J. Food and Dairy Sci., Mansoura Univ., Vol. 3 (2): 85 - 100, 2012

SEQUENCE ANALYSIS AND CHARACTERIZATION OF *Enterococcus deurans* PLASMID

Gomah, Nanis H.
Dairy Department, Assiut University, Assiut, Egypt

ABSTRACT

More than twenty different fragments of a plasmid pNG (size 7743- 19329 bp) found in *Enterococcus deurans* strain resistant to different antibiotics (cefotaxime CTX, kanamycin K, methicillin MET, streptomycin S) isolated from Egyptian hard cheese were successfully cloned with the blue script vector into E- coli XL1-blue, JM 101. The Sequencing was done for all different colons. Sequencing results were subjected to Bork Group's Advanced BLAST2 search Service at EMBL for making the identification of code regions with data base similarity.The results showed that the plasmid contained different genes identified as identical to hypothetical protein gene produces "hypothetical protein" found in *Entrococcus faecium* plasmid pRUM, GTP-binding protein gene from *Entrococcus faecium* V583 produces "GTP-binding protein", V-type sodium ATP synthase subunit A (EC 3.6.3.15) (Na(+) – translocating ATPase subunit A gene produces "Na+- ATPase alpha and beta subunit ", integral membrane protein gene:"ip-0259 " produces "integral membrane protein" from *Lactobacillus Plantarum* strain WCFS1 , putative RepA gene produces: " putative RepA " ;*Entrococcus faecium* plasmid pRUM, GTP cyclohydroase I from *Lactococcus lactis subsp. Lactis* (strain IL1403) , Protease gene: "sppA"; produces:"protease IV"from *Shigella flexneri 2a* str. 301 and other different genes. The complete sequence of the studied plasmid is now under investigation.
Keywords: *Enteroccus deurans*, plasmid, cloning, sequence analysis.

INTRODUCTION

Bacteria of the genus Enterococcus, or enterococci (formerly the ‘‘faecal’’ or Lancefield group D streptococci) are ubiquitous Gram-positive, catalase-negative cocci that often occur in large numbers in vegetables, plant material, and foods, especially those of animal origin such as dairy products (Franz *et al*., 1999). Enterococci are fit within the general definition of lactic acid bacteria. Modern classification techniques resulted in the transfer of some members of the genus *Streptococcus*, notably some of the Lancefield’s group D streptococci, to the new genus *Enterococcus*. In processed meat, enterococci may survive heat processing and cause spoilage; though in certain cheese types the growth of enterococci contributes to ripening and development of product flavour. Some enterococci of food origin produce bacteriocins that exert anti-*Listeria* activity.

Enterococci are used as probiotics to improve the microbial balance of the intestine, or as a treatment for gastroenteritis in humans and animals (franz *et. al*.1999).

Enterococci have important implications in the dairy industry. They occur as nonstarter lactic acid bacteria (NSLAB) in a variety of cheeses, especially artisan cheeses produced in southern Europe from raw or pasteurised milk, and in natural milk or whey starter cultures. They play an acknowledged role in the development of sensory characteristics during ripening of many cheeses and have been also used as components of

cheese starter cultures. The positive influence of enterococci on cheese seems due to specific biochemical traits such as lipolytic activity, citrate utilisation, and production of aromatic volatile compounds. There is evidence that enterococci, either added as adjunct starters or present as NSLAB, could find potential application in the processing of some fermented dairy products. (Giorgio Giraffa, 2003)

Enterococci were isolated, identified and typed using RAPD-PCR and PFGE (Gelsomino *et al*., 2001). *E. casseliflavus* dominated among the isolates of human faeces, milk and cheese. Genotypic characterisation showed that the same three clones, one of *E. faecalis* and two of *E. casseliflavus*, predominated among almost all of the milk, cheese and human faecesl isolates (Gelsomino *et al*., 2002). These clones were also isolated from bulk tanks and milking equipment. It was concluded that these clones had established themselves on the farm equipment and that this led to contamination of the milk, the curd and the cheese. The presence of these clones in the family's faecal was most likely from their consumption of the cheese. It was clearly demonstrated in that study that cows' faeces were not the source of enterococci in the cheese, because only strains of *E. faecium* and *Streptococcus bovis* were isolated from the cows' faeces (Gelsomino *et al*., 2001, 2002).

Enterococci can grow in the restrictive environment of high salt content and low pH (Ordon˜ez *et al*., 1978).

Because of their role in ripening and flavour development in cheeses, enterococci with desirable technological and metabolic traits have been proposed as part of defined starter cultures for different European cheeses (Litopoulou-Tzanetaki *et al*., 1993).

Enterococci are associated with traditional European cheeses manufactured in Mediterranean countries from raw or pasteurised milk (Ordon˜ez *et al*., 1978).

A benefit of enterococci in cheeses is that many strains produce bacteriocins. Adulteration of cheeses by foodborne pathogens, particularly *Listeria monocytogenes*, has in the past led to severe disease outbreaks. Bacteriocin production by enterococci isolated from dairy products has been investigated. They produce a variety of bacteriocins. Strains producing the broad-spectrum, plasmid-encoded, cyclic bacteriocin, AS-48 (Ga´lvez *et al*., 1986) have been found in raw milk and dairy products (Maisnier-Patin *et al*., 1996). Enterocins or starter cultures containing bacteriocin-producing enterococci have been used in model studies to improve safety of the cheeses (Sulzer and Busse, 1991).

Bacteriocin-like substance E204 is an antimicrobial compound produced by *Enterococcus durans* E204 isolated from camel milk of Morocco that shows a broad spectrum of inhibitory activity against taxonomically related microorganisms (El Quardy Khay *et al*., 2012)

The finding of strains with good acidifying and/or proteolytic properties within *E. faecium* and *E. faecalis* isolated from various cheeses (Wessels *et al*., 1990) and the frequent isolation of enterococci from natural starter cultures used for the manufacture of artisan cheeses (Villani and Coppola, 1994) encouraged some applications of these microorganisms as primary

starter cultures. However, from the early works on Cheddar cheese (Dahlberg and Kosikowski, 1948)

This research aims to identification of different genes encoded on the plasmid of *Enterococcus deurance* isolated from Egyptian hard cheese.

