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16S rRNA Sequencing of Lipase Producing Bacterial Strain Isolated from Fish

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Abstract: The sequencing analysis for 1500 bases of 16S rRNA gene of isolate CP5 of *Channa punctatus* was compared with the base pairs of reference *Proteus* sp. and found maximum matching similarity of 76% with that of *Proteus mirabilis*. Hence, the isolate can be identified as *P. mirabilis* based upon the available sequential data.

Keywords: *Channa punctatus*, 16S rRNA, BLAST, Sequencing, Bacterial strain

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Introduction

Enzymes extracted from microbial sources have benefit over those derived from animal or vegetable sources. The benefits include lower production costs, the possibility of large-scale industrial fermentation production, a broad assortment of physical and chemical characteristics, the possibility of genetic engineering, the absence of seasonal effects, the rapid growth of culture and the use of non-burdensome methods. Microbial enzymes are ideal biocatalysts for various industrial applications for all the above characteristics. Microorganisms facilitate the development of new enzymatic systems that cannot be produced from plants or animals, and they can help the food industry make significant progress (Ahmed *et al.*,

2015; Fatma, 2015; Mardina *et al.*, 2018). Microbial proteases are increasingly being used to treat a range of diseases, including tumours, inflammation, coronary disease, necrotic wounds, and so on (Rustad, 2003; Arruda *et al.*, 2007; Jayathilakan *et al.*, 2012). Proteases are immunostimulants (Shobana and Subash, 2013) and are commonly used in the pharmaceutical industry for the preparation of medical products such as wound debridement ointments. It is also used in contact lens enzyme cleaners and denture cleaners (Chaijaroen and Thongruang, 2016).

Lipase enzyme

Lipase is an ester hydrolysis which, in the presence of excess water (Prasertsan and

Prachumratana, 2008), catalyses the hydrolysis of long-chain triacylglycerols to form glycerol and fatty acids. Microbial lipases are one of the most essential extracellular enzymes that have drawn the interest of scientists for years due to their broad biotechnological applications (Mohanty *et al.*, 2020). Microbial enzymes have achieved worldwide recognition for their widespread use in diverse sectors of industry.

Lipases are used instead of conventional chemical techniques in the industrial food industry and are used in the manufacture of a variety of products, such as fruit juices, cooked goods, fermented fruits, cheeses, soups and sauces. These reactions are used in various areas, such as detergent additives and degreasing preparations, refining of fats and oils, synthesis of fine chemicals and pharmaceuticals, manufacture of paper and cosmetics, bioremediation of the atmosphere and molecular biology (Mardina *et al.*, 2018).

Due to reduced process time, low energy input intake, cost-effective, non-toxic and eco-friendly functionality, enzyme-mediated processes are quickly gaining attention (Tripathi *et al.*, 2014). Lipases are often used in the synthesis of fatty acids and alcohols, which are accepted as flavour and scent compounds (Yusof *et al.*, 2016), to provide food a special flavour and taste.

Lipases are present in all kingdoms of life, including prokaryotes (bacteria and archaea) and eukaryotes (plants, mammals, and fungi) (Xie *et al.*, 2018). Microbial lipases are more soluble than their respective plant and animal roots and are more easy, simpler to produce and can be obtained in bulk at a low cost (Bairagi *et al.*, 2002). Generally, because of higher activities and acidic or alkaline pH optima, we might conclude that bacterial enzymes are more favoured over fungal enzymes. Genetic and environmental manipulations can be conducted more effectively on bacterial cells to improve cell yields and enzymatic processes, or to generate altered enzymes, owing to their brief generation cycles,

simple nutritional requirements, and simple screening procedures for desired properties (Achouri *et al.*, 2020). *Bacillus* sp. is the most common lipase-producing bacteria, and a number of lipases are produced by both Gram-positive and Gram-negative bacteria, but Gram-negative bacteria produce the majority of bacterial lipases (Sun *et al.*, 2016).

The development by microbial cells of lipases depends on the presence in the culture medium of lipids such as olive oil or some other inducer, such as triacylglycerols, fatty acids and tweens (Cheng *et al.*, 2009). Its benefit includes high oleic acid concentrations and being more economical (Smichi *et al.*, 2017), olive oil is considered the most appropriate lipid substrate. Some researchers have studied the impact of some factors on the behaviour of crude and refined lipases. Lipase activity may be increased or decreased by a number of factors, including pH, temperature, metallic ions, and organic solvents [20]. The present research was therefore aimed to define the potent bacterial strain (obtained from gut of *Channa striatus* and skin of *Mystus bleekeri*) generating protease and lipase by 16S rRNA sequencing study.

