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REGULAR ARTICLE

THE POSSIBILITIES OF DETECTION OF PUTRESCINE PRODUCTION IN GRAM-NEGATIVE BACTERIA – A KICK-OFF STUDY

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ABSTRACT

Biogenic amines have several important physiological functions but also can cause wide range of health problems when are consuming in high amount in food. Among the most serious toxicological effects of biogenic amines are undoubtedly possible carcinogenic effects of some polyamines, particularly putrescine. Putrescine could be formed by many bacterial strains. Putrescine can be produced by different metabolic pathways involving a larger number of enzymes. Possible detection of all important pathways by detecting the corresponding gene by PCR has not been sufficiently studied yet. In this paper we present a possible solution to this problem.

Keywords: PCR, biogenic amines, methods of detection

INTRODUCTION

Biogenic amines (BA) are low-molecular nitrogenous basic compounds mainly produced by decarboxylation of certain amino acids (Santos, 1996), they can have aliphatic

(putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) structures (Lorenzo et al., 2007). Several authors had classified cadaverine, putrescine, spermine and spermidine among polyamines (Kalač et al., 1997). BA are presented in a wide range of food products – meat and meat products, fish and fish products, fermented vegetables and soy products, wine, beer and nuts (Santos, 1996). Some of BA plays a major role in many human and animal physiological functions; for example regulation of body temperature, increase or decrease of blood pressure, stomach volume, stomach pH and brain activity (Ten Brink, et al., 1990; Shalaby, 1996). On the other hand, the intake of food containing high concentrations of BA can cause several toxicological problems (Ten Brink et al., 1990; Shalaby, 1996).

The occurrence of BA in food is attributed to the decarboxylase activity of certain bacteria (Halász et al., 1994). The ability of BA formation has been described for several groups of microorganisms, mainly *Enterobacteriaceae*, *Pseudomonas* spp., enterococci and some lactic acid bacteria (Santos, 1996; Halász et al., 1994). Microorganisms with a decarboxylase activity can be contaminating (Ten Brink, et al., 1990) or starter microorganism (Fernandez-García, 2000).

BA (especially histamine and tyramine) have been involved in food poisoning incidences, usually from the consumption of fermented foods containing high amount of this substances (González de Llano et al., 1998). Two most known of them are “scombroid fish poisoning” (histamine poisoning) and “cheese reaction” (caused by high levels of tyramine). Histamine and tyramine can cause vasoactive and psychoactive health problems, including nausea, headache, hyper- or hypotension and allergies (Ten Brink et al., 1990; Halász et al., 1994; Ladero et al., 2010). These problems are especially severe in consumers with low levels of the enzymes involved in detoxification system (mono- and diamionooxidases), either by genetic disorders (Caston et al., 2002) or medical treatments (Halász et al., 1994). Moreover, other amines such as putrescine and cadaverine play important role in food poisoning as they can potentiate the toxicity of histamine and/or tyramine (Taylor et al., 1986). Furthermore, putrescine, cadaverine, spermine and spermidine are potential precursors of carcinogenic nitrosamines (Shalaby, 1996; Halász et al., 1994; Bover-Cid et al., 1999). The amount of BA is also considered to be a marker of spoilage of meat and fish products. The amount of histamine, putrescine and cadaverine is defined as BAI (Biogenic Amine Index) (Karmas et al., 1981). Thus, there are two reasons for early detection of BA-producing bacteria in foods: (i) the first is their potential toxicity which causes food safety; and (ii) the second is the possibility of using them as food quality markers (Önal, 2007).

Methods of detection of biogenic amines production

During the last two decades, methods for the detection of BA-producing bacteria have been developed. Many screening methods are based on the use of differential culture media containing pH indicator (Bover-Cid et al., 1999). Several chromatographic methods in various modifications have been described for identification and quantification of BA (Önal, 2007). Recently, several PCR based methods have been developed for the detection and quantification of genes encoding microbial decarboxylases responsible for the production of BA. Most oligonucleotide primers were designed for detection histidine decarboxylases (that produce histamine) (Coton et al., 2005; Fernandéz et al., 2006), tyramine decarboxylase (that produce tyramine) (Ladero et al., 2010; Coton et al., 2005), ornithine decarboxylases (that produce putrescine directly from ornithine) (de Las Rivas et al., 2005) and agmatine deiminase (that produce putrescine from extracellular agmatine) in lactic acid bacteria (Torriani et al., 2007). Some of them were used in multiplex PCR reactions for simultaneous detection more decarboxylases genes (de Las Rivas et al., 2005; Coton et al., 2010; Marcobal etal., 2005; Muñoz et al., 2004; Moon et. al, 2010;) and some authors used them in qPCR (Ladero et al., 2010; Nannelli et al., 2008). Not yet been developed primers for the detection of other metabolic pathways for the production of putrescine in gram-negative bacteria. The detection of putrescine producing gram-negative bacteria that is extremely important because of its potential contribution to the production of carcinogenic nitrosamines, and because of its negative impact on food quality. Occurrence of putrescine and agmatine was announced in much research.

