Measurement and Molecular Detection of Acid Phosphatase Enzyme in *Moraxella catarrhalis*

Juman Khalil Mohammad Sabri Safaa Sahib
College of Medicine, University of Babylon, Hilla, Iraq.

Abstract

Acid phosphatase is one of the most important autotransporter in *Moraxella catarrhalis* . So *Moraxella catarrhalis* isolates were undergone to produce extracellular acid phosphatase enzyme in the presence and absence of its own substrate (p-nitrophenyle phosphate) at two pH conditions (pH 6.0 & pH 7.0). It was shown that acid phosphatase was produced in the presence and also in the absence of substrate but the highest activity was observed at pH 7.0 in the presence of the substrate, this will give an indicator that this enzyme is produced constitutively and inducibly.

The *mapA* gene is one of essential encoding gene for the production of acid phosphatase. This gene was detected by specific PCR primer. The results showed that only 3 of *M. catarrhalis* isolates had positive result for this gene where as the rest did not posses it.

الخلاصة

تم التحري عن انتاج انزيم acid phosphatase الخارجي من قبل بكتريا *Moraxella catarrhalis* في وجود وعدم وجود المادة الاساس الخاصة به والتي هي (p- nitrophenyl phosphate) في ظرفي حموضة مختلفين (pH 6.0 & pH 7.0) . واظهرت النتائج قابلية البكتريا على انتاج هذا الانزيم بوجود وعدم وجود المادة الاساس ، وقد لوحظت ان اعلى فعالية لهذا الانزيم كانت عند pH 7.0 وبوجود المادة الاساس .

كما تم التحري عن الجين(*map A*) و المسؤول عن انتاج هذا الانزيم باستخدام بادئات نوعية خاصة وبواسطة تقنية PCR، و قد اظهرت النتائج امتلاك ثلاث من العزلات فقط لهذا الجين بينما اعطت بقية العزلات نتائج سالبة لامتلاك الجين المسؤول عن انتاج انزيم acid phosphatase .

Introduction

Moraxella catarrhalis is a Gram negative non-encapsulated diplococcal bacterium belonging to the family *Moraxellaceae* that was first described in 1896 [1]. Pfeiffer gave the organism its first name, *Micrococcus catarrhalis* [2]. For a long time *M . catarrhalis* was considered a relatively harmless commensal in the respiratory tract [3,4]. At present , it is consederd as a third most frequent cause of otitis media and also as a significant agent in sinusitis and lower respiratory tract infections in adults with pulmonary disease [5]. *Moraxella catarrhalis* is one of the most common inhabitants of the pharynx of healthy infants and children but decreases substantially in adulthood [6]. The isolation of *M . catarrhalis* from a variety sites has forced re-evaluation the clinical significance of this organism [7]. Its isolation from sites normally considered sterile support its pathogenic role [8]. the transition of this organism between commensalism and virulence are not yet fully understood [9].

However , many studies have been done on *Moraxella catarrhalis* in

Iraq but these studies did not include the molecular aspects the role of autotransporters in induction the diseases through production or regulation of virulence associated factors. So this study came to verify some aims that were not included in previous studies done in Iraq .

the aims of this study is to investigate and detect the *map A* gene as autotransporter with acid phosphatase production .

Materials and Methods

1-Material

A- ***Moraxella catarrhalis*** **isolates :** (110) ear swabs were collected from patients suffering from otitis media , and were submitted to Al-Husaini General Teaching Hospital (ENT unit) during a period of three months from (October 2011 to January 2012) . The patient's age ranged from (6 months - 78 years).

B- The main diagnostic kits and chemical materials , which used throughout this study, were listed in the Table (1).

Table 1 Main materials used in this study.

No.	*Materials*	*Company/country*
1	DNA extraction kit	Promega/USA
2	master mix	
3	DNA ladder 100bp	
4	Primer of *mapA*	Bioneer/USA
5	Acid phosphatase kit	Biolabo/France
6	Blood agar ,Nutrient agar ,Brain heart infusion agar and broth	Himedia /India

2- Methods

A- Acid Phosphatase Production:

The detection of this enzyme from the cells filtrate was carried out as mentioned by Kit supplemented by the manufacturing company (Biolabo/France).

B- DNA Extraction from Gram Negative Bacteria:

This method was made according to the genomic DNA purification Kit supplemented by (Promega, USA).

.

C- Detection of Primer Used in This Study:

DNA (extract from bacterial cells) was used as a template in specific PCR for the detection of this *Moraxella catarrhalis* virulence gene . The primer used for the amplification of a fragment gene was listed in the Table (2)

Table 2 Primer sequence and PCR condition .

