

**TRANSCRIPTOME ANALYSIS OF *Porphyromonas gingivalis*
IN RESPONSE TO INHIBITION WITH
Lactobacillus rhamnosus ATCC 7469 CELL FREE
SUPERNATANT**

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A thesis submitted in fulfilment for the degree of
MASTERS OF SCIENCE

**UNIVERSITI SAINS ISLAM MALAYSIA
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SEPTEMBER 2023

AUTHOR DECLARATION

I declare that this report is my original work and that all references have been cited adequately as required by the University. The report has not been accepted for any degree and is not currently submitted in the candidature of any other postgraduate study.

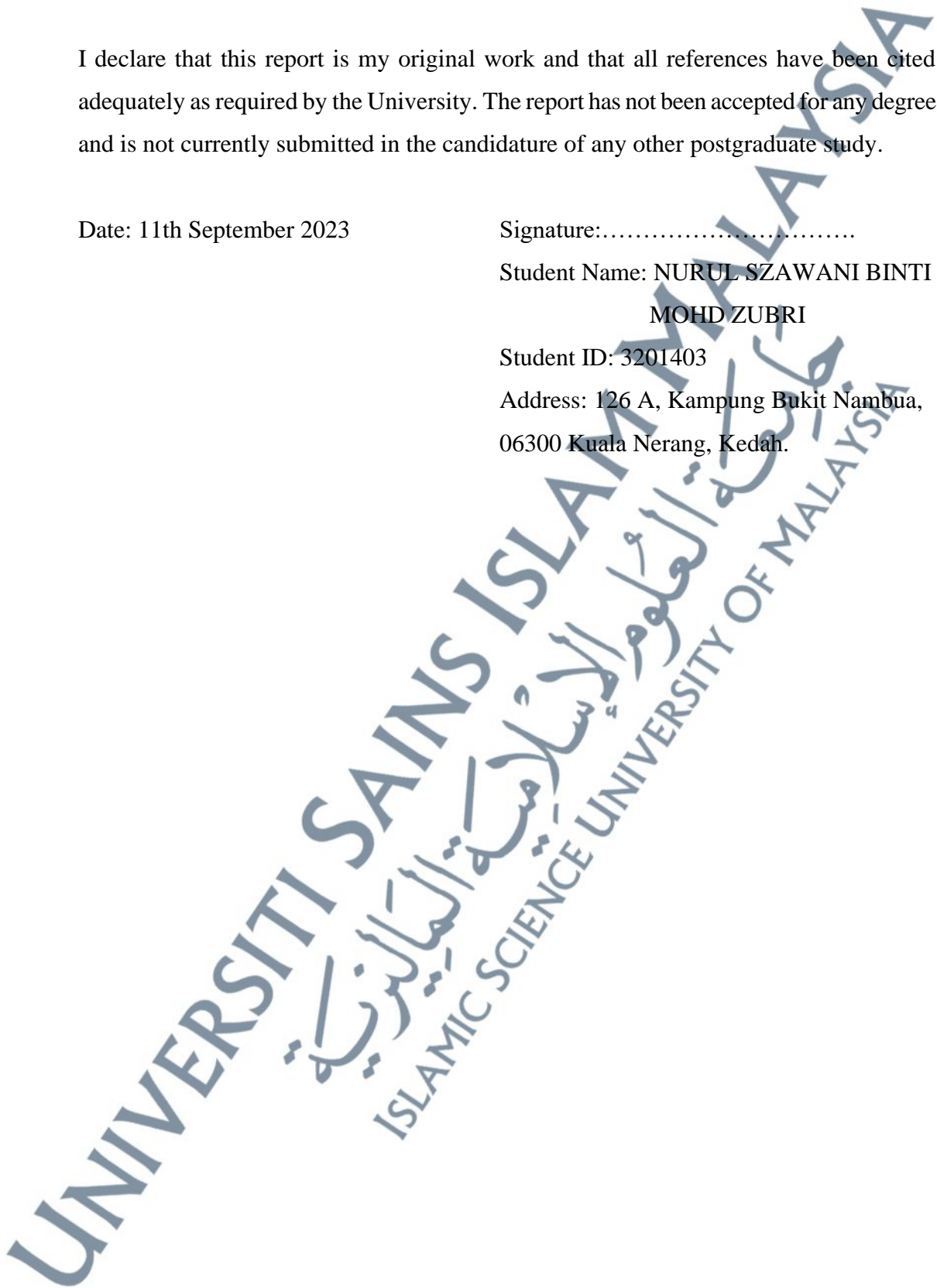
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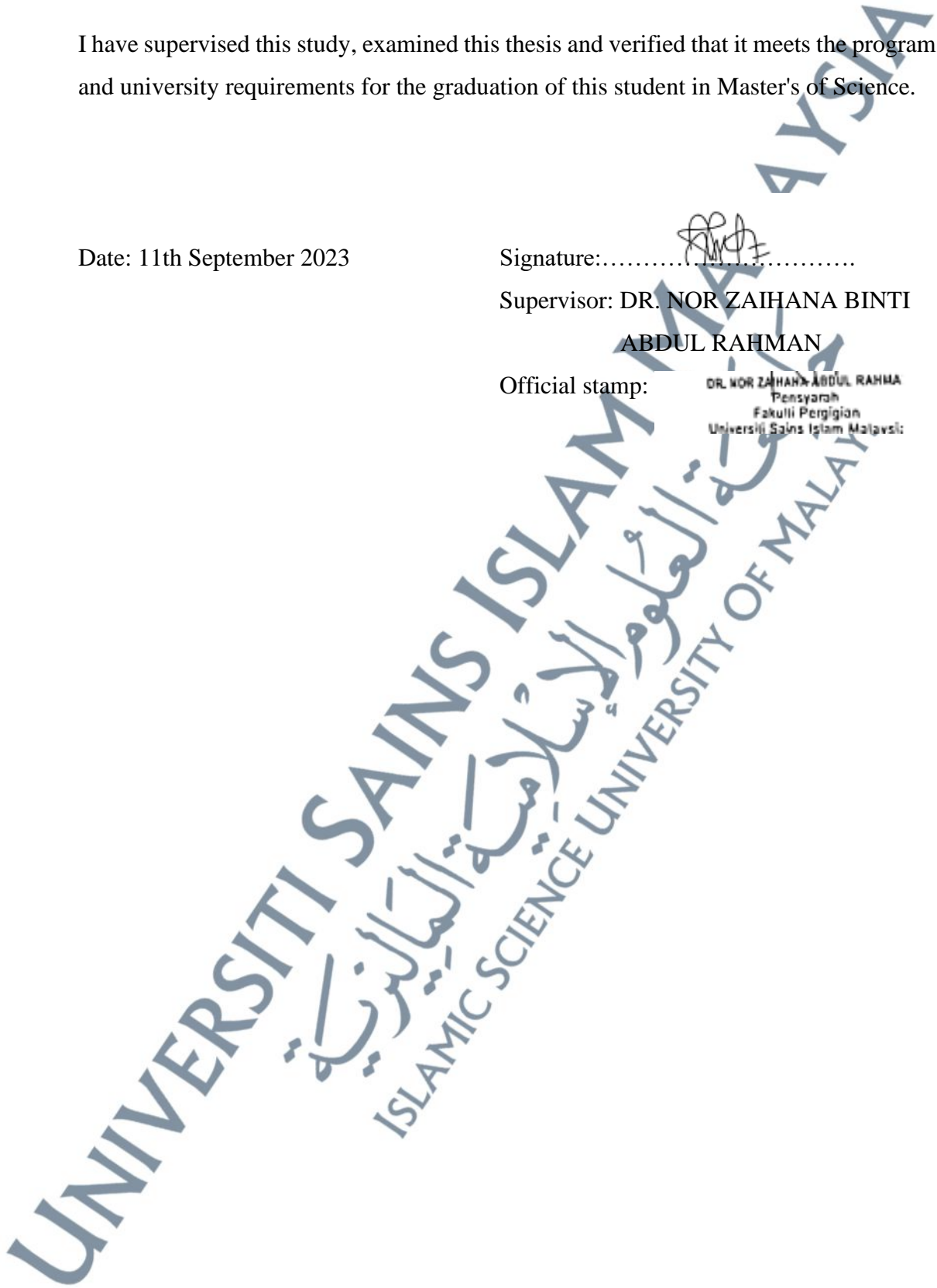
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DEDICATION

