Gene Technology
ISSN: 2329-6682
Ntougias. Gene Technology2012, 1:1
DOI: 10.4172/2329-6682.1000e102
Editorial
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Alkaliphilic Lactic Acid Bacteria: Novel Sources for Genetic Engineering and Biotechnology

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Lactic acid fermentation is an important process used in the production of several food and beverages. Optimization of lactic acid fermentation improves product quality by positively affecting preservation, organoleptic characteristics and nutritional value [1]. Numerous attempts were made to identify novel lactic acid bacteria as starters in order to achieve effective monitoring of the fermentation process. Starter cultures of lactic acid bacteria can provide long-term safe storage, contributing highly to the improvement of flavor and the development of pleasant organoleptic characteristics [2]. Lactic acid bacteria act protectively against food-borne pathogens, exhibiting probiotic properties [3,4]. Several criteria have been proposed for the selection of lactic acid bacteria as starters, e.g. fermentation type (homo and hetero-fermentation), halotolerance and bacteriocin production [2]. Besides, attention was drawn to the role of nonstarter lactic acid bacteria in ripening of fermented products [5]. Both *PEDIOCOCCI* and homofermentative *LACTOBACILLI*, as nonstarter lactic acid bacteria, were found to be involved in cheese ripening [6].

Lactic acid fermentation is achieved by lactic acid bacteria; *Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Enterococcus* and *Streptococcus* are common lactic acid bacteria, while others such as *Aerococcus*, *Carnobacterium*, *Lactococcus*, *Oenococcus*, *Tetragenococcus*, *Vagococcus* and *Weissella* have been also reported [7-9]. Typical lactic acid fermentation results in a pH drop below pH 4.5-5.0, owing to lactic acid production from sugars. Depending on the end products formed during the fermentation process, lactic acid bacteria are divided into homofermentative and heterofermentative. Homofermentative lactic acid bacteria produce almost exclusively lactic acid, whereas heterofermenters produce lactic acid as well as ethanol and/or acetic acid and CO_2 [7].

Since 2001, lactate fermentation was defined as an acidic microbial process, although the taxonomic description of the genus *Alkalibacterium* [10] revealed that lactate fermentation can be also achieved under alkaline conditions [11,12]. The members of the genus *Alkalibacterium* have the typical characteristics of lactic acid bacteria, i.e. they are Gram-positive, non-spore-forming, catalase-negative, aerotolerant, strictly fermentative, which lack cytochromes and produce lactate as fermentation end product [7], apart from the fact that, instead of acid-tolerant, are obligately (occasionally facultatively) alkaliphilic, extremely halotolerant/moderately halophilic bacteria [12]. The genus *Alkalibacterium* belongs to the family *Carnobacteriaceae* (order *Lactobacillales*) which consists exclusively of lactic acid bacteria. *Carnobacteriaceae* spp., such as *Carnobacterium divergens* and *C. maltaromaticum*, can produce bacteriocins and inhibit growth of spoilage bacteria, including the food-borne pathogen *Listeria monocytogenes* [13]. Nowadays, the genus *Alkalibacterium* includes 9 taxonomically-described species, showing species diversity almost equal to that of the genus *Carnobacterium*. The type species of the genus *Alkalibacterium* is *A. olivapovliticus* [10], a bacterium isolated from the alkaline wash-waters (lye) of Spanish-style green olives. The other taxonomically-described members of the genus were also isolated from fermentation sources and processes [12].

As reported above, *Alkalibacterium* [10] is the first discovered obligately alkaliphilic lactic acid bacterium, which together with its sister genus *Marinilactibacillus* (a member also of the family *Carnobacteriaceae*) [11] and the genus *Halolactibacillus* (family *Bacillaceae*) [14] are the only known microorganisms able to achieve lactate fermentation under highly alkaline conditions. Most of the isolation sources of these alkaliphilic and halotolerant lactic acid bacteria are fermented foods and samples from fermentation processes, e.g. olives, salted foods, marine decayed organisms and indigo fermentation. *Alkalibacterium*, primarily, and *Marinilactibacillus* spp. are also involved in the ripening of cheese. Both species have been identified as the predominant microbiota of several European mould-ripened cheeses [15]. Alkalibacteria were just recently found to inhibit *Listeria* growth in early ripening stages [16,17].

Apart from the beneficial role of *Alkalibacterium* spp. in the early ripening of cheese, the biotechnological potential of members of this genus is of high importance. *A. olivapovliticus* is able to degrade the phenolic content of edible olive wash-water [10], resulting in both the detoxification of the final brine and in affecting the organoleptic properties of the end-product which are linked to the presence of polyphenols. Olives fermented with starter cultures of lactic acid bacteria have been reported to be less bitter and more aromatic than those spontaneously fermented [18], providing evidence that early (alkaline) lactic acid fermentation may be also beneficial to edible olive quality and characteristics. Moreover, table olive wastewater is considered as an effluent of high toxicity due to its high polyphenolic and salt content [19,20]. *Alkalibacterium* spp. can contribute at the bioremediation of olive brine by reducing the polyphenolics of the table olive wash-waters and by increasing the amount of lactate produced, which is a favorable source for anaerobic digestion [21].

Alkaliphilic lactic acid bacteria with bacteriocidal activity could serve as gene pool for genetic engineering, in which novel bacteriocins-related genes may be used to prevent spoilage micro-organisms. New metabolic engineering and gene regulation strategies may be also revealed by exploiting the genetic basis of lactate fermentation occurring under strict alkaline conditions. Whole-genome sequencing of alkaliphilic lactic acid bacteria can provide new aspects of alkaline lactate fermentation, uncovering the biotechnological potential of those bacteria to be used for the production of pyruvate-dissipating end products, exopolysaccharides, vitamins, polyols and flavor compounds. Adaptation of alkaliphilic lactic acid bacteria to xenobiotics under alkaline conditions has been reported to occur through gene transfer

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Received October 30, 2012; **Accepted** November 01, 2012; **Published** November 08, 2012

Citation: Ntougias S (2012) Alkaliphilic Lactic Acid Bacteria: Novel Sources for Genetic Engineering and Biotechnology. Gene Technology1:e102. doi:10.4172/2329-6682.1000e102

mediated by plasmid conjugation [22], a phenomenon actually deserving further experimentation. New osmoregulatory mechanisms may be present in alkaliphilic lactic acid bacteria and novel genes encoding osmolytes might also be discovered since lactate fermentation is carried out under extremely alkaline and often saline conditions.

Further scientific knowledge on alkaline lactate fermentation will become applicable in industrial biotechnology and fermentation technology. Expanding the diversity of alkaliphilic lactic acid bacteria by applying both culture-dependent (e.g. development of novel selective media) and independent (such as functional and metagenomic analysis) approaches will enable a better understanding of the nature of lactate fermentation carried out by alkaliphilic lactic acid bacteria.

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