Materials and Methods

Molecular identification of alkaline protease and lipase producing isolates by 16S rRNA gene sequence analysis:

In addition to their 16S rRNA gene sequence analysis for recognition, the most promising two extracellular enzyme producing isolates were investigated by means of their quantitative enzyme assays (protease and lipase). PCR was used to amplify the gene encoding 16S rRNA from the isolates using universal bacterial primers. 27F (5'AGAGTTTGATCMTGGCTCAG-3') and the domain bacteria-specific primer 1492R (5'-TACGGYTACCTTGTTACGACTT-3'). The template DNA was obtained by using the GenElute Bacterial Genomic DNA Kit to remove genomic DNA (Sigma-Aldrich).

Results and Discussion

Molecular identification of Bacterial strain CP5 & MB3 by 16S rRNA Sequencing:

BLAST® » blastn suite » RID-Z5GJ6KXZ014

BLAST Results

Job title: ContigDeepaisolate CP

3RIDZ5GJ6KXZ014 Expires on 10-28 13:21 pm

Query ID|cl|Query_175207Database Name

DescriptionContigDeepa 1

Molecule typenucleic acid

Query Length 1520

[Graphic Summary](#)

Description Nucleotide collection nt

Program BLASTN 2.7.0+

Distribution of the top 166 Blast Hits on 100 subject sequences

Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Proteus mirabilis strain PM 01 16S ribosomal RNA gene, partial sequence	2808	2808	100%	0.0	100%	KY967315.1
Proteus sp. BAB-3406 16S ribosomal RNA gene, partial sequence	2687	2687	97%	0.0	99%	KF917137.1
Proteus mirabilis strain T18 chromosome, complete genome	2682	18738	97%	0.0	99%	CP017085.1
Proteus mirabilis strain T21, complete genome	2682	18743	97%	0.0	99%	CP017082.1
Proteus mirabilis strain AR_0156, complete genome	2682	18727	97%	0.0	99%	CP021852.1
Proteus mirabilis strain AR_0155, complete genome	2682	18734	97%	0.0	99%	CP021694.1
Proteus mirabilis strain AR_0159, complete genome	2682	18749	97%	0.0	99%	CP021550.1
Proteus mirabilis strain AR_0059, complete genome	2682	18716	97%	0.0	99%	CP020052.1
Bacillus sp. BAB-3447 16S ribosomal RNA gene, partial sequence	2682	2682	97%	0.0	99%	KF917177.1
Proteus sp. strain KR 249 16S ribosomal RNA gene, partial sequence	2678	2678	97%	0.0	99%	KY944568.1
Proteus mirabilis strain B1 16S ribosomal RNA gene, partial sequence	2678	2678	97%	0.0	99%	KC344360.1
Proteus mirabilis strain BAB-199 16S ribosomal RNA gene, partial sequence	2678	2678	97%	0.0	99%	KF535110.1
Proteus mirabilis strain ATCC 29906 16S ribosomal RNA gene, partial sequence	2678	2678	97%	0.0	99%	NR_114419.1
Proteus mirabilis strain SeY03 16S ribosomal RNA gene, partial sequence	2676	2676	97%	0.0	99%	MF942361.1
Proteus mirabilis strain PMB31 16S ribosomal RNA gene, partial sequence	2676	2676	97%	0.0	99%	KU378106.1
Proteus mirabilis strain AOUC-001, complete genome	2676	18684	97%	0.0	99%	CP015347.1
Proteus vulgaris strain CYPV1, complete genome	2676	18738	97%	0.0	99%	CP012675.1
Proteus mirabilis strain CYPM1, complete genome	2676	18738	97%	0.0	99%	CP012674.1

