Detection of hyaluronidase (hyase) gene in Propionibacterium acne that isolated in Acne vulgaris patients, Kirkuk-Iraq

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Abstract
The study which involved 200 patients with Acne vulgaris, was carried out in Kirkuk City from October 2021 to March 2022. The most common isolated bacteria P.acne 40.5%, followed by S.epidermidis 22.5%, S.aureus 19.5%, and 7% S.pyogenes . The results of our study show that all clinical isolates of P. acnes samples under study have the hyaluronidase gene, and most of them have the ability to degrade hyaluronic acid. The hyaluronidase gene, which was not shown in this study. The high prevalence of this gene in P. acnes isolates makes it interesting as a potential genetic target for the detection of P. acnes samples.
Keywords: Acne vulgaris, P.acne, hyaluronidase gene.

Introduction

The human skin microbiome has important roles in skin health and disease ,The diversity of the human microbiota at the strain level and its association with human health and disease are largely unknown. However, many studies have shown that microbe-related human diseases are often caused by certain strains of a species, rather than the entire species being pathogenic (Hansra and Shinkai, 2011).
Acne vulgaris (commonly called acne) is one of the most common skin diseases with a prevalence in up to 85% of teenagers and 11% of adults . Although the etiology and pathogenesis of acne are still unclear, microbial involvement is considered to be one of the main mechanisms contributing to the development of acne (Bojar and Holland, 2004). However, despite decades of study, it is still not clear how P. acnes contributes to acne pathogenesis while being a major commensal of the normal skin flora (Dominguez -Bello *et al.*, 2010).
Cutibacterium acnes, an anaerobic human skin bacterium, is known as an exacerbating factor of acne vulgaris. Imbalances of the skin microbiome may be related to the development of acne vulgaris; in fact, an overpopulation of *C. acnes* in acne pustules is known to induce inflammation (Ramasamy *et al.*, 2019).
P. acnes secretes a number of proteins , the *P. acnes* secretome includes 20 proteins. The identity and function of only a few of these proteins have been described and characterized. Among the proteins in the secretome are the enzymes hyaluronidase, chondroitin sulfatase, and gelatinase , Hyaluronidases are enzymes that degrade hyaluronic acid (Holland *et al.*,2010).
A component of the extracellular matrix. Hyaluronic acid is present in tissues throughout the body, including bones and joints, contributing to the viscosity of synovial fluid and the lubrication of joints. It is also a part of the extracellular structure of chondrocytes involved in tissue healing (Patil *et al.*,2019). The ability of bacteria to degrade hyaluronic acid may be a virulence factor, enabling penetration of hyaluronidase-producing organisms in to tissues rich in hyaluronic acid, including joints, creating an advantage for establishing growth in articular spaces. In addition to P. acnes, other Gram-positive pathogens, such as *Streptococcus pneumoniae*, *Streptococcus intermedius*, *Streptococcus constellatus*, *Streptococcus dysgalactiae*, *Staphylococcus aureus*, *Peptostreptococcus species*, *Clostridium perfringens*, and *Clostridium septicum*, as well as other species of *Propionibacterium*, including *P. granulosum*, produce hyaluronidase (Patil *et al.*,2021).

Materials and Methods

The study conducted during the period October 2020 to March 2021. Included 200 with Acne vulgaris disease was carried out in Kirkuk City , Patients ages ranged from 12 to 25 years.