MATERIALS AND METHODS

Enzymes: Restriction endonucleases were purchased from Eurogenetec (Belgium) and New England Bio labs Germany. The enzymes were used under the conditions recommended by the suppliers.

Plasmid isolation from gram positive bacteria: Isolation was Carried out according to NucleoSpin Kit (Mackery & Nagel, Germany) with modification of (Lick and Heller 1998)

Preparation of the plasmid for cloning: (El-Demerdash, 2003)

1- **Plasmid**: 100-µl plasmid DNA+ 20 µl buffer Enzyme+ 5 µl Enzyme+ 7.5 µl TE buffer

Vector: 50 µl vector + 10 µl buffer Enzyme + 2.5 µl Enzyme + 35.5 µl TE buffer

TE buffer: 10 mM Tris / HCL pH 8.0, 1.0mM EDTA,in distilled water.

Three different enzymes were used Cla1, Hind3, EcoR1 and the mixture incubated for 2 (h) at 37°C for digesting.

2- 600 µl yellow buffer (buffer QG solubilization buffer with pH indicator) was added to the mixture and transferred in a violet column for the Q/A quick ^{R} Spin (Q) A Quick Gel Extraction Kit and centrifuged for 15 Sec.

3- Add 700 µl PE buffer (wash buffer) and centrifuge for 15 Sec. discard the supernatant repeat this step to get red of all the Ethanol.

4- Transfer the column in a new 1.5 ml eppendorf and add 25 µl Elution buffer, let the column stand for 2 min and centrifuge for 1 min at the maximum high speed, this step can be repeated.

5- Use 5 µl for running on a gel electrophoresis.

6- Add 40 µl of the digested fragment of the plasmid to10 µl of the digested fragment of the vector which was cut with the same enzyme + 40 µl TE buffer + 10 µl $NaAc_3$ 1 M.

Fast centrifuge then add 250 µl 100% ethanol, the mixture was kept at – 80 °C for 3 h and centrifuged for 30 min at 4°C in a full speed discard, all the ethanol carefully.

7- Add 200µl ethanol 78% and centrifuge for 5 min at 4°C discard all the Ethanol carefully and let the eppendorf open until all the ethanol are evaporated.

8- To the dray DNA add 12.5 µl TE + 1.5 µl ligase buffer (10x+ATP) + 1 µl T4 ligase incubate the solution at the refrigerator over night, use 3 µl for gel running.

9- The rest of the solution must be filtered to clean it from the salt which comes from the buffers by using filter type VS0.025 µm Millipore. Filter summing on TE buffer 1X the ligase mix carefully dropped on the bright surface and leave it stand for 10-15 min. poll the DNA carefully with the pipette.

10- Transformation preparation was done with 50 µl of competent cells from *E-coli* XL1Blue and mixed with the DNA in a chilled micro centrifuged tube.
11- The mixture was transferred to a cold 0.1 cm cuvette, shacked to the bottom, and placed in the Gene pulsar Cuvette holder.
12- One pulse has been done on the cells at 1.25 KV and 25 µF. The pulse controller was set at 2100 Ω the time for the transformation 4-5m sec.
13- The cells were immediately diluted with 1 ml SOC media incubated at 37°C on a slowly shaker for 1 h.
14- Cells were plated on LB agar media + 2ml Am (20mg / ml) + 150 µl X Gal (0.5 g/ ml) + 100 µl IGPT (0.5 g/ ml) with different concentrations: undiluted, 0.1, 0.01
15- Plates were incubated at 37°C overnight and checked for the white colony's plasmid.

The sequencing was done using LI-COR DNA Sequencer 4000 and 4200 series.

Sequencing results was subjected to Bork Group's Advanced BLAST2 search Service at EMBL for making the identification of code regions with data base similarity.

RESULTS AND DISCUSSION

By using λ DNA. Restricted by Stye 1 enzyme as a marker to discover the size of the plasmid it appear that it is in between two pands the highest one equal's 19329 bp and the other is 7743 bp as shown in Figure (1).

λ marker **plasmid**

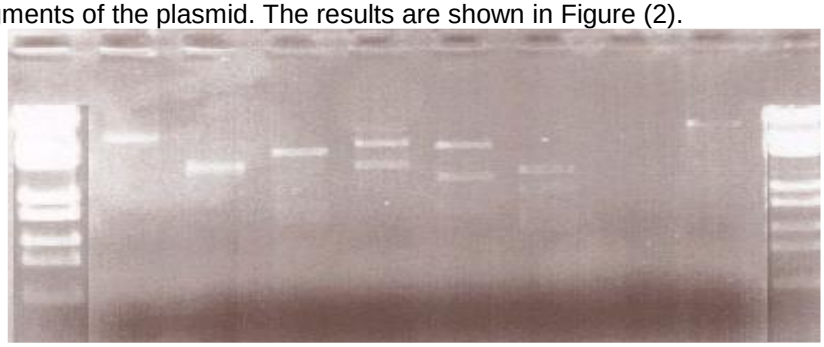
Figure (1): λ marker and gel electrophoresis for plasmid pNG

Restriction enzyme test for the plasmid (PNG):

The plasmid restricted by using different kind of restriction enzymes sty1, Hind 3, sac1, EcoR1, Cla1, Dpn2, Nhe1, Hinp2, Hpa1and Taq1.

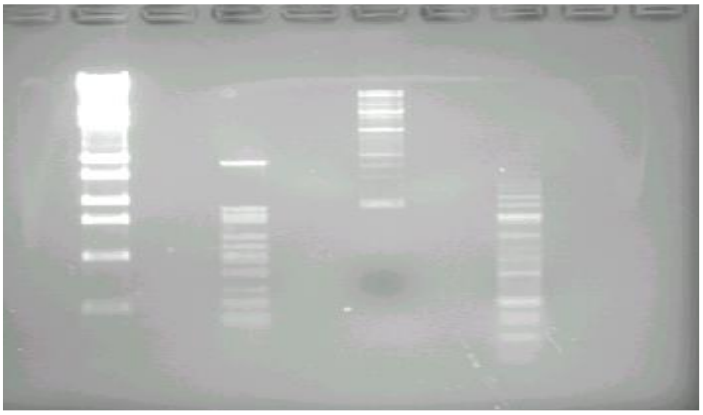
the blue script vector on *E-coli* XL1-blue was also cut by using the same restriction enzymes sty1, Hind 3, sac1, EcoR1, Cla1, Dpn2, Nhe1, Hinp2, Hpa1and Taq1.