>ContigDeepa CP5

```
CGGTGTCGCCTTTTTTAGATTTTTATTGCCCTGCTCAGATTGAACGCTGTCGGCAGGCCTAACACATGCAAG
TCGTGCGGTAACAGAGAAAAGCTTGCTTTCTTGCTGACGAGCGGCGGACGGGTGAGTAATGTATGGGGA
TCTGCCCGATAGAGGGGGATAACTACTGGAAACGGTGGCTAATACCGCATAATGTCTACGGACCAAAGCA
GGGGCTCTTCGGACCTTGCACTATCGGATGAACCCATATGGGATTAGCTAGTAGGTGGGGTAAAGGCTCA
CCTAGGCGACGATCTCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGGACTGAGACACGGCCCAGACT
CCTACGGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTAT
GAAGAAGGCCTTAGGGTTGTAAAGTACTTTTCAGCGGGGAGGAAGGTGATAAGGTTAATACCCTTATCAAT
TGACGTTACCCGAGAAGAAGCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAAGGGTGCAA
GCGTTAATCGGAATTACTGGGCGTAAAGCGCACGCAGGCGGTCAATTAAGTCAGATGTGAAAGCCCCGA
GCTTAACCTGGGAATTGCATCTGAAACTGGTTGGCTAGAGTCTTGTAGAGGGGGGGGTAGAATTCCATGTG
TAGCGGTGAAATGCGTAGAGATGTGGAGGAATACCGGTGGCGAAGGGCGGCCCCCTGGACAAAGACTG
ACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGATG
TCGATTTAGAGGTTGTGGTCTTGACCGTGGCTTCTGGAGCTAACGCGTTAAATCGACCGCCTGGGGAGTA
CGGCCGCAAGGTTAAACTCAAATGAATTGACGGGGGCCGCACAAGCGGTGGAGCATGTGGTTTAATTC
GATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAGCGAATCCTTTAAAGATAGAGGAGTGCCTTCGG
GAACGCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTGTTGTGAAATGTTGGGTAAAGTCCCGCAAC
GAGCGCAACCTTATCCTTTGTTGCCAGCACGTGATGGTGGGAAGTCAAAGGAGACTGCCGGTGATAAAC
CGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTAGGGCTACACACGTGCTACAATG
GCAGATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGAAGTCAATAAGTCTGTCGTAGTCCGGATTGG
AGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAGAATGCTACGGTGAATACGTT
CCCGGGCCTTGACACACCGCCCGTCACACCATGGGAGTGGGTTGCAAAGAAGTAGGTAGCTTAACCTT
CGGGAGGGCGCTTACCACTTTGTGATTCATGACTGGGGTGAAGTCGAACAGGGAA
```

Result: *Proteus mirabilis*

Fig. 1: Phylogenetic tree of the *Proteus mirabilis* (CP5).

BLAST® » [blastn suite](#) » RID-Z5H7HYXP014

BLAST Results

Job title: ContigDeepa Isolate MB3

RID [Z5H7HYXP014](#) Expires on 10-28 13:32 pm

Query ID cl|Query_224957

Description ContigDeepa Isolate MB3

Molecule type nucleic acid

Query Length 1518

Database Name nr

Description Nucleotide collectionsnt

Program BLASTN 2.7.0+

Graphic Summary

Distribution of the top 118 Blast Hits on 100 subject sequences

Descriptions

Sequences producing significant alignments:

Description	Max score	Total score	Query cover	E value	Ident	Accession
Providencia rettgeri strain PR 01 16S ribosomal RNA gene, partial sequence	2804	2804	100%	0.0	100%	KY967319.1
Providencia rettgeri strain RB151, complete genome	2527	17597	97%	0.0	97%	CP017671.1
Providencia rettgeri strain BFM1 16S ribosomal RNA gene, partial sequence	2525	2525	97%	0.0	97%	KU870748.1
Providencia vermicola strain CICR-SPBB 16S ribosomal RNA gene, partial sequence	2523	2523	98%	0.0	97%	JX645158.1
Providencia rettgeri strain OK-62_NRB-DRDO/MP 16S ribosomal RNA gene, partial sequence	2521	2521	95%	0.0	98%	KX289656.1
Providencia sp. SY-1 16S ribosomal RNA gene, partial sequence	2521	2521	97%	0.0	97%	JQ954968.1
Providencia rettgeri strain IITRP2 16S ribosomal RNA gene, partial sequence	2521	2521	97%	0.0	97%	GU193984.1
Providencia sp. AA7 16S ribosomal RNA gene, partial sequence	2519	2519	97%	0.0	97%	KR232641.1
Providencia sp. AA4 16S ribosomal RNA gene, partial sequence	2518	2518	97%	0.0	97%	KR232640.1
Providencia rettgeri strain SNCO1_1A 16S ribosomal RNA gene, partial sequence	2516	2516	97%	0.0	97%	JN644501.1
Providencia vermicola strain FFA6 16S ribosomal RNA gene, partial sequence	2516	2516	97%	0.0	97%	JN092794.1
Providencia rettgeri strain CT15 16S ribosomal RNA gene, partial sequence	2516	2516	95%	0.0	98%	EU660367.1
Providencia rettgeri strain CM7 16S ribosomal RNA gene, partial sequence	2516	2516	95%	0.0	98%	EU660316.1
Providencia sp. YRL09 16S ribosomal RNA gene, partial sequence	2516	2516	97%	0.0	97%	EU373416.1
Providencia sp. AA1 16S ribosomal RNA gene, partial sequence	2514	2514	97%	0.0	97%	KR232639.1

