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1 Innovative approaches to nisin production

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Abstract

Nisin is a bacteriocin which is produced by *Lactococcus lactis* and approved by FDA to be utilized as GRAS status food additive. Nisin has antimicrobial activity against *Listeria*, *Clostridium*, *Staphylococcus* and *Bacillus* species or spores. Also in some circumstances, it has an immune modulator role and a selective cytotoxic effect against cancer cells. However, it is notable that the high production cost of nisin is an important issue which restricts its intensive use. The major reason is the low nisin production yield of producer strains. In recent years, the production of nisin has been significantly improved by genetic modifications in nisin producer strains or also innovative applications in fermentation conditions. Recently, 15400 IU/mL nisin production has been achieved in *L. lactis* cells by genetic modifications with eliminating the factors that affect nisin biosynthesis or by increasing the density of the producing strains in the fermentation medium. In this review, the innovative approaches, which were applied in cell and fermentation systems where nisin production is increased, are comparatively discussed and interpreted regarding developing industrial nisin production.

Keywords: Nisin, innovative system, fermentation, bacteriocin

1. Introduction

Nisin is a bacteriocin of the type I lantibiotic group, which is produced by *Lactococcus lactis* (Hurst, 1981). This bacteriocin has a wide antimicrobial activity against the spores of Gram-positive bacteria, as well as especially the food pathogens *Listeria monocytogenes*, *Staphylococcus aureus*, *Clostridium* and *Bacillus* species. Nisin can show an inhibition effect on Gram-negative pathogen species (such as *Escherichia coli* and *Salmonella*) when used together with applications that disrupt the cell wall such as EDTA, thermal treatment and freezing (Belfiore et al. 2007). Nisin is a cationic, hydrophobic, heat-resistant peptide with a

molecular weight of 3500 Da. In the structure of this molecule, there are the dehydroalanine (DHA), dehydrobutyrine (DHB), lanthionine and β -methylanthionine amino acids that are scarce in nature and that are connected to each other by thioether bridges. To date, five variants have been characterized as follows: nisin A (Gross and Morell 1971), nisin Z (Graeffe et al. 1991; Mulders et al. 1991), nisin Q (Zendo et al. 2003), nisin U (Wirawan et al. 2006) and nisin F (Kwaadsteniet et al. 2008). Nisin A, Z and U producers have been isolated from milk and dairy products (Gross and Morell 1971, Graeffe et al. 1991; Mulders et al. 1991), nisin Q producer from river water (Zendo et al. 2003) and nisin F producer from catfish (Kwaadsteniet et al. 2008). Of these variants, only nisin U is produced by a bacteria (*Streptococcus uberis*) other than the *L. lactis* strains.

Nisin, as it has a wide antimicrobial spectrum and as it is decomposed by the digestive enzymes, has been defined and documented (E234) by the FDA (Food and Drug Administration) as a GRAS agent (Generally Recognized As Safe) and has been allowed to be used in food systems (Luck, 1995). Nisin was used in the 1950s to solve the problem arose from *Clostridium tyrobutyricum* in cheese. Currently, commercial nisin preparations are being produced in powder form of 2,5% purity and having 40×10^6 IU (International Unite) biological activity in one gram (Tramer and Fowler, 1964). Today, nisin is used in the production of various cheese, ready-made soups and canned foods.

The current industrial nisin production is carried out in batch fermentation systems by growing the nisin A producing *L. lactis* cells in supplemented whey or milk medium, and the product is subsequently partially purified. However, the high cost of industrial nisin production is an important issue that restricts widespread use of this bacteriocin in food systems due to the low production yield of the producer cells, the feedback inhibition factors depending on fermentation conditions and the sensitivity of the producers to nisin. Therefore many studies

have focused on the elimination of the factors that restrict the amount of production since the high commercial value of nisin. These studies have demonstrated that high amount of nisin production is closely related with the biomass amount as well as the genetic properties of the producer cells, the fermentation conditions and the fermentation metabolites which cause feedback inhibitions. In this respect, many innovative systems have been developed by the researchers to eliminate these restrictions such as, metabolic regulation at producers aiming the increase biomass and relatively nisin, high producer construct which could overcome the restrictions and fermentation optimization in term of pH, temperature, substrate and dissolved oxygen concentrations (Kong and Lu, 2014; Simsek, 2014; Zhu et al. 2015; Papagianni ve Avramidis, 2012; Hao et al. 2017; Ni et al. 2017; Liu et al. 2017; Zhang et al. 2014; Zhang et al. 2016; Kordikanlioglu et al. 2015; Zheng et al. 2015; Jiang et al. 2015; Ariana ve Hamedi, 2017).

