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"Assessment and Critical Evaluation of Antibiotic Resistance Transferability in Food Chain"

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ABSTRACT: *In the present study a total of five strains were isolated from human faecal samples and microbially and biochemically characterized based on typical selective characteristic of lactic acid bacteria. Systemic procedures were used to evaluate the properties of isolated lactic acid bacteria (LAB) strains. The selection of the isolates was based on their gram positivism non-spore forming rods and catalase negativity, acid and bile tolerance. Only five isolates, which passed their criteria, were selected. These selected five acid and bile tolerant LAB strains produced lactic acid in HPLC. The spent culture supernatants (SCS) of these LAB isolates exhibited high antagonistic effect towards enterotoxigenic pathogen such as E. coli, Staphylococcus aureus and two strains towards Salmonella typhimurium. The LAB isolates were more resistance to commonly used antibiotics amoxicillin, cephalexin and norflaxin than gentamicin. From the above studies, 5 new LAB isolates including one predominated LAB isolate IF4 was additionally identified to species level as Microbacterium flavescens MTCC 8096. It possesses probiotic properties and can be exploited as a potential probiotic strain by further well-controlled experimental in vivo studies.*

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ABSTRACT: The probiotic potential of two environmental isolates of Lactic acid Bacteria (LAB), Lactobacillus plantarum LR/14 and Enterococcus faecium LR/6 was investigated. The said assessment comprised cell surface hydrophobicity, acid and bile tolerance, bile salt hydrolyzing ability, removal of cholesterol and the production of antimicrobial compounds. Moreover, the survival of these strains in simulated stomach condition with and without the presence of antacids was also looked into. The strain L. plantarum LR/14 showed wide range of probiotic properties and appears to be a good candidate for in vivo testing. E. faecium LR/6, in comparison, was less promising but showed probiotic qualities in terms of acid and bile tolerance. Both the strains showed a good survival in simulated stomach conditions especially in the presence of antacids.

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207-212 A TWO SPECIES-SPECIFIC PRIMER SET TO IDENTIFY *LACTOBACILLUS RHAMNOSUS* GR-1 AND *LACTOBACILLUS REUTERI* RC-14 AFTER PROBIOTIC USE

KC Anukam and G Reid

ABSTRACT: The use of universal primers especially LGC-1, and LGC-2 GC specific for molecular identification and differentiation of Lactobacillus species has been shown to be unreliable, failing to detect many species. The aim of this study was to determine whether it was possible to design a primer that could identify both probiotic L. rhamnosus GR-1 and L. reuteri RC-14 in human samples. DNA was extracted from Lactobacillus GR-1 and RC-14. Two raw sequences were aligned using Clustal W 1.81 software multiple sequence alignment program. Using the PRIMER3 version 3.2 software, primers showed significant alignment to the sequences of the GR-1 and RC-14. The primer was chosen for PCR while the LGC-1 and LGC-2-GC primers served as control. SYBR Green supermix Real-Time PCR was used to quantify the DNA amplification. To test the clinical veracity of the primers, 66 vaginal swabs from women who took this probiotic were analyzed. The amplicons were visualized in a 1.5% agarose gel electrophoresis. The primer set was able to amplify Lactobacillus RC-14 and GR-1 together. Of 14 other Lactobacillus species including L rhamnosus ATCC 7469, only L reuteri ATCC 23272 was amplified. The LGC-1 and LGC-2-GC primers amplified all the samples including GR-1 and RC-14. The clinical vaginal swabs showed the presence of either GR-1 or RC-14 in 30.3% (20/66) of samples. This is the first report showing the use of one primer set for the detection of two Lactobacillus species at a human site. The method provides a means to track the organisms during human studies, without using the universal Lactobacillus primers and labor intensive DGGE.

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213-218 HEALTH STATUS OF BALB/C MICE ORALLY FED WITH *BIFIDOBACTERIUM PSEUDOCATENULATUM* G4

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ABSTRACT: Safety profiles of Bifidobacterium pseudocatenulatum G4 and commercial Bifidobacterium longum B536 were evaluated. Groups of BALB/c mice were orally administered sterile skim milk suspensions containing viable B. pseudocatenulatum G4 at 2 x 10^4, 1 x 10^8, or 1 x 10^{11} CFU/day and reference B. longum BB536 at 1 x 10^8 CFU/day for four weeks. None Bifidobacterium supplemented was used as control. No abnormal clinical signs were revealed during the assessment. There were no noticeable differences in food intake; water intake and weight gain between treatment groups. Feeding

with strain G4 did not cause any changes in blood biochemistry (Albumin, Glucose, Cholestrol, and Total protein) or haematological (Red blood cell (RBC), Protein cell volume (PCV), haemoglobin, Mean corpuscular volume (MCV), Mean corpuscular haemoglobin concentration (MCHC), White blood cell (WBC), Neutrophils, Lymphocytes, Monocytes, and Eosinophils) measurements. Hence, this strain of B. pseudocatenulatum evaluated during this study; did not adversely affect the health of the mice and is likely to share the safe status of probiotic bacterium for future application.

