Current Microbiology (2021) 78:2839–2849
https://doi.org/10.1007/s00284-021-02554-1

REVIEW ARTICLE

# Conjugal Transfer of Antibiotic Resistances in *Lactobacillus* spp.

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Received: 8 October 2020 / Accepted: 26 May 2021 / Published online: 2 June 2021


**Abstract**
Lactic acid bacteria (LAB) are a heterogeneous group of bacteria which are Gram-positive, facultative anaerobes and non-motile, non-spore forming, with varied shapes from cocci to coccobacilli and bacilli. *Lactobacillus* is the largest and most widely used bacterial species amongst LAB in fermented foods and beverages. The genus is a common member of human gut microbiome. Several species are known to provide benefits to the human gut via synergistic interactions with the gut microbiome and their ability to survive the gut environment. This ability to confer positive health effects provide them a status of generally recognized as safe (GRAS) microorganisms. Due to their various beneficial characteristics, other factors such as their resistance acquisition were overlooked. Overuse of antibiotics has made certain bacteria develop resistance against these drugs. Antibiotic resistance was found to be acquired mainly through conjugation which is a type of lateral gene transfer. Several in vitro methods of conjugation have been discussed previously depending on their success to transfer resistance. In this review, we have addressed methods that are employed to study the transfer of resistance genes using the conjugation phenomenon in lactobacilli.

## Introduction

Being the largest genus amongst the lactic acid bacteria (LAB) group, *Lactobacillus* is extensively used in many fermented foods such as fermented meats and fermented dairy products. These bacteria are Gram-positive, varying from long to short rods, sometimes curved appearance, sometimes coryneform coco-bacilli arranged in chains commonly without any endospore forming ability [1]. Till now about 237 species and 29 subspecies of this genus have been identified (http://www.bacterio.net/lactobacillus.html). *Lactobacillus* and other genera are also known to be a part of human gut microbiome [2]. As per culture independent DNA sequence analysis of distal gut, autochthonous *Lactobacillus* is considered to be at most 1% of the total bacterial population [3]. Various strains of *Lactobacillus* are used for improving digestion, and absorption of nutrients in humans and livestock [4, 5]. Members of the group have been given the generally recognized as safe (GRAS) status by the American FDA (Food and Drug Administration) due to frequent occurrence in fermented food [6].

Over the years the focus has been on the beneficial aspects of the probiotics due to their abundance in fermented foods, but the transmission of antibiotic resistance has been less considered [7]. Researchers have now shed light on the potential risks associated with antibiotic resistance gene transfer during continuous consumption of probiotics [8–10]. In recent times lactobacilli have been successfully used as probiotics and starters in industrial and agricultural applications. Probiotics are live microorganisms when consumed in a definite concentration confer a health benefit [11–14]. For any organism to be considered as probiotic certain in vitro and in vivo studies need to be undertaken [15, 16]. Each probiotic organism also needs to fulfill biosafety criteria including the absence of transferable antibiotic resistance elements due to their close proximity with other organisms in food chain as well as gastrointestinal tract where horizontal gene transfer can occur [17–21]. Therefore, the European Food Safety Authority (EFSA) has framed certain criteria with regards to the safety assessment of microbes used in fermented food. These are also known as qualified presumption of safety (QPS) where these

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microorganisms must meet all the condition to be considered safe for consumption [22–24].

Studies have revealed that the threat of resistance genes transfer by probiotics largely occur in countries with lenient guidelines and regulations [25]. According to EFSA, bacterial strains which have the ability to transfer the antibiotic resistance genes should be restricted for use in animal feeds and fermented foods. Thus non judicious use in animal feeds and veterinary applications could be hazardous [26, 27]. Antibiotic resistance in bacterial pathogens has been very well-studied and reported [28–30]. In the past decade researchers have started to focus on characterizing resistance mechanism employed by *Lactobacillus* [31–35]. Dissemination of intrinsic resistance and resistance through mutational gene variation are rarely seen but risk associated with horizontally transferred genes (acquired resistance) which are present in mobile genetic elements can bring about the acquisition of resistance genes by bacterial conjugation [36]. Therefore, antibiotic resistance acquired due to horizontal gene transfer in lactobacilli from food sources has become a matter of concern [37].

Many species of *Lactobacillus* are intrinsically resistant to aminoglycosides (gentamicin, kanamycin streptomycin and neomycin), quinolones (ciprofloxacin, norfloxacin and nalidixic acid) and nucleic acid inhibitor (trimethoprim) [21, 38]. Some are also naturally resistant to other antibiotics like vancomycin, bacitracin, cefoxitin, metronidazole and nitrofurantoin [39]. Acquired resistance to tetracycline, erythromycin, clindamycin and chloramphenicol has been associated with resistance through conjugative transfer mechanisms [40]. Since resistance through conjugative gene transfer is rarely reported in lactobacilli harbouring intrinsic gene of antibiotic drug resistance. But, resistant lactobacilli with acquired resistance genes may pose risk of transfer through conjugative means.

Conjugation is one of the prevalent and most significant methods of horizontal gene transfer adopted by species of *Lactobacillus*. Here the transfer of a resistance gene occurs through physical mating between two bacteria with the help of a conjugation pilus [41, 42].These genes could exist on mobile genetic elements like plasmids, transposons, insertion sequences and introns [43]. Species of *Lactobacillus* were found to be harboring the genes encoding antibiotic resistance against tetracycline: tet(M), tet(K) and tet(W) and erythromycin erm(B), erm(C) and erm(G) [21]. Since lactobacilli are potential reservoirs of antibiotic resistance genes, transfer to bacterial pathogens dwelling in food matrices or in GI environment is worrisome [44, 45].

Thus to study this (conjugation) method of transfer, in vitro studies have been carried out extensively by several group of investigators due to animal ethics associated with in vivoevaluation. However, in order to understand the resistance gene transfer conjugally in natural conditions many researchers have observed the role of *Lactobacillus* in transferring genes responsible for resistance to tetracycline, erythromycin, chloramphenicol and vancomycin [32, 33, 46–49]. Study of transfer of plasmid encoded vancomycin resistance gene between strains of a LAB group of *Enterococcus faecium* and *Lactobacillus acidophilus* in mice was performed successfully in in vivo conditions [50]. The transfer of resistance genes by some strains of *Lactobacillus* to pathogenic bacteria in the food or GI environment is alarming to food industries which incites them to ensure the safe use of these bacteria.

An unambiguous review is needed to club all experimental methods employed to observe the conjugal gene transfer from lactobacilli to other group of bacteria. In this review, different approaches (in vitro, in vivo, and in situ) undertaken by researchers to study conjugal transfer of antibiotic resistance genes in food organism, *Lactobacillus* are discussed. This review focuses not only the various methods used to study conjugal gene transfer in lactobacilli but also provides an insight into factors responsible for the success rate of transfer in species of lactobacilli. To best of our knowledge, none of the available literatures had discussed all these three methods of conjugal transfer e.g., in vitro, in vivo and in situ in lactobacilli.