A special dedication to my parents, my supervisors, my sibling, and to all my fellow friends. Thank you for everything.

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ABSTRACT

Periodontal disease is a chronic disease that is majorly caused by polymicrobial biofilm formation at the periodontal pocket. The important pathogen of this disease is *P. gingivalis* where it is involved in biofilm formation and virulent activity on the gingival tissue. Probiotics are suggested as an adjunct method to overcome this pathogen's colonization and biofilm formation. The main objective of this study is to investigate the molecular mechanism of antimicrobial and anti-biofilm properties of *L. rhamnosus* ATCC 7469 cell-free supernatant (CFS) against periodontal pathogen, *P. gingivalis*. To achieve the objective, disc diffusion assay, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were carried out to determine the antimicrobial properties while anti-biofilm assay were carried out with various concentration of *L. rhamnosus* ATCC 7469 CFS to assess the anti-biofilm activity. To identify the effect of *L. rhamnosus* ATCC 7469 on the gene expression of *P. gingivalis*, Next Generation Sequencing (NGS) were employed followed by RT-qPCR to validate the gene expressions. The antimicrobial screening revealed that *L. rhamnosus* ATCC 7469 CFS was able to inhibit *P. gingivalis* with the inhibition zone of 10.64 ± 0.44 mm with the MIC and MBC value of 25% CFS concentration. In addition, *L. rhamnosus* ATCC 7469 CFS able to reduce biofilm formation by 84.94% at the highest concentration of CFS as observed by the biofilm assay. The molecular investigation by NGS showed that various genes and pathways related to cell survival, metabolism, and virulent activity were differentially expressed. Then, the affected gene *mfa1*, *kgp*, and *rgp* with the addition of one more biofilm related gene, *FimA* were validated by RT-qPCR technique. The genes of interest expression were in agreement with the NGS outcomes. In conclusion, probiotics *L. rhamnosus* ATCC 7469 CFS possess antimicrobial and anti-biofilm properties against *P. gingivalis* and the effects of exposure to *L. rhamnosus* ATCC 7469 CFS are reflected in the gene expression of *P. gingivalis*.

