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API-ZYM Enzymatic Profile of *Shigella dysenteriae* IM Isolated from Drinking Water

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ABSTRACT

The enzymatic potential of bacterial species is an essential feature to carry out a wide range of biological and physiological processes, particularly involved in the hydrolysis of complex organic materials. Several pathogenic microbial species utilize enzymes as virulence factors to cause diseases. The aim of the present study was to evaluate enzymatic activities of the bacterial strain *Shigella dysenteriae* IM using API-ZYM test system. Out of nineteen different enzymes, *S. dysenteriae* IM strain was capable to poroduce nine enzymes, *i.e.,* alkaline-phosphatase, acidic-phosphatase, lipase, valine arylamidase, cystine arylamidase, napthol-AS-BI-phosphohydrolase, β-galactosidase, leucine arylamidase, and trypsin. While, esterase, esterase lipase, α-chymotrypsine, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl- β-glucosaminidase, α-mannosidase, and α-fucosidase were recorded as negative. Moreover, *S. dysenteriae* IM showed elevated alkaline-phosphatase, leucine arylamidase, trypsin, and acidic-phosphatase activities. The present study reveals that, the API-ZYM test is the convenient, reliable and inclusive alternative to recent biological methods. This method could be applied for the identification of various enzymes on routine basis directly in bacterial cultures and biological fluids.

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ANJ and SAT designed the study and analyzed the results. ANJ, SBM and ASQ performed the experiments. ANJ, SA, SBM, ASQ and MAB wrote the paper.

Key words
API-ZYM, Enzymes, Shigella dysenteriae.

INTRODUCTION

Enzymatic characterization of the microorganisms using a variety of synthetic substrates is an essential tool to substantiate the production of various enzymes. The enzyme production can be assayed within seconds to maximum of 4 h of incubation, thus, the identification of bacterial species based on enzymatic analysis yields quick and authentic results (Bascomb and Manafi, 1998; Bascomb, 1980; D'Amato, 1980). Enzymes have been shown to perform versatile range of applications in biomedical sciences, food industries, chemical industries, paper and pulp industry, lignocellulosic biorefinery, and feed additive (Pandey *et al*., 2000; Niehaus *et al*., 1999). A rapid enzymatic identification system, API-ZYM has been widely applied for the identification of various enzymes from a single substrate using bacterial culture or even biological fluids (Waitkins *et al*., 1980). This identification system comprises 19 substrates and the enzymatic identification can be achieved within 4 h of incubation.

The virulence of several bacterial species is due to coordinated activity of several secreted toxins and digestive enzymes as well as a large number of bacterial surface-exposed proteins that facilitate their binding to extracellular matrix and plasma proteins (Arvidson and Tegmark, 2001). The role of enzymes in the pathogenesis of infection has been well studied that suggests a direct relationship between the virulence characteristics of the bacterial strains and their ability to produce enzymes. *In vitro* studies have illustrated that protease enzymes secreted by some of the bacterial strains can cleave and degrade several important host proteins, including the heavy chains of all human immunoglobulin classes, plasma proteinase inhibitor, and elastin (Potempa *et al*., 1988), indicating its crucial involvement in virulence.

The *S. dysenteriae* is a Gram-negative, oxidase negative, non-motile and non-lactose fermenting bacterial species and belongs to family Enterobacteriaceae (Davis *et al*., 1988). *S. dysenteriae* is an etiological agent of shigellosis, a disease of high morbidity and mortality particularly affecting children (Maurelli *et al*., 1998; Kartz, 1986; Chen *et al*., 1980). Several bacterial toxins are to be activated by catalytically active polypeptide fragments (Gordon and Leppla, 1994). Interference with virulence expression of pathogenic microorganisms could potentially be predicted as a non-antibiotic disease control. However, the implementation of such measures needs to thoroughly understand the regulation of virulence factors by a wide range of enzymes in pathogenic bacterial species. Infections caused by *S. dysenteriae* frequently

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progress to the severe stages of dysentery and lead to life-threatening hurdles (Shears, 1996; Chen *et al*., 1980). In various developing countries with insufficient sanitation, fecal-contamination of water by enteric pathogenic is highly frequent. Hence, there is a dire need to understand whether *Shigella* species can endure and persist in different environments. Moreover, the development of rapid, reliable and more sensitive analytical methods such as API-ZYM based test system can be very essential for the correct and less time consuming enzymatic identification processes.

In this study, enzymatic characterization of bacterium *S. dysenteriae* IM was carried out using API-ZYM, a semi-quantitative analytical system. The bacterial strain *S. dysenteriae* IM was isolated from drinking water. Moreover, 19 different enzymes were screened and measured based on the color intensity.