DNA extraction and polymerase chain reaction (PCR)

Genomic DNA of all resistance isolates of *P. acnes* was extracted using Wizard genomic DNA Purification kit (Geneaid USA) as stated by manufacturer instruction. PCR reaction was performed to amplify hyaluronidase gene using specific primers in table (I).
Genotypic Detection of Hyaluronidase Gene. *P. acnes* isolates were grown under anaerobic conditions in 2 mL brain heart infusion broth supplemented with 1% glucose, with agitation. DNA was extracted using the PCR reagent Kit (Geneaid USA) with a final elution into 50 μL elution buffer. A 451-base-pair region of the hyaluronidase gene sequence (Fourie *et al.*,2020) was targeted by polymerase chain reaction (PCR) primers, forward primer 5` -TTTCGGGATCCTTGTGGTA-`3 , and

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reverse primer 5` -TCTGGACAACAAACCTGT-`3 . Primer sequences were selected from the published P. acnes hyaluronidase gene sequence (Fourie *et al.*,2020) Basic Local Assignment Search Tool (BLAST) analysis showed that the primer sequences had complete matches only with the two published P. acnes hyaluronidase gene sequences. 5 µL of isolated DNA was amplified using 42 cycles as follows: melting at 95◦ C for 30 seconds, annealing at 54◦ C for 60 seconds, and amplifying at 72◦ C for 120 seconds. The final product was run on a 1% agarose gel .

Table (I) The specific primers that used in PCR			
Primer Name	Seq	temperature	Product Size (bp)
hyase	F AATGGTAACCGCTTTCCTGTAAT	58	478
hyase	R ATTTCGGGATCCTTGTGGTAG		

Results

The results of the isolation showed that out of the 200 samples, 81 (40.5)%) were positive for *P.acnes*, 45 (22.5)%) *S.epidernidis* , 39 (19.5%) *S.aureus* , and 14 (7%) *S.pyogenes* as shown in table (2).
The study reported that 88.9% of *P.acnes* isolates and the results of the detection of the hyaluronidase gene in bacterial isolates p1, p2, p3, p4, p5, p6, p7 showed that all of them were positive for the hyaluronidase gene in a percentage (100%) as the bands which are 478 bp in size appear with high purity, which indicates the accuracy of the interaction For diagnosis, a DNA sequencing test was conducted to ensure the accuracy of the results. As shown in Figure (1).

Table (2) Rates of isolated bacteria in Acne vulgaris disease		
bacterial isolates	NO.	%
P.acnes	81	40.5%
S. epidermidis	45	22.5%
S. aureus	39	19.5%
S.pyogenes	14	7%
E.coli	6	3%
klebsiella oxytoca	4	2%
pantoea agglomerans	1	0.5%
No growth	10	5%
Total	200	100%

