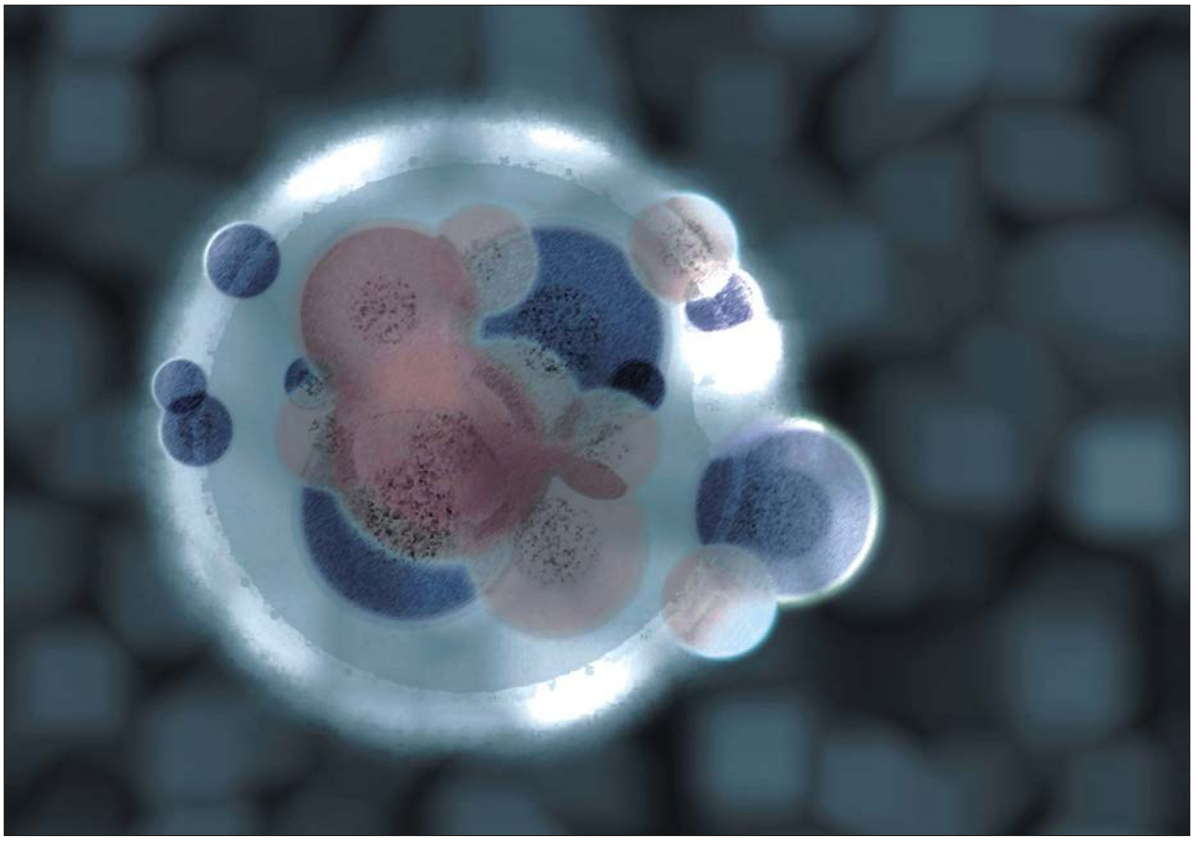

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Detection of Human Pathogenic Gram-negative Bacteria from Ornamental Goldfish Accordimg to Gene Sequence Alignment

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Abstract: Identification of bacterial infections involved in ornamental fishes has a great importance with considering the adverse effects on the aquatic health as well as the possibility of common diseases between human and fish. In the present study pathogenic bacteria were isolated and purified from skin, gill, kidney and brain of symptomatic goldfish samples from aquaculture ponds in Iran on the enriched culture media. The isolated bacteria have been first identified according to the morphological and biochemical characteristics. Molecular detection was done based on the amplification of specific genome fragments and sequencing of the amplified nucleotides. The isolated bacteria detected as *Pseudomonas mendosina* and *Aeromonas* spp. including *Aeromonas hydrophila* (the most sequence similarity), *Aeromonas veronii*, *Aeromonas sobria* and *Aeromonas salmonicida* (partial similarity in the genome sequence). The results of present study showed that gold fishes which are among the most favorable ornamental fishes, can be carriers of human pathogens from *Pseudomonas* and *Aeromonas* genera and this fact is necessary to considered in aquaculture healthcare systems.

