



DEPARTEMENT OF MICROBIOLOGY

N°/NLS/2025

DISSERTATION

Presented by

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For the award of the diploma of

MASTER

Branch : **Biology**

Specialty : **Microbiology and quality control**

THEME

**Optimization of hyaluronic acid production through bacterial
fermentation for industrial application**

Publicly defended on /06/2025

Before the jury

President :	- DJELLAL Nadjiha	MCB. UFA Setif 1
Promoter :	- BOUZID Djihane	MCA. UFA Setif 1
Examiner :	- ELKOLLI Meriem	Pr. UFA Setif 1

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Academic year : 2024/2025

Acknowledgments

First and foremost, we thank Allah, the Almighty, for granting us the strength, patience, and resources necessary to complete this modest project.

We wish to express our utmost respect to our supervisor, Dr. Bouzid Djihane, for his invaluable guidance, guidance, and support throughout the completion of this project.

Our sincere thanks also go to Dr. Djellal Nadjiha, for the honor she has bestowed upon us by agreeing to chair this jury, as well as to Dr. Elkolli Meriem, whom we thank for agreeing to review our work.

We also express our sincere gratitude to all our esteemed professors, our fellow students, and all those who contributed, directly or indirectly, to the success of this project.

Dedication

In the name of God, the Most Gracious, the Most Merciful

I praise and thank Almighty God for granting me the honor of attending my graduation ceremony and witnessing the results of my efforts, even the fruits of my hard work and dedication, after 17 years of challenges and successes. I express my deepest gratitude to my dear father, Yassine Boukazata, for his constant support and immense sacrifices, which have had a considerable impact on me reaching this stage. May God grant you long life. To the source of tenderness, to the one who sowed hope in me since my childhood, to my dear mother Nawal Allou, whose prayers have been the secret of my success, I extend to you all my gratitude and deep appreciation for the love, care, and constant support you have offered me. Your role has been essential in my academic journey. May God protect you and grant you long life. I cannot fail to express my deep gratitude to my two dear brothers, Yasser and Abd Elbasset, my companions and my support in all circumstances. Thank you for your constant support and your invaluable love. To my fiancé, Rami Laouici, who supported me through the ups and downs, always by my side and encouraged me at every step, I dedicate this humble work as a token of love and gratitude.

Thank you for your support and your patience. I also do not forget my companions throughout my university career: Oumaima Cheriet, a faithful friend with whom I shared every moment and every detail, as well as Chourouk, Hadjar Boumechouane and Imane, a lifelong friend, for their constant support and their presence at my side. Thank you all, from the bottom of my heart.

- *Kawthar* -

Thanks be to God, who gave me knowledge and granted me this accomplishment. Today, I reap the fruits of years of hard work. Praise be to God for His complete blessing and perfect grace.

A huge thank you to my father, Mr. Cheriet Salem, my first and constant supporter. Without him, after God, I would not be standing on the graduation podium today. May you always be my pride and honor. My testimony today is the work of my illiterate mother, who never held a pen, but she held my hand and pushed me towards success. My mother, Mrs. Baz Salima, is the true heroine of this success. May God grant you infinite happiness and eternal paradise. My husband and life partner, Mr. Anes Abchi, my accomplishment today bears your signature in every detail..., in your support, your love, and your trust, and even in your silence when I needed to express my pain, thank you for being life at a time when I felt nothing was worth continuing. Brotherhood is not just a blood bond, it is a spirit and unwavering support. My brothers Haitham, Adam, and my sister Taouba, my joy would not have been complete without you.

May God protect and take care of you, and may I see you reach the highest ranks of transcendence.

Friend of the path and partner in late nights and fatigue, Kawthar Boukazata, with you the road has become easier. Thank you from the bottom of my heart. My colleague Hajar Boumechouane, thank you for your friendship and your participation in this success. I will never forget this chance that brought us together. My faithful friend, Ghellab Hayet, safety did not reside anywhere, but rather in your presence, your words and your embrace, thank you from the bottom of my heart.

- *Oumáima* -

Praise be to God who blessed me and granted me success for this day and granted me success and arrival,

I dedicate my diploma and the harvest of what I sowed throughout my academic career to my grandparents who worked hard and made every effort until I reached this precious moment, to the one who harvested the thorns of my path to open the path of knowledge for me, my beloved and supportive father in life "Boumechouane Ammar" and to the one who breastfed me with love, tenderness and healing balm, my beloved mother "Menter Fahima" to the kind and innocent hearts of my dear brothers and sisters "Nawal, Maryam, Hayat, Abir and Idris" thank you for your constant support and encouragement that pushed me to arrive, and thank you to my second family "Omnia and Yousra" thank you for your love and support at every step, to those whom the days brought me together, my companions on the journey "Chriet Omaïma and Boukazata Kawthar" a big thank you to you for the days we have lived and for the experience we have lived together.

- *Hadjer* -

Abstract

This study aims to evaluate the potential for hyaluronic acid (HA) production and improve detection methods, using bacteria discovered by Dr. Bouzid Djihane, which have not yet been classified or identified. We performed several tests on these bacteria, including Gram staining, catalase testing, and India ink staining, to determine their characteristics and classify them. The bacteria were Gram-positive, appeared in chains, and possessed a capsule. To detect and purify hyaluronic acid, we used the Cetyltrimethylammonium bromide (CTAB) method and hyaluronidase enzyme assays.