MATERIALS AND METHODS

Bacterial strains and culture conditions

Bacterial strain *Shigella dysenteriae* IM was isolated from drinking water under aseptic conditions. Isolation was carried out using MacConkey agar, Xylose Lysine Deoxycholate (XLD) agar following incubation at 37 ºC for 24 h. All non-lactose fermenters were separated and subjected to oxidase test. Oxidase negative isolates were further processed using a number of biochemical tests to confirm the isolate (Qazilbash, 2002; Zareen *et al*., 2014).

Identification and Characterization of the S. dysenteriae *IM strain*

Identification and characterization of the isolates was carried out using standard methods (Cheesbrough, 1985; Cheesbrough *et al*., 1985). Initial characterization of isolates was accomplished based on colonial appearance (size, and shape), staining reactions and cultural characteristics using standard protocols (Collins *et al*., 1995). Further, characterization of the bacterial strain was performed based on oxidase test, hanging drop technique, mannitol fermentation test, nitrate reduction test, TSI test and methyl red test (Qazilbash, 2002).

Enzymatic analysis

The enzymatic characterization of *S. dysenteriae* IM was carried out using a semi-quantitative API-ZYM test system (BioMerieux). Out of twenty microcupules, nineteen were loaded with different dehydrated chromogenic-substrates in order to detect enzymatic activities, while number one cupule was designated as a negative control, without addition of substrate (Table I). The experiment was performed according to the manufacturer's instructions. Briefly, the microcupules of the API-ZYM strip were inoculated with 24 h old broth culture of *S. dysenteriae* IM and incubated at 30 ºC for 4 h. After incubation ZYM A and ZYM B reagents were added to each cupule. Finally API-ZYM strip was placed under 1000 W lamp for 10 seconds in order to prevent yellow color formation. The enzymatic activities were graded from 0-5 based on color formation intensity.

Table I.- Enzymes and substrates employed in API-ZYM test system.

Name of enzyme	Substrate
Control	-------
Alkaline phosphatase	2-napthyl phosphate
Esterase (C4)	2-napthyl butyrate
Esterase lipase (C8)	2-napthyl caprylate
Lipase (C14)	2-napthyl myristate
Leucine arylamidase	L-leucyl-2-napthylamide
Valine arylamidase	L-valyl-2-napthylamide
Cystine arylamidase	L-cystyl-2-napthylamide
Trypsin	N-benzoyl-DL-arginine-2-napthylamide
A-chymotrypsin	N-glutaryl-phenylalanine-2-napthylamide
Acid phosphatase	2-napthyl phosphate
Napthol-AS-BI-phosphohydrolase	Napthol-AS-BI-phosphate
α-galactosidase	6-Br-2-napthyl- αD-galactopyranoside
β-galactosidase	2-napthyl-βD-galactopyranoside
β-glucuronidase	Napthol-AS-BI- βD-glucuronide
α-glucosidase	2-napthyl- αD-glucopyranoside
β-glucosidase	6-Br-2-napthyl- βD-glucopyranoside
N-acetyl-β-glucosaminidase	1-napthyl-N-acetyl-βD-glucosaminidase
α-mannosidase	6-Br-2-napthyl- αD-mannopyranoside
α-fucosidase	2-napthyl- αL-fucopyranoside

RESULTS AND DISCUSSION

Bacterial strain isolated from drinking water was identified as *S. dysenteriae* IM based on colonial, cultural and biochemical tests. *S. dysenteriae* IM was Gram-negative, non-motile, non-spore-forming and rod-shaped. The biochemical characterization demonstrated that bacterium was oxidase negative and non-lactose fermenter. Over all, 19 different enzymes were measured using API-ZYM strips. API-ZYM is a semi-quantitative

system, applied for screening different enzymes produced by microorganisms. The enzymatic characterization by API-ZYM test system revealed alkaline-phosphatase, acidic-phosphatase, lipase, valine arylamidase, cystine arylamidase, naphthol-AS-BI-phosphohydrolase, β-galactosidase, leucine arylamidase, and trypsin as positive enzymes produced by *S. dysenteriae* IM. While, esterase, esterase lipase, α-chymotrypsin, α-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase were found negative (Table II). Alkaline-phosphatase, leucine arylamidase, trypsin, and acidic-phosphatase were recorded as highly positive and noted as grade level-5. Valine arylamidase and β-galactosidase were recorded as grade level-4. Whereas, lipase, cystine arylamidase and napthol-AS-BI-phosphohydrolase were found at low grade level-2 (Fig. 1).