In this review, the innovative approaches applied in cell and fermentation systems where nisin production was increased, were comparatively discussed and were interpreted concerning development of industrial nisin production. Firstly, the current commercial nisin production was evaluated, then the studies aiming for increasing the nisin production in cells and the applications in fermentation conditions were subsequently discussed. Thus, a perspective to improve industrial nisin production was tried to be established in this paper.

2. Commercial Production of Nisin

Commercial nisin has been produced in batch fermentation systems at industrial scale. Whole milk or skim milk is sterilized after the casein or para-casein fractions separated by enzymatically or acidification and subsequently the whey is used as a substrate to grow the producer *L. lactis* in large scale reactor systems. The fermentation conditions were kept under

89 promoting conditions whereas the pH is 6.0 and temperature is 30°C. In the optimum production
90 conditions, nisin producer *L. lactis* cells show common bacterial growth curve where the
91 highest nisin activity is measured after 8 h and reached maximum at 12 h of fermentation.
92 However, suprisingly nisin activity dramatically decreased at the following hours of
93 fermentation. Figure 1 shows the representative cell growth and relavant nisin production versus
94 to time scale. After fermentation, the foaming procedure is applied to the fermentate to separate
95 and partially to concentrate the produced nisin. Firstly, the fermentate pH is reduced to 4.50
96 that enables the precipitation of milk protein such as casein and serum proteins. Then, the fluid
97 is taken into the system which contains circulating vertical tubes. To ensure that the nisin-
98 containing liquid is foamed, 0.1% Tween is added; ventilating from the bottom, the foam
99 formed at the top is collected. At the last stage, sodium chloride and acetone are used to separate
100 nisin and the precipitate is dried. Thus, commercial nisin preparation that has a purity of 2.5%
101 and an activity of 40×10^6 IU in 1 gram is obtained (Patent, US2935503 A).

102 The main aim at nisin production is to increase the amount of active producing cells in
103 fermentation to achieve high nisin production yield. On the other hand, at nisin production, the
104 lactic acid produced by the *L. lactis* resulted feedback inhibition on the producer cells.
105 Accumulation of the lactate concentration in the medium accelerates protein denaturation in the
106 cells, it also causes the *L. lactis* cells to spend more energy to be able to tolerate the
107 unfavourable fermentation condition. These adversities lead to the disruption of active nisin
108 production phase in the *L. lactis* cells and even to a decrease in cell density. Another factor that
109 inhibits nisin production is a high concentration of nisin produced in the fermentation. The high
110 amount of nisin that is produced by *L. lactis* cells in turns inhbited the itself, although these
111 producer cells have a limiting or resistance level against nisin concentration (Kim et al. 1998;
112 Ra et al. 1996; Kim et al. 1998).

3. Innovative Nisin Production Systems

The innovative studies, such as constructing producer strains giving high yield through genetic manipulations in the producing cells to minimizing the factors affecting nisin production and re-routing the metabolic pathways in producing cells or ensuring special fermentation conditions are the main targets of the studies which aim increasing the nisin production. The innovative approaches applied have provided higher nisin production at various levels. In Table 1 recently high nisin production innovative systems are listed.

3.1. Recombinant Nisin Producers

Characterization the molecular mechanism of nisin biosynthesis and metabolic regulation on species level have opened the way to genetic regulation efforts to increase the yield in nisin production. Within this context, the genetic manipulations have focused on the problems that restrict nisin production, including i) the high nisin concentration in the medium, ii) high lactate accumulation and iii) low biomass formation.