## Mechanisms of Antibiotic Resistance in *Lactobacillus*

*Lactobacillus* adopts very sophisticated mechanisms to develop resistance against antibiotics. They can evade the effect of antibiotics by modifying their receptors without altering the antibiotic compound (passive effect) or modification of the compound by enzymes (active effect) [40, 41]. *Lactobacillus* group has transferrable antibiotic resistance genes against certain antibiotics which behave as carriers of such genes and confer these resistances to others which are not resistant. When these groups are exposed to antibiotics, they are compelled to develop mechanisms which make them resistant to these antimicrobial compounds. The mechanisms involved in antibiotic resistance development in bacteria have been discussed earlier [51]. The three main mechanisms involved are: (1) modification of the antibiotic by enzymatic complexes that prevent the antibiotic-target interaction, (2) degradation of intra or extracellular antibiotics by enzymatic action, and (3) the activation of flow pumps (such as efflux pumps) or change in the cell wall permeability leading to reduction in the antibiotic concentration inside the cell (Fig. 1).

Multidrug resistant (MDR) efflux pump has been identified as the major mechanism responsible for resistance in lactobacilli [52, 53]. In certain strains of *L. plantarum*, the ABC type transporter protein (*lmr*A gene) which is chromosomally encoded is responsible for multi drug resistance in these strains. Whereas, strains of *L. pentosus* possess

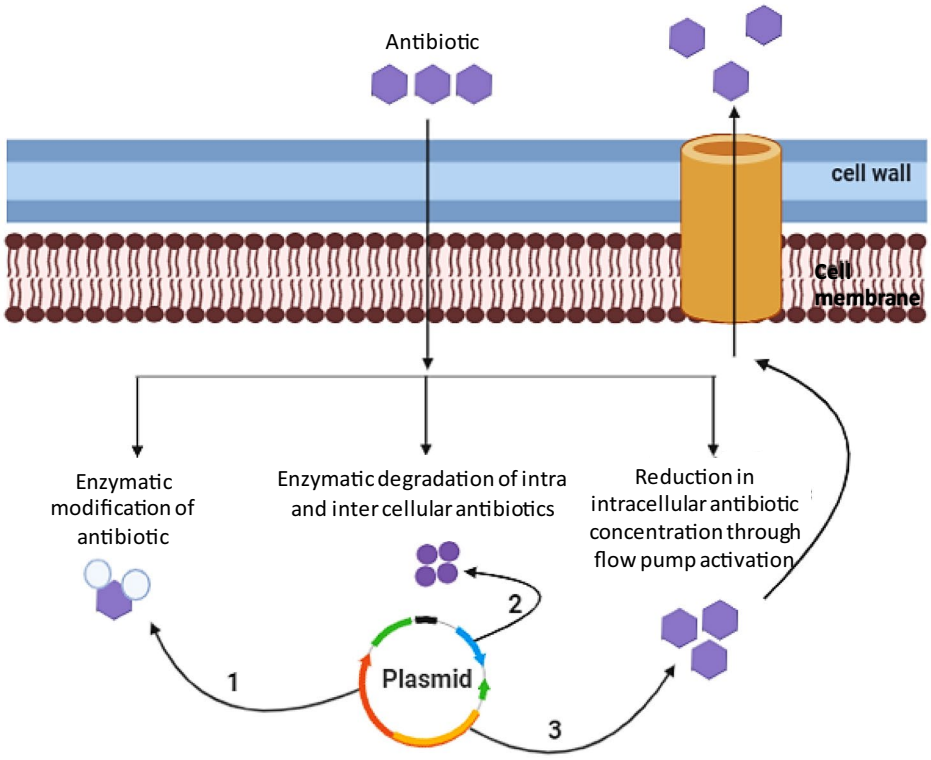


**Fig. 1** Antibiotic resistance mechanism in *Lactobacillus*

AcrAB-TolC like complex systems which causes resistance to various antibiotics such as β-lactams, chloramphenicol, tetracycline through efflux pump mechanisms [40].

## Approaches of Resistance Gene Transfer

There are mainly three strategies which bacteria adopt to transfer their resistance genes to other group of bacteria., e.g., transformation, transduction and conjugation [41, 54, 55].

In 1928, Fredrick Griffith, a British bacteriologist who illustrated transformation in *Streptococcus pneumonia* for the very first time [56]. Hotchkiss, in 1951 successfully induced resistance to penicillin and streptomycin in strains of *S. pneumonia* by exposing cells to DNA encoding resistance [57]. Transformation has been a major mechanism of resistance gene transfer [58]. For transformation of bacteria cells must be exposed to extracellular DNA (Fig. 2A). The recipient bacteria must attain the state of competence to acquire the foreign DNA. Bacterial species need environmental and cellular signals to induce competence except *Neisseria* spp. which are competent constitutively e.g. they do not require any such environmental or cellular signals to develop competence [59]. For transformation to occur, either the segments of genomic DNA or entire plasmid DNA uptake is required. In several human pathogenic bacteria such as species of *Campylobacter*, *Haemophilus*, *Helicobacter*, *Neisseria*, *Pseudomonas*, *Staphylococcus* and *Streptococcus*, natural transformation has been observed [60]. Efficient plasmid transformation by artificial means (electroporation) have been successful in species of *Lactobacillus* [61, 62].

In transduction, bacterial cell is invaded by bacteriophage where segment of bacterial DNA is removed by phage and then the recombinant phage infects other bacterial cells (Fig. 2B). Transduction can be of two types mainly: generalized transduction where phage DNA can be incorporated with any segment of bacterial DNA, and specialized transduction where only specific segment of DNA is incorporated. Due to their abundant occurrence in nature they can disseminate antibiotic resistance genes horizontally to several bacterial communities in microbial ecosystems [63]. Antimicrobial resistance gene transfer through transduction has been reported in various species of bacteria such as *S. pyogenes*, *E. coli*, *Salmonella* and Enterococci [64–68]. Many lactic acid bacteria like *E. faecalis* and species of *Lactococcus* have been found to harbor bacteriophages [69].