Table II.- Enzyme activities in *S. dysenteriae* IM isolated from drinking water.

Enzyme	Positive (+) / Negative (-)
Control	–
Alkaline phosphatase	+
Esterase (C4)	–
Esterase lipase (C8)	–
Lipase (C14)	+
Leucine arylamidase	+
Valine arylamidase	+
Cystine arylamidase	+
Trypsin	+
A-chymotrypsin	–
Acid phosphatase	+
Napthol-AS-BI-phosphohydrolase	+
α-galactosidase	–
β-galactosidase	+
β-glucuronidase	–
α-glucosidase	–
β-glucosidase	–
N-acetyl- β-glucosaminidase	–
α-mannosidase	–
α-fucosidase	–

The bacterium *S. dysenteriae* IM was characterized with two different pathogenic modes of action, for example, invasiveness and enterotoxin. However, epithelial penetration is the potential virulence property of *S. dysenteriae*. Along with various virulence factors and toxins, pathogenic bacterial species may also release a broad range of enzymes to bypass the immunological defense mechanisms of the host and contributing in virulence of the pathogenic microbes. The enzymatic activity by microbial populations is a crucial process in organic matter decomposition, nutrient recycling, and carbon and energy flow. The regulation of bacterial enzymatic activity is based on the ecosystem level, environmental factors and the microenvironment level by enzyme-substrate interactions. Enzyme production is regulated by several factors such as growth phase, environmental (temperature or pH) and nutritional factors. The API-ZYM test system has been proved as a simple, reliable and rapid method to identify various enzymes. This semi-quantitative enzymatic analysis method imitates a substantial saving incubation time; only 4 h of the incubation period are required as compared to conventional biochemical tests (Waitkins *et al*., 1980).

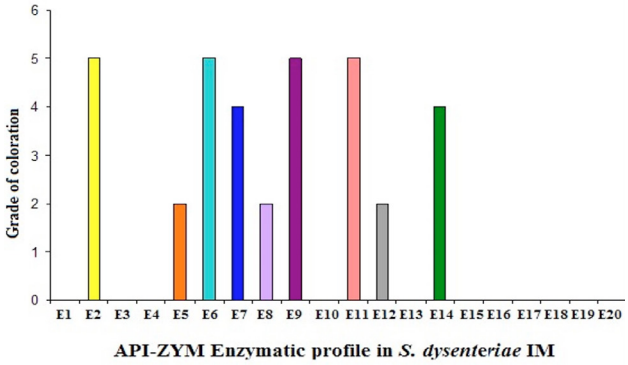

Fig 1. Grade level enzymatic activities in *S. dysenteriae* IM isolated from drinking water. E1 indicates microcupule marked as negative control without addition of any substrate, while E2-E20 are the microcupules loaded with specific substrates showing reactions of the different enzymes.

Generally, it was noted that *S. dysenteriae* IM showed high concentration of the phosphatase enzymes, for instance, both alkaline and acidic phosphatases classified on the basis of pH. Phosphatases catalyze the hydrolysis of C-O-P linkage of various phosphate esters (Karasu-Yalcim *et al*., 2012). While, extracellular proteases are known to be as the potential virulence factors in several microbial diseases (Zhao-lan *et al*., 2002). The production of high concentration of proteases by *S. dysenteriae* IM has also great concern due to the potential role of proteases in virulence of pathogenic bacteria. Moreover, several peptidases in disease causing bacteria are indicated to play a vital role in pathological actions together with digestion of host connective tissues and cytotoxicity to host cells (Miyoshi, 2013; Zhao-lan *et al*., 2002; Miyoshi and Shino, 2000). The positive reactions for arylamidases (proteolytic

enzymes) by *S. dysentriae* IM show that these enzymes may help the bacterial species to catalyze the hydrolysis of *N*-terminal aminoacids from peptide, amide and arylamides (Dodor and Tabatabai, 2007). The enzymatic activities by pathogenic microbes may play a broad range of pathological functions. These enzymes directly can digest many types of host proteins and also indirectly by processing many other toxic virulence factors.

CONCLUSION

The findings of the present study suggest that the enzymes produced by pathogenic bacterial species play a crucial role in pathogenesis of these bacteria. Since, Virulence of *Shigella* species depends upon the mucosal penetration and subsequent intraepithelial multiplication. Therefore, the public health authorities should ensure clean water supply, high-quality sewage management and hygienic environment.

Statement of conflict of interest

The authors have declared no conflict of interest.

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