The active nisin production is coded by the 14 kb site of the conjugative nisin-saccharose transposon that carries 11 genes (*nisA/Z/Q BTCIPRK FEG*). This gene cluster includes two operons (*nisA/Z/Q BTCIPRK* and *nisFEG*) and is transcribed as three different mRNA molecules (*nisA/Z/Q*, *nisBTCIPRK* and *nisFEG*). Of the genes in this site, *nisA/Z/Q* encodes for the synthesis of the pre-peptide; *nisB,C* encodes for the modification of the pre-peptide after translation; *nisP* encodes for the protease that allows the formation of the nisin peptide from pre-nisin; *nisT* encodes for the transfer of the prenisin molecule; *nisF,E,G* encodes for the resistance of the producing cell to nisin; *nisR,K* encodes for the transcriptional regulation of nisin production. The regulation of nisin production is ensured by a dual regulation system comprising histidine kinase (NisK) and its regulator (NisR) and nisin, in turn, induces the

transcription of the biosynthesis genes (Engelke et al., 1992; Kuipers et al., 1995; Ra et al., 1996).

Increasing the copy number of the key genes in nisin biosynthesis directly contributed to the increase of nisin production. Likewise, in the studies, increasing the copy number of the regulation and resistance genes (*nisRK*, *nisFEG*) involved in nisin biosynthesis in producing cells significantly improved nisin production (Kim et al., 1998; Cheigh et al. 2002; Simsek et al., 2009a,b; Ni et al. 2017). Although nisin is functional in its own biosynthesis as a regulator, nisin producing *L. lactis* cells are adversely affected by nisin. Particularly in the later times of fermentation, high nisin accumulation in the growing medium causes disruption on the cell membranes of the producer *L. lactis* strains. Although various proteins have been produced in *L. lactis* cells against the nisin are produced, the producers have different level of resistance against nisin. Thus, increasing the expression of nisin resistance genes (*nisI*, *nisF*, *nisE* and *nisG*) in producer *L. lactis* cells enhanced the resistance against nisin. Because, as elucidated, there is a nisin tolerance limit at each producer cells. A relevant study reported that by transferring *nisI* genes on a vector plasmid to the wild type producer strain and ensuring the expression of these genes resulted 20% increase in the amount of nisin production (Kim et al. 1998). Using the *nisI* gene led the way to increase the copy number of the other genes in the operon at the producer cells. In a study conducted on this basis, it was attempted to increase nisin Z production in an *L. lactis* subsp. *lactis* 164 strain by cloning the essential gene (*nisZ*), regulations genes (*nisR*, *nisK*) and the resistance genes (*nisF*, *nisE*, *nisG*). Nisin activity which was 16,000 AU ml⁻¹ in the control strain was improved to 25,000 AU ml⁻¹ by increasing the copy number of the regulatory genes. The transcription of the *nisZ* gene when the *nisR* and *nisK* genes were highly expressed and resulted favorable improvements (Cheigh et al. 2002). In another study, the copy number of the nisin regulation and resistance genes (*nisRKFE*) in the *L. lactis* LL27 strain was increased simultaneously which gained 45% increase in nisin

production compared to that of the wild type strain. In the same study, in the batch fermentation system of the recombinant strain, in which the copy number of the *nisRKFEg* genes were simultaneously increased, the dramatic losses experienced in the nisin activity of the control strain in the late hours of the fermentation was prevented (Simsek et al. 2009a). In nisin production in continuous nisin fermentation, increasing the copy number of the regulation and resistance genes simultaneously has allowed working at high dilution ratios (0.29 h^{-1}) of the producer strain. Thus, a significant increase was ensured in specific nisin production amount (Simsek et al. 2009b).

In industrial nisin production, the pH that decreases due to the production of lactic acid by *L. lactis* is neutralized by adding alkali to establish suitable physiological conditions for the cells in question. However, the lactate that accumulates in the medium at progressive hours of the fermentation inhibits cell proliferation, and consequently, there are losses in nisin production. Examining the nisin production graphs in batch systems, it is seen that nisin activity seriously decreases towards the end of the fermentation. It is discussed that in such a case, the cells undergo autolysis and release proteases and thus the nisin activity decreases. One of the first steps taken to solve this identified problem is cloning and expressing the pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) genes to direct the carbohydrate metabolism in *L. lactis* cells to ethanol production. As Wardani et al. (2006) pointed out nisin production in the cells in which the metabolic pathway is converted to heterofermentative has increased 1.7 times.