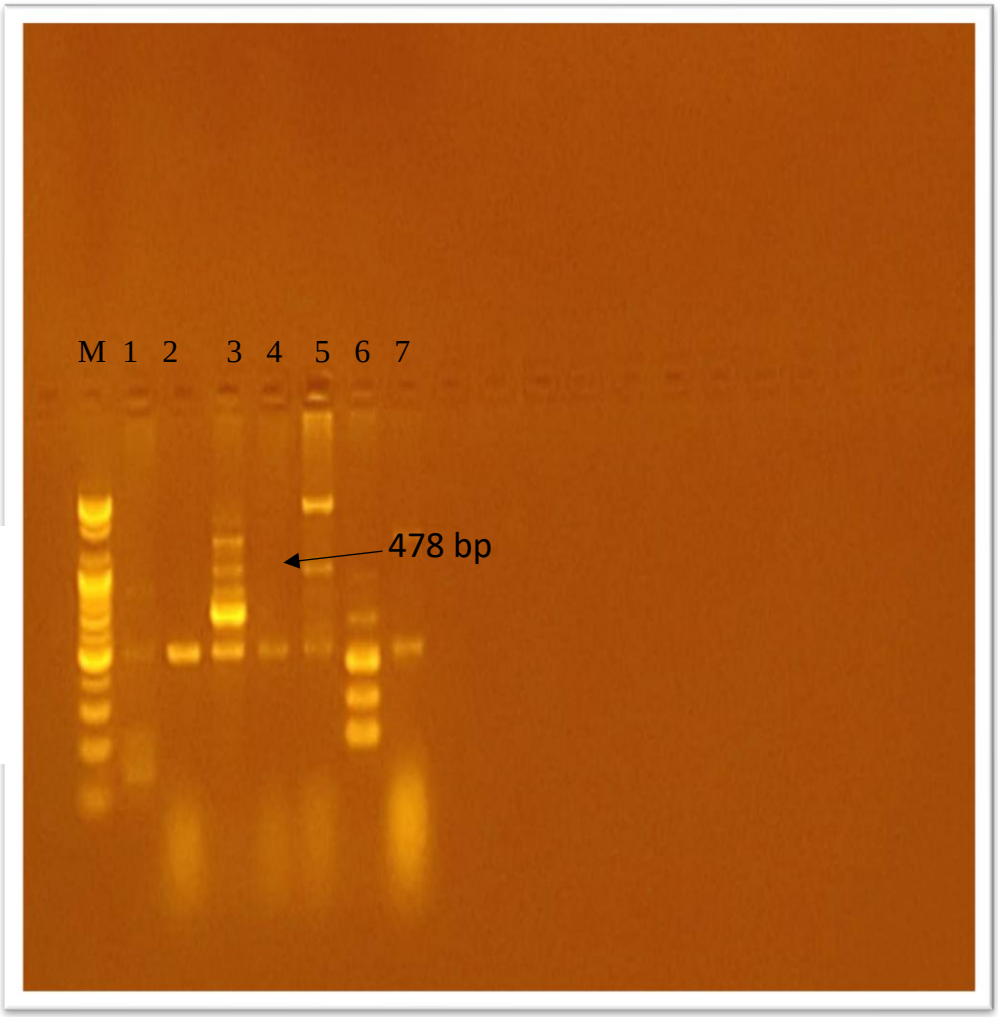

Figure (1) shows the result of a PCR reaction with a size of 478 bp for the genetic detection of P. acnes hyaluronidase gene, which was migrated in 2% agarose gel at a voltage of 70 V for 60 minutes. All isolates are shown 1, 2, 3, 4, 5, 6, 7 have the p. acnes hyaluronidase

Phylogenetic tree

Figure (2) shows the degrees of genetic proximity and genetic distance between samples of *Propionibacterium acnes* for the hyaluronidase (hyase) gene under study among them, in addition to the genetic dimension with its global isolates.