The mixture was then loaded into gel electrophoresis. To separate the fragment, the min Elute gel Extraction kit was used and the transformation experiment was repeated three times to get the different fragments of the plasmid. The results are shown in Figure (2).



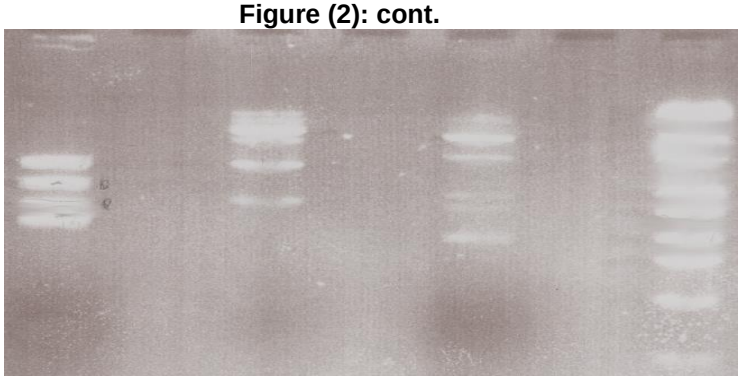
λ-*stay*1 Plasmid sty1 Hind 3 sac1 EcoR1 Cla1 Dpn2 Nhe1 λ-*stay*1

Figure (2): plasmid pNG restricted by different restriction enzymes.



λ-*stay*1 Hinp2 Hpa1 Taq1

Figure (2): cont.



Cla1 EcoR1 Hind3 λ-*stay*1

Figure (2): cont.

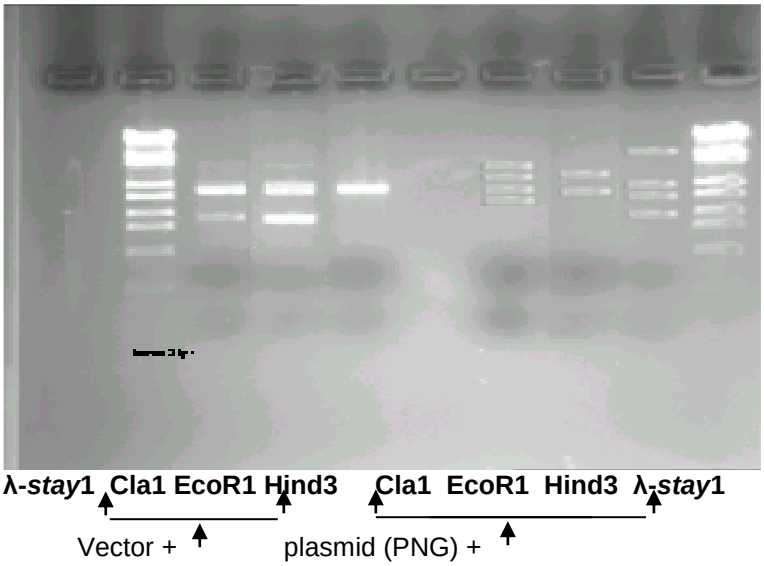


Figure (3): restriction of Vector and pNG plasmid by Cla1, EcoR1 and Hind3 enzymes.

Four different fragments of the plasmid restricted with Cla1 were inserted with the blue script vector into *E. Coli* JM 101 by using Transform Aid ™ Bacterial Transformation Kit the results are described as in the following figures this experiment was repeated with the different restricted enzyme.

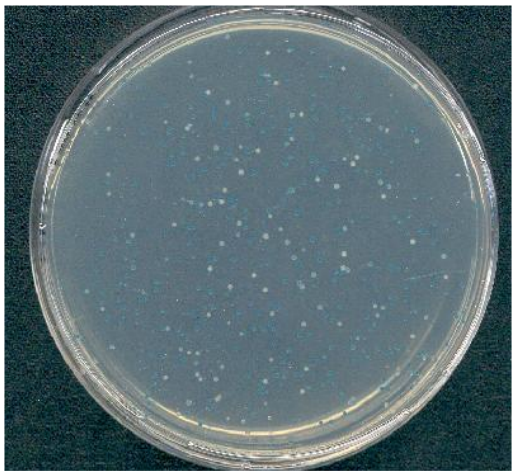


Figure (4): colonies of E-coli XL1-blue Cloned by the plasmid (PNG) with blue script vector