International Journal of Probiotics & Prebiotics 3(4): 219-230

219-230 NATURAL ANTIMICROBIALS AND THEIR ROLE IN VAGINAL HEALTH: A SHORT REVIEW S. E. Dover, A. A. Aroutcheva, S. Faro and M. L. Chikindas

ABSTRACT: *Lactobacillus species maintain the vaginal ecosystem in a healthy condition by production of antimicrobial substances. Depletion of lactobacilli in the vagina results in bacterial vaginosis (BV), where the normal flora is replaced by several bacterial pathogens, usually Gardnerella vaginalis and obligate anaerobes. BV may cause complications such as premature labor, low birth weight and increased risk of HIV acquisition. The currently recommended antibiotic treatments for BV are not always effective and often lead to reoccurrence of the infection. In many cases, this is due to the antibiotic-resistant forms of the pathogens. Therefore, there is an interest in the development of treatments using antimicrobials derived primarily from Lactobacillus spp., such as ribosomally produced antimicrobial peptides (bacteriocins) and lactic acid. These substances effectively inhibit pathogenic bacteria, are safe and do not pose any threat to healthy vaginal Lactobacillus spp. It may be possible to find an effective treatment against BV while reducing the infection's reoccurrence and the treatment-related complications through hurdle technology. This would be achieved by combining antimicrobials produced by Lactobacillus spp. with different natural antimicrobials obtained from plants or other non-pathogenic organisms.*

International Journal of Probiotics & Prebiotics 3(4): 231-238

231-238 ZYACTINASE STIMULATES THE PROBIOTIC GUT MICROFLORA WHILST INHIBITING PATHOGENIC MICROFLORA
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ABSTRACT: *Zyactinase a freeze-dried extract of Kiwifruit (Actinidia deliciosa) has been developed as a constipation relief product as well as for long-term gut health. Zyactinase contains a protease complex, fiber, pectin and fructo-oligosaccharides. Clinical Studies have proven that Zyactinase stimulates increased bowel movements and relieves the symptoms of irritable bowel syndrome. This was proposed to be partially due to the stimulation of the gut microflora. To investigate this, the effect of Zyactinase on the growth of the probiotic bacteria Lactobacillus reuteri, Lactobacillus acidophilus, Pediococcus acidilactici, and Lactobacillus planetarium was tested in vitro. Zyactinase was found to significantly increase the growth of these probiotic bacteria, in comparison to isomalt. Zyactinase was also tested in vitro against the pathogenic bacteria E. coli, Salmonella typhimurium, Staphylococcus aureus and Listeria monocytogenes. Zyactinase was found to significantly inhibit the growth of E.coli and Salmonella typhimurium with almost significant inhibition of Staphylococcus aureus. However, Zyactinase stimulated the growth of Listeria monocytogenes. Heating of the extract to deactivate the protease complex had no effect on the efficacy of Zyactinase to stimulate probiotic bacteria growth whilst inhibiting pathogenic bacterial growth. Therefore, it is likely that the probiotic-stimulating efficacy of Zyactinase originates from the fructo-oligosaccharides and pectin within Zyactinase.*

International Journal of Probiotics & Prebiotics 3(4): 241-246

241-246 INTRODUCING THE ACE-ART PROJECT: FROM THE EXPRESSION OF INTEREST TO 42 MONTHS OF RESEARCH
L. Morelli

ABSTRACT: *Despite concern that the use of antibiotics in the food chain contributes to the development of resistant bacteria, research has yet to provide the data necessary for the development of an effective risk management strategy. Risk assessment of antibiotic resistant, nonpathogenic bacteria present in the food chain requires data on the sources of these bacteria, their genetic composition and potential for resistance transfer. The European Union funded project named Assessment and Critical Evaluation of Antibiotic Resistance Transferability in Food Chain (ACE-ART) has addressed this problem; this project has been focused on non-pathogenic bacteria belonging to Lactobacillus, Bifidobacterium, Lactococcus and Streptococcus thermophilus, as they are the starter cultures for fermented foods. Within this project the importance of these bacteria as a source of antibiotic resistance genes has been assessed. The project has also examined the genetic basis supporting the phenotypic resistance as well as the potential of their horizontal transfer. An industrial platform provide by members of the European Food and Feed Cultures Association (EFFCA) have provided the link with the industrial world. Results achieved have been used by the European Food Safety Authority and by ISO to improve the approach to the risk management of these bacteria.*