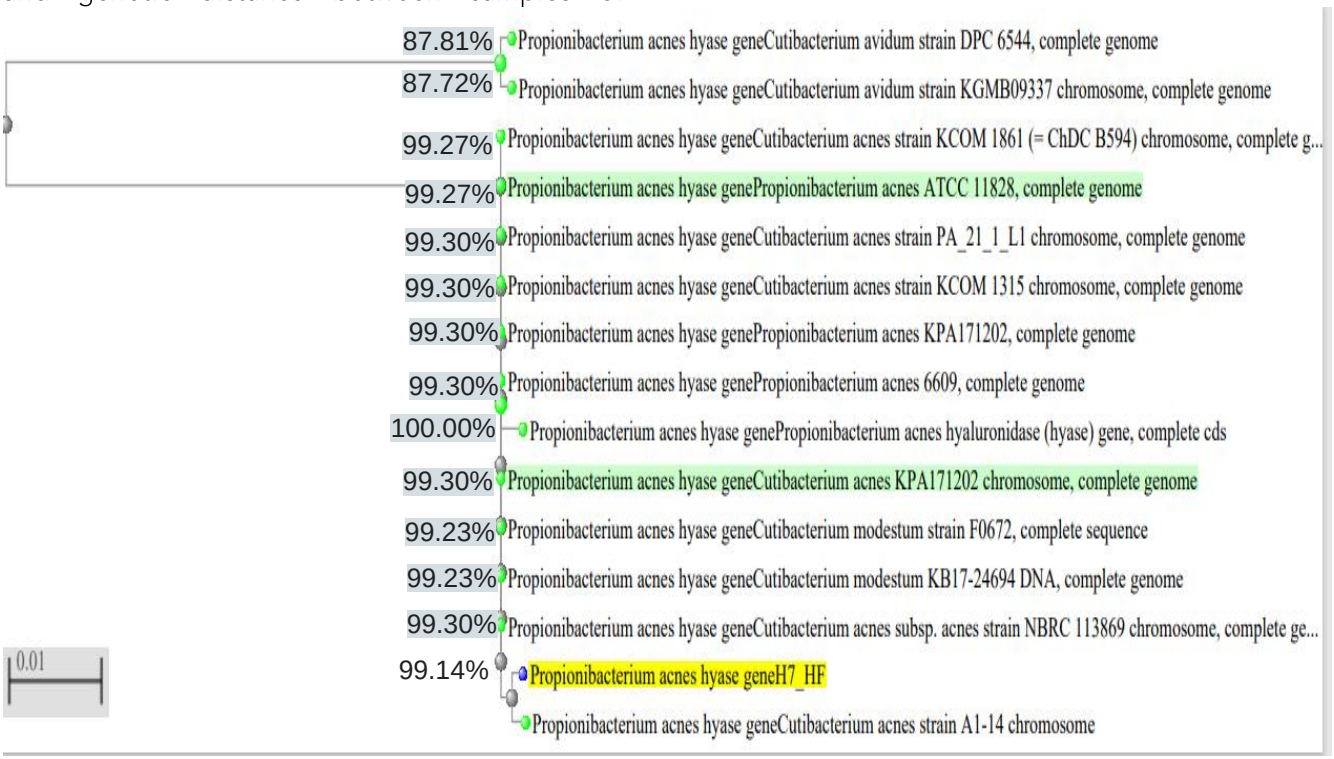

Figure (2) Phylogenetic tree of the hyaluronidase (hyase) gene

Discussion

The body's extracellular matrix and skin both contain hyaluronic acid, which is mostly found in soft tissue. Any enzyme that can cleave hyaluronic acid is known as a hyaluronidase. There are various different hyaluronidases, including ones that can be discovered in bacteria, leech saliva, and mammalian spermatozoa. Bacterial hyaluronidases are comparable to one another in that they both produce unsaturated disaccharides as their final product. This is different from parasite and mammalian hyaluronidase , Several Gram-positive human pathogens excrete hyaluronidase, a bacterial spread and tissue penetrating enzyme (Brown *et al.*,2017).

Along with *P. acnes*, these microorganisms also include the previously mentioned Peptostreptococcus, Staphylococcus, Streptococcus, and Clostridium species. However, unlike the enzyme generated by Gram-positive bacteria, which is excreted extracellularly, the hyaluronidase that Gram-negative bacteria produce is a periplasmic enzyme and as such is less likely to aid in tissue penetration. Although it is abundant in articular spaces as well, the skin contains about 50% of the body's hyaluronic acid. As a result, because of their improved capacity to enter these crevices, microbes that can cleave hyaluronic acid may be preferred as infection-causing agents in these places (Tyner and Patel,2015). Results of our investigation show that all tested clinical isolates of P. acnes have the described hyaluronidase gene, and most have the ability to cleave hyaluronic acid.

Through the analysis of the genetic tree of the hyaluronidase (hyase) gene, it was observed that the nitrogen bases of *Propionibacterium acnes* are similar to the samples registered globally. 1., CP002815.1, AE017283.1, and also a similarity of 99.27% to the sample with serial number CP012647.1, CP003084.1, and 99.23% to the sample with serial number CP017040.1, AP024747.1 and 91.14% to the sample with serial number Serial number CP013693.1, and 87.81% to the sample with serial number CP016954.1, CP068550.1 as shown in Figure (2).

The use of this technique aims to confirm the diagnosis of the species recorded in the current study with comparing them with international samples and identifying the sequence of nitrogenous bases for genes (Chai *at el.*,2015)

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