Key words: Human pathogens, ornamental goldfish, sequence alignment, *Pseudomonas* sp., *Aeromonas* sp.

INTRODUCTION

Different factors including poor quality water, sewage pollution, unhygienic handling of fishes and polluted aquaculture feeding have been reported as the entrance sources of potential pathogenic bacteria to cultured and environmental fishes (Reilly and Kaferstein, 1998; Kigigha *et al*., 2012).

Goldfish (*Carassius auratus*) is one of the most familiar and traditional ornamental fish in different countries. The fish is from cold water oviparous fishes (Geran, 1992). Because of the little importance of goldfish culturing as a food production system, the most infection hazard to human is from skin contact from injured surface tissues, especially in immunodeficient patients (Lewbart, 2001).

Different bacteria, fungi and viruses can cause infection in fishes (Noga, 2000) but the most common infectious diseases of ornamental fishes are caused by bacteria, particularly gram-negative species (Lewbart, 2001). Environmental factors (especially stress) and deficiency in immune system leads to increasing the susceptibility to infection (Toranzo *et al*., 2005).

The aim of present study was detection of human pathogenic gram-negative bacteria from ornamental goldfish based on genome sequence analysis of the isolated bacteria.

MATERIALS AND METHODS

Samples and isolation of the bacteria: Skin, gill, kidney and brain samples obtained from symptomatic goldfish in five aquaculture ponds in Iran in a period of time from January to September 2012. The symptoms were included: ulcer, pop eye, cloudy eye, dropsy, columnaris, mouth rot, tail rot and bloated stomach. The samples inoculated on enriched culture media including brain heart infusion broth, tripticase soy broth and blood agar. The culture media were incubated at 32°C for 24 h. Microscopic morphology of the isolated bacteria detected by gram staining and the colonies were transferred to blood agar and nutrient agar media for purification of the isolated bacteria. The purified isolates were detected according to morphological and biochemical characteristics (Brooks *et al*., 2010; Mahon *et al*., 2010).

DNA extraction and sequence amplification: DNA content of media was extracted using Roche Applied Science DNA extraction kit. Pairs of primers were used according to specific genome sequences of the biochemical detected bacteria. The characteristics of primers and information about amplification by polymerase chain reaction (PCR) are shown in Table 1. A mixture consists of 1X PCR buffer, 0.2 mM dNTP mix, 1.5 mM $MgCl_2$, 0.5 μM of each primer, 1 unit μL^{-1} Taq

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Table 1: Primer sequences and characteristics of amplification procedures

Isolated bacteria	Primer sequence	Amplification cycle	Amplified fragment	Reference
Pseudomonas spp.	TGC ATT CAA AAC TGA CTG AAT CAC ACC GTG GTA ACC G	Step 1: 94°C, 2 min; Step 2: 94°C, 55 sec; 39°C, 1 min; 72°C, 90 sec (5 repeats); Step 3: 94°C, 55 sec; 42°C, 1 min; 72°C, 2 min (35 repeats)	16S rDNA (850 bp)	Scarpellini *et al.* (2004)
Aeromonas spp.	AAC CTG GTT CCG CTC AAG CCG TTG TTG CTC GCC TCG GCC CAG CAG CT	Step 1: 94°C, 2 min; Step 2: 94°C, 1 min; 63°C, 1 min; 72°C, 1 min (35 repeats); Step 3: 72°C, 5 min	Lipase gene (760 bp)	Cascon *et al.* (1996)

Table 2: Morphological and biochemical characteristics of the isolated bacteria

Characteristics	Isolate No. 1	Isolate No. 2
Morphology and gram staining	Gram-negative coccobacilli	Gram-negative bacilli
Haemolysis on blood agar	Alpha	Beta
Indole production	+	-
Methyl red reaction	-	-
Voges-Proskauer reaction	+	+
Citrate utilization	-	+
Urease	-	-
SH_2 production	+	-
Motility	+	+
Catalase	+	+
Oxidase	+	+
Oxidative/Fermentative	Fermentative	Oxidative
Glucose fermentation	+	*
Xylose fermentation	-	*
Lactose fermentation	-	*
Sorbitol fermentation	+	*
Saccharose fermentation	+	*
Mannitol fermentation	+	*
Identified species	*Aeromonas hydrophila*	*Pseudomonas* sp.