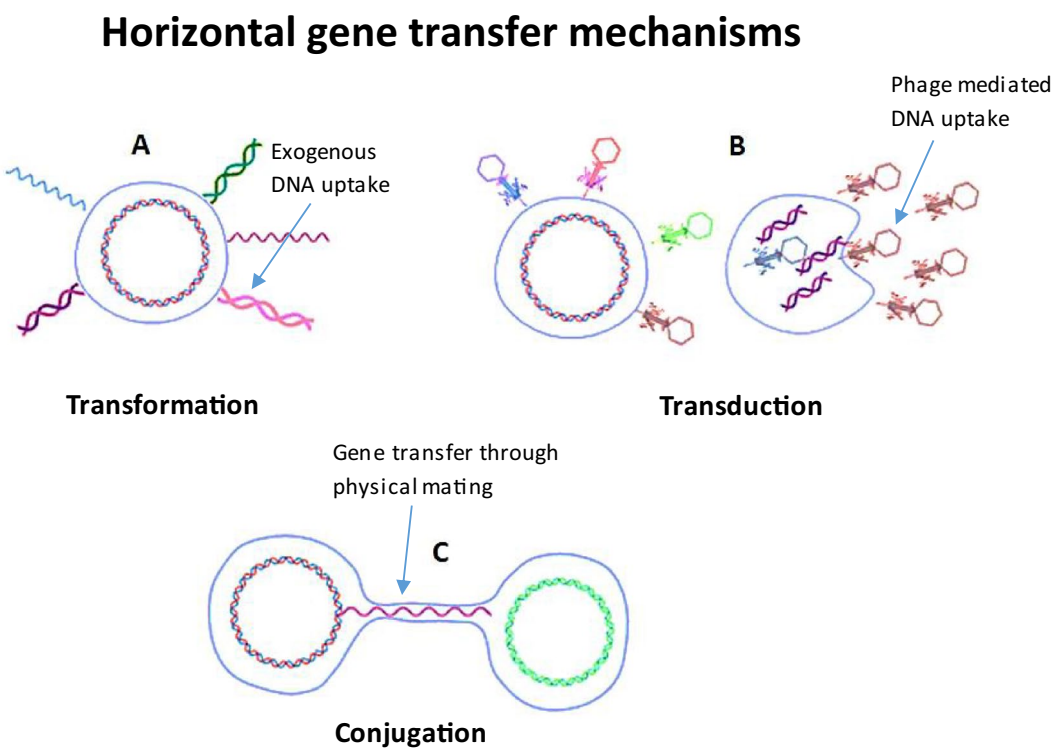


**Fig. 2** Horizontal gene transfer mechanisms

## Conjugation Mechanisms of Resistance Gene Transfer

Conjugation is a phenomenon of DNA translocation in bacteria through physical cell to cell contact using surface pilli protein (Fig. 2C). It is aided by conjugative machinery encoded either by autonomously replicating plasmid genes or by integrative conjugative elements in the chromosome [70, 71]. It was first reported by Lederberg and Tatum in 1946 [72]. Conjugation usually occurs in bacterial species acting as donor and recipient but as an exception in species *Agrobacterium*. Horizontal gene transfer also occurs in plant cells [73]. This method of gene transfer appears to be more efficient than transformation and transduction. The genetic material to be transferred is more secured and protected from their surroundings during conjugation than other two means of transfer. Conjugation allows the conjugally transferred elements such as plasmids or conjugative transposons to remain in new hosts without any large sequence similarity to integrate into the new host's genome. In species of lactobacilli, this type of gene transfer mechanism has been reported explicitly [32, 33, 46, 49].

While conjugation occurs widely in nature where bacteria remain in close proximity, in laboratory conditions, different approaches have been adopted to study the transfer of antibiotic resistance through conjugation.

## Conjugation Through In Vitro Filter Mating Technique

Conjugal transfer of resistance genes in lactobacilli can be observed in vitro through filter mating techniques[33, 46, 74]. Where in recipient (sensitive) and donor (resistant) strains are allowed to mate on 0.45 µm pore size sterile filter paper membrane and then allowed to grow on Brain Heart Infusion agar (BHI)/ Mueller Hinton Agar (MHA) media (Fig. 3). Detachment of cells is performed in peptone physiological saline (PPS) using a vortex and the suspension is serially diluted. Suitable dilutions are plated in selective media for donor, recipient and transconjugants (containing the acquired gene of antibiotic resistance) before selecting the probable transconjugants. This method of in vitro transfer has been highly constructive over the other methods. Using this filter mating technique, many investigators were able to successfully achieve the gene transfer [32, 46, 49, 75, 76]. According to Sasaki & group, filter mating is found to be the best method for conjugal transfer of pAMβ1 from *E. faecalis* to *L. plantarum* [77]. There were three different conjugation methods tested e.g., filter, solid and liquid mating

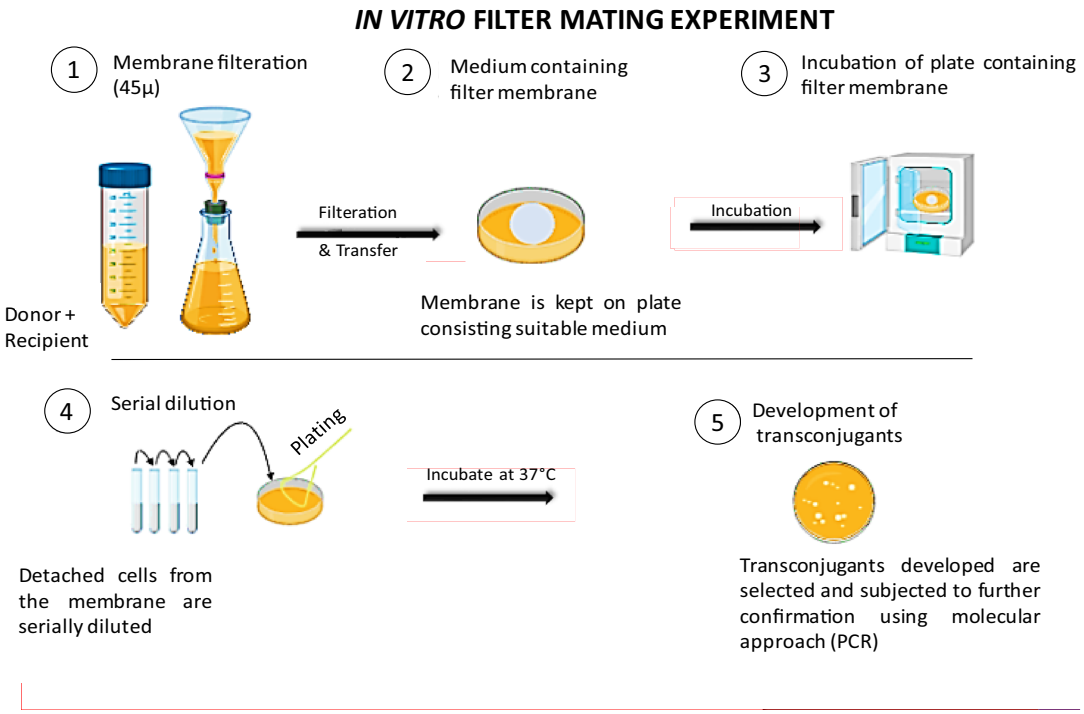


**Fig. 3** In vitro filter mating experiment

and the results obtained were method dependent when late exponentially grown 1:1donor/recipient ratio were used in different sets of experiments [80]. The transfer frequencies (Table 1) in filter mating were higher than both solid and liquid mating which indicates that transfer frequency is distinctly dependent on the growth phase of donor/recipient, their optimum ratio and the method of conjugative transfer.

## Conjugation Through In Vitro Solid and Liquid Mating

In 2008, Lampkowska and his team assessed conjugal transfer of resistance genes in LAB by using solid and liquid mating procedures. Briefly, equal ratio (1:1) of donor and recipient of late log phase growth was used to inoculate the solid agar media. Whereas in liquid mating, the mixture of donor and recipient is used to inoculate a broth medium [75]. In solid mating, 1 ml of physiological bacteriological solution (PBS) is used to suspend the grown transconjugants while in liquid mating, cells were collected through centrifugation and then suspended in 1 ml PBS. A series of tenfold dilution of both solid and liquid cells suspension is plated on a respective agar medium supplemented with antibiotics and incubated for 24–48 h at 30 °C. Other research groups employed the same method [49, 76]. This in vitro method of gene transfer has been quite facile to perform but this gives a low transfer frequency as compared to other methods such as filter mating and in vivo studies. Hence more research is needed to explore various strategies in order to make these methods effective and reproducible.

