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Design and construction of an expression plasmid containing mycobacterial ESAT-6, CFP-10 fusion protein cassette

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Abstract:

Background and Objectives *:Mycobacterium tuberculosis*, the causative agent of tuberculosis disease, has infected roughly one third of the world's population. Based on World Health Organization (WHO) reports, around 2 million people die due to this disease annually, necessitating development of an effective, rapid and precise diagnostic strategies to detect tuberculosis. Quantification of interferon-γ which has been secreted by lymphocytes exposed to mycobacterial ESAT-6 and CFP-10 proteins is the basis of a specific diagnostic test to detect latent tuberculosis infection (LTBI). This method is an appropriate replacement for tuberculin skin test (TST) without limitations related to BCG vaccinated individuals. The aim of our study was to create an expression construct which contains genes encoding two specific proteins from *M. tuberculosis*: ESAT-6 and CFP-10. Expressed fusion protein could be applied to design tuberculosis diagnostic kits.

Methods and Materials: Genomic DNA from *Mycobacterium tuberculosis* (H37Rv) was extracted and its concentration was determined. The primers were designed to amplify ESAT-6 , CFP-10 and overlap segment of these two genes. PCR1,2 for ESAT-6 and CFP-10 and finally Overlap PCR were performed. ESAT6-CFP10 fusion segment was cloned in pTZ57T/A cloning vector. pT-ESAT-CFP and PQE-60 plasmids were digested with *BamHI* and *NcoI* and ESAT-CFP DNA fragment and back bone of pQE60 were extracted from agarose gel. Then ESAT-CFP was sub-cloned into pQE60.The resulted colonies were analyzed by PCR, restriction endonuclease digestion and sequencing.

Results: The results of PCR, restriction enzyme digestion and sequencing for PCR products,pTZ clone and final plasmid, confirmed the authenticity of them.

Conclusion: The purified fusion protein that resulted from expression of this construct can be used in diagnostic kit for detecting latent tuberculosis infection (LTBI).

Key words: Latent tuberculosis, Fusion protein ESAT-6, CFP-10, QuantiFERON

Molecular identification and genotyping of *Mycoplasma genitalium* in women with genital infections by PCR-RFLP

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Abstract:

Background and Objectives: *Mycoplasma genitalium* is extremely difficult to be isolated from clinical specimens; thus, it may not be possible to type them on the base on phenotype techniques. However, the availability of complete genome sequence of bacteria has offered an opportunity to approach these goals. The aim of this study was molecular identification and genotyping of *Mycoplasma* using PCR-RFLP in women with genital infections.

Materials & Methods: Genital swabs were taken from 210 patients, who referred to gynecology clinic of Rasool hospital between years 2007 and 2008. Samples were transported to laboratory in PBS buffer. The PCR assays were performed, using a genus specific primer pair and amplified a 465 bp fragment of 16S rRNA. Samples containing bands were subjected to digestion with restriction enzymes (AluI,Taq I, CacI8, BbsI, EcoRI).

Results: Of the 210 genital swabs tested, 11 samples (5.2%) showed positive reactions in PCR for *M. genitalium*, that all showed the same genotype and sequence as strain *Mycoplasma genitalium* G37. It was also understood that only the restriction enzyme CacI8 can cut *M. genitalium* genome and so it can be used as a marker to identify and type the organism.

Conclusion: This study showed that *M. genitalium* can be isolated from genital tracts. It also suggested that there is no heterogeneity between the isolates as genomic enzyme patterns of isolates were the same. This probably means that all infections in individuals were originated from the same ancestor.

Key Words: *Mycoplasma genitalium*, genotyping, PCR-RFLP

Comparison of sugars fermentation in methicillin resistant and sensitive *Staphylococcus aureus* isolates

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Abstract

Background and objectives: The *mec A* gene in *Staphylococcus aureus* leads to production of new penicillin-binding protein called ***PBP2a***. This phenomenon may change some phenotypic properties. The aim of this study was the comparison of sugars fermentation in MRSA (Methicillin resistant *Staphylococcus aureus*) and MSSA (Methicillin sensitive *Staphylococcus aureus*) isolates.

Material and Methods: Total 206 MRSA and MSSA isolates (57 MRSA and 149 MSSA isolates) obtained from 120 inpatients and 86 healthy carriers in the Gorgan were tested. Sugar fermentation carried out in phenol red broth medium, containing glucose, galactose, arabinose, fructose, xylose, rhamnose, mannose, sucrose, trehalose, raffinose or maltose . Change in color after 24 hours incubation at 37°C were recorded. The data were analyzed by SPSS software, using Chi squared test and P<0.05 was considered as meaningful.

