Chapter 2

Literature reviews

1. Lactic acid bacteria (LAB)

1.1 Characteristic of LAB

Originally, the term lactic acid bacteria, dating back to the late nineteenth century, was a synonym of "milk-souring organisms". The first pure culture of LAB obtained in 1873 by Lister was *Bacterium lactis* (*Lactococcus lactis*) (Axelsson, 1998). LAB are Gram-positive, catalase negative, nonsporing, fastidious and acid tolerant cocci or rods, which produce lactic acid as the major end product during the fermentation of carbohydrates. Some LAB are also responsible for producing flavor compounds characteristic of fermented products such as diacetyl in cultured butter. Moreover, organic acids and additional metabolites produced during fermentation play an important role in food preservation. Lower food pH and bacteriocins produced inhibit growth of spoilage and pathogenic organisms, resulting in extending the shelf-life of fermented foods (Shareck *et al*., 2004).

Two main sugar fermentation pathways can be distinguished among lactic acid bacteria. Glycolysis (Embden-Meyerhof pathway) results in almost exclusively lactic acid as the end product under standard conditions. The metabolism is referred to as homolactic fermentation (Figure 2.1) while heterolactic fermentation uses 6-phosphogluconate/phosphoketolase pathway resulting in significant amounts of other end products, such as ethanol, acetate, and CO_2 in addition to lactic acid (Figure 2.2). Various growth conditions may significantly alter end-product formation by some

lactic acid bacteria. These changes can be attributed to an altered pyruvate metabolism and/or the use of external electron acceptors such as oxygen or organic compounds (Axelsson, 1993).

1.2 Classification of lactic acid bacteria

Orla-Jensen (1919) used a few characters as classification basis: morphology (cocci or rods, tetrad formation), mode of glucose fermentation (homo- or heterofermentation), growth at certain "cardinal" temperatures (10°C and 45°C) and isomeric form of lactic acid produced (D, L, or both). These characters are still very important in current LAB classification. After the work by Orla-Jensen, the view emerged that the core of LAB comprised four genera: *Lactobacillus, Leuconostoc, Pediococcus* and *Streptococcus*. The classification of LAB described above is largely based on phenotypical and biochemical characters. In practice, these characters may not be enough to definitely assign a strain to particular species. Thus, in some cases, DNA-DNA homology studies have been the only way to resolve identification problems (Kandler and Weiss, 1986).

Nowadays, based on 16S rRNA gene studies, LAB are divided into 12 genera (Wood and Holzafel, 1995) including *Aerococcus, Alloiococcus, Atopobium, Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Pediococcus, Streptococcus, Tetragenococcus* and *Vagococcus*.

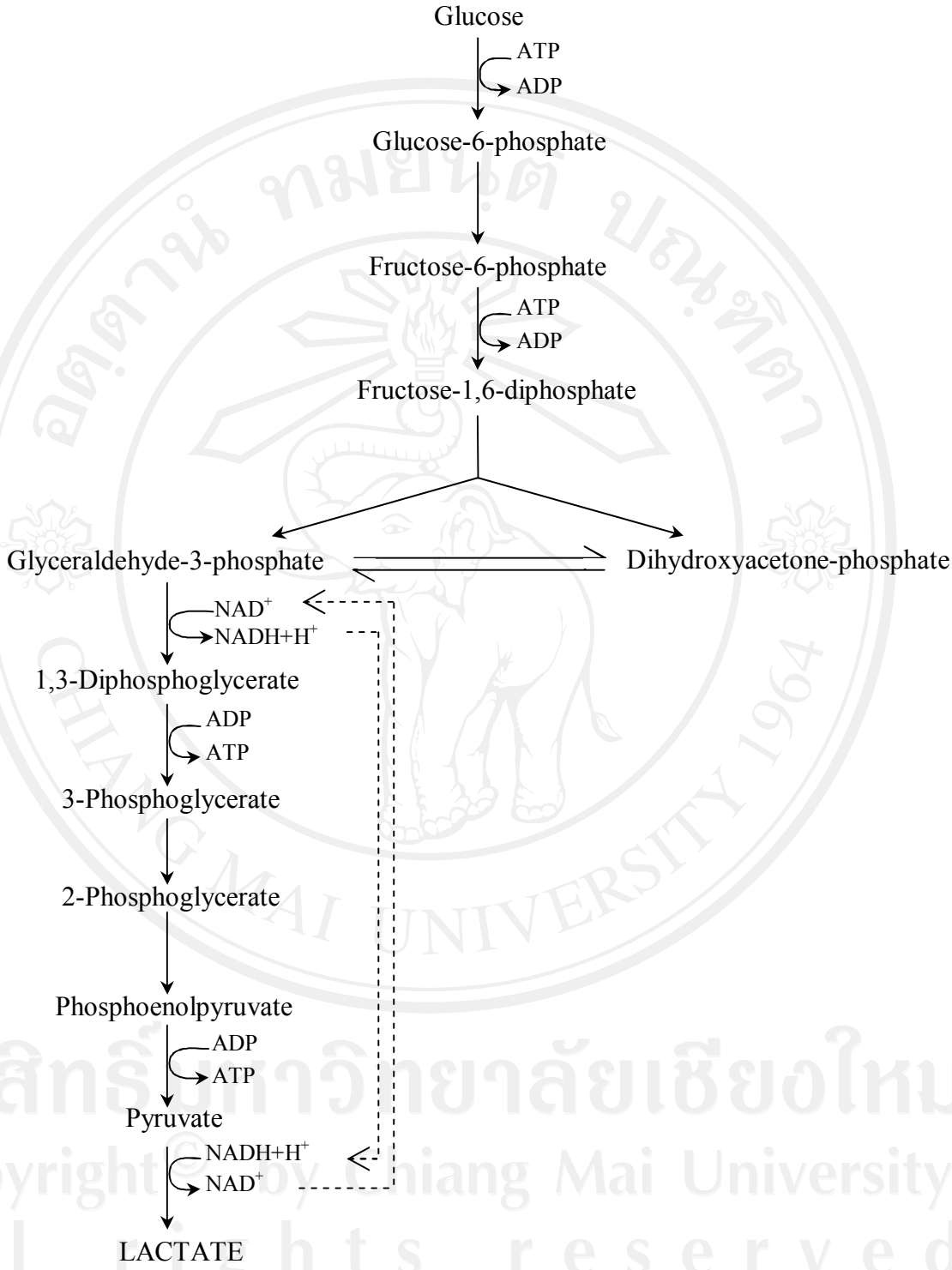

Figure 2.1 Homolactic fermentation (Axelsson, 1993)

