استمارة مستخلصات رسائل واطاريح الماجستير والدكتوراه في جامعة البصرة

الكلية: الطب البيطري **اسم الطالب: هبة علي ناصر خضير المنصوري**

القسم:الاحياء المجهرية و الطفيليات **اسم المشرف: عدنان موسى الروضان**

التخصص: الاحياء المجهرية **الشهادة: الماجستير**

عنوان الرسالة أو الأطروحة

االكشف الجزيئي للجراثيم المحبة للبرودة *Pseudomonas fluorescens* في الحليب الخام في محافظة البصرة

ملخص الرسالة او الاطروحة

الخلاصـــة

التقصي عن فعالية تحليل البروتين في جرثومة *Psuedomonas fluorescens* هو الهدف الاساسي لهذه الدراسة. لتحقيق *هذا الهدف استعملت* البادئات 16S rDNA و SM2F/SM3R في تضخيم الحامض النووي DNA المستخلص من العزلات الجرثومية للحليب الخام. في هذه الدراسة اخضعت للتشخيص المظهري والاحيائي الجزيئي 92 عزلة بكتيرية متكونة من42 عزلة حليب الابقار الخام و50 عزلة حليب الجاموس الخام حصل عليها من240 عزلة حليب خام للابقار و الجاموس. كشفت نتائج التشخيص المظهري عن ان اعلى نسبة (42% and 41.7%) لتشخيص العزلات البكتيرية المعتمد على الزرع بالاطباق والاختبارات الكيمياحيوية لوحظت في حليب الجاموس الخام بالمقارنة مع تلك النسب التي لوحظت في عزلات حليب الابقار البكتيرية (35% الزرع بالاطباق و33.3 الاختبارات الكيمياحيوية) مع ذلك ان الاختلاف بين الابقار والجاموس فيما يتعلق بنتائج التشخيص البكتيري لايعتبر ذو معنوية احصائية.(P>0.05). كشفت نتائج الPCR ان تاثير السلالة في حليب الابقار وتاثير العمر في الابقار والجاموس على نتائج تضخيم منتجات جينات (16SrDNA و(SM2F/SM3R) جرثومة *P. fluorescens* في حليب الاليقار و الجاموس الخام لا يعتبر ذو معنوية احصائية.(P>0.05). بالاعتماد على نتائج الPCR المعتمد على16SrDNA لوحظت اعلى نسبة (% 62.5) لتشخيص *P. fluorescens* في حليب الابقار المحلية الخام بالمقارنة مع نسب .(57.7%) العزلات البكتيرية لحليب الابقار المضربة . اظهرت نتائج ال PCRالمعتمد علىSM2F/SM3R ان 33.3% و 34% من عزلات *P. fluorescens* لحليب الابقار والجاموس الخام على التوالي لها فعالية في تحليل البروتين.

College: Colleg of Veterinar **Name of Student: Hiba Ali Nasir Khudheir AL_Mansory**

Dep.: Microbiology and Parasitology

Name of Supervisor: . Adnan Mousa AL-Rodhan

Certificatte: master **Specialization: Microbiology**

Tital of Thesis:

PCR-based Detection of psychrotrophic bacteria *Pseudomonas fluorescens* in raw milk in Basrah Province