One of the objectives of increasing nisin production is improving the tolerance of the cells to acidic conditions in the fermentation medium. In a study within this context, it was ensured that the asparagine synthetase (*asnH*) gene was expressed more to increase the ratio of D-Asp amidation in the cell wall composition of the *L. lactis* F44 strain. The tolerance of the

recombinant *L. lactis* F44A strain obtained was improved at a significant level, and consequently, it was determined that the F44A strain could produce more nisin in the fermentation media compared to the wild type strain. In both batch and fed-batch fermentation systems, 2884 and 3405 IU ml⁻¹ nisin were produced at wild type F44 strain where as the nisin production was enhanced to 3876 and 5346 IU ml⁻¹ nisin was achieved respectively at the acid-tolerant *L. lactis* F44A strain respectively (Hao et al. 2017). In another study with the parallel hypothesis, 17 genes giving acidic tolerance were transferred to *L. lactis* F44 sustaining the optimum pH value in the cell, Among these genes, the *hdeAB*, *Idh* and *murG* resulted the highest amount of nisin production at recombinant *L. lactis* cells (5560 IU ml⁻¹) (Zhang et al. 2016). These results are also the evidences showing the adverse effect of the lactate on nisin production.

The nisin production amount is closely dependent on the biomass yielded in the fermentation. Hence, the presence of active producer cells in the system promoted nisin production. Therefore, many studies aimed to increase the physiological wellness or the number of the active nisin-producing cells under fermentation conditions. Especially, increasing the energetic level of the producers is one of the most basic approach. For example, Papagianni and Avramidis (2012) provided the cells to be produced more energy with some manipulations promoting the oxidative respiration in the nisin producer, thus accelerating cell division and metabolism under fermentation conditions. In this study, Papagianni and Avramidis succeeded to clone the *aox1* gene from *Aspergillus niger* to the nisin producer *L. lactis* ATCC11454 and used it in a fermentation system consisted 90% dissolved oxygen and 10 g l⁻¹ glucose concentration. Accordingly, the biomass and nisin production of *L. lactis* ATCC11454 was 3.2 g l⁻¹ and 5900 IU ml⁻¹ while the *aox1* gene including *L. lactis* ATCC11454 produced 5.8 g l⁻¹ and 7900 IU ml⁻¹ biomass and nisin respectively. This enhancement revealed the necessity to express the *pfk* gene encoding the phosphofructokinase (pfk) together with the *aox1* gene in *L.*

210 *lactis* ATCC11454. Thereby, the *aoxI* gene was further cloned to the *L. lactis* ATCC11454
211 together with the *pfk13* ve *pkaC* genes responsible for the phosphofructokinase and AMP
212 protein kinase and it was demonstrated that the biomass and nisin production of the
213 recombinants including the *pfk13-pkaC-aoxI* genes in a fed-batch fermentation system
214 containing hemin reached 7.5 g L^{-1} and 14000 IU mL^{-1} the highest nisin activity ever reported
215 (Papagianni and Avramidis, 2012). In another similar study, the 8-phosphofructokinase *pfk*
216 gene was expressed in the *L. lactis* N8 strain and 20% more nisin yield was obtained in the
217 recombinant strain after 10 hours of fermentation (Zhu et al. 2015).

218 Well organized and optimized fermentation systems enable to produce high amount of nisin.
219 Thus increase at nisin production and yield was been hypothesized for continuous fermentation
220 if the nisin producer *L. lactis* cells could be kept in reactor against dilution. In this respect, the
221 gene encoding the chitin binding domain of chitinase, one of the enzyme cleaving the chitin
222 which is the abundant polysaccharide in nature, was successfully cloned and expressed in nisin
223 producer *L. lactis* N8. Subsequently, the chitin which the cells be intact was used in the
224 continuous fermentation system of the study to prevent the cell outflow. In the system, the nisin
225 producers that can adhere to chitin, remain in the continuous fermentation system (CICON-
226 FER), and a nisin production over 10000 IU mL^{-1} was able to be produced with 0.9 h^{-1} dilution
227 rate (Simsek, 2014).