Genes	*Primer sequence (5´- 3´)*	*Size bp*	*PCR conditions*	*Ref.*
mapA F *mapA R*	ATAGGATCCGCACCAGCCTCATCAAAT AATGGATCCTTGTGCCAGTGCCATTT	140	94ºC 4min 1x 94ºC 2min 55ºC 1min 30x 72ºC 1min 72ºC 8min 1x	[10]

D- Detection of Amplified Product by Agarose Gel Electrophoresis

Results and Discussion

1 - Detection of Acid Phosphatase Enzyme as an Autotransporter Protein :

Moraxella catarrhalis isolates were subjected to produce extracellular acid phosphatase enzyme in the presence and absence of its own substrate (p- nitrophenyle phosphate) under two pH conditions (pH 6.0 and pH 7.0) .

It was shown that acid phosphatase was produced in the presence and also in the absence of substrate and the highest activity was observed at pH 7.0 in the presence of the substrate (Table 4) . So, acid phosphatase could be synthesized at two levels one of them is constitutively produced and the other level is through inducible manner . Over all , the production at (pH 7.0) was totally higher than the production at (pH 6.0).

Acid phosphatases catalyze the hydrolysis of phosphomonoesters at an acidic pH . The cleavage and release of phosphate from organic sources by this acid phosphatase may be necessary for the uptake of an essential nutrient [11].

Table 3 Acid phosphatase production by *Moraxella catarrhalis*

Isolate No.	*OD (405nm) pH (7)*		*OD (405nm) pH (6)*	
	Without substrate	*With substrate*	*Without substrate*	*With substrat*
1	46.6	71.9	44.2	67.3
2	44.0	63.8	39.2	52.0
3	48.6	67.1	39.7	57.2
4	54.2	76.8	37.5	58.4
5	52.5	82.8	36.8	68.2
6	53.2	98.4	45.1	55.7
7	46.1	87.8	39.0	58.0
Control	0.0	0.0	0.0	0.0

The ability of *Moraxella catarrhalis* to produce constitutive and inducible acid phosphatase at various pH conditions may give an indication that there are at least two isoenzymes in which they will play a role in its pathoginicity in various environmental factors .

This study is identical to the study [10] which observed that acid phosphatase has been produced by *Moraxella* isolated from clinical cases particularly from otitis media .

Generally, In gram-negative bacteria , acid phosphatase enzyme was considered as an autotransporter that make up the largest family of outer membrane porins involved in protein translocation [12].

In *Moraxella catarrhalis*, some studies have pointed out that acid phosphatase might play a role as autotransporter [10,13]. Where this enzyme has a role in many bacterial activities such as cell-to-cell aggregation, biofilm formation, serum resistance and adherence to host epithelial cells [14].

These acid phosphatase enzymes can purified from different types of bacteria like *Bacills anthracis* [15], *Staphyllococcus aureus* [16] and *Clostridium perfringens*, in which this enzyme play a critical role in the survival of this bacteria in its environment [13].

On the other hand it was reported that the physiological function of the class A Non Specific Acid Phosphatase (NSAPs) remains to be determined [17].

2- Detection of *mapA* Marker

The *mapA* gene is one of essential genetic factors encoding the production of acid phosphatase .

This gene was detected by specific PCR primer .It was found that only 3 isolates of *M. catarrhalis* showed their possession of *map A* gene where as the rest did not have this gene Figure below . A previous study [10] has indicated that *mapA* gene plays a role in acid phosphatase.