+: Positive reaction, -: Negative reaction, *: Not valuable to perform

DNA polymerase and 1 μg of the extracted DNA was used for each amplification reaction. The products analyzed by gel electrophoresis using 1% agarose.

Sequence analysis of the amplified fragments: The final products have been sequenced by ABI3730XL system (Bioneer corporation, Korea).

RESULTS

Isolated bacteria: Two isolates belonging to *Aeromonas* sp. and *Pseudomonas* sp. were detected by morphological and biochemical analysis. The isolated *Aeromonas* showed the most similarity to *Aeromonas hydrophila* but the isolated *Pseudomonas* had characteristics similarity to *Pseudomonas fluorescens* and *Pseudomonas aeruginosa* (Table 2). Because of the mentioned similarities, specific primers used for amplification of *Aeromonas hydrophila* lipase gene (Cascon *et al*., 1996) and the sequence of 16SrDNA was amplified for detection of *Pseudomonas* spp. (Scarpellini *et al*., 2004).

Molecular detection: As the results of molecular amplification a 760 bp fragment for *Aeromonas hydrophila* and an 850 bp fragment for *Pseudomonas* sp. were obtained (Fig. 1).

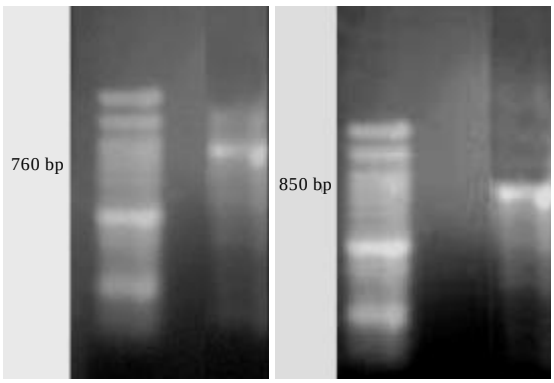

Fig. 1: The amplified fragment for *Aeromonas hydrophila* lipase gene (760 bp) and *Pseudomonas* sp. 16S rDNA (850 bp)

Sequenceing analysis: Alignment of the amplified regions in BLAST data base showed that the isolated *Pseudomonas* sp. Belongs to *Pseudomonas mendosina*. The isolated *Aeromonas* sp. showed the most sequence similarity to *Aeromonas hydrophila*. Also this fragment had partial similarity to *Aeromonas veronii*, *Aeromonas sobria* and *Aeromonas salmonicida*. The nucleotide sequences of the amplified fragments and the organism's reports are shown in Table 3 and 4.

Table 3: The nucleotide sequence and taxonomy report of fragments amplified from *Aeromonas* spp.

Sequence based on the amplification with forward primer

GGGGTGGCGAGGCGGCCCCCTGGACAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG
TAAACGATGTCGATTTGGAGGCTGTGTCCTTGAGACGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAAGGT
TAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTGACA
TGTCTGGAATCCTGTAGAGATACGGGAGTGCCTTCGGGAATCAGAACACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCCTGTCCTTTGTTGCCAGCACGTAATGGTGGGAACTCAAGGGAGACTGCCGGTGATAAACCGGAGGA
AGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGCTACACACGTGCTACAATGGCGCGTACAGAGGGCTGCAAGCTAGCGATA
GTGAGCGAATCCCAAAAAGCGCGTCGTAGTCCGGATCGGAGTCTGCAACTCGACTCCGTGAACTCGGTCCCCCTTTAGTAAAGA

Sequence based on the amplification with reverse primer

TTAAGTTGGCTTACGCGTCCCCCTGGACAAGACTGACGCTCAGGTGCTAAAGCGTGGGGAGCTAACAGGATTAGATACCCTGGTTGTCCACG
CCGTAAACGATGTCGATTTGGAGGCTGTGTCCTTGAGACGTGGCTTCCGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTACGGCCGCAA
GGTTAAAACTCAAATGAATTGACGGGGGCCCGCACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTGGCCTTG
ACTTGTCTGGAATCCTGTAGAGATACGGAAGTGCCTTCGGGAATCAGAACACAGGTGCTGCATGGATGTCGTCAGCTCTTGTCGTGAGATGT
TGGGTTAAGTCCCGCAACGAGCGCAACCCCTGTCCTTTGTTGCCAGCACGTAATGGTGGGAACTCAAGGGAGACTGCCGGTGATAAACCGGA
GGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGGCCAGGGTTACACACGTGCTACAATGGCGCGTACATAGGGCTGCAAGCTATCG
ATATTTGAGCGAATCCCAAAAAGCGCGTCGTAGTCCGGATCGGAGTCTGCAACTCTACTCCGTGAACTCTGAATCTCTTAGTAGAAGGAAAA

