Molecular Detection of *nan*, *tly* and *dsA* gene of *Propionibacterium acnes* Isolated from acne vulgaris in Babylon Province

Rabab J H AL-Hasseny[1], Lamees A. Abdul-Lateef [2], Hussein Abbas AL-Sultany[3]

[1]Assist. Lect. Microbiology / College of Food Science /AL-Qasim Green University, Iraq, [2]Prof. Department of Microbiology, College of Medicine, University of Babylon, Iraq, [3]Prof. Venereology and dermatology, College of Medicine, University of Babylon, Iraq

Abstract

Propionibacterium acnes virulence factors can be divided into chromosomal, plasmid and bacteriophages encoded virulence factors. Many of the *Propionibacterium acnes* virulence factors, are *camp5*, *gehA*, *tly*, sialidases, neuraminidases, endoglycoceramidases, lipases, and hemolysins genes are clustered in certain areas of the chromosome. A total of 200 clinical acne samples were collected during this study which obtained from patient Suffering from **acne vulgaris** who to the out-patient clinics and delivery dermatology unite of acne, in hospitals of Babylon Province: AL-Hashmeia general Hospital and Marjan hospital, during the period from April to November 2019. Out of 200 specimens **14 (7.7%)**were detected by culture and vitek2 compact**9 (64.3%)**of them were confirmed by PCR using target gene of *Propionibacterium acnes* PCR was conducted to determine the some virulence genes of the isolates by using primers ***nan, tly and dsA gene***. The PCR amplification products were visualized by electrophoresis on 1% agarose gels for 35min at 70v. The sizes of the amplicons were determined by comparison to the 200 bp allelic ladder. Among isolates studies it was found that *nan* gene present in 6/9(66.7%). isolates, *dsA* gene it was found in only 3isolates (33.4%) while *tly* gene it was found in all isolates(100%) .

Keywords: *Propionibacterium, camp5, gehA, tly, PCR, Isolate.*

Introduction

Propionibacterium acnes is a nonspore- forming, gram- positive, anaerobic, pleomorphic rod whose end products of fermentation include propionic acid [(1)]. *P.acnes* is considered an opportunistic pathogen, causing a range of infections as well as being associated with a number of inflammatory conditions. Itis primarily recognized for its role in acne vulgaris where it is thought to contribute to the inflammatory phase of the condition [(2)].

Propionibacterium acnes, which are a normal inhabitant of the skin, produce fatty acids that inhibit the growth of fungi on the skin. [(3)]. However, when it becomes trapped inside the hair follicle, it may grow and cause inflammation and acne infection [(4)].

The virulence genes involved in the pathogenesis of acne are *camp5*, *gehA*, *tly*, sialidases, neuraminidases, endoglycoceramidases, lipases, and hemolysins . The lipoglycan-based cell envelope and their extracellular secreted lipase, particularly triacylglycerol lipase, encoded by the *gehA* gene assists in the adherence and the colonization of the bacterium to the sebaceous follicle. The other product which aids in the acne process by destroying the host tissue includes porphyrins, hyaluronate lyase, endoglycoceramidase, sialidases/ neuramidase, cardiolipin synthetase, and calicineurin like phosphoesterase [(5)] .

Materials and Methods

Samples Collection:

The study involved (200) patients were subjected for sampling which include both skin sites (comedon and pustule) for the sampling were forehead, cheek, forearm, axilla, sole from both sexes and the age of patients ranged from 13 to 30 years. These patients

were diagnosed by dermatology physician , according to the signs and symptoms,, in addition to be having risk factors that were determined by the information about patients . In this study, patients with recent usage of local antibiotic treatment and usage cosmetic material were excluded from sampling

Culturing of Samples:

Spread a 10 µl(loop full) from the inoculated and incubate thioglygolay agar and blood agar plates after add0.01 ml from Tween-80 and incubate at 37ºC at 4-7 days and read plates of *Propionibacterium acnes* suspect colonies thioglygolay agar and blood agar onto non-selective media, (nutrient agar)plates for morphology and biochemical confirmation of *Propionibacterium acnes*. After culturing of sample used vitek 2 compact system to detection of *Propionibacterium acnes*.

DNA Extraction for Gram POSITIVE Bacteria

DNA extraction was carried out according to the genomic DNA purification kit supplemented by manufactured company (Gene aid, UK).

Confirmed Detection *Propionibacterium acnes* by PCR using Specific Primer

The one aim of this study was to develop a rapid molecular diagnostic test to identify and purity of *P.acnes* isolates based on the specific primer(*par* gene). The primer flanking portion length 1202bp were selected[6]

Molecular Detection of *Salmonella Typhimurium* and *Salmonella Enteritidis* Group using Multiplex PCR

PCR mixture was prepared by adding 25 µl of Green master mix (2x) promaga, 8 µl template DNA, 3 µl from forward primer and 3 µl from each four revers primer, final volume was completed to 50 µl by adding nucleuse free water.