ABSTRAK

Penyakit periodontal adalah penyakit kronik yang sebahagian besarnya disebabkan oleh pembentukan biofilm polimikrob pada poket periodontal. Patogen penting penyakit ini ialah *P. gingivalis* di mana ia terlibat dalam pembentukan biofilm dan aktiviti virulen pada tisu gingiva. Probiotik dicadangkan sebagai kaedah tambahan untuk mengatasi penjajahan patogen ini dan pembentukan biofilm. Objektif utama kajian ini adalah untuk menyiasat mekanisme molekul sifat antimikrob dan anti-biofilem *L. rhamnosus* ATCC 7469 supernatan bebas sel (CFS) terhadap patogen periodontal, *P. gingivalis*. Untuk mencapai objektif, ujian resapan cakera, kepekatan perencatan minimum (MIC) dan kepekatan pembasmian minimum (MBC) telah dijalankan untuk menentukan sifat antimikrob manakala ujian anti-biofilem dijalankan dengan kepekatan *L. rhamnosus* ATCC 7469 CFS yang berbeza untuk menilai aktiviti anti-biofilem. Untuk mengenal pasti kesan *L. rhamnosus* ATCC 7469 pada ekspresi gen *P. gingivalis*, teknik NGS telah digunakan dan kemudian diikuti oleh RT-qPCR untuk mengesahkan ekspresi gen. Saringan antimikrobial mendedahkan bahawa *L. rhamnosus* ATCC 7469 CFS mampu menghalang *P. gingivalis* dengan zon perencatan 10.64 ± 0.44 mm dengan nilai MIC dan MBC sebanyak 25% kepekatan CFS. Selain itu, *L. rhamnosus* ATCC 7469 CFS telah mampu mengurangkan pembentukan biofilem oleh *P. gingivalis* sebanyak 84.94% pada kepekatan tertinggi CFS seperti yang diperhatikan oleh ujian biofilm. Penyiasatan molekul menggunakan teknik NGS menunjukkan bahawa pelbagai gen dan laluan yang berkaitan dengan survival sel, metabolisme, dan aktiviti virulen dinyatakan secara berbeza. Kemudian, gen yang terjejas *mfaI*, *kgp*, dan *rgp* dengan penambahan satu lagi gen berkaitan biofilm, *FimA* telah disahkan oleh teknik RT-Qpcr. Ekspresi gen yang penting adalah selari dengan hasil keputusan NGS. Kesimpulannya, probiotik *L. rhamnosus* ATCC 7469 CFS mampu mempamerkan sifat antimikrob dan anti-biofilem terhadap *P. gingivalis* dan kesan pendedahan kepada *L. rhamnosus* ATCC 7469 CFS dicerminkan dalam ekspresi gen *P. gingivalis*.

ملخص

أمراض اللثة هي اضطراب التهابي مزمن ناتج عن تكوين غشاء حيوي متعدد الميكروبات على الجيب اللثوي. المرض الرئيسي لهذا المرض هو البورفيروموناس اللثوي *P. gingivalis* الذي يشارك في تكوين الأغشية الحيوية والنشاط الضار على أنسجة اللثة. للتغلب على الاستعمار وتكوين الأغشية الحيوية لهذا العامل الممرض ، يُقترح البروبيوتيك كعلاج مساعد محتمل يعتمد على مضادات الميكروبات ومضاد الغشاء الحيوي ضد العديد من مسببات الأمراض عن طريق الفم. الهدف الرئيسي من هذه الدراسة هو دراسة الآلية الجزيئية للنشاط المضاد للميكروبات من *L. rhamnosus* ATCC 7469 الخالي من الخلايا الطافية ضد مسببات أمراض اللثة *P. gingivalis*. لتحقيق الأهداف ، تم إجراء اختبار انتشار القرص وفحص التخفيف الدقيق للمرق للتحقيق في الخصائص المضادة للميكروبات لـ *L. rhamnosus* ATCC 7469 الخالي من الخلايا الطافية. تم إجراء فحص الأغشية الخلوية لـ *L. rhamnosus* ATCC 7469 الخالي من الخلايا الطافية بتركيز مختلف لتقييم النشاط المضاد للغشاء الحيوي للعينة. تم استخدام مرق MRS المعقم و 0.2% كلورهيكسيدين كعناصر تحكم سلبية وإيجابية على التوالي في الفحوصات المذكورة. لفهم الآلية الأساسية لخصائص مضادات الميكروبات ومضادات الغشاء الحيوي ، تم استخدام تقنية تسلسل الجيل التالي (NGS) للكشف عن الجينات المصابة متنوعة بإجراءات تفاعل البوليمراز المتسلسل بالزمن الحقيقي (RT-qPCR) للتحقق من صحة التعبير الجيني. كشف فحص مضادات الميكروبات التي تم إجراؤها أن لـ *L. rhamnosus* الخالي من الخلايا الطافية من قدرة على تثبيط *P. gingivalis* مع منطقة التثبيط 10.64 ± 0.44 ملليمتر مع تركيز الحد الأدنى المثبط و الحد الأدنى مبيد الجراثيم (MBC\MIC) بنسبة 25% للمواد الطافية الخالية من الخلايا. بالإضافة إلى ذلك ، تمكنت *L. rhamnosus* ATCC 7469 الخالي من الخلايا الطافية من تقليل تكوين الأغشية الحيوية بواسطة *P. gingivalis* بنسبة اختزال بلغت 84.94% عند أعلى تركيز طاف كما لوحظ في اختبار الأغشية الخلوية. أظهر الفحص الجزيئي باستخدام تقنية تسلسل الجيل التالي NGS أن تعرض مزرعة *P. gingivalis* للمواد الطافية الخالية من الخلايا لـ *L. rhamnosus* ATCC 7469 أعاق تنظيم العديد من الجينات المهمة التي تشارك في تكوين الأغشية الحيوية. الجينات المهمة التي تأثرت هي جين *gipA* ، تم التحقق من صحة جينات *mfa1* و *kgp* و *Rgp* التي تم تعطيلها كما لوحظ في تقنية تسلسل الجيل التالي (NGS) مع إضافة جين واحد مرتبط بتكوين الأغشية الحيوية ، *FimA* باستخدام تقنية تفاعل البوليمراز المتسلسل بالزمن الحقيقي (RT-qPCR). كان التعبير عن الجينات موضع الاهتمام متفقا مع نتائج تسلسل الجيل التالي (NGS). في الختام ، أظهرت البروبيوتيك *L. rhamnosus* ATCC 7469 الخالي من الخلايا الطافية خصائص مضادة للميكروبات ومضادة للأغشية الحيوية ضد *P. gingivalis* والآليات الكامنة وراء عملها تنعكس في التعبير الجيني لـ *P. gingivalis*.