228 In the another innovative study, to increase nisin Z production in the *L. lactis* YF11 strain,
229 genome shuffling was applied using repeated protoplast fusion method. In genome shuffling,
230 ultraviolet radiation and diethyl sulfate mutagenesis were used for template line production.
231 After 4 rounds of shuffling, an F44 strain that can tolerate both high glucose (8% to 15% w/v)
232 and high nisin concentrations ($5000\text{-}14000 \text{ IU mL}^{-1}$) was obtained. This recombinant nisin
233 producer produced 2.4 fold more (4023 IU mL^{-1}) nisin in the fed-batch fermentation system

comparing to the YF11 control strain. The findings showed that the transcription levels of *nisZ* and *nisI*, the structural genes of nisin, in the F44 were higher (48% and 130% respectively) compared to those of the control strain (Zhang et al. 2014).

3.2. Modified Nisin Fermentation Systems

Nisin production was first carried out in batch systems, then, fed-batch fermentation systems were used. Relevant studies have demonstrated that the factors limiting nisin production are: i) inhibition of producers by high substrate concentration, ii) lactate repression on producers, iii) nisin feedback inhibition on producers, and iv) nisin degradation by the proteases released from the cells. To eliminate these disadvantages, various innovative modifications have been made in fermentation systems which have contributed to some extent to the improvement of nisin production (e.g., de Vuyst 1992; Pongtharangkul et al. 2006; Simsek et al. 2009).

One of the fermentation trials carried out for nisin production was cycling the active nisin producer cells that cultivated in the batch system into the fresh substrate. This application sustained the active physiological state of the logarithmic phase cells in the batch system maintaining the high nisin production characteristics. Bertrand et al. (2001) transferred 10^{11} CFU *g*⁻¹ *L. lactis* subsp. *lactis* biovar. *diacetylactis* cells, immobilized on k-carrageenan/legume gum onto a fresh medium every one hour and achieved 8200 IU ml⁻¹ total nisin activity and 5730 IU ml⁻¹ h⁻¹ volumetric nisin activity. However, since the cells were stabilized in solid phase, the substrate access and also the high nisin concentration exposed to cells were the adverse factors. In another similar study, to increase the specific nisin production, *L. lactis* N8 and LAC48 strains were grown in the batch system and every 30, 60 and 120 min cells were separated and suspended in the fresh substrate. Thus, nisin production was sustained continuously in active phase. Although high nisin productivity was attained in 60 min cycles,

the cellular stability could be maintained more in 30 min cycles. However the cell stability decreased within 120 min at nisin producers significantly after 5th cycle. In this study, 60 min cycle was offered continuously produce nisin with high productivity (Şimşek et al. 2009).

The high substrate concentrations in the fermentation medium adversely affect the nisin producers. Therefore, fed-batch fermentation systems are recommended in nisin production instead of batch fermentation systems. However, some studies to prevent the high substrate inhibition in fed-batch fermentation systems are still carried out. Malvido et al. (2016), re-alkalized the medium in line according to the pH reduction rate of the cells in the fermentor and glucose was added depending on the amount of spent NaOH. In this fermentation system, glucose was used by monitoring the activity of the *L. lactis* CECT539 cells. As a result, it was reported that use of 400 g L⁻¹ glucose in fed-batch fermentation system, where the medium was re-alkalined increased the production and this system was established an alternative for economic nisin production.

Nisin production is closely associated with the number and the physiological stability of the producing cells in the fermentor medium. It is notable that particularly a high number of active producing cells in the fermentor significantly increase the nisin production. It is known that bacterial activity is related with the energetic level. Therefore, nisin producer *L. lactis* cells were promoted to oxidative respiration in fermentor systems inducing heterofermentative instead of homofermentative metabolism which resulted the cells have high energy by activating the ETS system. Figure 2 is showing heterofermentative metabolism of a *L. lactis* when the aerobic fermentation is applied in the presence of hemin. Additionally, this reroute of metabolism also resulted higher biomass after fermentation. Because acetone, diacetyl and ethanol were produced instead of lactic acid which avoided lactate accumulation. Thereby lactate inhibition could be also eliminated by heterofermentative metabolism.