Table 1: ***The primer sequences and PCR conditions of Propionibacterium acnes.***

gene	Primer sequence (5′-3′)	BP	PCR condition	Reference
par gene	F-AGCTCGGTGGGGTTCTCTCATC-3’ R-GCTTCCTCATACCACTGGTCA TC-3	1201	94ºC 3min 94ºC 30sec 65ºC 45min 72ºC 1.30min 72ºC 10min 1x	(Naghdi1 *and* Ghan (2014)

Detection of Some Virulence Gene Markers by PCR

The primers and PCR conditions used to amplify genes encoding virulence factors with PCR are listed in Table (2). The primer includes *tly* gene, *dsA* gene and *nan* gene. Each 25µl of PCR reaction contained 5µl of each upstream and downstream primer, 5 µl of free nuclease water, 2.5µl of DNA extraction and 12.5 µl of master mix. The PCR amplification products were visualized by electrophoresis on 1% agarose ladder (promega, USA).

Table 2: Virulence factor primers sequences with their amplicon size Base pair (bp) and their condition

Genes	Primer sequence (5′-3′)	Size (bp)	PCR condition	Reference
Tly	F5-CAGGACGTGATGGCAATGCGA-3′) R5-TCGTTCACAAGACCACAGTAGC-3′)	909	94ºC 10min 1x 94ºC 2min 55ºC 1min 40x 72ºC 1min 72ºC 10min 1x	In this study procedure designed
nan	F-5-CATCGACCGACAATGGACAC R-5-TCGGAATAGATCGACTGGGC	196	94ºC 10min 1x 94ºC 1min 51ºC 1min 35x 72ºC 1min 72ºC 10min 1x	
dsa	F-ACCATCAACCATCACCGACT R-TTCGGATGAGAAGAGCTGCT	325	94ºC 10min 1x 94ºC 1min 51ºC 1min 35x 72ºC 1min	

Results and Discussion

The one aim of this study was to develop a rapid molecular diagnostic test to identify and purity of *P.acnes* isolates based on the specific primer(*par* gene). The primer flanking portion length 1202bp were selected. The result was 9 (64.3%) isolation from *P.acnes* as the following Figure (1).

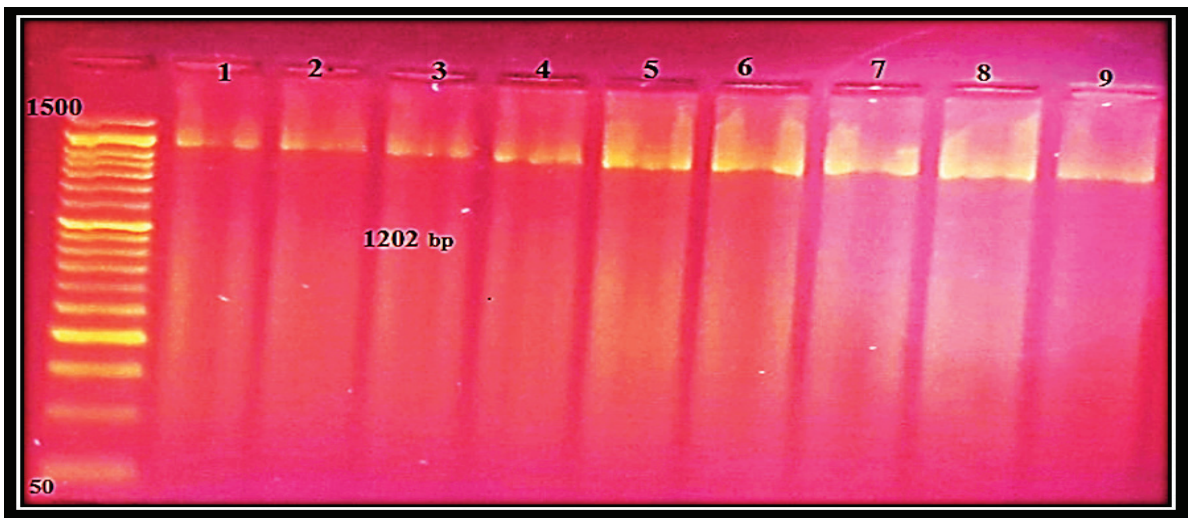

Figure (1):1% Agarose gel electrophoresis at 70 volt for 50 min for par gene PCR products visualized under U.V light at 280 nm after staining with ethidium bromide. L50:1500bp ladder; lane (1-9) were positive for this gene, the size of product is 1202bp.