## Conjugation Through In Vivo Mating

To find a real delineation of the transfer of resistance genes, in vivo conjugal mating has been used by few researchers [49, 78–80]. Investigation of in vivo transfer of plasmids containing *tet*(M) and *erm*(B) resistance genes from food strains of *L. plantarum* to *E. faecalis* in the GI tract of gnotobiotic rats was done previously [78]. Streptomycin treated gnotobiotic mice model was used to study the transfer and establishment of a *L. plantarum* resistance plasmid in controlled GI environment [79]. Transferability of erythromycin, tetracycline and vancomycin resistance genes in *Enterococcus* and *Lactobacillus* spp. were studied by Preethi and co-workers [80]. Further a group of researchers, using conjugal method also observed transfer of *erm*(B) and multiple *tet* genes from *Lactobacillus* spp. to bacterial pathogens in animal gut [49]. Briefly to perform the experiment of conjugation, the animal gut was allowed to colonize with the recipient strain at a concentration of $10^{10}$ CFU/ml for a week (1–7 days) and colonization was checked with the faecal sample on a medium supplemented with antibiotics (selective to the recipient). After a week (8th day) of colonization of the recipient, donor strains were administered through

**Table 1** Gene transfer using different conjugation-based methods in *Lactobacillus* and other LAB strains

| Conjugation Transfer method | Donor organism | Recipient organism | Transfer frequency (TC/recipient) | References |
|---|---|---|---|---|
| 1. In vitro filter mating | *Streptococcus faecalis* JH2-2(pAMβ1) | *Lactobacillus plantarum* JCM1149 | $1.5\times10^{-6}$ to $8.6\times10^{-7}$ | [77] |
| | *Lactobacillus plantarum* | *Enterococcus faecalis* JH2-2 | $10^{-4}$ to $10^{-6}$ | [46] |
| | *L. alimentarius* | *Enterococcus faecalis* JH2-2 | $10^{-4}$ to $10^{-6}$ | [46] |
| | *L. sakei* subsp. *sakei* | *Enterococcus faecalis* JH2-2 | $10^{-4}$ to $10^{-6}$ | [46] |
| | *Lactococcus lactis* SH4174 (pAMβ1) | *Lactococcus lactis* Bu2-60 | $1.09\times10^{-2}$ | [75] |
| | *Lactococcus lactis* SH4174 | *Listeria monocytogenes* (H7) | $5.1\times10^{-4}$ | [83] |
| | *Streptococcus thermophilus* E2 | *L. monocytogenes* (H7) | $3.1\times10^{-6}$ | [83] |
| | *S. thermophilus* E2 | *Listeria welshimeri* | $2.1\times10^{-8}$ | [83] |
| | *Lactococcus lactis* SH4174 | *Lactococcus lactis* Bu2-60 | $1.1\times10^{-3}$ | [83] |
| | *L. lactis* 477 | *L. lactis* Bu2–60 | $4.1\times10^{-5}$ | [83] |
| | *L. lactis* 477 | *Enterococcus faecalis* | $6.4\times10^{-7}$ | [83] |
| | *L. fermentum* NWL24 | *E. faecalis* 181 | $2.62\times10^{-5}$ | [32] |
| | *L. salivarius* NWL33 | *E. faecalis* 181 | $2.9\times10^{-6}$ | [32] |
| | *L. plantarum* NWL22 | *E. faecalis* 181 | $1.39\times10^{-5}$ | [32] |
| | *L. brevis* NWL59 | *E. faecalis* 181 | $2.1\times10^{-6}$ | [32] |
| | *L. salivarius* CHS-1E | *E. faecalis* JH2-2 | $1\times10^{-4}$ | [49] |
| | *L. salivarius* CH7-1E | *E. faecalis* JH2-2 | $3.8\times10^{-3}$ | [49] |
| | *L. reuteri* CH2–2 | *E. faecalis* JH2-2 | $2.0\times10^{-3}$ | [49] |
| 2. In vitro solid mating | *Lactococcus lactis* SH4174 (pAMβ1) | *Lactococcus lactis* Bu2-60 | $1.35\times10^{-3}$ | [75] |
| | *Lactococcus lactis* SH4174 | *Listeria monocytogenes* (H7) | $6\times10^{-7}$ | [83] |
| | *Streptococcus thermophilus* E2 | *L. monocytogenes* (H7) | $4\times10^{-7}$ | [83] |
| | *S. thermophilus* E2 | *Listeria welshimeri* | $1.2\times10^{-7}$ | [83] |
| | *Lactococcus lactis* SH4174 | *Lactococcus lactis* Bu2-60 | $9.1\times10^{-4}$ | [83] |
| | *L. lactis* 477 | *L. lactis* Bu2–60 | $9.6\times10^{-4}$ | [83] |
| 3. In vitro liquid mating | *Lactococcus lactis* SH4174 (pAMβ1) | *Lactococcus lactis* Bu2-60 | $2.3\times10^{-7}$ | [75] |
| | *L. lactis* 477 | *L. lactis* Bu2–60 | $2.6\times10^{-7}$ | [83] |
| 4. In vivo mating | *L. plantarum* DG 522 (LMG 21,687) | *E. faecalis* JH2-2 | NR | [78] |
| | *L. plantarum* M345 | *E. faecalis* JH2-2 | $10^{-4}$ | [79] |
| | *E. faecium* M3G | *E. faecalis* JH2-2 | $1.70\times10^{-4}$ | [80] |
| | *L. plantarum* S11T | *E. faecalis* JH2-2 | $2.01\times10^{-5}$ | [79] |
| | *L. salivarius* CHS-7E | *E. faecalis* JH2-2 | NR | [49] |
| | *L. reuteri* CH2-2 | *E. faecalis* JH2-2 | NR | [49] |
| 5. In situ mating | *L. lactis* 477 | *L. lactis* Bu2–60 | $2.6\times10^{-7}$ | [83] |
| | *L. salivarius* CHS-1E | *Listeria monocytogenes* Scott A | $1.9\times10^{-6}$ | [49] |
| | *L. salivarius* CH7-1E | *Yersinia enterocolitica* MTCC859 | $0.8\times10^{-8}$ | [49] |
| | | *E. faecalis* JH2–2 | NR | [49] |
| | | *Listeria monocytogenes* Scott A | $4.1\times10^{-8}$ | [49] |
| | | *Yersinia enterocolitica* MTCC859 | $1.9\times10^{-7}$ | [49] |
| | | *E. faecalis* JH2–2 | NR | [49] |
| | *L. reuteri* CH2–2 | *Listeria monocytogenes* Scott A | $2.9\times10^{-7}$ | [49] |
| | | *Yersinia enterocolitica* MTCC859 | $0.8\times10^{-6}$ | [49] |
| | | *E. faecalis* JH2–2 | NR | [49] |

*NR* Not reported

oral gavage in a concentration of $10^9$ CFU/ml. Faecal samples were collected during the colonization of the recipient and after the administration of donor strains (8–17 days). The animals were sacrificed at the end of experiment (after 17 days) and faecal or intestinal samples were collected and homogenized in normal saline water (0.85% NaCl). A suitable dilution series was made and ten-fold dilutions were plated onto an appropriate antibiotic supplemented agar medium in order to enumerate TCs, recipients and donors (Fig. 4).