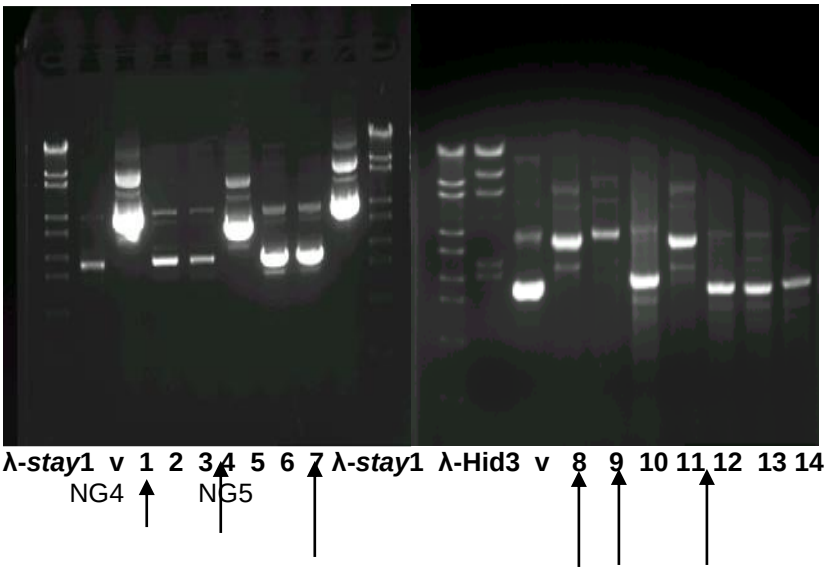
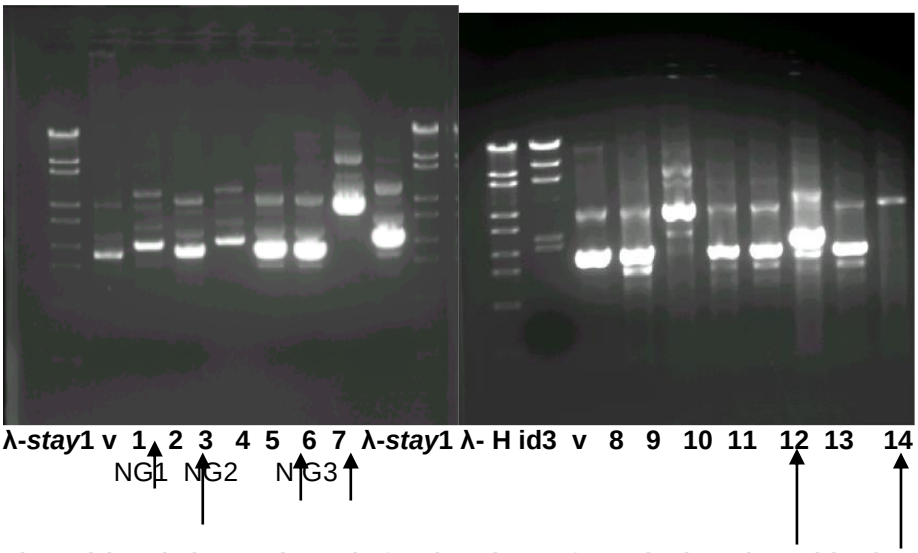


Figure (5): Gel electrophoresis for the clones from the legation with Hind 3.



Figure(6):Gel electrophoresis for the Clones from the legation with Cla 1

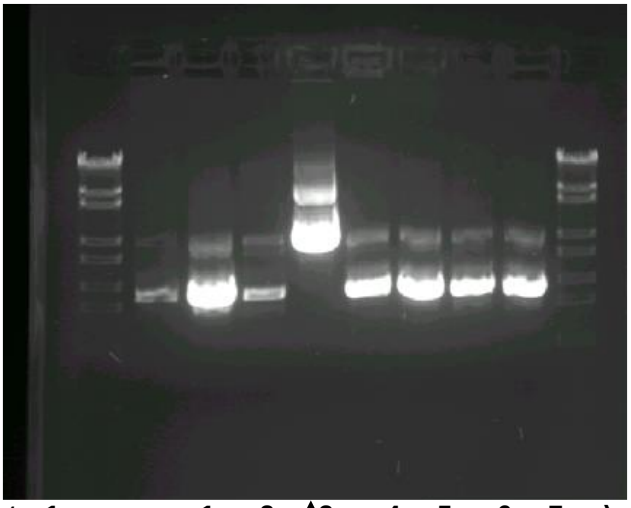


Figure (7): Gel electrophoresis for the clones from the legation with EcoR1.

All the clones were tested by cutting with the same Enzyms which were used previous and the results are presented in figure (8),

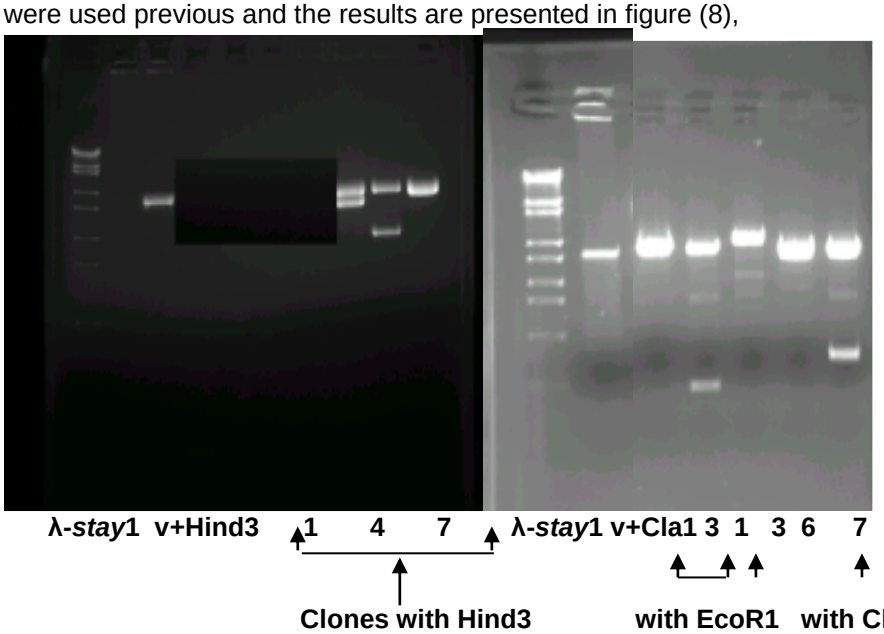


Figure (8): Gel electrophoresis for the clones restricted by the same enzyme which were used for the plasmid pNG (EcoR1 and Cla1)

From this experiment we got five different clones, three of them were from Cla1 enzyme and two from Hind3 enzyme. All clones were given new names as the following :

1 Cla1 = NG1
3 Cla1 = NG2
7 Cla1 = NG3
1 Hind3 = NG4
4 Hind3 = NG5

The experiment was repeated with other types of restriction enzymes, the plasmid and the blue script vector were restricted with Taq1, Hinp2.as follow:

42µl from the DNA fragment + 43 µl TE buffer + 110 µl 10x buffer from Alkalin phosphates + 5 µl Alkaline phosphates. The mixture was incubated for 30 min at 37°C, then at 65°C for 15 min. The DNA was cleaned from the salts by using the yellow buffer and the purple column as described before. The obtained results are shown in the following figures.