Propionibacterium acnes on the other hand were considered as normal flora in the skin , however their role in acne is still vague. So this study may concern on this bacteria because there is no molecular studies performed on *Propionibacterium acnes*, also there is no molecular research in Iraq which covered the importance and pathogenesis of this through its ability to produce and detection certain virulence factor .

N- acytalnuramendas gene (nan gene) was investigated by PCR technique using specific primers for this gene. The result of this experiment indicate for positive amplification as shown in figure (2). . Not all samples were gave positive results for nan gene. the size of this genes of product is 196 bp. It was found that nan marker was observed in 6/9 isolates of Propionibacterium acnes (66.7%). .

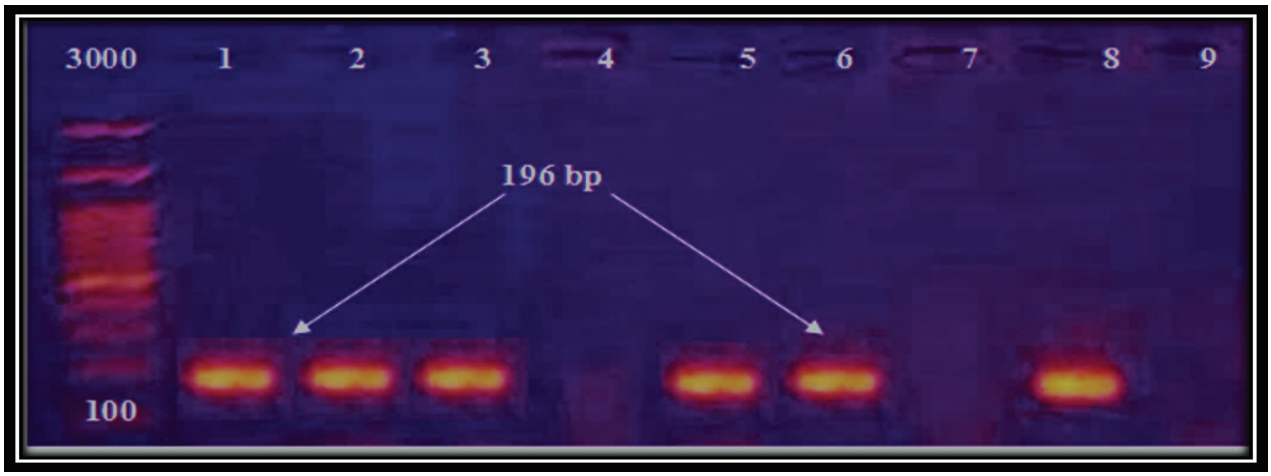

Figure (2): Agarose gel electrophoresis at 82 volt for 30 min for par gene PCR products visualized under U.V light at 280 nm after staining with ethidium bromide L100- 3000 bP ladder(abm); lane [1, 2, 3, 5, 6 and 8] were positive for this genes *nan gene* the size of product is 196bp.

The *nanA* gene was found to be conserved and sialidase activity was found in *P.acnes* isolated over a period of 50 years from various geographical locations. The analyzed the sialidase activity of the NanA protein of *P.acnes* and cloned the sialidase gene *nanA*. Sialidase is encoded as a precursor protein of 722 amino acids with a 26 amino acid signal peptide. The mature sialidase has a calculated molecular mass of 81 kDa and contains the carbohydrate binding module 32. Sialidase activity does not require the CBM32 domain. The NanA protein is secreted by *P.acnes* as a dimer [7]

Molecular studies of *dsA* gene were done for all *Propionibacterium acnes* isolates by using specific PCR markers. But in present study not all samples were gave positive results for *dsA* gene. 3 (33.4%) samples were positive for the *dsA* genes the size of product is 325bp as shown in figure(3).

