Short Communication

Use of Maldi-Tof MS biosensor in microbial assessment of Brazilian kefir grains[1]

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ABSTRACT

The aim of this study was to evaluate the use of Maldi-Tof MS biosensor in microbial assessment of Brazilian kefir grains. Maldi-Tof MS is a new methodology for the rapid diagnosis of microorganisms. A total of 358 microorganisms were isolated, 31 were yeasts and 327 were bacteria (divided into lactic and acetic bacteria). Microbial colonies were grown in Luria-Bertani agar medium and incubated at 35 °C for 18h and used in the identification of species by Maldi-Tof MS. The microbial population identified in Brazilian kefir grains was *Lactobacillus paracasei, Saccharomyces cerevisiae, Lactobacillus plantarum, Acetobacter pasteurianus*, and *Acetobacter syzygii*. This study demonstrated a rapid and accurate identification of the Brazilian kefir grains microorganisms using the Maldi-Tof MS biosensor. In conclusion, the Maldi-Tof MS technology can facilitate the microbiological control in a fermentation process using kefir grains as starter cultures.

Keywords: microorganisms; bacteria's; yeast; biological sensor.

RESUMO

Utilização do biossensor Maldi-Tof MS na avaliação de micro-organismos dos grãos de kefir brasileiro

O objetivo deste estudo foi avaliar o uso do biossensor de Maldi-Tof MS na avaliação microbiana de grãos de kefir. Maldi-Tof MS é uma nova metodologia para o diagnóstico rápido de micro-organismos. Um total de 358 micro-organismos foram isolados, 31 foram leveduras e 327 foram bactérias (divididas em bactérias láticas e acéticas). Colônias microbianas foram cultivadas em meio Luria-Bertani (LB) e incubadas a 35 °C por 18h e posteriormente utilizadas na identificação a nível de espécies pelo Maldi-Tof MS. A população microbiana identificada nos grãos de kefir brasileiros foi *Lactobacillus paracasei, Saccharomyces cerevisiae, Lactobacillus plantarum, Acetobacter pasteurianus* e *Acetobacter syzygii*. Este estudo demonstrou uma identificação rápida e precisa dos micro-organismos de grãos de kefir brasileiros utilizando o biossensor Maldi-Tof MS. Em conclusão, a tecnologia Maldi-Tof MS pode facilitar o controle microbiológico em um processo de fermentação usando grãos de kefir como culturas iniciadoras.

Palavras-chave: micro-organismos; bactérias; levedura; sensor biológico.

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INTRODUCTION

Kefir is a polysaccharide grain containing microorganisms that produce fermented beverages, such as the traditional Turkish beverage also named "kefir", which is from milk, and has low alcohol content (Simova *et al.*, 2002; Guzel-Seydim *et al.*, 2005; Irigoyen *et al.*, 2005; Magalhães *et al.*, 2011a, 2011b, 2011c; Puerari *et al.*, 2012; Cho *et al.*, 2018). Kefir is a mixed culture of various yeasts and bacteria species combined in a matrix of proteins and polysaccharide "kefiran", which are formed during cell growth under aerobic conditions (Magalhães *et al.*, 2010a, 2010b). The kefir grains are irregularly shaped and hard granules, with yellowish-white colour, which resemble miniature cauliflower blossoms (Corona *et al.*, 2016).

In Brazil, kefir grains are used in private households (Magalhães *et al.*, 2011c) and are added to different types of milk, such as cow, goat, or sheep, coconut, rice, and soy (Irigoyen *et al.*, 2005; Magalhães *et al.*, 2011c). The grains are responsible for the fermentation that produce lactic acid, acetic acid, CO_2, alcohol (ethyl 2 alcohol), and aromatic compounds. These compounds provide the unique sensory characteristics of kefir: fizzy, acid taste, and tart and refreshing flavour (Corona *et al.*, 2016).

The kefir beverage contains vitamins, minerals, and essential amino acids that help the body with healing and maintenance functions and contains easily digestible complete proteins (Roos *et al.*, 2018). In accordance with Medrano *et al.* (2008), the benefits of consuming kefir in the diet are numerous, such as its antitumoral, (Vinderola *et al.*, 2005), antimicrobial (Rodrigues *et al.*, 2005), antiinflammatory, and antiallergical (Lee *et al.*, 2007) activities. The composition of microbial species is an important factor to characterize the kefir therapeutic benefits (Simova *et al.*, 2002; Guzel-Seydim *et al.*, 2005; Irigoyen *et al.*, 2005; Magalhães *et al.*, 2011a, 2011b, 2011c; Cho *et al.*, 2018). A rapid identification of the microorganisms is necessary in the fermentative process of kefir grains, which facilitates the microbiological control of fermentative processes on a large scale.