International Journal of Probiotics & Prebiotics 3(4): 247-248

247-248 THE DEVELOPMENT OF AN INTERNATIONAL ISO/IDF STANDRD OR SUSCEPTIBILITY TESTING OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA BASED ON CONTRIBUTIONS FROM PROSAFE AND ACE ART
M. Danielsen and J. Seifer

ABSTRACT: *A working group under the International Dairy Federation (IDF) has been working on an international ISO/IDF standard on antibiotic susceptibility testing of lactic acid bacteria and bifidobacteria. The work was based upon the results achieved in the two EU-projects PROSAFE and ACE-ART. The background for this work, the organization of it within IDF, and the process of it are described in this paper.*

International Journal of Probiotics & Prebiotics 3(4): 249-256

249-256 RESISTANCE-SUSCEPTIBILITY PROFILES OF *LACTOCOCCUS LACTIS* AND *STREPTOCOCCUS THERMOPHILUS* STRAINS TO EIGHT ANTIBIOTICS AND PROPOSITION OF NEW CUT-OFFS

Ana Belén Flórez, Lorenzo Tosi, Morten Danielsen, Atte von Wright, Jacek Bardowski, Lorenzo Morelli, and Baltasar Mayo

ABSTRACT: *The minimum inhibitory concentration (MIC) of ampicillin, chloramphenicol, clindamycin, erythromycin, gentamicin, streptomycin, tetracycline, and vancomycin was determined in 89 different Lactococcus lactis and in 64 Streptococcus thermophilus strains in order to establish resistance-susceptibility cut-off values in these dairy important organisms. Cut-offs were defined on the basis of the distribution of the MICs, which in the absence of acquired determinants should approach to a normal statistical distribution. In general, the new cut-off values proposed in this study are higher than previously defined (European Commission, 2005. The EFSA Journal 223, 1-12). Based on these new values, all tested strains were either susceptible (ampicillin, chloramphenicol, gentamicine, and vancomycin) or intrinsically resistant (streptomycin) to most antibiotics. However, 11 L. lactis strains (around 7%) (Six strains have been previously selected as tetracycline resistant among 500 isolates) were considered resistant to tetracycline, and 8 S. thermophilus strains (around 11%) were considered resistant to tetracycline, erythromycin or clindamycin. Of these, three S. thermophilus strains proved to be resistant to both tetracycline and erythromycin, and a further strain resistant to tetracycline, erythromycin and clindamycin. By PCR, tet (M) and tet (S) genes were amplified from the L. lactis tetracycline resistant strains. An erm (B) was identified as the genetic baseis of erythromycin resistance in all four S. thermophilus strains.*

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257-268 ANTIMICROBIAL SUSCEPTIBILITY AND PROPOSED MICROBIOLOGICAL CUT-OFF VALUES OF LACTOBACILLI BY PHENOTYPIC DETERMINATION

J.M. Korhonen, M. Danielsen, B. Mayo, M. Egervärn, L. Axelsson, G. Huys. and A von Wright

ABSTRACT: *This article summarizes published and unpublished data of antibiotic resistances in strains (n = 675) of fourteen Lactobacillus species. Minimum inhibitory concentrations (MICs) were determined against antibiotics representing different classes, namely ampicillin (penicillins), tetracycline (tetracyclines), gentamicin and streptomycin (aminoglycosides), and erythromycin and clindamycin (macrolide-lincosamide-and streptogramin B group, MLS_B).The Lactobacillus species under study included the L. delbrueckii group (n = 218), L. plantarum (n = 121), L. sakei (n = 83), L. rhamnosus (n = 75), L. paracasei (n = 66, including one L. casei), L. reuteri (n = 56) and L. fermentum (n = 56). The strains were of human, animal and food origin. The MICs were determined with microdilution and/or E-test methods using LSM (Lactic acid bacteria susceptibility test medium) agar and broth. In general, the results obtained with the microdilution method were one or two dilution steps higher than with the E-test. Accordingly, tentative microbiological cut-off values are given separately according to the method and species used. A vast majority of the strains were phenotypically susceptible to ampicillin and erythromycin, whereas many species showed a wide range of MICs for tetracycline. A broad range in distribution of MICs was also observed with aminoglycosides and clindamycin, especially in L. plantarum.*

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269-270 SUSCEPTIBILITY OF BIFIDOBACTERIA ORIGINATING FROM DIFFERENT SOURCES TO TETRACYCLINE, ERYTHROMYCIN, STREPTOMYCIN AND VANCOMYCIN