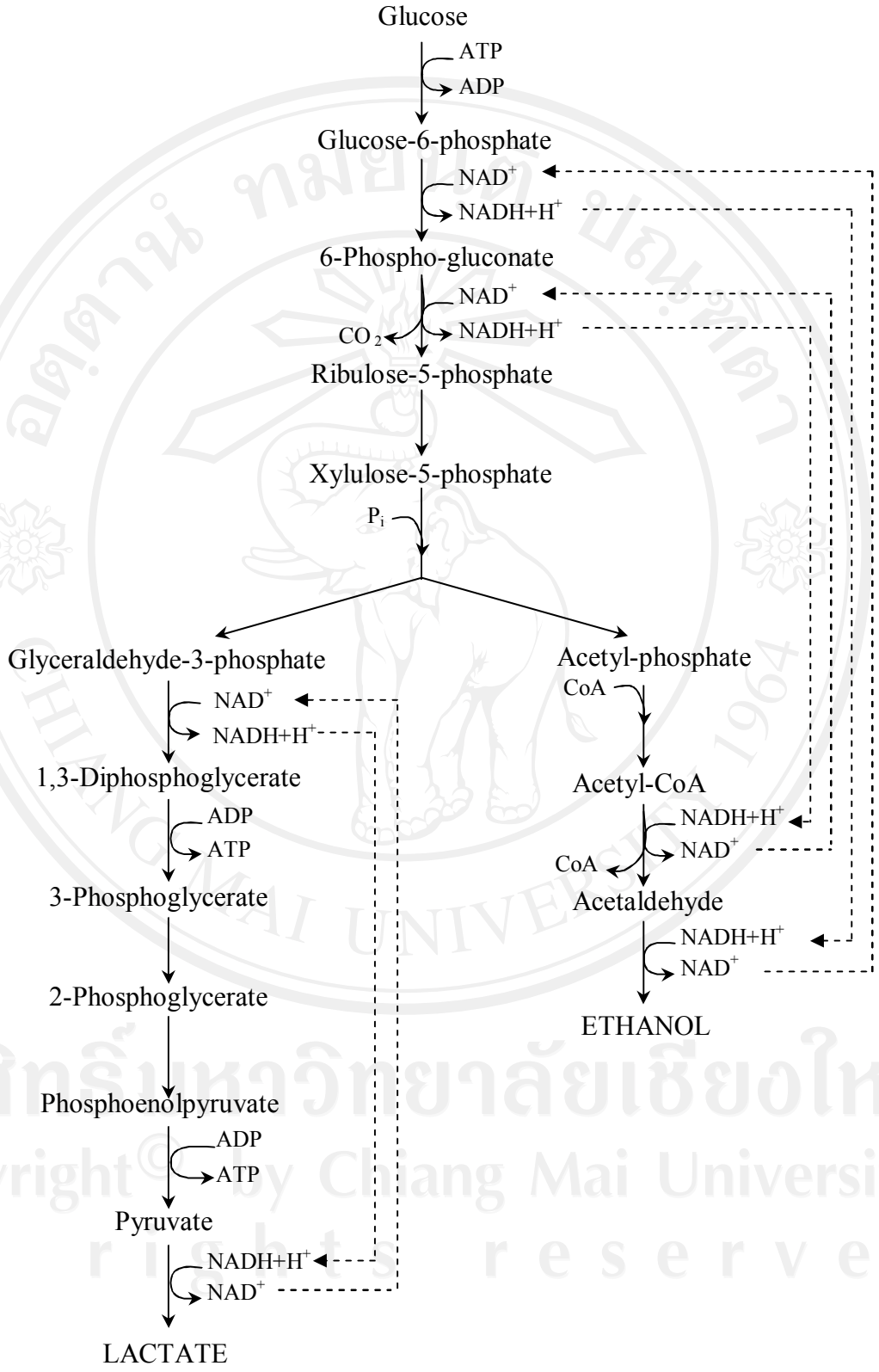

Figure 2.2 Heterolactic fermentation (Axelsson, 1993)

2. Lactic acid

Lactic acid (2-hydroxypropionic acid or 2-hydroxypropanoic acid, $CH_3CHOHCOOH$) is the most widely utilized organic acid in food, pharmaceutical, textile, leather and other chemical industries (Vickroy, 1985; Wee *et al*., 2006; John *et al*., 2007). The first report on isolation of lactic acid from milk can be found as early as 1780 by a Swedish chemist, Carl Wilhelm Scheele (Holten *et al*., 1971). Lactic acid exists naturally in two optical isomers. One is L-(+)-lactic acid and its mirror image, D-(-)-lactic acid (Figure 2.3).

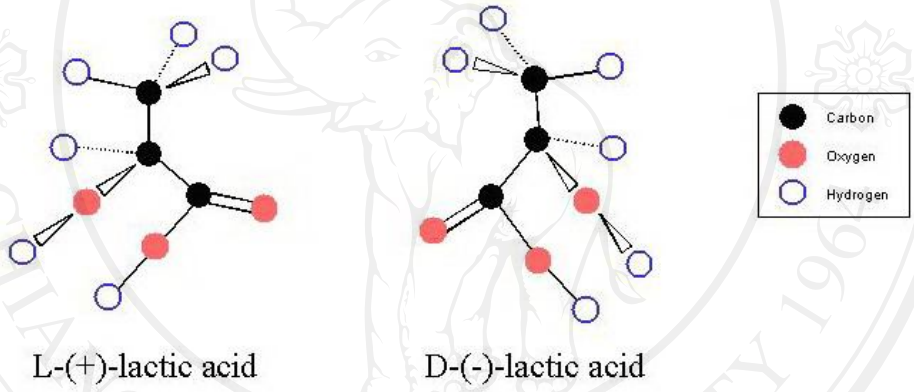

Figure 2.3 Structural formula of lactic acid (Anonymous 1, 2010)

L-(+)-lactic acid, the biological important isomer, is found in liver, kidney, blood, muscle, thymus gland and body fluids of humans and animals. Its production can be increased in muscle and blood after severe exercises. D-(-)-lactic acid is found only in metabolisms of microorganisms, and harmful to humans (Expert committee on food additives, 1967). Properties of lactic acid are explained (Table 2.1).