Abstract of Thesis

Summary

Investigations of the *Psuedomonas fluorescens* proteolysis activity was the basal objective of this study. To achieve this objective, 16S rDNA and aprX gene were used in the amplification of DNA extracted from raw milk bacterial isolates. In the present study, the 92 bacterial isolates including 42(cows raw milk isolates) and 50 (buffaloes raw milk isolates) were obtained from 240 cows and buffaloes raw milk samples (120 for each) previously refrigerated for 72 hr. and subjected to phenotypic and molecular identification of *P. fluorescens*. Selective plating ,morphological, biochemical characterization and PCR were done. The results of PCR based aprX gene was similar to biochemical tests concerning the detection ratio of *P. fluorescens* (33.3 %) in cows milk but this ratio differs in case of buffaloes milk as PCR based aprX gene ratio was lower (34%) than biochemical tests detection ratio (42%). There was high significant differences ($p<0.001$) between the three applied methods in case of buffaloes raw milk testing. Also high significant differences ($p< 0.01$) was observed between the results of these three methods in the testing of cows raw milk. The results of phenotypic identification concerning the selective plating revealed that different percent of the *pseudomonas* isolates was recovered from cows and buffaloes milk samples. Triptyc soy agar was more productive for isolation of this bacteria in 35% (cows) and 41.7% (buffaloes)milk samples respectively), compared to *Pseudomonas* f agar (30.8% and 38.3% of cows and buffaloes milk respectively) and Violet Red Bile Glucose Agar (8.3% and 10. 8% of cows and buffaloes milk respectively) .High significant difference ($P<0.01$) was observed among these media concerning their isolation productivity. Distribution of *Pseudomonas* isolates in cows (42) and buffaloes (50) raw milk samples isolates according to age groups, breed (in cows only), Basrah districts and months of sampling was investigated. Concerning the age of tested cows the results of morphological characterization revealed that the higher rate of pseudomonas contamination of raw milk was observed in cows (36.6%) at 1st age group (>1-4years) and of buffaloes (73.9%) at 2nd age group (>4-8year). There was high significant differences ($p<0.01$) between the two age groups of buffaloes. Also significant differences were observed between cows and buffaloes at both age groups ($p< 0.05$). According to the Biochemical characterization results the higher ratio of raw milk pseudomonas contamination was 40 and 47.1% in cows and buffaloes at 2nd age group respectively. significant differences ($p< 0.05$) were observed between cows and buffaloes at both age groups and between buffaloes two age groups. According to Basrah districts ;the highest ratio of cows raw milk *pseudomonas* contamination was found in AL-Qurna (52.9%); the lowest was found in Basrah center (14.3%). In buffalo, the highest ratio of raw milk pseudomonas contamination was found in Abi- Elkhasib (54.1%) and the lowest was in Basrah center (30.8%). The differenc among Basrah districts concerning raw milk *pseudomonas* contamination was not significant ($p>0.05$) Concerning the months of sampling; the highest ratio of cows raw milk *pseudomonas* contamination was found in October, 2014 (52.9%); the lowest was found in January 2015 (14.3%). In buffaloes , the highest ratio of raw milk *pseudomonas* contamination was found in January (54%) and the lowest was in February (30.7%).The differenc among months of sampling concerning raw milk *pseudomonas* contamination was not significant ($p>0.05$) in both cows and buffalo milk. The effect of cows breed on raw milk *pseudomonas* contamination was not significant ($p>0.05$). In morphological characterization, the higher rate of *pseudomonas* contamination of raw milk was observed in native cows (35.6%) while Biochemical Characterization revealed that higher rate of *pseudomonas* contamination of raw milk was observed in (38.5%) of crossbred cows. The molecular detection for presence and proteolysis ability of *P. fluorescens* in cow and buffalo raw milk, was done by the PCR based 16S Rdna (850 -bp) and aprX gene (900-bp) primers. The results revealed that PCR with DNA isolated from cows and buffaloes raw milk bacteria led to one main product of the expected size with each primer pair(16SrDNA and SM2F/SM3R) in (59.5) and (33.3%) of cows raw milk bacterial isolates respectively and in (68) and (34%) of buffaloes raw milk bacterial isolates respectively. The effect of age and breed (cows only) on the PCR amplification results was investigated. The current results revealed that the effect of these factors considered to be not statistically significant ($P>0.05$).According to cow breed 16SrDNA and aprX gene based PCR analysis showed higher ratio of *P. fluorescens* identification (62.5 %) (16SrDNA) and (34.6%) (SM2F/SM3R)} in the native and crossbred cows raw milk respectively. Concerning the effect of age; cows at first age group(>1-4 years) showed higher ratio of 16SrDNA(64.9 %) and aprX gene (35.1%) based PCR concerning presence and proteolysis activity of *P. fluorescens* in raw milk , While buffaloes at second age group(>4-8years) showed higher ratio of 16SrDNA(70.6 %) and SM2F/SM3R (29.4%) based PCR concerning presence and proteolysis activity of *P. fluorescens* in raw milk.