Results: The fermentation of glucose at the 4th hour of incubation happened in 10(17.5%) and 11(7.4%) of MRSA and MSSA isolates respectively (P=0.03). The ability ferment rhamnose and xylose in MRSA isolates were 11 (19.3%) and 6 (10.5%), and in MSSA strains were 3 (2%) and 4 (2.7%) respectively. The differences between MRSA and MSSA isolates was statistically significant (P<0.05). The highest difference between *S.aureus* which isolated from patients and carriers were seen in fermentation of fructose [120 (100%) vs 80 (93%)], raffinose [46 (38.3%) vs 2 (2.3%)] and rhamnose [14 (11.7%) vs 0 (0%)] (P<0.005). The ability of *S.aureus* isolates with ability of raffinose fermentation isolated from urinary tract infections was 23 (%56.1), which is significant (P=0.03).

Conclusion: Methicillin resistant in *Staphylococcus aureus* isolates is accompanied with increased ability of sugars fermentation. The ability of sugars fermentation in *Staphylococcus aureus* isolated from patients is generally more than healthy carriers.

Keyword: *Staphylococcus aureus*, fermentation of sugar, MRSA, MSSA

HBV genotype and subtypes in HBV infected patients with and without HIV infection

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Abstract:

Background and Objective: The infections caused by hepatitis B virus (HBV) and HIV are a part of world health complications. Chronic hepatitis B affects nearly 10% of HIV-infected patients. Four million people are estimated to have HBV/HIV co-infection worldwide. The aim of the study was to determine the prevalence of HBV genotypes and subtypes in Iranian HBV infected patients with or without HIV coinfection.

Material and Methods: Sera from 58 Hepatitis B patients including 30(51.7%) with HBV infection and 28 (48.3%) with HIV/HBV co-infection were collected from medical centers in Tehran, Fars and Hormozgan provinces in years 2007-2009. DNA was extracted and surface gene (S region) was amplified by Nested PCR and subjected to sequencing. Phylogenic analysis was done using Mega4 software and drawing phylogenetic tree.

Results: Genotype D was the only genotype detected in both groups of patients.
HBV subtypes observed in patients without HIV coinfection were ayw2 (27.90, 30%), ayw3(1.30, 3/3%) and ayw4 (2.6, 30.7%) while the subtypes found in HBV patients coinfected with HIV were included ayw2 (10.28, 7.35%), ayw3 (11.28, 39.3%) and ayw4 (7.28, 25%). The findings revealed a significant correlation between the types of infection (HBV/HIV co-infection or HBV mono infection) and the subtypes ($P<0.001$).

Conclusion: The individuals With HBV infection that carry ayw4 and ayw3 subtypes are more likely to be infected with HIV.

Keywords: HBV/HIV, HBV, Genotype, Subtype, Iran

Cloning of G subunit's of V-ATPase gene from *Trychophyton rubrum* for use the proteins

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Abstract:

Background & objective: *Trichophyton rubrum* is the most common etiologic agent of chronic dermatophytosis of body in the world. Therapy of this disease is not complete and the disease may relapse after stopping medicine consumption. Recently it is suggested that V-ATPase may work as a target for antifungal drugs development. In this study we cloned G subunit of V-ATPase gene from *T.rubrum* into *E.coli* to produce the relevant protein in high density, save it and then study it.

Material and methods: DNA and RNA was extracted from *Trichophyton rubrum* . Then, the gene was replicated using PCR & RT-PCR. We inserted cDNA in pTG19-T and cloned it in *E.coli* white using hit shock method. Then PCR was done with the same protocol.

Results: DNA has 750 bp and cDNA has 700 bp**.** When E. coli was cultured on Luria agar we found both blue and white colonies and by doing PCR it was shown that the 5 colony of *E.coli* had this gene

Conclusion: It seems the gene V-ATPase has intron. It is also suggests that the white colonies have probably the gene. The product of latest PCR indicated that the cloning was done successfully.

Key words: Cloning, G subunit, V-ATPase, *Trichophyton rubrum*, PCR

Antimicrobial activity of Nisin on *Bacillus cereus* and *Bacillus subtilis* in a food model and their ultra structural investigation

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Abstract:

Background and objectives: Nisin is the only bacteriocin confirmed by the World Health Organization (WHO) and is used as a natural food preservative. Nisin is classified as a safe component. The study was conducted to evaluate the antibacterial effectivness of nisin on *Bacillus subtilis* and *Bacillus cereus* in the commercial barley soup under different temperature conditions. Additionally, using electron microscopy, cellular structures of the concerned bacteria affected by nisin were studied.