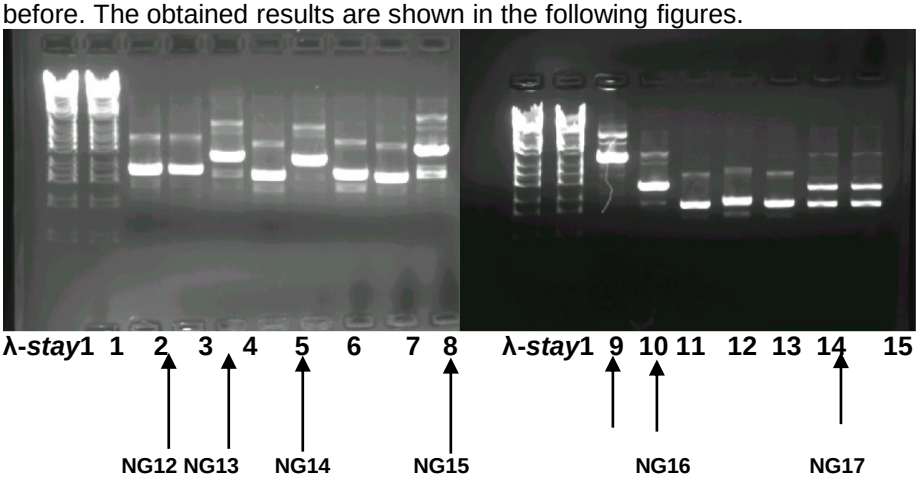


Figure (9):Gel electrophoresis for the clones from the legation with Hinp2

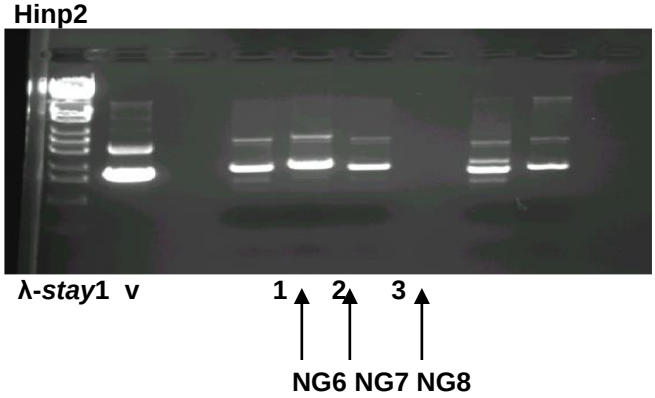


Figure (10): Gel electrophoresis for the clones from the legation with Taq1

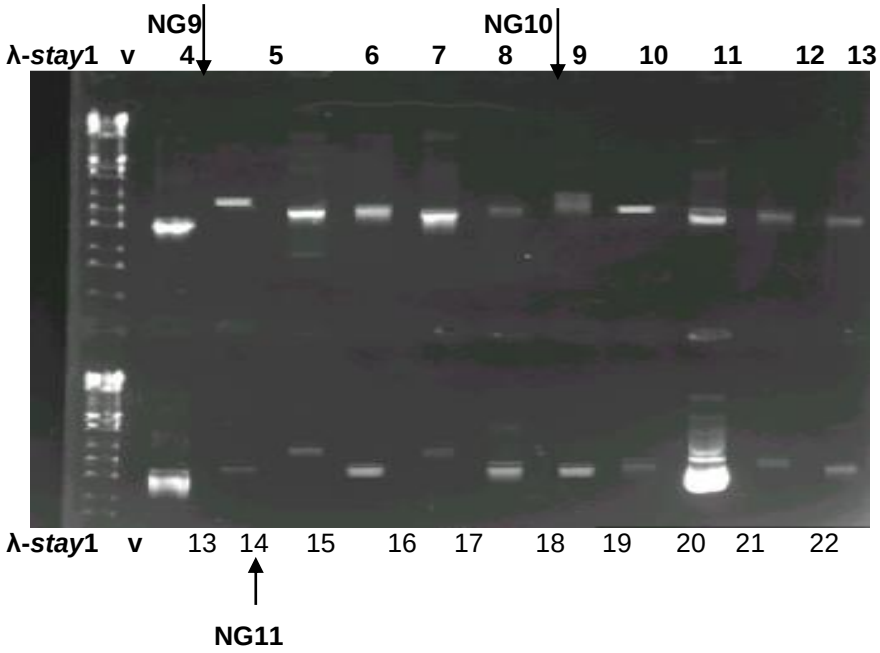


Figure (11): Gel electrophoresis for the clones from the legation with Taq1

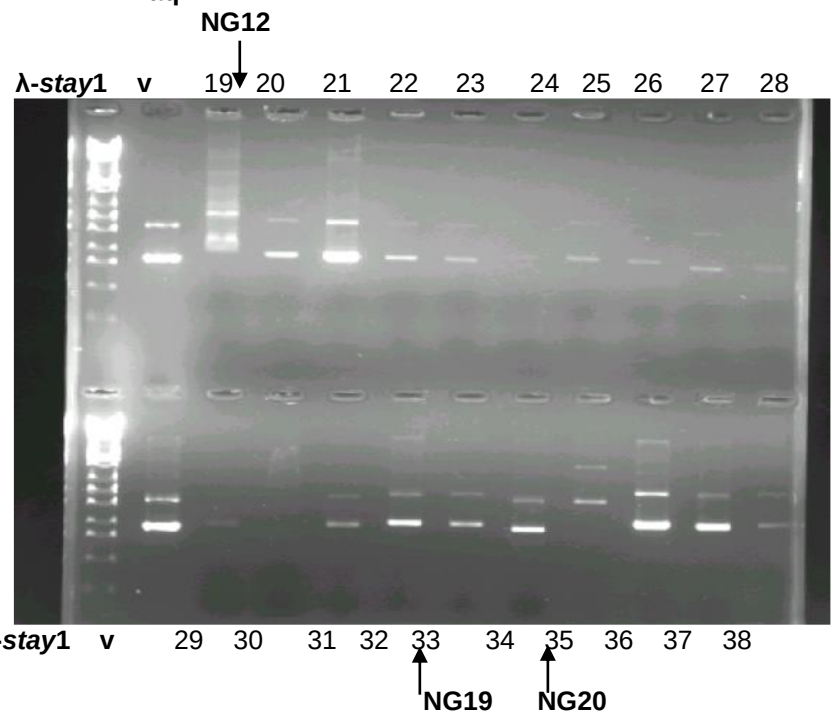


Figure (12): Gel electrophoresis for the clones from the legation with Hinp2

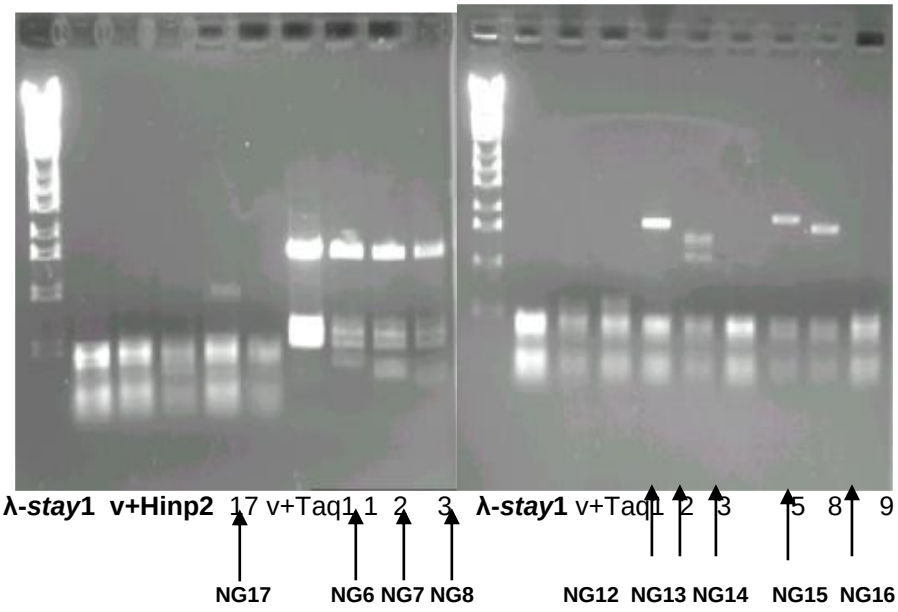


Figure (13): Gel electrophoresis for the clones restricted by the same enzyme which was used for the plasmid pNG (Taq1 and Hinp2).

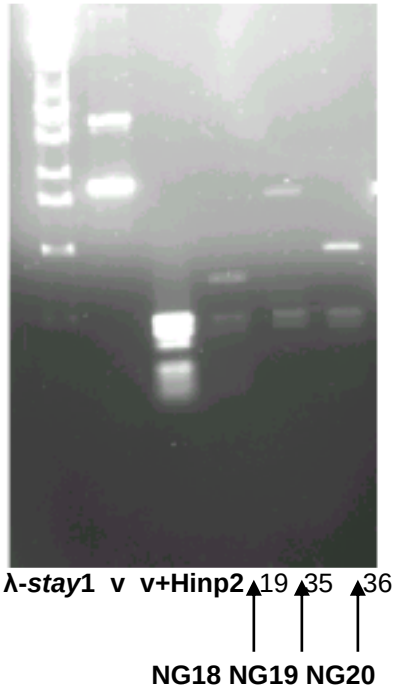


Figure (13): cont.

Most of these clones were sequenced by using the technique of sequencing brochure by MWG-Biotech Europe with the Li – COR DNA sequencer 4000 and 4200 series (sequencing data are not shown)

The obtained sequence analyses of different clones was subjected to Bork Group's Advanced BLAST2 search Service at EMBL for making the identification of code regions with data base similarity. The search resulted on the following encoded genes for the different clones :-

pNG1:-

A part identified (98%) as hypothetical protein.//:tremblnew |AF507977|AF507977_30 gene product: "hypothetical protein";*Entrococcus faecium* plasmid pRUM, complete sequence also anther part as putative GTP-binding protein.//: tremblnew|AE016951|AE016951_260 gene: "EF1527";*Entrococcus faecium* V583, secration 5 of 11 of complete genome product from Streptococcus pyogenes M1 GAS strain SF370 *another part identified as GTP-binding protein from* Enteroococus Faecium.