281 Respiration of facultative anaerobic *L.lactis* cells is only possible by the activation of
282 cytochromes in the fermentation media where hemin and hemin-menachinon are present
283 (Bryan-jones et al. 1969; Sijpesteijn 1970; Whittenbury et al. 1978; Lechardeur et al. 2004;
284 Brooijmans et al. 2009; Pedersen et al. 2012). However, since lactic acid bacteria do not have
285 the enzymes to carry out hemin biosynthesis, adding hemin into the medium is necessary to
286 start respiration in these strains.

287 There is only one type of cytochrome oxidase (CydAB) enzyme in all lactic acid bacteria. This
288 enzyme complex can function in media containing oxygen and contributes to the oxygen
289 tolerance of the bacterial cell (Rezaiki et al. 2004). The cytochromes inducing the proton motive
290 force by being final proton acceptor in membranes warrant the cell to be able to respire in media
291 even with low oxygen concentration (Brooijmans et al. 2009). *L. lactis* that is known for its
292 fermentative metabolism can respire under aerobic conditions in the presence of hemin since it
293 has the *cydAB* gene. Bolotin et al. (1999) reported that the *cydA* gene is present in the genome
294 of the *L. lactis* IL1403 strain responsible for the respiration encoded by cytochrome bd oxidase.
295 During respiration, the *cydA* gene play an active role in the electron transfer system and enable
296 ATP production with transferring the electrons to the oxygen.

297 In the fed-batch fermentation system, where the glucose, hemin and dissolved oxygen
298 concentrations were optimized and the *L. lactis* N8 induced for respiration was used, nisin
299 production was 3.1 times more than the control group fermented without hemin and a maximum
300 nisin production of 5410 IU ml⁻¹ was achieved. It was reported that this increase in nisin
301 production was attributed to the increase in cell biomass within the energetic level in the
302 producing strain and to the minimizing the feedback inhibition by the lactate accumulated in
303 the medium (Kordikanlioglu et al. 2015).

Nisin also has antimicrobial activity on itself. Although there are immunity proteins and various proteases in the cell wall to protect from nisin, the high concentration accumulated in the fermentation seriously affect the producer strains. Therefore, one of the issues on which researchers focus on is to online separation of nisin from the fermentation system. Indeed, this success will also allow the nisin to be concentrated and pure. The foam fractionation method is an innovative method for obtaining nisin and similar surfactant compounds from media at low cost and high concentration. As reported in many studies, aeration of the fermentation medium aiming to optimize the percentage of dissolved oxygen in the medium has a stimulating effect on nisin production (Amiali et al. 1998; Cabo et al. 2001; Kordikanlioglu et al. 2015). In a study examining the effectiveness of the foam fractionation method to improve nisin production in the fermentation medium, the fermentation medium was subjected to foam fractionation with sterile air inlet at flow rate of 30 ml min⁻¹ while the producer cell was in exponential growth phase. In this relevant study, maximum total nisin activity was measured as 4657 IU ml⁻¹. This strategy resulted in a 36.2% increase in the total nisin activity measured in the control group fermentation performed with the conventional batch system (Zheng et al. 2015). The aerotolerance of microorganisms is related to superoxide dismutase enzyme activity and the ability to induce NADH. Since *L. lactis* species are catalase negative, fermentation media is generally anaerobic. In fact, the aeration of the fermentation medium has an effect on promoting the growth and nisin production of *L. lactis* cells (Jiang et al. 2015; Kordikanliglu et al. 2015). In a study, which the relation between the aeration of the fermentation media and nisin production was determined, 10700 IU ml⁻¹ nisin amount was produced in the anaerobic condition, where the production amount was 15400 IU ml⁻¹ in the aerobic condition (Jiang et al. 2015).