## Conjugation Through In Situ Mating

Conjugal mating in fermented chicken sausages was described to study in situ mating of resistance gene transfer [81]. Similar methods of conjugative transfer of resistance genes were adopted [49, 76], using directly fermentable food as a co-culturing medium for donor and recipient. Conjugation in food matrix was achieved in actual condition where donor and recipient inoculum were prepared in the food to be fermented. In case of fermented milk, 1 ml ($10^9$ CFU/ml) of donor and recipient each were mixed together and then centrifuged to pellet down the cells. These cell pellets were dissolved in 1 ml of skim milk and whole suspension was inoculated in bulk skim milk in order to allow the fermentation of milk (42 °C) incubated at 42 °C. After the milk was coagulated, it was cooled and stored at 4 °C for 2 days. Tenfold serial dilutions of fermented milk were plated on selective media supplemented with antibiotics to obtain the growth of donor, recipient and transconjugants separately. Similar procedures were followed to obtain conjugation in other food matrices with some modifications [48, 83].

## Factors Affecting Transfer Frequency

Several methods (in vitro, in vivo and in situ) have been used by researchers the world over to study antibiotic resistance in lactic acid bacteria including lactobacilli. There is no comprehensive literature available to compare all methods in similar conditions but in vitro method of conjugal gene transfer has been quite promising as far as the success of transfer frequency is concerned. There are various factors which affect the transfer frequency rate in the in vitro conjugation. Type of membrane filter and age of donor and recipient used could be the important factors responsible for success rate of transfer frequency [46]. Sasaki and co-workers reported that highest transfer frequencies were observed when a sponge membrane with a pore size 0.45 µm and front side up was used [77]. This allowed the trapping of cells more tightly in spongy network of membrane upon passing of sterile water or buffer through the filter membrane. In 2008, Lampkowska and team assessed several factors affecting conjugal gene transfer in lactococcal species [75]. They reported a significant effect of the factors like growth

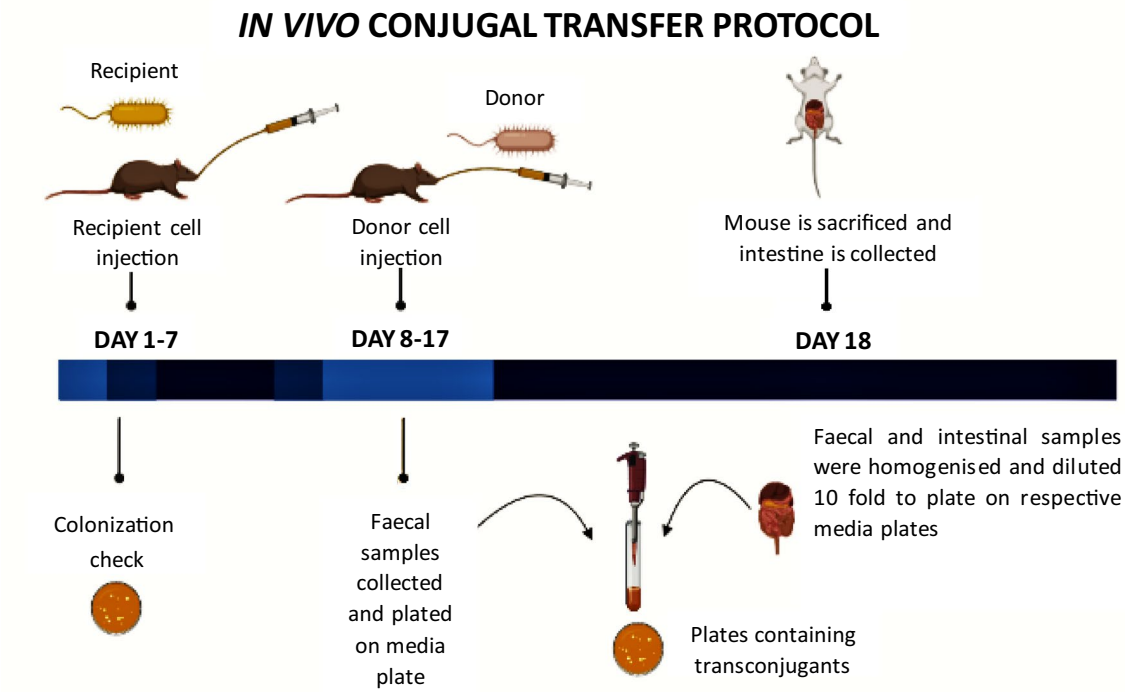


**Fig. 4** In vivo conjugal transfer protocol

phase, donor/recipient ratio and conjugal protocol. Effect of erythromycin concentration on transfer frequency was investigated where a highest transfer frequency ($2.0 \times 10^{-6}$ Transconjugant/Recipient) was reported at a concentration of 0.50 mg/L, indicating that antibiotic concentration can also show effect to the transconjugants formation during the experiment [79]. Since transfer frequency is dependent on the growth of transconjugants, hence use of optimal antibiotic concentration can also be a decisive factor affecting transconjugant/recipient ratio. Transfer frequency was also found to be dependent upon the growth kinetics of donor/recipient strains which were influenced by pH and temperature of the food system used for the transfer [49, 81, 82].

## Conclusions

Food safety has been of prime focus in fermented food industry due to the natural ecological habitat for lactobacilli which can inherently or non-inherently transfer resistance genes. Conjugation is the most frequently employed mechanism by lactobacilli to transfer the resistance genes can be made to occur in lab conditions using modern microbiological and molecular methods. To study gene transfer in bacteria, in vitro models of conjugation are frequently used due to our limited understanding of the in vivo environment. However, to determine the true transfer of resistance genes in lactobacilli strains, natural condition-based studies are needed to assess [83] the transfer frequency in a substantial manner such as in vivo or in situ studies. Despite the challenge to achieve the formation of transconjugants, efforts are being made to overcome the problems faced in conjugal transfer under in vitro conditions. Factors responsible for increment of transfer frequency must be taken into consideration to optimize the in vitro transfer approaches. Still more research data are required to fully understand conjugal gene transfer in *Lactobacillus*, especially in food matrices where there is a greater concern for human safety.

**Author Contributions** AKO contributed in conceiving, writing the manuscript, prepared figures. NPS contributed in refining the manuscript and VM conceived, revised the manuscript. All authors read and approved the manuscript.

**Funding** The work is funded by National Institute of Food Technology Entrepreneurship and Management (NIFTEM) Kundli, Sonepat (India).