Material and Methods: The logarithm number of vegetative form of *Bacillus cereus* and *Bacillus subtilis* treated with different concentrations of Nisin (0.5, 0.25, 0.125 µg/ml) were analysed during 21 days incubation in a commercial barley soup in temperatures 8 and 25 °C. The exams were repeated three times. The cell structure of studied bacteria were analysed under treating with highest concentration of nisin. The logarithm number of bacteria was evaluated under effectiveness of different concentration of nisin, temperature, and the time of incubation using analysis of varians (ANOVA).

Results: The result showed that the growth and survival of the bacteria in barley soup were affected by all nisin concentrations. The bacteria were inhibited at 8 °C greater than to that of 25 °C ($p<0.05$). Pictures of Electron microscopy indicated different degrees of structural damages in cytoplasmic membrane of the bacteria treated with nisin.

Conclusion: Nisin shows antimicrobial activity and inhibit the growth of food-born bacterial pathogens in low temperature.

Key Words: Nisin, *Bacillus cereus*, *Bacillus subtilis*, commercial barley soup

A survey on *Enterococci* contamination of non-pasteurized ice-creams in Ahvaz using reference and impedance-splitting methods and determining the correlation between the methods

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Abstract:

Background and Objectives: Enterococci are known as indicator microorganisms for food safety in many countries. The presence of enterococci in excessive number in foods means undesirable sanitary situation during production and distribution of foods. The aim of this study was to detect enterococcal contamination of non-pasteurized traditional ice-cream during cold and warm seasons with standard reference and impedance-splitting methods and determination the rate of correlation between the two methods.

Material and Methods: 100 samples of non-pasteurized traditional ice-cream during cold and warm seasons were collected from five different areas of Ahvaz and tested for enteroccocci enumeration with both standard reference and impedance-splitting methods. The reference standard method was based on the Standard Institute and Industrial Research (ISIRI) of Iran's recommendation. The impedance method was carried out by measuring the each 10 minutes variation of electrical resistance of selective broth media (M-value) for enteroccocci which used in this method. The rate of concordance and correlation between the two methods was determined using Excel software and the data were analysed using student t-test and Chi squared test.

Results: The result showed that 75 percents of samples in standard method and 79 percent of the samples in impedance-splitting method were contaminated with enteroccocci. Also the minimum and maximum spending time for obtaining results in impedance technique was 6.72 and 22.81 hours respectively which in comparing to conventional standard methods is enormously faster. The rate of correlation between impedance method and the standard reference method in non-pasteurized traditional ice-cream was 87.85 %.

Conclusion: In attention to this fact that impedance-splitting method could examine more samples in shorter time, so this method could be used as a reliable and suitable technique instead of standard conventional methods in quality control of foods.

Key words: *Enterococcus*, Ice-cream, Impedance, Standard reference method, Ahvaz

Evaluation of the survival *Listeria monocytogenes* during the manufacture, ripening, and storage process of probiotic Iranian white cheese

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Abstract

Background and Objectives: The increasing incidence of food borne diseases, coupled with the resultant social and economic implications means there is a constant striving both to produce safer food and to develop new antimicrobial agents. In this regard, the use of bacteriocins or bacteriocin-producing lactic acid bacteria as biological additives has been extensively studied. This study was conducted to evaluation the growth of *Listeria monocytogenes* and survivability of *lactobacillus casei* in Probiotic Iranian white cheese.

Material and Methods: The growth of *L. monocytogenes* and survivability of *lactobacillus casei* were determined by evaluation of the microorganism growth on the selective media (PALCAM Listeria agar, MRS agar) in laboratory in different interval of production and preservation of Probiotic Iranian white cheese.

Results: Results indicated that the cheese with only probiotic bacteria in comparison with other samples had the highest inhibitory effect on the growth of *L. monocytogenes.* The rate of bacteria decreasing at the end of conservation period was 2.96 log more than that of the control group. Besides, the survival of probiotic bacteria in the control cheese was higher than that of cheese with *L. monocytogenes.*

Conclusion: During the ripening of Probiotic Iranian white cheeses the numbers of *L. monocytogenes* decreased but it did not disappear completely. The population of *lactobacillus casei* also decreased gradually during processing and storage of this cheese but not lower than 10^6 cfu/g, which is in concordance with the concentration recommended by food sanitation agencies.

Keywords: Iranian White Cheese, *Listeria monocytogenes*, Probiotic, Starter. *lactobacillus casei*