Detection of putrescine production

There is a particular problem in detection of putrescine-producing bacteria. Histamine, cadaverine, tyramine etc. are produced by direct decarboxylation of the corresponding amino acid by substrate-specific decarboxylase enzymes, but putrescine can be produced by three different metabolic pathways in gram-negative bacteria. Putrescine can be synthetized either (i) by ornithine decarboxylase (ODC, the *speC* product) directly from ornithine (ODC pathway); or (ii) indirectly from L-arginine by arginine decarboxylase (ADC) via agmatine (ADC pathway) (Fig.1). Both this pathways operate simultaneously in many bacteria (Cunnin et al., 1986; Tabor et al.,1972). Furthermore, there are two variations of the ADC pathway. In both cases L-arginine is first converted to agmatine by ADC. However, in enterobacteria

agmatine is converted directly to putrescine by the enzyme agmatinase (the *speB* product), while in *Pseudomonas* spp. and *Aeromonas* spp. agmatine is first hydrolyzed by agmatine deiminase AgDI (the *aguA* product) into *N*-carbamoylputrescine and ammonia, and putrescine is formed by removal of the ureido group from *N*-carbamoylputrescine by the enzyme *N*-carbamoylputrescine amidohydrolase N-CPAH (the *aguB* product).

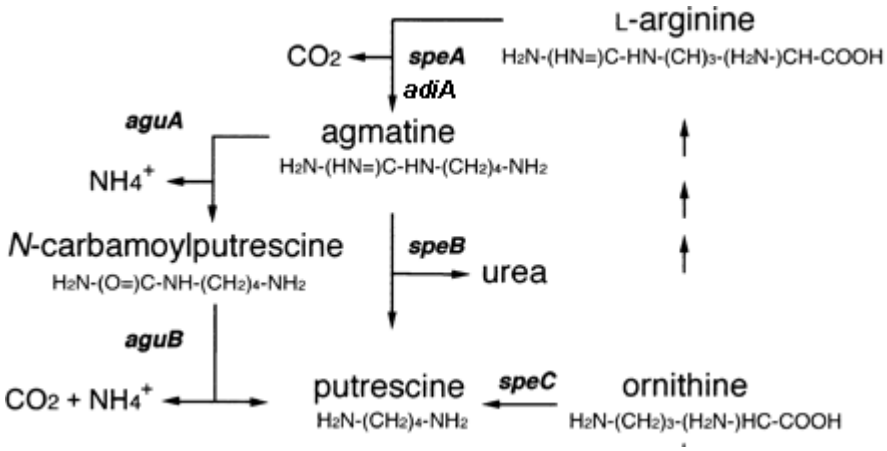

Figure 1 Schematic pathways of production putrescine. Enzymes encoded by *adiA, speA, speC, aguA* and *aguB* genes are described in the text (Nakada et al., 2003)

Thus there are three pathways by which putrescine could be synthetized in gram-negative bacteria. Moreover, in many gram-negative bacteria can be found two forms of ADC: biosyntetic ADC encoded by *speA* genes and biodegradative ADC encoded by *adiA* genes. In the detection of putrescine-producing bacteria have been developed and tested so far only the primers for the detection of ODC in gram-negative bacteria and detection of AgDI at lactic acid bacteria. No further research on observations of the remaining metabolic pathways leading to the production of putrescine, namely metabolic pathways via arginine decarboxylase.

To detect gram-negative bacteria that could produce putrescine we must have more than only two sets of primers for detection of ODC and AgDI. For complete detection of all pathways is necessary to have other five sets of primers for detection: ADC biosythetic (*speA*) and biodegradative (*adiA*), agmatinase (*speB*), AgDI (*aguA*) and N-CPAH (*aguB*). Design of these sets of primers would improve possibilities of food quality protection.

CONCLUSION

We would like to design these new sets of primers by using programme Gene-fisher2 (http://bibiserv.techfak.uni-bielefeld.de/genefisher2/) to align nucleotide sequences for all targeting genes available in gen databases. These sets of primers then will be checked by program Blast that simulates PCR reaction with all known DNA sequences from the gen bank and the best sets of primers subsequently will be tested in real PCR with putrescine-producing bacterial strains. These primers will contribute great benefits for detecting of putrescine producers in the food products.

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