## Declarations

**Conflicts of interest** There is no declared conflict of interest.

**Ethical Approval** The paper does not contain any study on human participants or animals.

## References

1. Zheng J, Wittouck S, Salvetti E, Franz CM, Harris HM, Mattarelli P, O'Toole PW, Pot B, Vandamme P, Walter J, Watanabe K (2020) A taxonomic note on the genus *Lactobacillus*: description of 23 novel genera, emended description of the genus *Lactobacillus* Beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int J Sys Evol Microbiol 70(4):2782–2858. https://doi.org/10.1099/ijsem.0.004107
2. Walter J (2008) Ecological role of lactobacilli in the gastrointestinal tract: implications for fundamental and biomedical research. Appl Environ Microbiol 74(16):4985–4996. https://doi.org/10.1128/AEM.00753-08
3. Rossi F, Amadoro C, Colavita G (2019) Members of the *Lactobacillus* genus complex (LGC) as opportunistic pathogens: a review. Microorganisms 7(5):126. https://doi.org/10.3390/microorganisms7050126
4. Tellez G, Laukova A, Latorre JD, Hernandez-Velasco X, Hargis BM, Callaway T (2015) Food-producing animals and their health in relation to human health. Microb Ecol Health Dis 26:25876. https://doi.org/10.3402/mehd.v26.25876
5. Zommiti M, Chikindas ML, Ferchichi M (2020) Probiotics—live biotherapeutics: a story of success, limitations, and future prospects—not only for humans. Probiotics Antimicrob Proteins 12:1266–1289. https://doi.org/10.1007/s12602-019-09570-5
6. FDA (2010) Generally Recognized as Safe (GRAS). Notifications FDA. https://www.fda.gov/food/generally-recognized-safe-gras/microorganisms-microbial-derivedingredients-used-food-partial-list. Accessed 1 Oct 2019
7. Mishra V, Upgade A, Sharma KP (2009) Role of food chain containing lactic acid bacteria in antibiotic resistance. Curr Science 97(12):1703
8. Doron S, Snydman DR (2015) Risk and safety of probiotics. Clin Infect Dis 60(Suppl 2):S129–S134. https://doi.org/10.1093/cid/civ085
9. Sanders ME, Merenstein DJ, Ouwehand AC, Reid G, Salminen S, Cabana MD, Paraskevakos G, Leyer G (2016) Probiotic use in at-risk populations. J Am Pharm Assoc 56:680–686. https://doi.org/10.1016/j.japh.2016.07.001
10. Maragkoudakis PA, Zoumpopoulou G, Miaris C, Kalantzopoulos G, Pot B, Tsakalidou E (2006) Probiotic potential of Lactobacillus strains isolated from dairy products. Int Dairy J 16:189–199. https://doi.org/10.1016/j.idairyj.2005.02.009
11. Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, Morelli L, Canani RB, Flint HJ, Salminen S, Calder PC (2014) Expert consensus document: The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroentero Hepat 11(8):506. https://doi.org/10.1038/nrgastro.2014.66
12. Suez J, Zmora N, Segal E, Elinav E (2019) The pros, cons, and many unknowns of probiotics. Nat Med 25(5):716–729. https://doi.org/10.1038/s41591-019-0439-x
13. Khalesi S, Bellissimo N, Vandelanotte C, Williams S, Stanley D, Irwin C (2019) A review of probiotic supplementation in healthy adults: helpful or hype? Eur J of Clin Nutri 73(1):24–37. https://doi.org/10.1038/s41430-018-0135-9
14. WHO/FAO (2006) Probiotics in food health and nutritional properties and guidelines for evaluation. Food and Agriculture Organization of the United Nations, Rome
15. Mishra V, Prasad DN (2005) Application of in vitro methods for selection of *Lactobacillus casei* strains as potential probiotics. Int J of Food Microbiol 103(1):109–115. https://doi.org/10.1016/j.ijfoodmicro.2004.10.047
16. Bhushan B, Sakhare SM, Narayan KS, Kumari M, Mishra V, Dicks LMT (2020) Characterization of riboflavin-producing strains of

