2022 Nisin in Dairy Systems

Request: We are interested in some information on nisin, particularly related to dairy applications.

1. When in the growth cycle nisin is produced?
2. Do the presence of particular nutrients increase / decrease nisin production?
3. What levels of nisin are inhibitory to what species of bacteria?

Response:

Question 1:

- From a recent review (Khelissa et al., 2021): "For a long time, it was believed that nisin is synthesized during the stationary phase when nutrients are exhausted. However, it has been reported that in batch fermentation, the nisin production follows a primary metabolite kinetic (Guerra et al. 2007): a production during the exponential growth phase and a full stop when the bacteria enter the stationary phase. It was observed that nisin was detected in the growth medium during the exponential phase and its production rate peaked at the end of this phase which confrms that the synthesis of this peptide follows a primary metabolite kinetic (Chinachoti et al. 1998; Zhu 2017).
- Nisin expressed *in situ* by a costarter culture during the making of a hard Graviera cheese was investigated in Noutsopoulos (2017). Nisin was expressed to the highest level on Day 1 of fermentation (Figure 2), with "increasing nis A expression by the NisA+ costarter strain M78 during the exponential phase of microbial growth".
- Another paper from the Samelis research group also looked at nisin expression in a co-culture of the *L. lactis* M78 (NisA + strain) with *Streptococcus thermophilus* in milk and looked how *S. thermophilus* inoculation level affected M78 growth and nisin A expression and activity vs. *L. monocytogenes*. A fairly low inoculation level of the *S. thermophilus* strain (5-log) together with a moderate level of the M78 NisA+ starter (6 log inoculation) was best to produce sufficient nisin A to provide good activity in milk (Samelis and Kakouri, 2020).
- Commercial production of nisin in a *L. lactis* monoculture in milk is usually optimized at pH 6.0 and 30°C. Fed batch systems are generally better than using high substrate concentrations in the media, and lactate accumulation can also adversely affect nisin production in *L. lactis*. Under such conditions, the highest nisin activity is generally found after 8-10 hours of fermentation. Nisin activity decreases dramatically after that, probably due to proteolytic degradation or adsorption of the nisin onto producer cells (Özel et al., 2018).

Question 2:

- Factors in culture media that influence bacteriocin production in LAB have been reviewed in several recent papers (Abbasiliasi et al., 2017; Khelissa et al., 2021; Peng et al., 2022):
  - A carbon source is essential, with glucose promoting bacteriocin production.
  - A nitrogen source is also essential, with yeast extract preferable to peptone or meat extract for bacteriocin production. Yeast extract may have more absorbable amino acids and short peptides (as well as more growth factors, minerals, and vitamins).
  - Potassium ion at 2 g/L has been found to promote bacteriocin production in MRS medium but has a negative effect in M17 medium.
  - Sodium decreases bacteriocin production in *L. lactis* subsp. lactis and other LAB.
  - Manganese is frequently reported to promote bacteriocin production (0.01 to 0.2 g/L).
  - Magnesium did not appear to stimulate or reduce bacteriocin production, while no conclusions can be drawn from other metal ions.

  - An initial pH of 6-7 and temperature of 30-37°C is optimal for bacteriocin production in LAB. Oxygen is reported to stimulate the production of nisin.
- The discussion (Noutsopoulos et al., 2017) reviews factors that reduce nisin production by *L. lactis* during cofermentation hard cheese making (deprivation of sugars, amino acids, and other essential nutrients; the possibility of other bacteria excreting proteases, effects of temperature, pH, salt concentration and water activity, etc.
- A recent book chapter by Singh discusses nutrients and growth conditions for optimizing nisin production in media and in commercial applications (Singh, 2019).
- Another paper reviews other work which examined the effects of various factors on nisin production(Garcia-Parra et al., 2011). The paper itself tests various factors on nisin production in a new *L. lactis* strain and found various factors that improved production.

Question 3:

Bacillus cereus

- The MIC for nisin against B. cereus **in milk** at 21°C was 32 IU/mL.

Clostridium butyricum

- "Nisin, at concentrations of 2.50–6.24 µg/g, was shown to be extremely effective in preventing the growth of relevant Clostridium spp. inoculated in processed cheese and cheese spreads (with or without ham) incubated at 37°C. This effectiveness was also reported by Plocková et al. (1996) who found that similar nisin levels inhibited Clostridium and Bacillus in processed cheese during a 3-month storage period".
- MICs for nisin in *C. tyrobutyricum, C. butyricum*, and other dairy-related clostridia ranged from 0.1 to 6.25 µg/mL for vegetative cells and 0.20 to 25 µg/mL for spores (Ávila et al., 2014).

Clostridium perfringens

- Nisin (MIC values 0.78 to 12.5 µg/ml) was also effective against vegetative cells and spores of *C. perfringens* in media and milk after 7 days at 37°C under anaerobic conditions (Garde et al., 2014). The paper also discusses a number of other papers that determined MIC values for nisin against *C. perfringens* in milk or media.
- Against *C. perfringens* vegetative cells, nisin was reported to lengthen the lag phase (Garde et al., 2014).

Listeria

- The MIC for nisin against *L. monocytogenes* in milk at 21°C was 16 IU/mL; at 30°C, it was 48 IU/mL.
- Against *L. monocytogenes* in cuajada cheese, nisin initially reduced growth of the pathogen (2.74 log CFU/g lower counts than control cheese after 3 days at 10°C); however, the extension of lag phase was followed by growth from days 3-9 similar to those found in the control cheese without nisin (Arques et al., 2008)

Staphs

- The MIC of nisin against various *S. aureus* strains was reviewed and ranged between 6.4 and 12.8 µg/mL.

- One study added nisin to a raw-milk Mexican cheese contaminated with *S. aureus*: "Addition of nisin during cheese production reduced (P≤0.05) S. aureus counts compared to a nisin-free control; at 24 h, nisin lowered *S. aureus* counts 2.3 log cycles in the 500 IU/kg nisin treatment and 1.9 log cycles in the 625 IU/kg treatment". (Alvarez Badel et al., 2022)
- Nisin alone resulted in a significantly lower levels (~ 1-2 log) of *S. aureus* in inoculated pasteurized milk after 24 hours of storage at 25 or 4°C (Li et al., 2022).
- Against *S. aureus* in milk, nisin was most effective during lag phase; log phase was not affected (Felicio et al., 2015). Similarly, Yehia (2022) and Jensen (2020) found that MRSA in culture was most sensitive early in the growth curve, and the loss in sensitively after 4 hours of nisin exposure was not due to stable mutations (Yehia et al., 2022) (Jensen et al., 2020).
- In cheese curd and whey, nisin (which partitioned into both phases) was most effective in reducing high initial *S. aureus* counts in highly contaminated milk (Felicio et al., 2015).

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