Table 2.1 Properties of lactic acid (Vickroy, 1985)

Properties	
Molecular weight	90.08
Melting point	L : 53.0°C
	D : 53.0°C
	D/L : 16.8°C
Boiling point	82.0 at 0.5 mm Hg
	122.0 at 14 mm Hg
Acidity (pK_a at 25°C)	1.37×10^{-4}
Heat of combustion	1361 KJ/mol
Specific heat (C_p at 20°C)	190 J/mol °C

Lactic acid is considered as generally recognized as safe (GRAS) for use as food additives by the regulatory agencies like Food and Drug Administration (FDA) in USA. It is used as acidulant, flavoring or buffering agent or inhibitor of bacterial spoilage in a wide variety of processed foods, such as candy, bread and bakery products, soft drinks, soups, sherbets, dairy products, beer, jam and jellies, mayonnaise and processed eggs, often in conjuction with other acidulants (Datta *et al*., 1995; John *et al*., 2007). The water-retaining capacity of lactic acid makes it suitable for use as moisturizer in cosmetic formulations. The ability of lactic acid to suppress the formation of tyrosinase is responsible for its effects as skin lightening and rejuvenation (Datta *et al*., 1995). Lactic acid has long been used in pharmaceutical formulations, mainly in topical ointments, lotions and parenteral solution. It also finds applications in the preparation of biodegradable polymers for

medical uses such as surgical sutures, prostheses and controlled drug delivery systems (Wee *et al*., 2006).

Technical-grade lactic acid is extensively used in leather tanning industries as an acidulant for deliming hides and in vegetable tanning as well as descaling agent, solvent, cleaning agent, slow acid-releasing agent and humectants in a variety of technical processes. The demand for lactic acid has been increasing considerably, owing to the promising applications of its polymer as an environment-friendly alternative to petrochemicals plastics. The lactic acid polymers and co-polymers, with tremendous advantages like biodegradability, thermoplasticity and high strength have potentially large markets. Lactic acid could be potentially used for the manufacturing of large-volume oxygenated chemicals, such as propylene glycol, propylene oxide, acrylic acid and acrylate esters, and other chemical intermediates such as lactate ester plasticizers. The advances made in hydrogenolysis technology can be further developed and integrated to make propylene glycol from lactic acid in the future (Datta and Henry, 2006).

Lactic acid can be produced by chemical synthesis or fermentation. Chemical synthesis of lactic acid is mainly based on hydrolysis of lactonitrile, derived from acetaldehyde and hydrogen cyanide, by strong acids (Figure 2.4). This petrochemical process yields a racemic mixture of the two isomers while the fermentation process provide both optically pure form of lactic acid and racemate depending on type of microorganisms, substrates and fermentation conditions employed in the production process (Yin *et al*., 1997; Akerberg *et al*., 1998; Huang *et al*., 2003). Besides the high product specificity as it produces a desired optically pure L-(+)- or D-(−)-lactic acid, the biotechnological production of lactic acid offers several advantages over chemical

synthesis such as low cost of substrates, low production temperature and low energy consumption (John *et al*., 2007). Biotechnologically produced lactic acid can be obtained from cheap raw materials such as molasses, starchy waste, cellulosic and other carbohydrate rich materials (Anuradha *et al*., 1999; Vishnu *et al*., 2000). Raw material cost is one of the major factors to be considered in economic production of lactic acid.

$$HCN + CH_3CHO \rightarrow CH_3CH(OH)CN \text{ (lactonitrile)}$$

$$CH_3CH(OH)CN + 2H_2O + HCl \rightarrow CH_3CH(OH)COOH + NH_4Cl$$

Figure 2.4 Chemical synthesis of lactic acid

3. The genus *Lactobacillus*

Lactobacillus is the largest genus of LAB. It is very heterogeneous and encompassing species with a large variety of phenotypic, biochemical and physiological properties. The heterogeneity is reflected by the range of DNA mol% G+C which is between 32-53%, as twice as the span usually accepted for a single genus (Schleifer and Stackebrandt, 1983).

Lactobacilli are strictly fermentative, aero-tolerant or anaerobic, aciduric or acidophilic and have complex nutritional requirements (such as carbohydrates, amino acids, peptides, fatty acid esters, salts, nucleic acid derivatives and vitamins). Cultivated with glucose as a carbon source, lactobacilli may be either homofermentative, which produced more than 85% lactic acid, or heterofermentative,

which produced lactic acid, CO_2, ethanol and/or acetic acid in equimolar amounts (Wang and Lee, 1997).

Lactobacilli are widespread in nature. Many lactobacilli have been used in food fermentation processes including milk, meat and plant materials. In addition, a few species of *Lactobacillus* are used as probiotic microorganisms in functional foods. Lactobacilli are found where rich carbohydrate-containing substrates are available such as mucosal membranes of man and animal (oral cavity, intestine and vagina), plants or materials of plant origin, manure and man-made habitats such as sewage and fermenting or spoiling foods (Hammes and Vogel, 1995).

The classical ways of distinguishing among species of lactobacilli are carbohydrate fermentation patterns, configuration of lactic acid produced, hydrolysis of arginine, growth requirements and growth at certain temperatures (Sharpe, 1979, 1981). These characters are still useful, but proper classification may also require analysis of the peptidoglycan, electrophoretic mobility of the lactate dehydrogenase, mol% G+C of the DNA, and DNA-DNA homology studies (Kandler, 1984; Kandler and Weiss, 1986). Hammes and Vogel (1995) divided lactobacilli into 3 groups:

Group A: Obligately homofermentative lactobacilli. Hexoses are almost exclusively (>85%) fermented to lactic acid by the Embden-Meyerhof-Parnas (EMP) pathway. The organisms possess fructose-1,6-bisphosphate-aldolase but lack phosphoketolase, therefore, neither gluconate nor pentoses are fermented.

Group B: Facultatively heterofermentative lactobacilli. Hexoses are almost exclusively fermented to lactic acid by the EMP pathway. The organisms possess both aldolase and phosphoketolase, therefore, not only ferment hexose but

also pentoses (and often gluconate). In the presence of glucose, the enzymes of the phosphogluconate pathway are repressed.

Group C: Obligately heterofermentative lactobacilli. Hexoses are fermented by the phosphogluconate pathway yielding lactate, ethanol, acetic acid and CO_2 in equimolar amounts. Pentoses enter this pathway and may be fermented.

Table 2.2 summarizes the characters used to distinguish among the three groups and some of the well-known species included in each group.