*Lactobacillus plantarum* as potential probiotic candidate through in vitro assessment and principal component analysis. Probiotics Antimicrob Proteins. https://doi.org/10.1007/s12602-020-09696-x
17. EFSA (2008) Technical guidance-update of the criteria used in the assessment of bacterial resistance to antibiotics of human or veterinary importance. EFSA J 732:1–15. https://doi.org/10.2903/j.efsa.2008.732
18. Liu L, Chen X, Skogerbo G, Zhang P, Chen R, He S, Huang DW (2012) The human microbiome: a hot spot of microbial horizontal gene transfer. Genomics 100:265–270. https://doi.org/10.1016/j.ygeno.2012.07.012
19. Founou LL, Founou RC, Essack SY (2016) Antibiotic resistance in the food chain: a developing country-perspective. Front Microbiol 7:1881. https://doi.org/10.3389/fmicb.2016.01881
20. Lerner A, Matthias T, Aminov R (2017) Potential effects of horizontal gene exchange in the human gut. Front Immunol 8:1630. https://doi.org/10.3389/fimmu.2017.01630
21. Zarzecka U, Zadernowska A, Chajęcka-Wierzchowska W (2020) Starter cultures as a reservoir of antibiotic resistant microorganisms. LWT 19:109424. https://doi.org/10.1016/j.lwt.2020.109424
22. Herman L, Chemaly M, Cocconcelli PS, Fernandez P, Klein G, Peixe L, Prieto M, Querol A, Suarez JE, Sundh I, Vlak J (2019) The qualified presumption of safety assessment and its role in EFSA risk evaluations: 15 years past. FEMS Microbiol Lett 366(1):17–23. https://doi.org/10.1093/femsle/fny260
23. Leuschner RGK, Robinson TP, Hugas M et al (2010) Qualified presumption of safety (QPS): a generic risk assessment approach for biological agents notified to the European Food Safety Authority (EFSA). Trends Food Sci Tech 21:425–435
24. European Food Safety Authority (EFSA) Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) (2012) Guidance on the assessment of bacterial susceptibility to antimicrobials of human and veterinary importance. EFSA J 10:2740–2750
25. Imperial IC, Ibana JA (2016) Addressing the antibiotic resistance problem with probiotics: reducing the risk of its double-edged sword effect. Front Microbiol 7:1983. https://doi.org/10.3389/fmicb.2016.01983
26. Ammor MS, Florez AB, Mayo B (2007) Antibiotic resistance in non-enterococcal lactic acid bacteria and bifidobacteria. Food Microbiol 24:559–570. https://doi.org/10.1016/j.fm.2006.11.001
27. EFSA, ECDC, (2018) The European Union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2016. EFSA J 16:5182. https://doi.org/10.2903/j.efsa.2018.5182
28. Frieri M, Kumar K, Boutin A (2017) Antibiotic resistance. J Infect Public Health 10(4):369–378. https://doi.org/10.1016/j.jiph.2016.08.007
29. Akova M (2016) Epidemiology of antimicrobial resistance in bloodstream infections. Virulence 7(3):252–266. https://doi.org/10.1080/21505594.2016.1159366
30. Aslam B, Wang W, Arshad MI, Khurshid M, Muzammil S, Rasool MH, Nisar MA, Alvi RF, Aslam MA, Qamar MU, Salamat M, Baloch Z (2018) Antibiotic resistance: a rundown of a global crisis. Infect Drug Resist 11:1645–1658. https://doi.org/10.2147/IDR.S173867
31. Belletti N, Gatti M, Bottari B, Neviani E, Tabanelli G, Gardini F (2009) Antibiotic resistance of lactobacilli isolated from two Italian hard cheeses. J Food Prot 72:2162–2169. https://doi.org/10.4315/0362-028X-72.10.2162
32. Nawaz M, Wang J, Zhou A, Ma C, Wu X, Moore JE, Millar BC, Xu J (2011) Characterization and transfer of antibiotic resistance in lactic acid bacteria from fermented food products. Curr Microbiol 62:1081–1089. https://doi.org/10.1007/s00284-010-9856-2
33. Guo HL, Pan L, Li LN, Lu J, Kwok L, Menghe B, Zhang HP, Zhang WY (2017) Characterization of antibiotic resistance genes from *Lactobacillus* isolated from traditional dairy products. J Food Sci 82:724–730. https://doi.org/10.1111/1750-3841.13645
34. Ledina T, Mohar-Lorberg P, Golob M, Djordjevic J, Bogovic-Matijasic B, Bulajic S (2018) Tetracycline resistance in lactobacilli isolated from Serbian traditional raw milk cheeses. J Food Sci Technol 55:1426–1434. https://doi.org/10.1007/s13197-018-3057-6
35. Rozman V, Lorbeg PM, Accetto T, Matijasic BB (2020) Characterization of antimicrobial resistance in lactobacilli and bifidobacteria used as probiotics or starter cultures based on integration of phenotypic and in silico data. Int J Food Microbiol 314:108388. https://doi.org/10.1016/j.ijfoodmicro.2019.108388
36. Dec M, Nowaczek A, Stępien-Pysniak D, Wawrzykowski J, Urban-Chmiel R (2018) Identification and antibiotic susceptibility of lactobacilli isolated from turkeys. BMC Microbiol 18(1):1–14. https://doi.org/10.1186/s12866-018-1269-6
37. Abriouel H, CasadoMunoz MDC, Lavilla Lerma L, Perez Montoro B, Bockelmann W, Pichner R, Kabisch J, Cho GS, Franz CMAP, Galvez A, Benomar N (2015) New insights in antibiotic resistance of *Lactobacillus* species from fermented foods. Food Res Int 78:465–481. https://doi.org/10.1016/j.foodres.2015.09.016
38. Campedelli I, Mathur H, Salvetti E, Clarke S, Rea MC, Torriani S, Ross RP, Hill C, O'Toole PW (2019) Genus-wide assessment of antibiotic resistance in *Lactobacillus* spp. Appl Environ Microbiol. https://doi.org/10.1128/AEM.01738-18
39. Bulajic S, Mijacevic Z (2011) Antimicrobial susceptibility of lactic acid bacteria isolated from Sombor cheese. Acta Veterinaria-Beograd 61(2–3):247–258. https://doi.org/10.2298/AVB1103247B
40. Alvarez-Cisneros YM, Ponce-Alquicira E (2018) Antibiotic resistance in lactic acid bacteria. Antimicrob Resist-A Glob Threat. https://doi.org/10.5772/intechopen.80624
41. Soucy SM, Huang J, Gogarten JP (2015) Horizontal gene transfer: building the web of life. Nat Rev Genet 16(8):472–482
42. Munita JM, Arias CA (2016) Mechanisms of antibiotic resistance. Virulence Mech Bact Pathog. https://doi.org/10.1128/9781555819286.ch17
43. Mathur S, Singh R (2005) Antibiotic resistance in food lactic acid bacteria—a review. Int J Food Microbiol 105:281–295. https://doi.org/10.1016/j.ijfoodmicro.2005.03.008
44. Salvetti E, O'Toole PW (2017) When regulation challenges innovation: the case of the genus *Lactobacillus*. Trends Food Sci Technol 66:187–194. https://doi.org/10.1016/j.tifs.2017.05.009
45. CDC Antimicrobial Resistance (2017). www.cdc.gov/ncidod/aip/research/ar.html
46. Gevers D, Huys G, Swings J (2003) In vitro conjugal transfer of tetracycline resistance from *Lactobacillus* isolates to other Gram-positive bacteria. FEMS Microbiol Lett 225:125–130. https://doi.org/10.1016/S0378-1097(03)00505-6
47. Huys G, D'Haene K, Collard JM, Swings J (2004) Prevalence and molecular characterization of tetracycline resistance in *Enterococcus* isolates from food. Appl Environ Microbiol 70:1555–1562. https://doi.org/10.1128/AEM.70.3.1555-1562.2004
48. Toomey N, Bolton D, Fanning S (2010) Characterization of antibiotic resistance genes from lactic acid bacteria isolated from Irish pork and beef abattoirs. Res Microbiol 161:127–135. https://doi.org/10.1016/j.resmic.2009.12.010
49. Thumu SCR, Halami PM (2019) Conjugal transfer of *erm*(B) and multiple *tet* genes from *Lactobacilllus* spp. to bacterial pathogens in animal gut, in vitro and during food fermentation. Food Res Int 116:1066–1075. https://doi.org/10.1016/j.foodres.2018.09.046
50. Mater DD, Langella P, Corthier G, Flores MJ (2007) A probiotic *Lactobacillus* strain can acquire vancomycin resistance during digestive transit in mice. J Mol Microbiol Biotechnol 14:123–127. https://doi.org/10.1159/000106091