pNG2:-

97% identified as Protease IV.//:tremble|AE015169|AE015169_5 gene: "sppA"; product:"protease IV"; Shigella flexneri 2a str. 301 secration132 of 412 of the complete genome another part identified as Signal peptide peptidase SppA protein.//:tremble |AB063521|AB063521_107 gene: "sppA",Wigglesworthia brevipapalpisDNA, complete genome, section1/2. product:"signal peptide peptidase SppA (71%) positives.

pNG3:-

90% identities to V-type sodium ATP synthase subunit A (EC 3.6.3.15) (Na(+) – translocating ATPase subunit A gene : “ntpA”; prouduct : ´´Na+- ATPase alpha and beta subunit ``, *Enterococcus. hirae*

Putative heme lyase subunit, cytochrome c-type biogenesis.//:tremble |AE008877|AE008877_18 gene: "ccmH", product:" Putative heme lyase subunit", Salmonella typhimurium LT2, secration 181 of 220 of the complete genome(91%) positives.

pNG4:-

identities to Dihydroperoate synthase (EC 2.5.1.15) gene ´´folP``, product :´´dihydroperoate synthase (EC 2.5.1.15)``; *Lactococcus lactis subsp. lactis* 72%positives.and another identities to FolKE protein gene; product : ´´FolKE protein ``; Lactococcus Lactis subsp. cremoris strain MG1363 folate gene cluster , partial sequence(70%) positives.

pNG5:-

identities to N- formylglutamate amidohydrolase gene.//tremble |AF032970 | AF032970_3 gene : ´´hutG``; product: N- formylglutamate amidohydrolase; *Pseudomonas putida* inducible histidine transporter (hutT)imidazolone propionate hydrolase (hutI), and N-formylglutamate amidohydrolase (hutG)genes, complete cds, and praline iminopeptidase (pip1) gene, partial cds.//:gp |AF032970| 2642341 N-formylglutamata amidohydrolase (50%) positives.