An optical technique with potential use to rapidly identify the microorganisms is the Maldi-Tof MS (Matrix Assisted Laser Desorption/Ionisation - Time of Flight Mass Spectrometry) (Microflex-Bruker Daltonics/BioTyper™) (Chang *et al.*, 2016; Pasternak *et al.*, 2012; Gaudreau *et al.*, 2018; Mayoral *et al.*, 2018). In this technique, the sample is uniformly mixed in a large quantity of matrix. The matrix absorbs the ultraviolet light (nitrogen laser light, wavelength 337 nm) and converts it to heat energy. A small part of the matrix (down to 100 nm from the top outer surface of the Analyte in the diagram) heats rapidly (in several nano seconds) and is vaporized, together with the sample. Charged ions of various sizes are generated on the sample spot (Figure 1). A potential difference V0 between the sample slide and ground attracts the ions in the direction shown in the diagram. The velocity of the attracted ions v is determined by the law of conservation of energy. As the potential difference V0 is constant with respect to all ions, ions with smaller m/z value (lighter ions) and more highly charged ions move faster through the drift space until they reach the detector. Consequently, the time of ion flight differs according to the mass-to-charge ratio (m/z) value of the ion. The method of mass spectrometry that exploits this phenomenon is called Time of Flight Mass Spectrometry (Pasternak *et al.*, 2012; Mayoral *et al.*, 2018).

The aim of this study was to evaluate the use of Maldi-ToF biosensor in the microbial assessment of Brazilian kefir grains, as well as to identify the microorganisms associated with them.

MATERIAL AND METHODS

Kefir grains

Brazilian kefir grains (Stock-culture of the Microbiology laboratory of the Federal University of Lavras, Brazil) were used in the experiments.

Microbiological analysis

We used Maldi-Tof MS biosensor (Microflex-Bruker Daltonics/BioTyper™) for the microbiological analysis. A total of 358 isolates were identified.

The isolation of microorganisms in kefir grains was performed according to Magalhães *et al.* (2011c). Bacteria and yeasts were enumerated by the surface spread technique, plating 100 μL of each diluted sample in triplicate. Enumeration of microorganisms was carried out using four different culture media. Lactic acid bacteria (LAB) were enumerated on Nutrient Agar (Oxoid, S/P, Brazil), De Man, Rogosa, and Sharpe Agar (MRS) (Oxoid, S/P, Brazil) media. Acetic acid bacteria (AAB) were enumerated on 135 medium (DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Germany). All media for bacterial enumeration were supplemented with 0.4 mg mL^{-1} nystatin (Sigma, St. Louis, USA). Yeasts were enumerated on yeast extract peptone glucose (YEPG) agar containing 100 mg chloramphenicol (Sigma, St. Louis, USA) and 50 mg chlortetracycline (Sigma, St. Louis, USA) to inhibit bacterial growth. After spreading, plates were incubated at 28 °C for 48 h for bacteria and for five days for yeasts, colony forming units (log10 CFU mL^{-1}) were quantified. For each type of medium containing isolated colonies, the square root of the number of colonies was taken at random for identification.

For identification of microbial species of kefir grains by Maldi-Tof MS, the *Escherichia coli* K12 strain was used as external standard for calibration biosensor following the method described by Chang *et al.* (2016). Cell colonies were grown in Luria-Bertani (LB) agar medium and incubated at 35 °C for 18 h. The microbial colonies were used in the identification by Maldi-Tof MS.

RESULTS AND DISCUSSION

Rapid diagnosis of microorganisms is decisive to guarantee adequate identification in biological samples. Biochemical methods are precise and sensitive, but rather slow. New resources are available to enable faster diagnosis, and the most promising is Maldi-Tof MS technology, applied to microbiological diagnosis by rapid method using the microbial colonies.

The microbial population count of the kefir grains performed in this study Found: lactic acid bacteria with a population of 8.10 log CFU mL^{-1} (approximately 10^8 cells); AAB in a low population of 4.01 log CFU mL^{-1} (approximately 10^4 cells); and yeasts in population of 6.01 log CFU mL^{-1} (approximately 10^6 cells). This microbial population is expected in the kefir grains because they are considered probiotic (Simova *et al.*, 2002; Guzel-Seydim *et al.*, 2005; Irigoyen *et al.*, 2005; Magalhães *et al.*, 2011a, 2011b, 2011c; Puerari *et al.*, 2012; Cho *et al.*, 2018).

A total of 358 microorganisms were isolated, 31 yeasts and 327 bacteria (divided into lactic and acetic bacteria). The isolates were identified by Maldi-Tof MS technique (Figure 1). The results showed higher population of *Lactobacillus paracasei* (10^6 CFU mL^{-1}); *Saccharomyces cerevisiae, Lactobacillus plantarum, Acetobacter pasteurianus*, and *Acetobacter syzygii* were found in smaller quantities (10^4 CFU mL^{-1}) (Figure 2).

Quintilla *et al.* (2018) evaluated the efficiency of Maldi-Tof MS to identify foodborn yeasts. The identified yeasts were named as *Rhodotorula babjevae*, *Meyerozyma caribbica*, *Clavispora lusitaniae*, *Debaryomyces hansenii*, *Candida oleophila*, *Pichia membranifaciens*, *Kazachstania telluris*, and *Mrakia frigida*. Authors showed that Maldi-Tof MS is applicable for routine identification and validation of foodborne yeasts.

