) without a permanent adverse influence on the resident microbial community.

16. Foght JM, Westlake DW. Transposon and spontaneous deletion mutants of plasmid-borne genes encoding polycyclic aromatic hydrocarbon degradation by a strain of Pseudomonas fluorescens. Biodegradation. 1996 Aug;7(4):353-66.
Pseudomonas fluorescens strain LP6a, isolated from petroleum condensate-contaminated soil, utilizes the polycyclic aromatic hydrocarbons (PAHs) naphthalene, phenanthrene, anthracene and 2-methylnaphthalene as sole carbon and energy sources. The isolate also co-metabolically transforms a suite of PAHs and heterocycles including fluorene, biphenyl, acenaphthene, 1-methylnaphthalene, indole, benzothiophene, dibenzothiophene and dibenzofuran, producing a variety of oxidized metabolites. A 63 kb plasmid (pLP6a) carries genes encoding enzymes necessary for the PAH-degrading phenotype of P. fluorescens LP6a. This plasmid hybridizes to the classical naphthalene degradative plasmids NAH7 and pWW60, but has different restriction endonuclease patterns. In contrast, plasmid pLP6a failed to hybridize to plasmids isolated from several phenanthrene-utilizing strains which cannot utilize naphthalene. Plasmid pLP6a exhibits reproducible spontaneous deletions of a 38 kb region containing the degradative genes. Two gene clusters corresponding to the archetypal naphthalene

degradation upper and lower pathway operons, separated by a cryptic region of 18 kb, were defined by transposon mutagenesis. Gas chromatographic-mass spectrometric analysis of metabolites accumulated by selected transposon mutants indicates that the degradative enzymes encoded by genes on pLP6a have a broad specificity permitting the oxidation of a suite of polycyclic aromatic and heterocyclic substrates.

17. Telang AJ, Voordouw G, Ebert S, Sifeldeen N, Foght JM, Fedorak PM, Westlake DW. Characterization of the diversity of sulfate-reducing bacteria in soil and mining waste water environments by nucleic acid hybridization techniques. Can J Microbiol. 1994 Nov;40(11):955-64. Nucleic acid hybridization techniques were used to characterize the sulfate-reducing bacterial communities at seven waste water and two soil sites in Canada. Genomic DNA was obtained from liquid enrichment cultures of samples taken from these nine sites. The liquid enrichment protocol favored growth of the sulfate-reducing bacterial component of the communities at these sites. The genomic DNA preparations were analyzed with (i) a specific gene probe aimed at a single genus (Desulfovibrio), (ii) a general 16S rRNA gene probe aimed at all genera of sulfate-reducing bacteria and other bacteria, and (iii) whole genome probes aimed at specific bacteria. This three-pronged approach provided information on the sulfate-reducing bacterial community structures for the nine sites. These were compared with each other and with the sulfate-reducing bacterial communities of western Canadian oil field production waters, studied previously. It was found that there is considerable diversity in the sulfate-reducing bacterial community at each site. Most sulfate-reducing bacteria isolated from distinct sites are genomically different and differ also from sulfate-reducing bacteria found in oil field production waters.

18. Foght, J.M. and D.W.S. Westlake. 1991. Cross hybridization of plasmid and genomic DNA from aromatic and polycyclic aromatic hydrocarbon degrading bacteria. Can. J. Microbiol. 37:924-932 [abstract not available]