Purity tests using a NanoDrop spectrophotometer showed a 260/280 ratio of 2.2, indicating no protein contamination. The 260/230 ratio was approximately 0.9 for both commercial and bacterially produced HA, consistent across three replicates. Enzymatic degradation tests showed that the bacterial HA degraded slightly slower than commercial HA, possibly indicating a higher molecular weight. The specific activity of hyaluronidase on HA was confirmed, demonstrating its ability to cleave β -1,4-glycosidic bonds in the HA polymer.

Importantly, this study confirmed that the newly discovered bacterial strain is capable of producing hyaluronic acid, and it provides a detailed description of improved HA production, detection, and purification methods, a confirmation that opens promising perspectives for biotechnological and medical applications.

Keywords: Hyaluronic acid, Gram stain, Cetyltrimethylammonium bromide (CTAB), hyaluronidase, NanoDrop

الملخص

تهدف هذه الدراسة إلى تقييم إمكانية إنتاج حمض الهيالورونيك (HA) وتحسين بعض طرق الكشف عنه، وذلك باستخدام بكتيريا اكتشفتها الدكتور بوزيد جيهان، والتي لم يتم تصنيفها أو التعرف عليها بعد. أجرينا عدة اختبارات على هذه البكتيريا، من بينها صبغة غرام، اختبار الكاتالاز، وصبغة حبر الصين، لتحديد خصائصها وتصنيفها. كانت البكتيريا موجبة لصبغة غرام، تظهر على شكل سلاسل وتمتلك كبسولة. للكشف وتنقية حمض الهيالورونيك، استخدمنا طريقة بروميد سيتيل ثلاثي ميثيل الأمونيوم (CTAB) واختبارات باستخدام إنزيم الهيالورونيداز.

أظهرت اختبارات النقاء باستخدام جهاز NanoDrop أن النسبة 280/260 كانت 2.2، مما يشير إلى عدم وجود تلوث بالبروتينات. وكانت النسبة 230/260 حوالي 0.9 لكل من الحمض الهيالورونيك التجاري والمستخلص، مع تكرار النتائج ثلاث مرات. كما أظهرت اختبارات التحلل الإنزيمي أن الحمض الهيالورونيك المنتج من البكتيريا يتحلل بشكل أبطأ قليلاً من التجاري، مما قد يشير إلى وزن جزيئي أعلى. وتم تأكيد النشاط النوعي لإنزيم الهيالورونيداز على الحمض الهيالورونيك، حيث يقوم بقطع الروابط الجليكوسيدية β -1,4 في جزيء الحمض

من المهم أن هذه الدراسة أكدت أن السلالة البكتيرية المكتشفة حديثاً قادرة على إنتاج حمض الهيالورونيك، وقدمت وصفاً تفصيلياً لطرق محسنة لإنتاج واكتشاف وتنقية هذا الحمض، وهو تأكيد يفتح آفاقاً واعدة في التطبيقات الطبية والتقنية الحيوية.

الكلمات المفتاحية: حمض الهيالورونيك، صبغة غرام، بروميد سيتيل ثلاثي ميثيل الأمونيوم (CTAB)، هيالورونيداز، NanoDrop

Résumé

Cette étude vise à évaluer le potentiel de production d'acide hyaluronique (AH) et à améliorer certaines méthodes de détection, en utilisant des bactéries découvertes par le Dr Bouzid Djihane, qui n'ont pas encore été classées ni identifiées. Plusieurs tests ont été effectués, notamment la coloration de Gram, le test de catalase et la coloration à l'encre de Chine, afin de caractériser ces bactéries. Elles sont Gram positives, apparaissent en chaînes et possèdent une capsule. Pour détecter et purifier l'acide hyaluronique, nous avons utilisé la méthode au bromure de cetyltriméthylammonium (CTAB) et des tests enzymatiques avec l'hyaluronidase.

Les tests de pureté réalisés avec un spectrophotomètre NanoDrop ont montré un ratio 260/280 de 2,2, indiquant l'absence de contamination protéique. Le ratio 260/230 était d'environ 0,9, identique pour l'acide hyaluronique commercial et celui extrait, confirmé sur trois répétitions. Les tests de dégradation enzymatique ont montré que l'acide hyaluronique bactérien se dégrade légèrement plus lentement que le commercial, ce qui peut indiquer un poids moléculaire plus élevé. L'activité spécifique de l'hyaluronidase sur l'acide hyaluronique a été confirmée, démontrant sa capacité à cliver les liaisons β -1,4-glycosidiques dans le polymère.

De manière significative, cette étude a confirmé que la souche bactérienne nouvellement découverte est capable de produire de l'acide hyaluronique, et elle fournit une description détaillée des méthodes améliorées de production, de détection et de purification de l'AH, une confirmation qui ouvre des perspectives prometteuses pour les applications biotechnologiques et médicales.

Mots clés : Acide hyaluronique, coloration de Gram, bromure de cetyltriméthylammonium (CTAB), hyaluronidase, NanoDrop