Table 2.2 Grouping of the genus *Lactobacillus* (Adapted from Sharpe (1981) and Kandler and Weiss (1986))

Characteristic	Group A: Obligately homofermentative	Group B: Facultatively heterofermentative	Group C: Obligately heterofermentative
Pentose fermentation	-	+	+
CO_2 from glucose	-	-	+
CO_2 from gluconate	-	+[a]	+[a]
FDP aldolase present	+	+	-
Phosphoketolase present	-	+[b]	+
	Lb. acidophilus	*Lb. casei*	*Lb. brevis*
	Lb. delbruckii	*Lb. curvatus*	*Lb. buchneri*
	Lb. helveticus	*Lb. plantarum*	*Lb. fermentum*
	Lb. salivarius	*Lb. sake*	*Lb. reuteri*

[a] When fermented
[b] Inducible by pentoses

4. Plasmid DNA

Many important bacterial genes are not part of the main chromosome but are on separate circles of DNA called plasmids. Plasmids have been found in species of the domains Archaea, Bacteria and Eukarya (Woese *et al*., 1990). The term "plasmid" was first introduced by an American molecular biologist Joshua Lederberg in 1952 (Lederberg, 1952). A plasmid is a molecule which can be stably inherited

without being linked to the chromosome (Figure 2.5). It can be replicated independent of the host genome. Bacterial plasmids are generally defined as circular double-stranded DNA molecules. No RNA bacterial plasmids have been found, although several fungal plasmids and numerous bacteriophage chromosomes are composed of RNA. All bacterial plasmids examined so far exist predominantly as circular molecules in their host cells (Hardy, 1981).

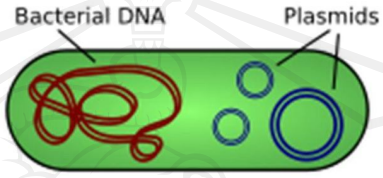

Figure 2.5 Illustration of a bacterium with plasmid enclosed showing chromosomal DNA and plasmids (Anonymous 2, 2010)

Plasmid size varies from 1 to over 1,000 kb. The number of identical plasmids within a single cell can range anywhere from one to even thousands under some circumstances. Plasmids may carry genes that provide resistance to naturally occurring antibiotics in a competitive environmental niche, or alternatively the proteins produced may act as toxins under similar circumstances. Plasmids also can provide bacteria with an ability to fix elemental nitrogen or to degrade calcitrant organic compounds which provide an advantage under conditions of nutrient deprivation (Lipps, 2008).

4.1 Structure of plasmids

Most of the plasmid DNA inside bacteria is in the form of a covalently-closed circle (CCC), meaning that there are no breaks in either of the two polynucleotide

strands which comprise the double-helix. Most of the CCC plasmid molecules isolated from bacteria are twisted to form supercoiled molecules which have superhelical twists. Plasmids isolated from bacteria often appear in the electron microscope as tightly coiled and branched structures because of these superhelical twists.

If one of the two polynucleotide strands in a closed-circular plasmid is broken or nicked, an open-circle is formed. If the CCC molecule was previously supercoiled, the superhelical twists are lost. Thus, the molecule unwinds and becomes relaxed. When both polynucleotide strands are broken, a linear molecule is formed if the two breaks are either exactly opposite, or close together. The hydrogen bonds between the intervening complementary bases are not strong enough to hold the two strands together (Figure 2.6). The small proportions of open-circular and linear molecules found in cell extracts may be derived in part from CCC molecules which are nicked when the cells are broken. Some of the very large plasmids are particularly difficult to keep in the CCC form during isolation and purification. Other forms including dimers, trimers and other multimers of plasmid DNA also occur in cell lysates (Hardy, 1981).

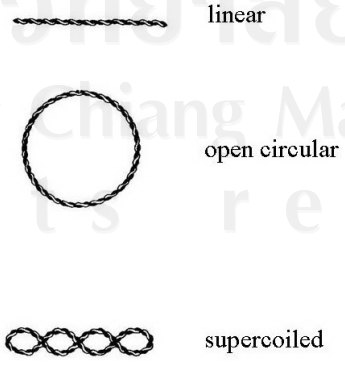

Figure 2.6 Forms of plasmids (Hardy, 1981)

4.2 Plasmid replication

All plasmids replicate in a semi-conservative manner, one strand of the DNA molecule serving as a template for the synthesis of a complementary strand by DNA polymerase. As autonomous genetic elements, plasmids contain genes essential for their replication including genes controlling the initiation and control of replication. Nevertheless, the process of plasmid replication is still highly dependent on host cells.

Plasmids replicate by one of two mechanisms, theta or rolling circle replication (also known as sigma-replication). Theta-replicating plasmids tend to be medium- and large-size plasmids (Shareck *et al*., 2004). Theta replication has three key components: (1) an initiator protein (Rep) necessary for strand opening, (2) an origin of replication (*ori*) with specific DNA structural organization for strand opening and initiator-protein binding and (3) a host-encoded polymerase I for nascent strand DNA synthesis (del Solar *et al*., 1998; Alpert *et al*., 2003). During theta replication, the two strand of the DNA double helix separate at the origin of replication, and the DNA synthesis proceeds in either one or both directions (Figure 2.7). Leading and lagging strand syntheses occur simultaneously during theta replication and is primed at closely located sites within the origin of replication. The plasmids molecule retains a circular conformation throughout the replicative process, and the DNA remains supercoiled ahead of the replication fork. On completion of the replication cycles, DNA gyrase nicks one of the circles to release a daughter plasmid molecule. The nicked plasmid is sealed and subsequently supercoiled (Rixon and Warner, 2003).

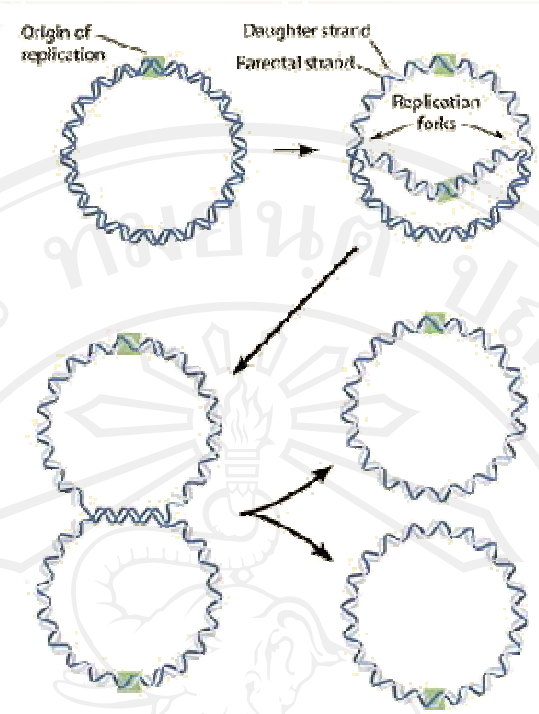

Figure 2.7 Theta replication (Anonymous 3, 2010)

The rolling circle replication (RCR) (Figure 2.8) seems to be restricted to relatively small cryptic plasmids. RCR plasmids constitute a group of small, multicopy replicons that are widely spread among bacteria. Genetic elements that are involved in RC replication are the *rep* gene that encodes the replication initiation protein (Rep) controlled by a repressor and its target site, the plus origin of replication or double-stranded origin (*dso*). Additionally, most RCR plasmid have a minus origin of replication or single strand origin (*sso*), a specific sequence that enables the conversion of ssDNA intermediates into double-stranded DNA (dsDNA) molecules (Gruss and Ehrlich, 1989).