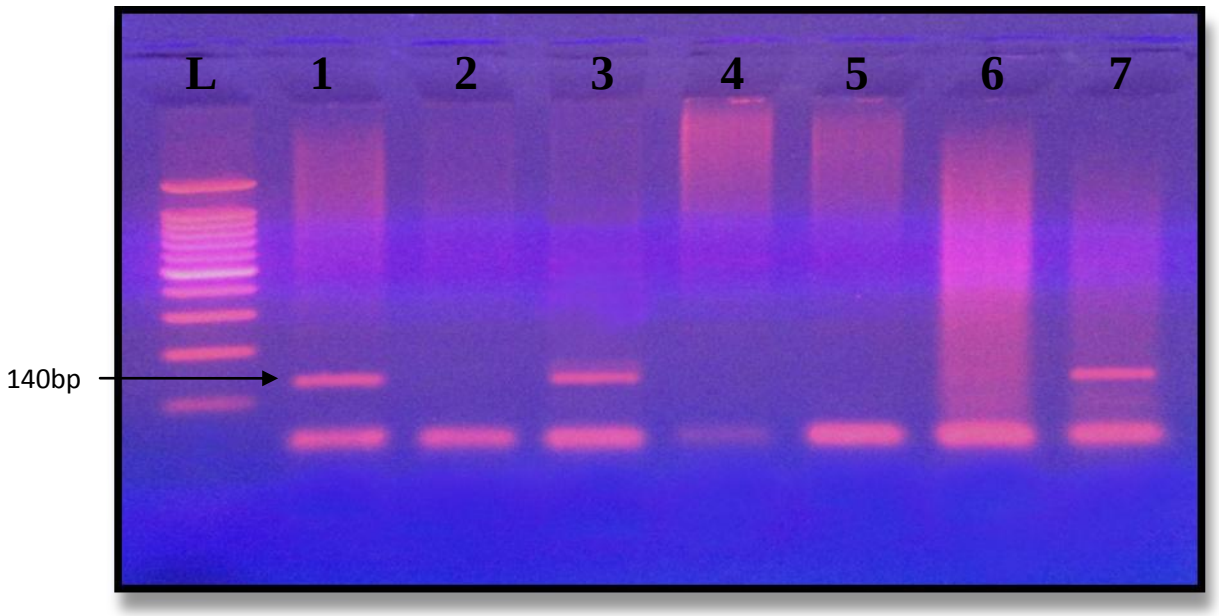

Figure Gel electrophoresis of PCR product of *mapA*
(1,3 and 7) isolates with positive result for *MapA*
(2,4,5 and 6) with negative result
L= ladder

Enzyme production in *M . catarrhalis* strains. But other studies stated that this enzyme can be produced by different types of bacteria like *Salmonella enterica* and *Escherichia spp.* [18,17].

However, phenotypically all *M . catarrhalis* isolates were found to be able to produce acid phosphatase extracellularly that will give an interpretation that this enzyme may be encoded by more than one genetic loci or due to may have more than one isoenzymes.

It wes referred to that Despite having a common functional identity, the isoenzymes of acid phosphatases differ widely regarding to the chromosomal origin, molecular weight, amino acid homology and sequence length [11]. The ability of *M . catarrhalis* to produce this enzyme regardless of presence or absence of *map A* gene, may also give an impression that this enzyme may be encoded by another genes available in *Moraxella* genome .

The fact that *MapA* is expressed at a relatively low level in comparison to other *M . catarrhalis* autotransporters (e.g., UspA2) may be related to the fact that its enzymatic function is catalytic and does not require abundant protein expression [10].

References

(1) Hays , J. P. (2009) . Journal of Pediatric Infectious Diseases 4:211.
(2)Pearson , M .M . (2004) . ph.D thesis ,University of Texas.
(3)Hays , J. P. (2011). landes bioscience J. 1(2):155.
(4)Attia , A. S. , Sedillo , J. L. , Hoopman ,T. D. , Liu , W. , Liu , L. , Brautigam,
C.A. and Hansen,E.J.(2009). BMC Microbiology 9:207.
(5)Forsgren, A. ,Brant, M. , Mollenkvist, A. , Muyombwe , A. , Janson, H. , Woin , N.
and Riesbeck , K . (2001) . J. Immunol. 167: 2112.
(6) Goldstein , E. J. , Editor , C. , Murphy , T. F. and Parameswaran, G. I. (2009).
Clin. Infect. Dis. 49 (1) : 124 .
(7) McGregor , K. , B. J. Chang , Mee , B. J. and Riley , T. V. (1998). Eur. J. Clin.
Microbiol. Infect. Dis.17:219-234.
(8)Ahmad , N . ,Meng , Y. and Tahir , H . M . (1994) . Malaysian J. Pathol. 16(1): 63.
(9)Seib , K. L. , Peak , I. R. A. and Jennings , M. P. (2002) . FEMS Immunol.
and Medical Microbiol. J. 32:159.
(10)Hoopman , T. C. , Wang , W. , Brautigam , C. A ., Sedillo , J. L. , Reilly , T. J.
and Hansen , E. J.(2008). Journal of bactriology 190 (4):1459.
(11)Bull, H., Murray, P. G., Thomas , D. , Fraser , A. M. and Nelson , P. N. (2002).
Mol. Pathol. 55:65.
(12)Desvaux , M . , Parham , N. J. , and Henderson , I. R . (2004) .Res .Microbiol.
155**:** 53.
(13)Reilly , T. J. ,Chance , D. L. ,Calcutt , M. J. ,Tanner , J. J., Felts , R. L. ,Waller,
S.C. ,Henzl ,M.T.,Mawhinney ,T. P.,Ganjam ,I. K. and Fales ,W. H. (2009).Appl.
Environ. Microbiol.75(11) : 3745.
(14) Henderson , I. R. , Navarro-Garcia , F . , Desvaux , M . , Fernandez , R. C. ,
Ala'Aldeen , D. (2004) . Microbiol. Mol. Biol. Rev . 68(4): 692.
(15)Felts , R. L. , Reilly , T. J. , Calcutt , M. J. and Tanner , J. J. (2006). Acta
Crystallogr. Sect. F 62:705.
(16)du Plessis , E. M. ,Theron , J. , Joubert , L. , Lotter , T. and Watson , T.G. (2002).
Syst. Appl. Microbiol. 25:21.
(17) Ishikawa , K . , Mihara , Y . , Gondoh , K . , Suzuki , E . and Asano , Y .
(2000) . The EMBO Journal 19:2412.
(18)Grose , J. H.,Bergthorsson , U . , Xu , Y . , Sterneckert , J ., Khodaverdian ,
B. and Roth , J. R . (2005) . J. Bacteriol. 187(13): 4521.