Gaudreau *et al.* (2018) evaluated Maldi-Tof MS to identify bacteria from biofilms. They compared three sample preparation procedures on biofilms grown *in vitro*. The extended direct transfer method was able to identify 13 isolates out of 18 (72%) at the species level and 15 out of 18 (83%) at the genus level.

Mayoral *et al.* (2018) reported two new cases involving immunocompetent girls with cervicofacial lymphadenitis due to *Mycobacterium mantenii*, and the reliability of Maldi-Tof MS for identifying *Mycobacterium mantenii* was efficient.

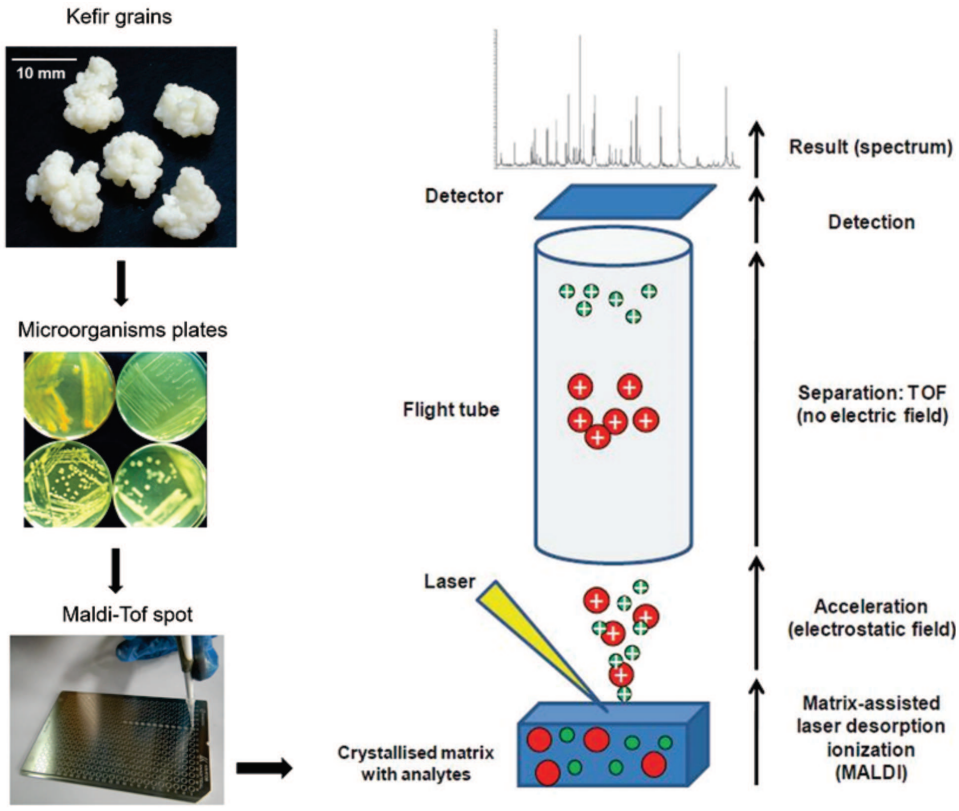

Figure 1: Brazilian kefir micoorganisms analysis methodology in the Maldi-Tof MS biosensor.

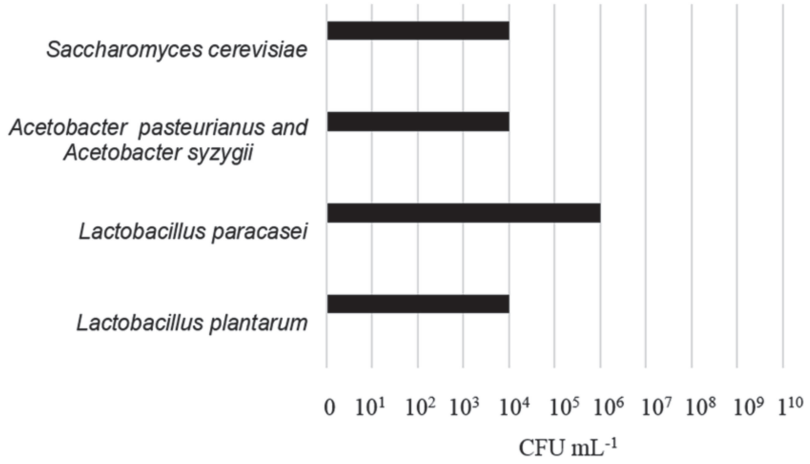

Figure 2: Microbial identification of Brazilian kefir grains by Maldi-Tof MS technique.

CONCLUSION

The Maldi-Tof MS technology proved to be useful for diagnosis of kefir microorganisms, allowing a fast and safe diagnosis for the scientific environment. The technology can facilitate the microbiological control in a fermentation process using kefir grains as starter cultures. The microorganisms identified in the Brazilian kefir are the same commonly found in kefir grains, with predominance of *Lactobacillus paracasei.*

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