The Rep protein is a site-specific nuclease, which produces a single stranded nick at the plus origin (*dso*), initiating positive strand replication and terminating it when a leading strand (ssDNA) is synthesized (Gruss and Ehrlich, 1989). The leading

strand replication generates: (1) a dsDNA molecule constituted by the parental [-] strand and (2) a ssDNA intermediate that corresponds to the parental [+] strand. Generation of ssDNA is the trademark characteristic of RC replication. Finally, lagging strand synthesis occurs and ssDNA intermediates are converted to dsDNA at the minus origin (*sso*). The last step involves supercoiling of the replicated DNA by the host DNA gyrase (Espinosa *et al*., 2000).

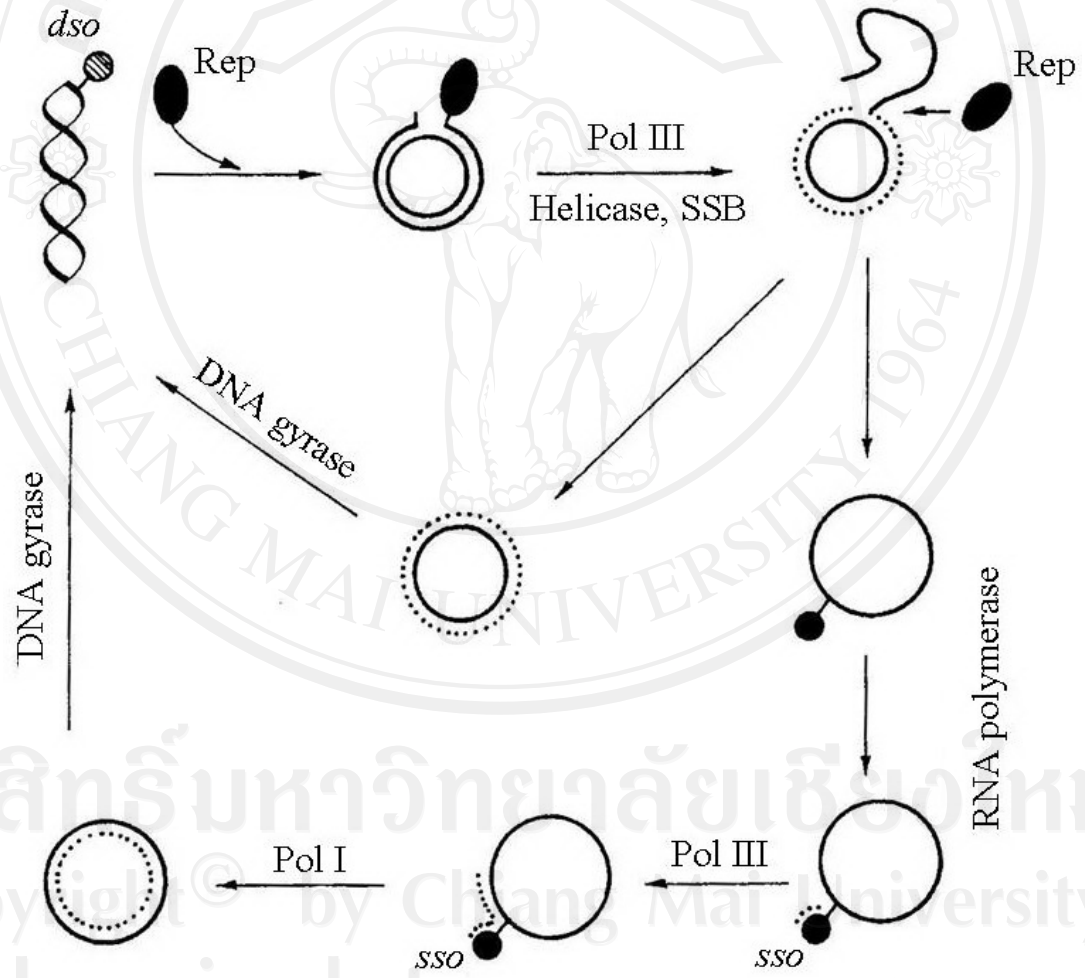

Figure 2.8 The rolling circle replication (RCR) (Espinosa *et al*., 1995)

4.3 Classification of plasmids (Hardy, 1981)

Various criteria are used to classify plasmids. The most conspicuous or the most important characteristic is used to provide one type of classification. Thus R plasmids confer resistance to one or more antibacterial drugs, and Col plasmids code for antibacterial proteins called colicins. Degradative plasmids code for a variety of catabolic enzymes, and virulence plasmids increase the pathogenicity of bacteria in various ways. Each type of plasmid can be found in a wide range of bacterial genera. Apart from the common feature which is used as a criterion for the classification, many plasmids within each group appear to be totally unrelated to each other. Some belong to several of these groups, some virulence plasmids specify drug-resistance, and many R plasmids also code for colicins.

Plasmids can be either conjugative or nonconjugative. Conjugative plasmids transfer copies of themselves from one bacterium to another and many of them are known to code for protein tubes called sex pili. DNA is believed to pass through these tubes from one cell to another. Conjugative plasmids can also transfer pieces of chromosomal DNA between bacteria, therefore sometimes referred to as sex factors. These plasmids are very useful for mapping the positions of chromosomal genes.

4.4 Plasmids and genetic engineering

Plasmids are used as vectors to clone DNA molecules. Restriction endonucleases, ligase and other enzymes can be used to add pieces of foreign DNA, called inserts, to plasmid molecules *in vitro*. Recombinant plasmid can be put back into a suitable host bacterium. The insert is added at an unessential site in the vector so that the recombinant plasmid can replicate in the bacterium (Hardy, 1981).

The main steps in using plasmid vectors to clone DNA molecules are as follows (Figure 2.9):

1. Isolation of circular plasmid DNA molecules and preparation of the DNA insert. The insert may be a piece of chromosomal DNA from animals, plants or other microorganisms, a cDNA (complementary DNA) molecule derived from mRNA, or a chemically synthesized sequence such as PCR product.