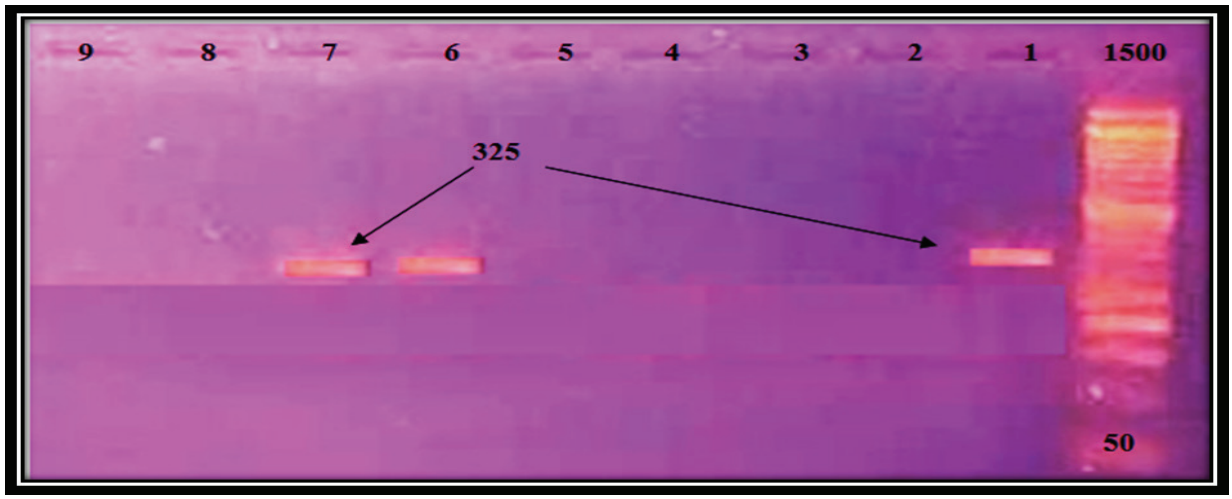

Figure (3): Agarose gel electrophoresis at 82 volt for 30 min for par gene PCR products visualized under U.V light at 280 nm after staining with ethidium bromide L50 - 1500 bP ladder (abm); lane [1, 6, 7] dermatan sulphate-binding adhesins (*dsA*) gene the size of product is 325bp.

This result was agreement with [8]who detected same strains of *Propionibacterium acnes* identified two dermatan-sulphate-binding proteins (DsA1& DsA2) with putative phase/antigenic variation signatures and the expression of these proteins by type IA organisms contributes to their role in the pathophysiology of acne and helps explain the recurrent nature of the disease.

Potential Effect of the DsA protein in *Propionibacterium acnes* are Colonization, adhesion, Fibrinogen-binding and inflammation [4]

The notable virulence gene involved in the pathogenesis of acne was *tly* gene responsible of damage the blood cells. So , this gene was very important virulence factor for bacteria associated with acne vulgaris. The molecular detection of *tly* gene was done by using specific primer. It was found that *tly* gene detected 9 (100%) isolates of *Propionibacterium acnes* isolates with long length 909 bp as shown in figure (4).

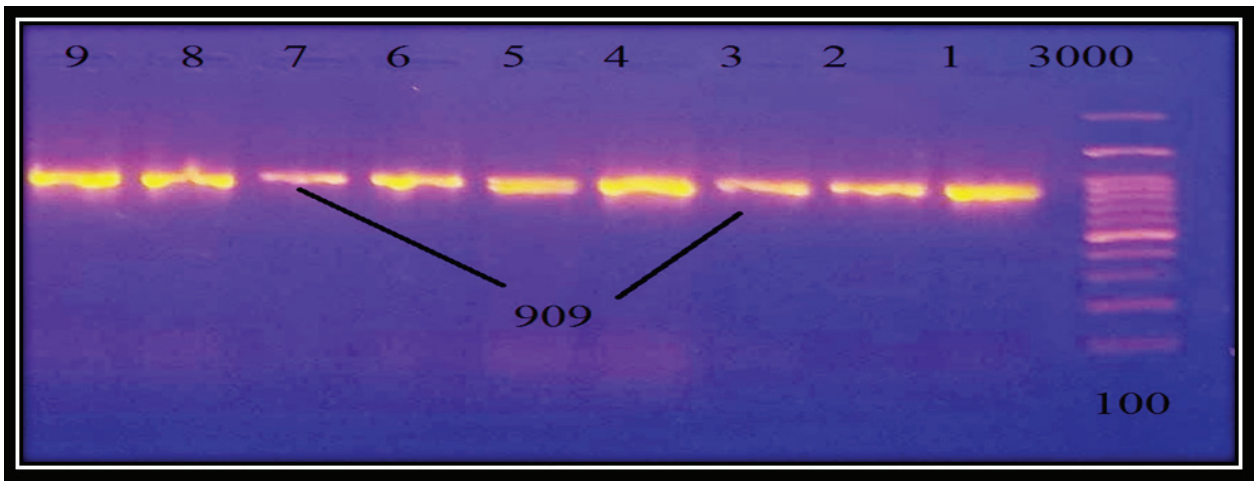

Figure (4) : Agarose gel electrophoresis at 82 volt for 30 min for par gene PCR products visualized under U.V light at 280 nm after staining with ethidium bromide L50 - 1500 bP ladder(abm); lane all *P.acnes* isolates were positive for this genes *Tly* gene the size of product is 909bp.

The result correlated with [9] who was showed a basically clonal population structure correlated with allelic variation in the virulence gene *tly*.

The result correlated with [10] who reporters the 98% identity sequenced of the *tly* gene from a further 19 *P.acnes* strains isolated from different sources and selected to represent types I and II. he showed that the differences between type I and II were based on type-specific polymorphisms in *tly* gene

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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