THE USE OF A FLOROGENIC METHOD TO DETERMINE THE BACTERIOCIN ACTIVITY OF SAMPLES OBTAINED OF DAIRY PRODUCTS

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Introduction. Bacteriocins are antimicrobial peptides synthesized ribosomally by bacteria (1). There are different methods for detecting bacteriocins activity, but the most common is the well-diffusion method. With this protocol ~ 3 days are used to obtain an inhibitory effect. Recently, we reported an innovative and rapid fluorogenic method for detecting bacteriocin activity *versus* Gram-positive and Gram-negative bacteria based on berberine fluorescence following its influx into compromised susceptible cells. With this method no more of one hour is required to observe a result of inhibitory activity (2, 3). Alternatively, Nisin is a bacteriocin produced by *Lactococcus lactis*, it has the GRAS status and is used commercially in various countries. Activity of this bacteriocin in food is regularly tested by the well-diffusion method.

In this work, our objective was to use the fluorogenic method in order to expand the utility of this protocol to detect rapidly the presence Nisin or other bacteriocin in dairy products. The importance of this method will be discussed.

Methods. Before testing the inhibitory effect by the well-diffusion and fluorogenic methods, these protocols were standardized testing *Micrococcus luteus* as indicator bacterium *versus* different concentrations (IU) of Nisin. Dairy products were treated with HCl 0.02 mol/L (pH 2), then heated, centrifuged, filtered and supernatans used for assaying the inhibitory activity with the well-diffusion (1) according with the Britanic standar determination. Presence of Nisin in dairy products was corraborated by PCR using specific oligonucleotides. To perform the fluorogenic method, supernatants obtained from treated dairy products were mixed with the indicator bacterium and berberine according with the the protocol previouly reported (2), and fluorescence was measured with a fluorometer and observed by fluorescence microscopy.

Results. When activity (UI) of purified Nisin was compared with the diamenter (mm) of inhibition and also with the relative fluorescens, they showed correlation of ~ 1. Nisin activity was determined in eleven dairy products by the well-diffusion method, and concentrations of antimicrobial peptide was detected in the range of 2 to 50 IU/ml. The fewer and higher activities were observed with respectively yogurts and cheeses (see resume, Table 1). When *M. luteus* was tested with purified Nisin, fluorescence was observed with a fluorometer and also with the fluorescense microscopy, indicatig that Nisin damage membrane, allowing the entrance of the berberine that fluoresce inside the cells by interaction with the cellular components. Interesting, when samples of Nisin obtained from dairy products were tested by the fluorogenic method, a detectable fluorescence of cells was observed by microscopy indicating the membrane damage. However, when fluorescence was measured with the fluorometer, it was too low, that was impractical to be used as a parameter to measure Nisin activity in dairy products. Currently, we are modifying the protocol reported by De Fuente-Salcido et al (2) in order to adapt it and obtain detectable fluorescence that can be used to evaluate the inhibitory effect of Nisin present in dairy products.

Table 1. Inhibitory activity and concentration of Nisin detected in dairy products.

Samples	Inhibition (mm)	IU
Yogurth	7	2
Cream	12	10
Panela cheese	22	50

Conclusions. The well-diffusion method is useful to detect Nisin of samples obtained from dairy products, but this spend ~ 3 days The fluorogenic method is a rapid protocol to detect inhibitory effect of purified or partially purified antimicrobial peptides obtained from an axenic bacterial culture. However, if we used the protocol with the same conditions as reported by De la Fuente-Salcido et al (2), the utility of the fluorogenic method to detect Nisin from dairy products is low. This result could be by the presence of food molecules or residues that mask the fluorescence emitted by berberine. Finally, it will be necessary to review step by step the original protocol in order to modify and adapt it to detect bacteriocins obtained from dairy products, activity that currently we are working.

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