2. Insertion of the foreign DNA into plasmid. There are several ways of doing this, but they all involve breaking a circular plasmid at a specific point with a restriction endonuclease to convert it into a linear molecule. The cleaved plasmid is mixed with the DNA to be inserted (the ends of the insert are usually made homologous to the ends of the vector) and the two are ligated together by DNA ligase to form a circular molecule.

3. Addition of recombinant plasmids to host cell. Several methods such as transformation, electroporation or transfection are used.

4. Identification of transformants which have recombinant plasmids. There are many ways to identify the transformants. For examples, α-complemetation, hybridization, restriction analysis, insertional inactivation and product assays.

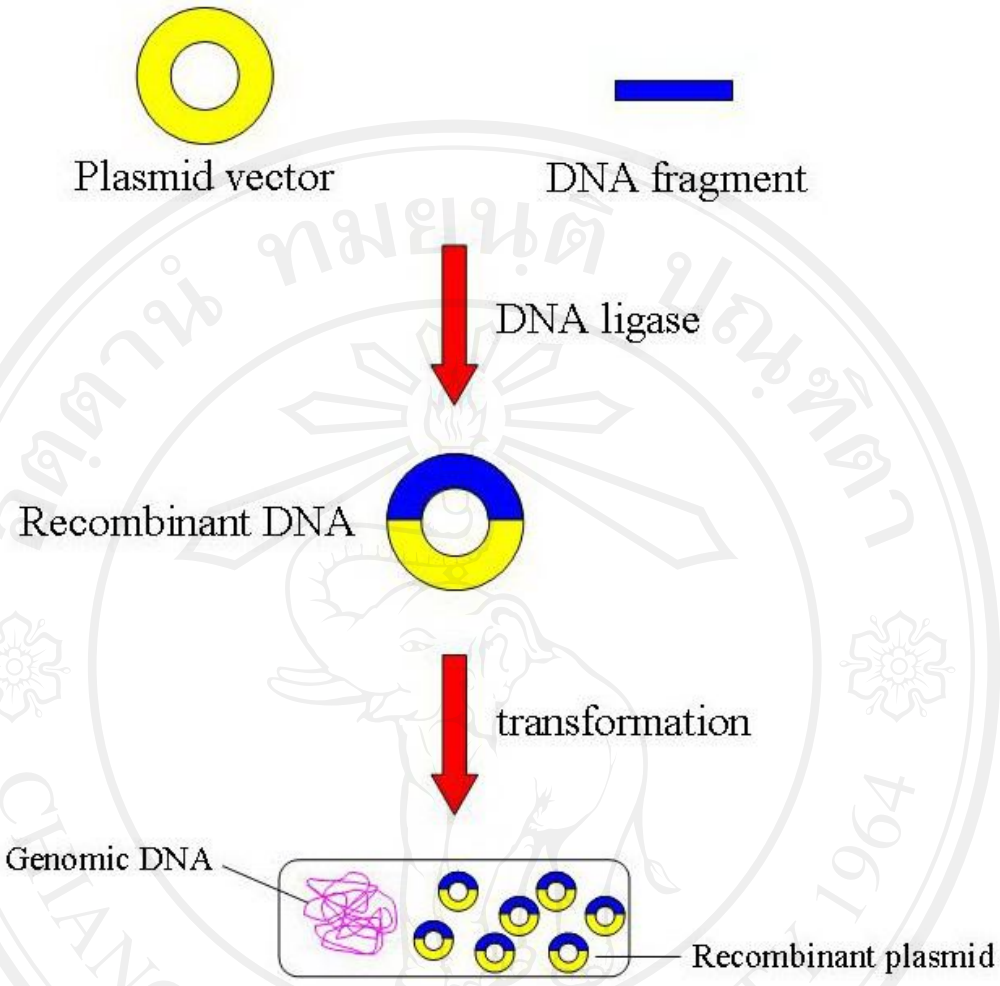

Figure 2.9 The main steps in using plasmid vectors to clone DNA molecules

5. Plasmids in *Lactobacillus*

For the last 20 years, *Lactobacillus* plasmids have been studied in several aspects, for examples, plasmid detection and isolation, investigation of structure, replication, function and stability of plasmids and construction of *Lactobacillus* vectors based on plasmids and development of transformation methods (Wang and Lee, 1997). One way to genetically improve lactobacilli is to clone and express a

foreign gene with beneficial features in lactobacilli using a cloning vector derived from a stable cryptic *Lactobacillus* plasmid (Pavlova *et al*., 2002).

5.1 Plasmid distribution

Nowadays, with the development of molecular tools and genetic characterization, the new species of lactobacilli were discovered by several research groups. For example, 44 *Lactobacillus* species and 11 subspecies were described in 1984 (Kandler and Weiss, 1986), 88 and 15 in 2003 (Coeuret *et al*., 2003) and 135 and 27 in January 2007 (Bernardeau *et al*., 2008) resulting that many new native plasmids from lactobacilli were increasingly detected. *Lactobacillus* plasmids were first isolated from *Lactobacillus casei* (Chassy *et al*., 1976). Mostly *Lactobacillus* plasmids were detected from *L. plantarum*, *L. acidophilus, L. casei* and *L. helveticus* (Wang and Lee, 1997).

Lactobacillus appears to contain one or more (usually from 1 to 10) different plasmids, but *L. plantarum* LPC25 had 16 plasmids (Ruiz-Barba *et al*., 1991). The known plasmids are ranging from 1.2 kb to 169 kb which isolated from *L. plantarum* LL31 and LL2, respectively (Mayo *et al*., 1989). The plasmids found are usually in a circular form, however, two linear plasmids from *L. gasseri* CNRZ222 (150 kb) and IP102991 (50 kb), have been identified (Roussel *et al*., 1993).

Studies of DNA hybridization and sequences have shown that certain homology existed in some plasmids from the same strains, species, genus and even intergenus. For example, plasmid p1 and p3 from the same strain *L. acidophilus* 168S showed a high DNA homology (Damiani *et al*., 1987). These phenomena strongly suggest that there is considerable plasmid recombination among *Lactobacillus*

plasmids and/or horizontal transfer of *Lactobacillus* plasmids occurs among intraspecies, interspecies, and with other genera, or these plasmids have evolved from common ancestors (Wang and Lee, 1997).