There are also different ways of reducing the accumulation of lactate that adversely affect nisin production in *L.lactis*. Extraction of lactate in the medium using solvent or neutralization by

alkalisation can be given as examples. Another innovative approach, unlike all these, is the mixed culture fermentation technique. The microorganism to be selected as an adjuvant to *L. lactis* in the mixed culture fermentation technique should meet certain criteria such as i) it should not have the ability to use the main carbon source, ii) must have the ability to use lactate, an inhibitor metabolite produced by *L. lactis*, iii) do not adversely affect the nisin produced by *L. lactis*, iv) increase the production of the desired target product, stimulate it. *Yarrowia lipolytica* that consumes lactate as a substrate source and the lactate producer *L. lactis* were simultaneously cultured in a fermentation medium that contains molasses and 50% increase was achieved in this mixed culture fermentation in nisin production compared to the control group where only *L. lactis* was used. While the nisin concentration in the control group was 170 mg l⁻¹, it was 270 mg l⁻¹ in the mixed culture (Ariana and Hamedi, 2017).

4. Conclusion

Nisin is the most widely known and applied lantibiotic and is the first and only bacteriocin allowed for use in food. Widespread use of nisin in biomedical applications, its therapeutic characteristic and as well as its production at industrial scale are the justifications for many studies to focus on to enhance its production and thereby reduce the cost. To date, many recombinant strains have been developed with different metabolic regulation to increase yields in producer strains. In non-molecular innovative approaches, the alternative ways have been developed to improve the adaptation capabilities of native strains to the conditions of the medium and such studies have been accelerated. It is inevitable that future research on nisin might be the fermentations used together with such innovative systems. When the innovative systems addressed above are upscaled to industrial scales, the factors that have limited nisin use will be tolerated to a great extent.

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488 **Table 1.** Recent innovative approaches resulting high nisin production.

<i>Nisin Producers</i>	<i>Nisin production</i>	<i>Innovative Approaches</i>	<i>Reference</i>
<i>L. lactis</i> ATCC11454	7900 IU ml ⁻¹	<i>aox1</i> gene was cloned and the rekombinant nisin producer was induced to oxidative respiration.	Papagianni et al. 2012
<i>L. lactis</i> ATCC11454	14000 IU ml ⁻¹	<i>aox1</i> gene was cloned together with <i>pfk13</i> and <i>pkaC</i> genes to increase the glikolitic activity as well as the oxidative respiration	Papagianni et al. 2012
<i>L. lactis</i> PLAC7	10500 IU ml ⁻¹	Chitin Binding Domain was cloned in producer <i>L. lactis</i> and this was used in continuous fermentation system in presence of chitin	Şimşek, 2014
<i>L. lactis</i> YF11	4023 IU ml ⁻¹	Genome shuffling was applied to improve nisin Z production of <i>L. lactis</i> ssp. <i>lactis</i> YF11 via recursive protoplast fusion.	Zhang et al. 2014
<i>L. lactis</i> N8	5410 IU ml ⁻¹	Fed-batch fermentation carried out with hemin under aerobic conditions.	Kordikanlioglu et al. 2015
<i>L. lactis</i> ATCC11454	4657 IU ml ⁻¹	Nisin was online recovered with foaming fractionation from the fermentation.	Zheng et al. 2015
<i>L. lactis</i> LD2	15400 IU ml ⁻¹	Nisin was produced in a aerated fed-batch fermentation system with a variable feeding rate.	Jiang et al. 2015
<i>L. lactis</i> F44	5560 IU ml ⁻¹	<i>hdeAB</i> , <i>Idh</i> and <i>murF</i> genes were cloned and expressed simultaneously to enhance the acidic tolerance of the producer <i>L. lactis</i> .	Zhang et al. 2016
<i>L. lactis</i> F44A	5346 IU ml ⁻¹	Acidic tolerance was enhanced by overexpression the <i>asnH</i> gene at nisin producer <i>L. lactis</i>	Hao et al. 2017
<i>L. lactis</i> UTMC106	10800 IU ml ⁻¹	This producer was used together with <i>Yarrowia lipolytica</i> ATCC18942 in fermentation system	Ariana and Hamed, 2017

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Figure1. Representative nisin production and biomass amount of nisin producer *L. lactis* in batch fermentation systems.

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502 **Figure 2.** The metabolic pathway followed by the *L.lactis* bacteria induced for oxidative
503 respiration.