M. Saarela, S. Mayrhofer, K. J. Domig, M. Danielsen, B. Mayo and J. Mättö

ABSTRACT *: MIC distributions of 365 Bifidobacterium spp. strains were determined for tetracycline, erythromycin, streptomycin and vancomycin. Susceptibility testing was performed either by Etest or broth microdilution (MDIL) using VetMIC panels. The tetracycline susceptible wild type population isolates of the human/probiotic Bifidobacterium spp., tested by Etest, had MICs ≤ 1µg/ml (B. adolescentis group, B. bifidum, B. catenulatum) or ≤ 4 µg/ml (B. longum group). All tetracycline susceptible wild type populations of the animal bifidobacteria, tested by broth microdilution, had MICs ≤ 4 µg/ml (B. pseudolongum) or ≤ 8 µg/ml (B. thermophilum). Acquired resistance to erythromycin was rare in bifidobacteria. B. adolescentis group, B. bifidum and B. pseudolongum showed a unimodal distribution consisting of only susceptible wild type population (cut-offs ≤ 0.25 µg/ml, ≤ 0.12 µg/ml, and ≤ 0.5 µg/ml, respectively). Eleven potentially erythromycin resistant strains representing B. animalis, B. longum group, B. catenulatum group and B. thermophilum were detected (cut-off for the groups being ≤ 0.25 µg/ml, ≤ 0.25 µg/ml, ≤ 0.25 µg/ml, and ≤ 1 µg/ml, respectively). All studied Bifidobacterium species/groups were intrinsically resistant to streptomycin and susceptible to vancomycin (cut-offs for B. adolescentis group, B. catenulatum group, B. animalis strains being ≤ 1 µg/ml, for B. longum group and B. bifidum ≤ 2 µg/ml, and for B. thermophilum, B.pseudolongum ≤ 1 µg/ml vancomycin).*

International Journal of Probiotics & Prebiotics 3(4): 271-280

271-280 MOLECULAR ASSESSMENT OF ERYTHROMYCIN AND TETRACYCLINE ANTIBIOTIC RESISTANCE GENES IN LACTIC ACID BACTERIA AND BIFIDOBACTERIA AND THEIR RELATION TO THE OBSERVED RESISTANCE

Angela H. A. M. van Hoek, Abelardo Margolles, Konrad J. Domig, Jenni M. Korhonen, Joanna Życka-Krzesińska, Jacek K. Bardowski, Morten Danielsen, Geert Huys, L. Morelli and Henk J. M. Aarts

ABSTRACT: This paper describes the molecular assessment of antibiotic resistance (AR) determinants in a large collection of Lactic Acid Bacteria (LAB) and bifidobacterial strains isolated from various sources and multiple decades. The investigations were focused on the most frequently identified resistances in the genera studied, i.e. erythromycin and tetracycline. In total 367 isolates were analyzed by various tools and the most commonly identified antibiotic resistance gene was tet(W), encoding for a tetracycline ribosomal protection protein. Furthermore, tet(M) and tet(S) besides the erythromycin resistance determinant erm(B) were also frequently characterized. However, some AR genes were demonstrated for the first time in the LAB and bifidobacteria investigated e.g. erm(X) in Bifidobacterium sp., mosaic tet genes in Bifidobacterium thermophilum and Lactobacillus johnsonii and erm(A) in Lactobacillus amylovorus. Multiple tet determinants, in various pairs, were found in several individual bacteria, but unique results were obtained analyzing two Lb. johnsonii strains both containing tet(L), tet(M) and tet(W) and one Bifidobacterium thermophilum harboring tet(L) and 2 different mosaic tet genes. The absence and/or presence of the erythromycin and tetracycline resistance genes will be discussed in relation to the displayed phenotype of the isolates

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281-286 THE ACE-ART DATABASE – ANTIBIOTIC SUSCEPTIBILITY OF LACTIC ACID BACTERIA AND BIFIDOBACTERIA IN RELATION TO THEIR BIOLOGICAL, GEOGRAPHICAL AND CHRONOLOGICAL ORIGIN

Konrad J. Domig, Sigrid Mayrhofer, Geert Huys, Lorenzo Tosi, Morten Danielsen, Baltasar Mayo, Lars Axelsson, Jenni M. Korhonen, Maria Egervärn, Jacek K. Bardowski, Maria Saarela, Lorenzo Morelli and Wolfgang Kneifel

ABSTRACT: *Within the EU funded project ACE-ART antibiotic susceptibility data of Lactobacillus, Lactococcus, Bifidobacterium and S. thermophilus strains isolated from different habitats were analysed, taking into consideration different time periods and from geographically distant regions. Besides the phenotypic data also the genetic basis of antibiotic resistance phenomena in selected strains and the possible horizontal transfer were examined. Due to the high number of strains, the multiple data sets and the necessity to share information with the project partners, a data sharing tool was implemented to accomplish further analysis (e.g. molecular biological analysis, gene transfer experiments) during the project. In addition, the prevalence of resistant strains in certain habitats was analysed. This paper reports data base related deliverables and complements the publications with species specific antibiotic susceptibility data and therefore contributes to the critical evaluation of the role of antibiotics in the feed and food chain and its impact on the level of antibiotic resistance in related bacteria.*