5.2 Plasmid structure and replication

Although many *Lactobacillus* plasmids have been found, very little is known about their structures. The comparison of DNA sequences and putatively translated amino acid sequences of *Lactobacillus* plasmids show that they have highly homologous DNA sequences with plasmids pC194 (Horinouchi and Weisblum, 1982a), pE194 (Horinouchi and Weisblum, 1982b), pSN2 (Khan and Novick, 1982) and pT181 (Khan and Novick, 1983) from other Gram positive bacteria. Plasmids pC194, pE194, pSN2 and pT181 amplify themselves by a model of rolling circle replication (RCR) and produce single-stranded DNA (ssDNA) as intermediate (Gruss and Ehrlich, 1989). For the RCR model, there are at least three plasmid-borne elements involved: (1) a gene (*rep*) encoding a replication initiation protein (REP), (2) a plus origin, the target site of REP that first nicks the positive strand of plasmid DNA for replication initiation, and then terminates the replication when a leading strand (ssDNA) is synthesized, and (3) a minus origin, characterized by a long, imperfect inverted repeat, which enables the conversion of the ssDNA intermediate into the dsDNA molecule. The DNA fragment containing plus origin and *rep* is called a replicon. In addition, some other Gram positive bacterial plasmids contain extra elements such as a specific cointegration site (RS_A) at which plasmid recombination occurs (Gruss and Ehrlich, 1989), a DNA region directing countertranscript-RNA (CT-RNA), and an open reading frame (ORF) encoding a repressor that accounts for

controlling the copy number of plasmid. On the basis of similarities in the structure of REP and plus origin, the RCR plasmids could be classified into four groups: pC194, pE194, pT181 and pSN2, which are regarded as typical representatives (Gruss and Ehrlich, 1989; Pouwels and Leer, 1993; Seery *et al*., 1993).

Generally, the *Lactobacillus* plasmids hypothetically consist of one or more ORFs, plus origin, inverted repeat sequences, minus origin, RS_A and CT-RNA (Wang and Lee, 1997) (Figure 2.10).

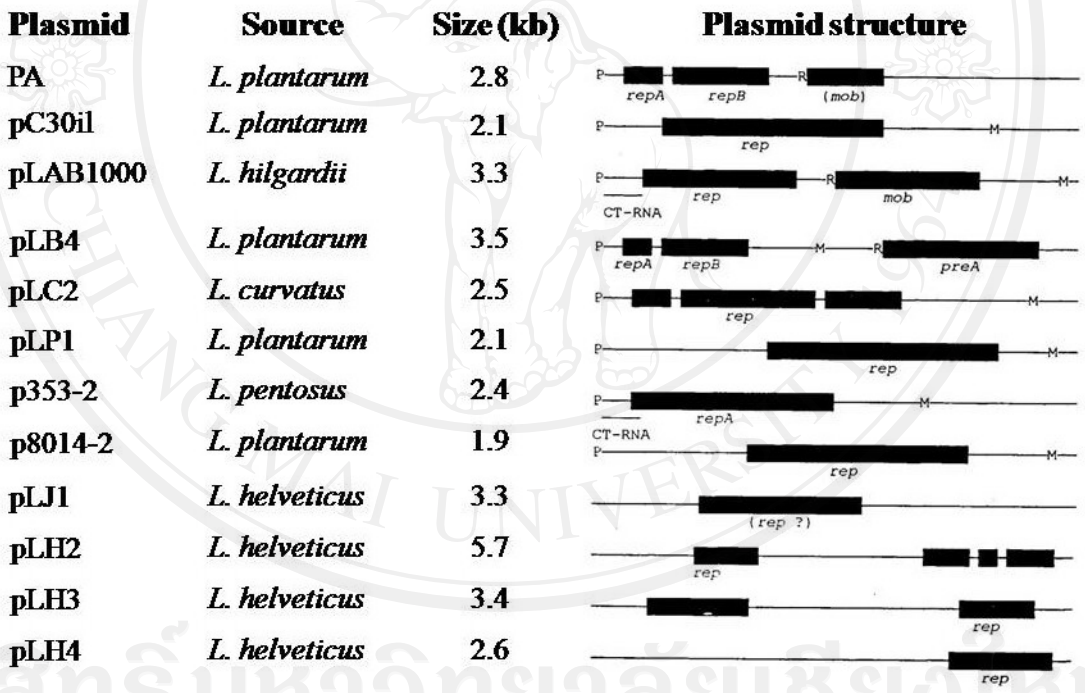

Figure 2.10 Comparison of *Lactobacillus* plasmids. (Symbols: black solid box, open reading frame (ORF); M, minus origin; P, plus origin; R, RS_A site; CT-RNA, the region coding countertranscript RNA. Inverted repeat sequences are not shown) (Wang and Lee, 1997).

5.3 Plasmid Functions

The procedures used to study the function of a native plasmid include curing the plasmid from a host, transferring the plasmid into a suitable host and comparing the physiological properties or phenotypes between the cured and uncured hosts. However, the technique provides an indirect evidence associated with plasmid functions. As a direct technique of investigating plasmid functions, some structural genes directing certain functions from the *Lactobacillus* plasmids have been cloned and studied. To this date, many lactobacilli plasmids have been found, but most remain cryptic. However, some functions have been found to be plasmid-encoded that relate to lactose metabolism, antibiotic resistance, bacteriocin production and immunity, DNA restriction or modification (R-M), exopolysaccharide production, N-acetyl glucosamine fermentation and certain amino acid (cysteine) transport (Pouwels and Leer, 1993; Arihara and Luchansky, 1994). Hence, plasmid functions can be divided into four main groups (Wang and Lee, 1997).

1. Hydrolysis of proteins

L. helveticus ssp. *jugurti* S36.2 increased the lactic acid production due to the proteolytic activity from the plasmid pLHJ1 (de Rossi *et al*., 1989).

2. Metabolism of carbohydrates, amino acid and citrate

2.1 Metabolism of carbohydrates

Some *Lactobacillus* plasmids can offer their hosts ability to digest carbohydrates. More than 10 *Lactobacillus* plasmids are known to be associated with lactose metabolism such as pLA102 from *L. acidophilus* (Kanatani *et al*., 1991), pLY101 (Shimizu-Kadota, 1987), pLZ14, pLZ15, pLZ18A, pLZ19A, pLZ61 (Lee-Wickner and Chassy, 1985) from *L. casei* and a plasmid from *L. plantarum* (Mayo *et*

al., 1994). In addition, *Lactobacillus* plasmids are correlated with galactose metabolism, such as pLA101 from *L. acidophilus* TK8912 (Kanatani *et al*., 1991), maltose utilization such as pML291 (Liu *et al*., 1988). Sorbitol fermentation was linked to a *Lactobacillus* plasmid of 60 kb (Ahrne *et al*., 1989).