>ContigDeepa Isolate MB3

```
GGGTGGGGTTTTTTTTTTAGTTTTATTTTCTCGGCTCAGATTACACGCTCGCCACACGTATCACACGTGCATGTC
GCGCTATCACAGAGGAAGCTTGTCTCTCGCTGACGAGCGGCGGACGGGTGAGTAATGTATGGGGATCTGCCC
GATAGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAATCTCTCAGGAGCAAAGCAGGGGACCTT
CGGTCCTTGCCTATCGGATGAACCCATATGGGATTAGCTAGTAGGTGAGGTAATGGCTCACCTAGGCGACGA
TCCCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAAGTGAAGACACGGTCCAGACTCCTACGGGAGGCAG
CAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTAGGGT
TGTAAGTACTTTTCACTGAGGAGGAAGGCGTTGATGCTAATATCATCAACGATTGACGTTACTGACAGAAGAA
```

GCACCGGCTAACTCCGTGCCAGCAGCCGCGGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGT
AAAGCGCACGCAGGCGGTTGGTTAAGTTAGATGTGAAATCCCCGGGCTCAACCTGGGAATGGCATCTAAGAC
TGGTCAGCTAGAGTCTTGTAGAGGGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATGTGGAGG
AATACCGGTGGCGAAGGCGGCCCTGGACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAG
GATTAGATACCCTGGTAGTCCACGCCGTAAACGATGTCGATTTGAAGGTTGTGCCCTTGAGGACGTGGCTTCC
GGAGCTAACGCGTTAAAGTCGACCGCCTGGGGAGTACGGCCGCAAGGTTAAAACTCAAATGAATTGACGGGG
GCCCCGACAAGCGGTGGAGCATGTGGTTTAATTCGATGCAACGCGAAGAACCTTACCTACTCTTGACATCCAG
AGAACTTAGCAGAGATGCTTTGGTGCCTTCGGGAACTCTGAGACAGGTGCTGCATGGCTGTCGTCAGCTCGTG
TTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTATCCTTTGTTGCCAGCGATTAGGTCGGGAACT
CAAAGGAGACTGCCGGTGATAAACCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCCCTTACGAGTA
GGGCTACACACGTGCTACAATGGCGTATACAAAGAGAAGCGACCTCGCGAGAGCAAGCGGACCTCATAAAGT
ACGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAAT
GCTACGGTGAATACGTTCCCGGGCCTTGACACACCGCCGTCACACCATGGGAGTGGGTTGCAAAGAAGTA
GGTAGCTTAACCTTCGGGAGGGCGCTTACCACCTTGTGATTCATGACTGGGGTGAAGTCGTAACAGGTA

Result: *Providencia rettgeri*

Fig. 2: Phylogenetic tree of the *Providencia rettgeri* (MB3).

The aforesaid experiment clearly indicates that the enzyme activity was found prominent in two isolates i.e., isolate CP5 obtained from gut of *Channa punctatus* and isolate MB3 obtained from skin of *Mustus bleekeri*. To identify the strain of the above said isolates 16S rRNA was performed by constructing suitable primer. The sequencing of 16S rRNA analyses was shown in Figures 1 and 2. The sequencing analysis for 1500 bases of 16S rRNA gene of isolate CP5 of *C. punctatus* was compared with the base pairs of reference *Proteus* sp. and found maximum matching similarity of 76% with that of *Proteus mirabilis*. Hence, the

isolate can be identified as *P. mirabilis* based upon the available sequential data (Fig. 1). Similarly the isolate obtained from *M. bleekeri* was identified as *P. rettgeri* (Fig. 2).

The studies made by several authors support the present investigation of identification of bacterial strains by using the technique of 16S rRNA. Similar studies were carried out by Clarridge (2004), Janda and Abbott (2007) and Suganthi *et al.* (2013). Lipolytic bacterial isolates were identified as highest lipase producers and were further characterized by 16S rDNA sequencing the best lipase producers.

Staphylococcus saprophyticus and *Pseudomonas otidigenus* were further characterized by 16S rDNA sequencing and identified. Salamon *et al.* (2018) screened the potential gelatinase, protease, amylase and lipase producing bacteria as *Bacillus* sp. by 16S rRNA gene analysis and it has been revealed that, the isolated bacteria showed a close resemblance to *B. fluxus* and *B. cereus*.

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