Organism report (obtained from BLAST)

Aeromonas hydrophila [g-proteobacteria] taxid 644
Aeromonas veronii [g-proteobacteria] taxid 654
Aeromonas sobria [g-proteobacteria] taxid 646
Aeromonas salmonicida subsp. *salmonicida* [g-proteobacteria] taxid 29491

Table 4: The nucleotide sequence and taxonomy report of fragments amplified from *Pseudomonas* spp.

Sequence based on the amplification with forward primer

TGATATGATTAGAGGCAGCAAACCATACAGCGAGTTACCACCAAAGTTCGTATCGGGCGTTACCAAACCGGCCAACGCGCCGCTGCGGTTGT
AAGCCGTATTGCCGCCATCGGCGAACAGGTCGGTCTCCGAGAAATAAGTACCGACCGGATCGACCGCAGTTTCTTTCCAGTTGAACTGATAG
AAGGTTTCCATCGAAAGGTTATCGGTAAGACCGATGTTGAAGCTAAGCGCCTCGACCGGCACCAGCACTTCTTTCAGTTCAGATCCTGGCAG
GCGGAACTGCGCAGCATTCAGCGGGTTGGTGGTGTTGATACCGCCACGATAAAAAATCCCTTCGCCCCAGTTGAATACCTGCTTACCGAAAC
GAGCCGTGACCGGCATCTCGGCCACATCCCAGTTGCCATAAACGTAAGCATCAAGAATCTGCGCATCACGCCCGGCCTTGTGCCGTGTTTCG
TGCGTAAAGCTGCTGTCGCGCGGCAGGTTCTGGCTCGGCTCATAGGCGCCGGCCCCAAGGAAATCATTGCGTTTGTCCATGATCTGGGTGTC
ATAAAACGCAGTGCCACGAACGAACATGCCGTAGTTCTGGTAGGACATTTCCAAATCGGAAGTCACCTTGAACACTTCGGAAACCAGGCCGG
TATCAAAGTTACGGACCGGGGGGTTGTATGTATGATGATTTA

Sequence based on the amplification with reverse primer

AGGGACTGAGTTTCGAGTGTTCAGGTGACTTCCGATTTGGAAATGTCCTACCAGAACTACGGCATGTTCGTTCGTGGCACTGCGTTTTATGA
CACCCAGATCATGGACAAACGCAATGATTTCCTTGGGGCCGGCGCCTATGAGCCGAGCCAGAACCTGCCGCGCGACAGCAGCTTTACGCACG
AAACACGGCACAAGGCCGGGCGTGATGCGCAGATTCTTGATGCTTACGTTTATGGCAACTGGGATGTGGCCGAGATGCCGGTCACGGCTCGT
TTCGGTAAGCAGGTATTCAACTGGGGCGAAGGGATTTTTTATCGTGGCGGTATCAACACCACCAACCCGCTGAATGCTGCGCAGTTCCGCCT
GCCAGGATCTGAACTGAAAGAAGTGCTGGTGCCGGTCGAGGCGCTTAGCTTCAACATCGGTCTTACCGATAACCTTTCGATGGAAACCTTCT
ATCAGTTCAACTGGAAAGAAACTGCGGTCGATCCGGTCGGTACTTATTTCTCGGAGACCGACCTGTTCGCCGATGGCGGCAATACGGCTTAC
AACCGCAGCGGCGCGTTGGCCGGTTTGGTAACGCCCGATACGAACTTTGGTGGTAACTCGCTGTATGGTTTGCTTGCCTCTAACCCTGCATC
CGGTATCGTCGGAGGTTTTTTTTTTTAAAAAAAAAA

Organism report (obtained from BLAST)

Pseudomonas mendocina ymp [g-proteobacteria] taxid 399739

DISCUSSION

Gram-negative pathogenic bacteria often cause severe symptoms such as septicemia in aquatic organisms. These bacteria can be transmitted and are resources of human pathogenicity (Geran, 1992).