51. Sharma P, Tomar SK, Goswami P, Sangwan V, Singh R (2014) Antibiotic resistance among commercially available probiotics. Food Res Int 57:176–195. https://doi.org/10.1016/j.foodres.2014.01.025
52. Gueimonde M, Sanchez B, de los Reyes-Gavilan CG, Margolles A (2013) Antibiotic resistance in probiotic bacteria. Front Microbiol 14:202. https://doi.org/10.3389/fmicb.2013.00202
53. Fraqueza MJ (2015) Antibiotic resistance of lactic acid bacteria isolated from dry-fermented sausages. Int J Food Microbiol 212:76–88. https://doi.org/10.1016/j.ijfoodmicro.2015.04.035
54. Von Wintersdorff CJ, Penders J, Van Niekerk JM, Mills ND, Majumder S, Van Alphen LB, Wolffs PF (2016) Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front Microbiol 7:173. https://doi.org/10.3389/fmicb.2016.00173
55. García-Aljaro C, Balleste E, Muniesa M (2017) Beyond the canonical strategies of horizontal gene transfer in prokaryotes. Curr Opin Microbiol 38:95–105. https://doi.org/10.1016/j.mib.2017.04.011
56. Griffith F (1928) The significance of pneumococcal types. J Hyg (Lond) 27:113–159. https://doi.org/10.1017/S0022172400031879
57. Hotchkiss RD (1951) Transfer of penicillin resistance in pneumococci by the deoxyribonucleate derived from resistant cultures. Cold Spring Harb Symp Quant Biol 16:457–461. https://doi.org/10.1101/SQB.1951.016.01.032
58. Huddleston JR (2014) Horizontal gene transfer in the human gastrointestinal tract: potential spread of antibiotic resistance genes. Infect Drug Resist 7:167–176. https://doi.org/10.2147/IDR.S48820
59. Johnston C, Martin B, Fichant G, Polard P, Claverys JP (2014) Bacterial transformation: distribution, shared mechanisms and divergent control. Nat Rev Microbiol 12:181–196. https://doi.org/10.1038/nrmicro3199
60. Lorenz MG, Wackernagel W (1994) Bacterial gene transfer by natural genetic transformation in the environment. Microbiol Mol Biol Rev 58:563–602
61. Landete JM, Arqués JL, Peirotén Á, Langa S, Medina M (2014) An improved method for the electrotransformation of lactic acid bacteria: a comparative survey. J Microbiol Methods 105:130–133. https://doi.org/10.1016/j.mimet.2014.07.022
62. Kathiriya MR, Gawai KM, Prajapati JB (2019) Transformation of lactobacilli plasmid by electroporation into probiotic strain *Lactobacillus helveticus* MTCC 5463. Int J of Ferment Foods 8:33–40. https://doi.org/10.30954/2321-712X.01.2019.3
63. von Wintersdorff CJ, Penders J, van Niekerk JM, Mills ND, Majumder S, van Alphen LB, Savelkoul PH, Wolffs PF (2016) Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. Front Microbiol 7:173. https://doi.org/10.3389/fmicb.2016.00173
64. Ubukata K, Konno M, Fujii R (1975) Transduction of drug resistance to tetracycline, chloramphenicol, macrolides, lincomycin and clindamycin with phages induced from *Streptococcus pyogenes*. J Antibiot 28:681–688. https://doi.org/10.7164/antibiotics.28.681
65. Mazaheri Nezhad Fard R, Barton MD, Heuzenroeder MW (2011) Bacteriophage-mediated transduction of antibiotic resistance in enterococci. Lett Appl Microbiol 52:559–564. https://doi.org/10.1111/j.1472-765X.2011.03043.x
66. Billard-Pomares T, Fouteau S, Jacquet ME, Roche D, Barbe V, Castellanos M, Bouet JY, Cruveiller S, Médigue C, Blanco J, Clermont O (2014) Characterization of a P1-like bacteriophage carrying an SHV- 2 extended-spectrum β-lactamase from an *Escherichia coli* strain. Antimicrob Agents Chemother 58:6550–6557. https://doi.org/10.1128/AAC.03183-14
67. Schmieger H, Schicklmaier P (1999) Transduction of multiple drug resistance of *Salmonella enteric* serovar*typhimurium*DT104. FEMS Microbiol Lett 170:251–256. https://doi.org/10.1111/j.1574-6968.1999.tb13381.x
68. Varga M, Kuntova L, Pantucek R, Maslanova I, Ruzickova V, Doskar J (2012) Efficient transfer of antibiotic resistance plasmids by transduction within methicillin-resistant *Staphylococcus aureus* USA 300 clone. FEMS Microbiol Lett 332:146–152. https://doi.org/10.1111/j.1574-6968.2012.02589.x
69. Yasmin A, Kenny JG, Shankar J, Darby AC, Hall N, Edwards C, Horsburgh MJ (2010) Comparative genomics and transduction potential of *Enterococcus faecalis* temperate bacteriophages. J Bacteriol 192:1122–1130. https://doi.org/10.1128/JB.01293-09
70. Smillie C, Garcillan-Barcia MP, Francia MV, Rocha EP, delaCruz F, (2010) Mobility of plasmids. Microbiol Mol Biol Rev 74:434–452. https://doi.org/10.1128/MMBR.00020-10
71. Wozniak RA, Waldor MK (2010) Integrative and conjugative elements mosaic mobile genetic elements enabling dynamic lateral gene flow. Nat Rev Microbiol 8:552–563. https://doi.org/10.1038/nrmicro2382
72. Lederberg J, Tatum EL (1946) Gene recombination in *E. coli*. Nature 158:558–558. https://doi.org/10.1038/158558a0
73. Kyndt T, Quispe D, Zhai H, Jarret R, Ghislain M, Liu Q, Gheysen G, Kreuze JF (2015) The genome of cultivated sweet potato contains *Agrobacterium* T-DNAs with expressed genes: an example of a naturally transgenic food crop. Proc Natl Acad Sci USA 112:5844–5849. https://doi.org/10.1073/pnas.1419685112
74. Anisimova E, Yarullina D (2018) Characterization of erythromycin and tetracycline resistance in *Lactobacillus fermentum* strains. Int J Microbiol 2018:3912326. https://doi.org/10.1155/2018/3912326
75. Lampkowska J, Feld L, Monaghan A, Toomey N, Schjørring S, Jacobsen B, van der Voet H, Andersen SR, Bolton D, Aarts H, Krogfelt KA (2008) A standardized conjugation protocol to assess antibiotic resistance transfer between lactococcal species. Int J Food Microbiol 127:172–175. https://doi.org/10.1016/j.ijfoodmicro.2008.06.017
76. Toomey N, Monaghan A, Fanning S, Bolton D (2009) Assessment of antimicrobial resistance transfer between lactic acid bacteria and potential foodborne pathogens using in vitro methods and mating in food matrix. Foodborne Pathog Dis 6:925–933. https://doi.org/10.1089/fpd.2009.0278
77. Sasaki Y, Taketomo N, Sasaki T (1988) Factors affecting transfer frequency of pAMß1 from *Streptococcus faecalis* to *Lactobacillus plantarum*. J Bacteriol 170:5939–5942. https://doi.org/10.1128/jb.170.12.5939-5942.1988
78. Jacobsen L, Wilcks A, Hammer K, Huys G, Gevers D, Andersen SR (2007) Horizontal transfer of *tet*(M) and *erm*(B) resistance plasmids from food strains of *Lactobacillus plantarum* to E*nterococcus faecalis* JH2-2 in the gastrointestinal tract of gnotobiotic rats. FEMS Microbiol Ecol 59:158–166. https://doi.org/10.1111/j.1574-6941.2006.00212.x
79. Feld F, Schjorring S, Hammer K, Licht TR, Danielsen M, Krogfelt K, Wilcks S (2008) Selective pressure affects transfer and establishment of a *Lactobacillus plantarum* resistance plasmid in the gastrointestinal environment. J Antimicrob Chemother 61:845–852. https://doi.org/10.1093/jac/dkn033
80. Preethi C, Thumu SCR, Halami PM (2017) Occurrence and distribution of multiple antibiotic-resistant *Enterococcus* and *Lactobacillus* spp. from Indian poultry: in vivo transferability of their erythromycin tetracycline and vancomycin resistance. Ann Microbiol 67:395–404. https://doi.org/10.1007/s13213-017-1270-6
81. Gazzola S, Fontana C, Bassi D, Cocconcelli PS (2012) Assessment of tetracycline and erythromycin resistance transfer during sausage fermentation by culture-dependent and independent methods. Food Microbiol 30:348–354. https://doi.org/10.1016/j.fm.2011.12.005

82. Cocconcelli PS, Cattivelli D, Gazzola S (2003) Gene transfer of vancomycin and tetracycline resistances among *Enterococcus faecalis* during cheese and sausage fermentations. Int J Food Microbiol 88:315–323. https://doi.org/10.1016/s0168-1605(03)00194-6
83. Toomey N, Monaghan A, Fanning S, Bolton D (2009) Transfer of antibiotic resistance marker genes between lactic acid bacteria in model rumen and plant environments. Appl Environ Microbiol 75:3146–3152. https://doi.org/10.1128/AEM.02471-08