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LIST OF ABBREVIATIONS

AMP	Adenosine monophosphate
ANOVA	Analysis of variance
APDT	Antibacterial photodynamic therapy
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
BP	Binding process
CC	Cellular components
cDNA	Complimentary DNA
CFS	Cell-free supernatant
CLSI	Clinical Laboratory Standard Institute
CMU	Cheonnam Medical University
Ct	Cycle threshold
dGTP	Deoxyguanosinetriphosphate
DNA	Deoxyribonucleic acid
EPS	Exopolysaccharides
FPKM	Fragments per kilobase of exon per million mapped fragments
GC	Gas chromatography
GMP	Guanosine monophosphate
GMSC	Gingival mesenchymal stem cell
GO	Gene Ontology
GTP	Guanosine triphosphate

HPLC	High-performance liquid chromatography
IBD	Inflammatory bowel disease
IgE	Immunoglobulin E
IgG	Immunoglobulin G
IL	Interleukins
IRF	Interferon responses factor
KEGG	Kyoto Encyclopedia of Genes and Genomes
KgP	Lysine-gingipain
LAB	Lactic Acid Bacteria
LPS	Lipopolysaccharides
MBC	Minimum Bactericidal Concentration
MF	Molecular function
MIC	Minimum Inhibitory Concentration
MRS	De Man, Rogosa and Sharpe
MSC	Mesenchymal stem cell
MTT	-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NAD	Nicotinamide adenine dinucleotide
NGS	Next Gene Sequencing
OPG	Osteoprotegerin
PBS	Phosphate Buffer Saline
PCR	Polymerase chain reaction
PFOR	Pyruvate-ferredoxin oxidoreductase
PLP	Pyridoxal-5'-phosphate

PMN	Polymorphonuclear
PPD	Pocket Probing Depth
RANKL	Receptor activator of NF- κ B ligand
RgP	Arginine- gingipains
RNA	Ribonucleic acid
rpm	Revolution per minute
RT - qPCR	Real-Time Quantitative PCR
SAM	S-Adenosyl methionine
SEM	Scanning electron microscope
TLR	Toll-like receptors
TNF- α	Tumor necrosis factor-alpha
WHO	World Health Organization

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