pNG6:-

95% identities to putative Rep A. //: tremblnew |AF507977| AF507977_29 product: "putative RepA", Enterococcus Faecium plasmid pRUM, complete sequence.

pNG7:-

Identités to folypolyglutamate synthase (EC 6.3.2.17) (FOlylpoly-gamma-glutamate synthetase)(FPGS).//tremblnew|z99118|BSUB0015_73 gene: "folC", product:"foly-polyglutamate synthetase", *Bacillus subtilis* complte genome, secration 8/10 (62%) positives.

pNG8 :-

Identities to hypothetical protein ´´ylgG``. //: tremble| AE006347 |AE006347_12 gene:"ylgG"; product : hypothetical protein; *Lactococcus lactis subsp. lactis* IL1403 section 109 of 218 of the complete genome (58%) positives.

another part identities to Putative replication protein RepA.//tremblnew |AF507977|AF507977_29product: "putative RepA", Enterococcus Faecium plasmid pRUM, complete sequence.

backword sequence 80% identities to PutativeRepA product: ´´PutativeRepA``; *Enterococcus faecium* plasmid pRum, complete sequence.

pNG9, pNG10, pNG11, pNG12: are not identifiable.

pNG13:-

identities to putative DNA – invertase.//: tremblnew |AE016843| AE016843_42 gene: ´´t2667``, product: putative DNA – invertase; *Salmonella enterica subsp.enterica serovar typhi* Ty2 section 10 of 16 of the complete genome positives (68%).

pNG14:-

Identities to integral membrane protein.//:tremble |AL935252|AL935252_214gene: "ip_0259", product:: integral membrane protein from *Lactobacillus Plantarum* strain WCFS1 complete genome positives (72%).

another part identities to Deoxynucleoside kinase (EC2.7.1.113) .//:tremble |AE006285|AE006285_1gene: "dukA" ; product: Deoxynucleoside kinase(EC2.7.1.113); from *lactococcus lactis subsp. lactis* IL1403 secration 47 of 218 of the complete genome positives (68%).

pNG15:-

Identities to Transposase .//:tremble|AE002565|AE002565_8 gene :"EP0008"; product Transposase ; *Enterococcus faecalis* plasmid pAM373,complete sequence positives (73%).

Other part 92% identities hypothetical protein.//:tremblnew |AF507977|AF507977_30 gene product: "hypothetical protein";*Entrococcus faecium* plasmid pRUM, complete sequence also anther part as putative GTP-binding protein.//: tremblnew |AE016951|AE016951_260 gene: "EF1527";*Entrococcus faecium* V583, secration 5 of 11 of complete genome product from Streptococcus pyogenes M1 GAS strain SF370.

pNG16:-

Identities to putative secreted proline-rich protein gene:´´SCO0931``; ´´SCM10.19``; product:´´ putative secreted proline-rich protein``; *Streptomyces coelicolor* A3(2)complete genome positives (57%).

pNG18:-

91% identities to putative RepA .//: tremblnew |AF507977 |AF507977_ 29product: "putative RepA", Enterococcus Faecium plasmid pRUM, complete sequence.

Another part identifies to gene : "lacZa", product: "beta-glactosidase alpha peptide" , integration vector pCD11PSK chloramphenicol transacetylase (cat) ans beta-glactosidase alpha peptide (lacZA) genes,completeds. //:tremble|AF178453|AF178453_2 gene: "lacZa", product: "beta-galactosidase alpha peptide" positives(48%)

pNG19:-

54%identities to Transposase Transposase .//: tremble | AE002565 |AE002565_8 gene :"EP0008"; product Transposase ; *Enterococcus faecalis* plasmid pAM373,complete sequence positives (69 %).

pNG20:-

DNA- directed RNA polymerase beta chain (EC2.7.7.6).//: tremble |X95275|PFCOMPIRA_4 gene : "rpoB", Plasmodium falciparum complete gene map of plastid-like DNA (IR-A)//:gp|x75544|XPFRNAPOL_1 gene for beta subunit RNA polymerase positives (52%).

Another part unnamed ORF; sequence 1903 from patent EP1270724.//:gp |AX647711|29802155 unnamed protein product. Homo spiens positives (59 %).

Brigitta Kurenbach et al. (2003) completed the sequence of Enterococcus faecalis plasmid pIP501. The 8629-bp DNA sequence encodes 10 open reading frames (orf), 9 of them are possibly involved in pIP501 conjugative transfer. The putative pIP501 tra gene products show highest similarity to the respective ORFs of the conjugative Enterococcus faecalis plasmids pRE25 and pAMb1, and the Streptococcus pyogenes plasmid pSM19035, respectively. ORF7 and ORF10 encode putative homologues of type IV secretion systems involved in transport of effector molecules from pathogens to host cells and in conjugative plasmid transfer in Gram-negative (G)) bacteria. pIP501 mobilized non-selftransmissible plasmids such as pMV158 between different E. faecalis strains and from E. faecalis to *Bacillus subtilis*.

It was demonstrated that the enterococci contribute to the ripening and aroma development milk products due to their proteolytic and esterolytic activities, as well as the production of diacetyl (Jensen *et al*., 1975; Sarantinopoulous *et al*., 2002).

Acknowledgement: Grateful thanks are attended to Prof. Dr. Knut Heller institute for milk microbiology, Keil, Germany for his support and providing with the enzymes, chemicals and kits**.**

REFERENCES

Dahlberg, A.C., Kosikowsky, F.V., (1948). The development of flavor in American Cheddar cheese made from pasteurized milk with *Streptococcus faecalis* starter. Journal of Dairy Science 31, 275–284.

El-Demerdash, H. A. M. (2003). New Techniques for food biotechnology : development of Food – Grade Vectors for improvement of thermophilic Starter Culture Performance. Ph.D.Thesis, Institute of Microbiology, Kiel, Germany.