In the present study, the specific genes of gram-negative bacteria isolated from ornamental goldfish were amplified and sequenced for confirmed identification of the human pathogenic isolated species.

The sequencing of the amplified fragments showed most similarity to *Peudomonas mendosina* and did not accept the presence of *Pseudomonas fluorescens*. *Peudomonas mendosina* was first isolated in 1970 from soil and water (Palleroni *et al*., 1970). Aragone *et al*. (1992) first reported the endocarditis caused by this bacterium in humans.

Generally, the genus *Pseudomonas* particularly *Pseudomonas aeruginosa* which has the most similarity to *Peudomonas mendosina* is important cause of septicemia in fish but there is not direct report of *Pseudomonas mendosina* which has been found in goldfish. So this animal and human pathogenic bacterium is reporting in the present study for the first time in aquatic organisms.

Aeromonas hydrophila can produce heat-labile enterotoxins, which has hemolytic and cytotoxic effects (Adamski *et al*., 2006). The infection symptoms of *Aeromonas* in humans include gastrointestinal tract syndromes, infection of wound and soft tissues and blood borne dyscrasias (Janda and Abbott, 2010).

The present study as well as the previous reports, shows that aquatic organisms can be carriers of human pathogens, this fact is important to be mentioned in aquaculture healthcare systems.

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REFERENCES

Adamski, J., M. Koivuranta and E. Leppanen, 2006. Fatal case of myonecrosis and septicaemia caused by *Aeromonas hydrophila* in Finland. Scand. J. Infect. Dis., 38: 1117-1119.

Aragone, M.R., D.M. Maurizi, L.O. Clara, J.L. Navarro Estrada and A. Ascione, 1992. *Pseudomonas mendocina*, an environmental bacterium isolated from a patient with human infective endocarditis. J. Clin. Microbiol., 30: 1583-1584.

Brooks, G.F., J.S. Butel and S.A. Morse, 2010. Jawets Melnick and Adelbergs Medical Microbiology. 24th Edn., McGraw Hill, USA.

Cascon, A., J. Anguita, C. Hernanz, M. Sanchez, M. Fernandez and G. Naharro, 1996. Identification of *Aeromonas hydrophila* hybridization group 1 by PCR assays. Appl. Environ. Microbiol., 62: 1167-1170.

Geran, J., 1992. The Proper Care of Goldfish. 1st Edn., TFH Publications, New Jersey, ISBN: 079383158X.

Janda, J.M. and S.L. Abbott, 2010. The genus *Aeromonas*: taxonomy, pathogenicity and infection. Clin. Microbiol. Rev., 23: 35-73.

Kigigha, L.T., I.Y. Oku and A.S. Ojesanmi, 2012. Enumeration and characterization of bacteria associated with backwater fish species in Wilberforce island Bayelsa state Nigeria. Continental J. Biol. Sci., 5: 32-37.

Lewbart, G.A., 2001. Bacteria and ornamental fish. Semin. Avian. Exotic. Pet. Med., 10: 48-56.

Mahon, M.R., D.C. Lehman and G. Manuselis, 2010. Textbook of Diagnostic Microbiology. 4th Edn., W.B. Saunders Publication, ISBN: 9781416061656, USA.

Noga, E.J., 2000. Fish Disease: Diagnosis and Treatment. 1st Edn., Iowa State University Press, Ames, Iowa, USA., Pages: 367.

Palleroni, N.J., M. Doudoroff, R.Y. Stanier, R.E. Solanes and M. Mandel, 1970. Taxonomy of the aerobic pseudomonads: The properties of the *Pseudomonas stutzeri* group. J. Gen. Microbiol., 60: 215-231.

Reilly, A. and F. Kaferstein, 1998. Food safety and products from aquaculture. J. Applied Microbiol., 85: 249S-257S.

Scarpellini, M., L. Franzetti and A. Galli, 2004. Development of PCR assay to identify *Pseudomonas fluorescens* and its biotype. FEMS Microbiol. Lett., 236: 257-260.

Toranzo, A.E., B. Magarinos and J.L. Romalde, 2005. A review of the main bacterial fish diseases in mariculture systems. Aquaculture, 246: 37-61.