2.2 Metabolism of amino acid

Plasmids of *L. sake* L13 are related to a cysteine uptake system (Shay *et al*., 1988).

2.3 Metabolism of citrate

A 10.8 kb plasmid from *L. plantarum* IFO3070 was found to be associated with the production of citrate permease required for the citrate fermentation (Nakamura *et al*., 1991).

3. Production of bacteriocins, exopolysaccharides and pigment

3.1 Production of bacteriocins

Like other lactic acid bacteria, *Lactobacillus* are able to produce various bacteriocins to inhibit growth of related strains and even to far related species (Klaenhammer, 1988). At least 28 kinds of bacteriocins are produced by *Lactobacillus* species (Klaenhammer, 1993; Nettles and Barefoot, 1993). Among them, the production of 9 *Lactobacillus* bacteriocins is related to plasmids, for examples, acidocin A, acidocin B, acidocin 8912 encoded in pLA9201, pCV461 and pLA103, respectively, of *L. acidophilus* (Kanatani *et al*., 1992, 1995; van der Vossen *et al*., 1994), brevicin 27 from a 4.6 kb plasmid of *L. brevis* (Benoit *et al*., 1994), curvacin A from a 60 kb plasmid of *L. curvatus* (Tichaczek *et al*., 1992). The *Lactobacillus* plasmid-encoded bacteriocins not only inhibit growth of related species,

but also inhibit growth of opportunistic food-borne pathogens such as *Clostridium sporogenes, Enterococcus faecalis, Listeria monocytogenes* and others (Table 2.3).

Table 2.3 Bacteriocins produced by *Lactobacillus* plasmids and their activity spectra

Bacteriocins	Source	Inhibiting strain spectra[a]	Ref.
Acidocin A	*L. acidophilus*	*En. faecalis, L. acidophilus, L. brevis, L. casei, L. fermentum, Lc. lactis, Ls. monocytogenes, Pd. pentosaceus, Pd. halophilus, St. bovis, St. thermophilus, Propionibacterium*	Kanatani *et al*., 1995
Acidocin B	*L. acidophilus*	*Br. thermosphacta, Cl. sporogenes, L. fermentum, Ls. monocytogenes*	van der Vossen *et al*., 1994
Acidocin 8912	*L. acidophilus*	*L. acidophilus, L. amylophilus, L. casei, L. plantarum, Lc. lactis*	Kanatani *et al*., 1992
Brevicin 27	*L. brevis*	*B. coagulans, B. megaterium, B. stearothermophilus, L. buchneri, L. plantarum, L. sake, Le. paramesenteriodes , Pd. damnosus, Pd. pentosaceus*	Benoit *et al*., 1994
Curvacin A	*L. curvatus*	*C. divergens, C. piscicola, En. faecalis, L. curvatus, L. fructivorans, L. sake, Ls. monocytogenes, Ls. ivanovii*	Tichaczek *et al*., 1992
Lactacin F	*L. acidophilus*	*En. faecalis, L. acidophilus, L. delbrueckii* ssp. *lactis, L. fermentum, L. helveticus, L. lactis, L. leichmannii*	Muriana and Klaenhammer, 1987, 1991
Lactacin S	*L. sake*	*L. acidophilus, L. lactis, L. pentosaceus, L. plantarum, L. reuteri, Le. mesenteroides, Pd. acidilactici, Pd. Pentosaceus*	Mortvedt and Nes, 1990

Table 2.3 Bacteriocins produced by *Lactobacillus* plasmids and their activity spectra (continued)

Bacteriocins	Source	Inhibiting strain spectra[a]	Ref.
Sakacin A	*L. sake*	*C. piscicola, En. faecium, En. faecalis, L. alimentarius, L. curvatus, L. sake, Le. paramesenteriodes, Ls. monocytogenes*	Schillinger and Lucke, 1989
Plantacin 154	*L. plantarum*	*En. faecalis, L. acidophilus, L. brevis, L. casei, L. fermentum, L. plantarum, Lc. lactis, Pd. acidilactici, Pd. pentosaceus, Pp. acidipropionici, Pp. jensenii, Pp. theoenii, St. thermophilus*	Kanatani and Oshimura, 1994

[a] *B: Bacillus; Br: Brochotrix; C: Carnobacterium; Cl: Clostridium; En: Enterococcus; Lc: Lactococcus; Le: Leuconostoc; Ls: Listeria; Pd: Pediococcus; Pp: Propiniobacterium; St. Streptococcus*

3.2 Production of exopolysaccharides

Some *Lactobacillus* plasmids are responsible for exopolysaccharides production which can be used to improve the texture of products such as yogurt in the dairy industry (Cerning *et al*., 1986). Three *Lactobacillus* plasmids, one is 6.9 kb from *L. casei* ssp. *casei* NCIB4114 (Vescovo *et al*., 1989), another 11.2 kb from *Lactobacillus* sp. (Ahrne *et al*., 1989) and the other 30 kb from *L. casei* CG11 (Kojic *et al*., 1992) can produce exopolysaccharides.

3.3 Production of pigment

Plasmid pRL512 (12.6 kb) of *L. plantarum* L6222 isolated from fermented rice noodles was shown to produce an orange pigment (Takao *et al*., 1992).

4. Resistance to antibiotics, heavy metals and phages

4.1 Resistance to antibiotics

More than 10 identified *Lactobacillus* plasmids that confer resistance to antibiotics such as chloramphenicol, erythromycin, kanamycin, streptomycin and tetracycline are known, for examples**,** pBS195 from *Lactobacillus* sp. 195 resists to both kanamycin and streptomycin (Kozlova *et al*., 1991), a 53.9 plasmid from *L. acidophilus* resists to chloramphenicol (Ahn *et al*., 1992), pGT633 from *L. reuteri* resists to erythromycin (Tannock *et al*., 1994) and pLY2 from *L. fermentum* resists to tetracycline (Ishiwa and Iwata, 1980). Meanwhile, plasmids present in *L. delbruckii* ssp. *bulgaricus* and *L. helveticus* resist to gentamicin, neomycin, polymyxin B, rifamycin, streptomycin and terramycin (Morelli *et al*., 1983).

4.2 Resistance to heavy metals and phages

The plasmid associated with arsenate resistance was found in *L. helveticus* ATCC 15009 (Fortina *et al*., 1990). A 34 kb plasmid from *L. helveticus* CNRZ1094, 1905 and 1906 was correlated with resistance to two bacteriophage, 328-B1 and hv, respectively (de Los Reyes-Gavilan *et al*., 1990).