El-Quardy Khay, Lorenzo Miguel Pastrana Castro, Paula Fajardo Bernárdez, Nadia Skali Senhaji, Mohamed Idaomar and Jamal Abrini (2012). Growth of *Enterococcus durans* E204 producing bacteriocin-like substance in MRS Broth: Description of the growth and quantification of the bacteriocin-like substance. African Journal of Biotechnology 11, 659-665

Franz, C.M.A.P., Holzapfel, W.H., and Stiles, M.E., (1999). Enterococci at the crossroads of food safety. Int. J. Food Microbiol. 47, 1 –24.

Ga´lvez, A., Maqueda, M., Martı´nez-Bueno, M., Valdivia, E., (1996). The broad spectrum antimicrobial peptide AS-48 from *Enterococcus faecalis*. In: Pandalai, G. (Ed.), Recent Research Developments in Antimicrobial Agents and Chemotherapy. Research Signpost, Trivandrum, India, pp. 135– 147.

Ga´lvez, A., Maqueda, M., Valdivia, E., Quesada, A. and Montoya, E., (1986). Characterization and partial purification of a broad spectrum antibiotic AS-48 produced by *Streptococcus faecalis*. Can. J. Microbiol. 32, 765–771.

Gelsomino, R., Vancanneyt, M., Cogan, T.M., Condon, S. and Swings, J., (2002). Source of enterococci in a farmhouse raw-milk cheese. Appl. Environ. Microbiol. 68, 3560–3565.

Gelsomino, R., Vancanneyt, M., Condon, S., Swings, J. and Cogan, T.M., (2001). Enterococcal diversity in the environment of an Irish Cheddar-type cheese making factory. Int. J. Food Microbiol. 71, 177–188.

Giorgio Giraffa (2003) Functionality of enterococci in dairy products. International Journal of Food Microbiology 88,215– 222

Jensen, J.P., Reinbold, G.W., Washam, C.J. and Vedamuthu, E.R., (1975). Role of enterococci in Cheddar cheese: organoleptic considerations. J. Milk Food Technol. 38, 142–145.

Lick, S and Heller K.J. (1998) Quantitation by PCR of yoghurt starters in model yoghurt produced with a genetically modified streptococcus thermophilus. Milchwissenschaft 53, 671-675

Litopoulou-Tzanetaki, E., Tzanetakis, N. and Vafopoulou-Mastrojiannaki, A., (1993). Effect of type of lactic starter on microbiological, chemical and sensory characteristics of Feta cheese. Food Microbiol. 10, 31– 41.

Maisnier-Patin, S., Forni, E. and Richard, J. (1996). Purification, partial characterisation and mode of action of enterococcin EFS2, an antilisterial bacteriocin produced by a strain of *Enterococcus faecalis* isolated from cheese. Int. J. Food Microbiol. 30, 255– 270.

Ordon˜ez, J.A., Barneto, R. and Ramos, M., (1978). Studies on Manchego cheese ripened in olive oil. Milchwissenschaft 33, 609–612.

Sarantinopoulous, P., Kalantzopoulous, G. and Tsakalidou, E., (2002). Effect of *Enterococcus faecium* on microbiological, physicochemical and sensory characteristics of Greek Feta cheese. Int. J. Food Microbiol. 76, 93– 105.

Sulzer, G. and Busse, M., (1991). Growth inhibition of Listeria spp. On Camembert cheese by bacteria producing inhibitory substances. Int. J. Food Microbiol. 14, 287– 296.

Villani, F. and Coppola, S., (1994). Selection of enterococcal strains for water-buffalo mozzarella cheese manufacture. Ann. Microbiol. Enzimol. 44, 97– 105.

Wessels, D., Jooste, P.J. and Mostert, J.F., (1990). Technologically important characteristics of Enterococcus isolates from milk and dairy products. Int. J. Food Microbiol. 10, 349– 352.

تحليل التتابع الجيني وخصائص البلازميد الخاص ببكتريا انتيروكوكس ديورانس

نانيس حسنين جمعة

قسم الألبان- جامعة أسيوط – أسيوط – جمهورية مصر العربية

اكثر من عشرين شظية من البلازميد ب ن ج حجمه يتراوح بين ٧٧٤٣-١٩٣٢٩ زوج من القواعد النيتروجينيه والذي وجد انة يمتلك مقاومة لمضادات حيوية مختلفة مثل (cefotaxime CTX, kanamycin K, methicillin MET, streptomycin S) والمتواجد في ببكتريا انتيروكوكس ديورانس السابق عزلها من الجبن الجاف المصري قد تم ادخالها بنجاح فى خلايا السلالة اشريشيا كولاى XL1-blue, JM 101 . وقد تم تحليل تتابع التراكيب الوراثية لجميع الخلايا الجديدة المتحصل عليها وبمقارنة نتائج التتابع بقواعد البيانات المتخصصة لتعريف تلك الجينات امكن التعرف علي عدد كبير من الجينات ذات الخصائص الوراثية المفيدة مثل:

Hypothetical protein gene produces "hypothetical protein" found in *Entrococcus faecium* plasmid pRUM, GTP-binding protein gene from *Entrococcus faecium* V583 produces "GTP-binding protein", V-type sodium ATP synthase subunit A (EC 3.6.3.15) (Na(+) – translocating ATPase subunit A gene produces "Na+- ATPase alpha and beta subunit ", integral membrane protein gene:"ip_0259 " produces "integral membrane protein" from *Lactobacillus Plantarum* strain WCFS1 , putative RepA gene produces: " putative RepA " ;*Entrococcus faecium* plasmid pRUM, GTP cyclohydroase I from *Lactococcus lactis subsp. Lactis* (strain IL1403) , Protease gene: "sppA"; produces:"protease IV"from *Shigella flexneri 2a* str. 301

بالاضافة الي جينات اخري مختلفة تم استعراضها خلال البحث ولا زال التتابع الوراثي الكامل للبلازميد تحت الدراسة

قام بتحكيم البحث

أ.د / طه عبد الحليم نصيب	**كلية الزراعة – جامعة المنصورة**
أ.د / على اسماعيل احمد	**كلية الزراعة – جامعة اسيوط**