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Screening of Lactic Acid Bacteria from "*TapaiPulut*" for Biosynthesis of Glutamic Acid

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Abstract: Glutamic acid (glutamate) is an amino acid used in human body to build proteins. Glutamate can be converted to glutamine via glutamine synthetase, and to γ — aminobutyric acid (GABA) via glutamate decarboxylase. The objective of this study is to screen potential of lactic acid bacteria from "tapaiubi" for biosynthesis of glutamic acid. The traditional fermented food, "tapaipulut" was used as a source of lactic acid bacteria (LAB). The sample was cultivated on DeMan-Rogosa-Sharpe MRS agar containing 0.16% bromocresol purple and placed under anaerobic condition at 37°C for 48 hours. A total of 25 strains were isolated with 8 strains were determined to be LAB based on their morphology. The 8 strains were inoculated into 8 bottles of fermentation medium (4% v/v) in duplicate determinations and fermented in rotary shaker (180 rpm, 37°C, 4 days). After 18 hours, the fermented media were centrifuged at 10000 rpm (4°C) for 10 minutes and the supernatants were derivatised at 100°C for 10 minutes. Glutamic acid concentrations were determined by using HPLC with fluorescence detector (C18S) on a 250 × 4.6 mm column at a flow rate of 1.2 ml/min. Cut of 25 strains, 8 strains were shown to have the capability of producing glutamic acid. Five strains, including (TUS 25 (18.409 μg/ml), TUS 17 (18.437 μg/ml), TUS 7 (17.228 μg/ml), TUS 8 (14.033 μg/ml), TUS 3 (7.810 μg/ml) showed the capability of producing glutamic acid after 72 hours fermentation. While the other three strains TUS 11 (4.894 μg/ml), TUS 2 (0.0245 μg/ml), TUS 20 (0.405 μg/ml) were become 48 hours and 72 hours fermentation. The highest glutamate was registered after 48 hours and 96 hours. This study shows that LAB from "tapaipulut" has the capability of producing glutamic acid.

Key words: glutamic acid, LAB, tapaipulut

1. Introduction

Glutamic acid is an amino acid found in abundance in both plant and animal proteins. In humans it is a non-essential amino acid, i.e., the body is capable of producing its own glutamic acid, and is not dependent on glutamic acid from ingested food. L-glutamic acid (GA) is a ubiquitous amino acid present in most foods either in free form or bound to peptides and proteins. The metabolism of GA in the central nervous system in relation to nitrogen metabolism and its role as a neurotransmitter and as a precursor to another neurotransmitter (γ-amino butyric acid, GABA) has been studied extensively. GA is the most widespread excitatory transmitter system in the vertebrate central nervous system and in addition to its actions as a synaptic transmitter it produces long-lasting changes in neuronal excitability, synaptic structure and function, neuronal migration during development, and neuronal viability Monosodium glutamate, the sodium salt of GA is used commercially as a flavour enhancer. Usually in combination with nucleotides inosinate to provide an extension of taste in processed food such as soups, biscuits, noodles, meat and vegetable processing etc. Glutamic acid mother liquor in monosodium glutamate production is being used in the manufacture of sauce and as soil conditioner, fertilizer etc.

Lactic acid bacteria (LAB) are of great importance because of their beneficial roles in the human body and in a variety of food and feed fermentations [1]. LAB

generally requires amino acids for growth and their requirements for amino acids vary with species and between strains within species [2]. LAB as probiotic are known to produce a wide variety of antibacterial substances; as well as inhibitory primary metabolites like acetic acid, lactic acid, propionic acid, ethanol, diacetyl, hydrogen peroxide, bacteriocins and antibiotics-like substances with activity against Gram-negative bacteria [3].

Today LAB are used as probiotics. Although LAB are widespread in the natural environment and are largely implicated in the production of many fermented foods of both plant (pickled vegetables, silage, sourdough) and animal origin (dry fermented sausages, fermented fish, cheese). Moreover, some of these organisms have beneficial effects on human health and have been proposed as emerging probiotics. The present study was undertaken with the aim to screen potential lactic acid bacteria from "*tapaipulut*" for biosynthesis of glutamic acid. Attempts were also made to isolate and quantify the glutamic acid concentration using high performance liquid chromatography (HPLC).

2. Methodology

2.1 Sources of Lactic Acid Bacteria

Sources of indigenous lactic acid bacteria were isolated from local fermented foods such as "*tapaipulut*". The "*tapaipulut*" purchased in one batch consisted of material in small plastic bags tied using rubber band from wet market in Kuantan, Pahang Malaysia in December, 2016 and stored at 4°C before analysis.

2.2 Isolation of Lactic Acid Bacteria (LAB)

The medium used in the isolation process was MRS agar. 25 g of "*tapaipulut*" was aseptically transferred into sterile plastic bag and homogenized for 2 minutes in 225 ml of sterile 0.85% NaCl solution with Colworth Stomacher 400. Five 10-fold dilutions of the homogenates prepared and from that 0.1 ml random selections of 10^{-4} and 10^{-6} dilutions applied onto a MRS agar plates containing 0.16% bromocresol purple respectively and spreaded evenly with a sterile spreader. The MRS agar plates were anaerobically incubated for 2 days at 37°C. Bacterial colonies that developed on the plates turned into yellow colour were randomly picked and streaked on fresh MRS agar plates to obtain single colonies. This procedure repeated in order to purify the isolates, which were maintained on MRS agar slants and kept in chiller at 4°C for immediate use.

2.3 Cultivation of Lactic Acid Bacteria

Lactic acid bacteria were cultivated in fermentation medium following the methods of Jyothi et al. (2005) [4] was employed. In this procedure, 18 hours old lactic acid bacteria cultures of each isolate were inoculated into 8 universal bottles of fermentation medium containing 10 ml of de DeMan-Rogosa-Sharpe (1960) [5] (MRS) broth in duplicate determinations. The medium were incubated at 37°C for 4 days in incubator shaker (Classic C24, New Brunswick Scientific Co., Ins.,) with agitation of 180 rpm.

2.4 Quantification of Glutamic Acid

Samples were taken out within 24 hours intervals for consecutive 4 days to study the influence of time on level of glutamic acid in the fermentation medium. Each sample was centrifuged at 10000 rpm for minutes at 4°C and the supernatant stored at -20°C until derivatisation for glutamate analysis. The stock standards were prepared and stored at -20°C until analysis carried out. The derivatisation protocol based on method described. Briefly, 10 µl of either standard mix or sample supernatant, 50 µl of the buffer (4.0 g/L sodium hydrogen carbonate pH 10.5) solution were added to Pierce 1 ml Reacti-Vial, followed by 100 µl of dansyl chloride solution. Solution was mixed vigorously. Then, the vial was placed in boiling water bath at 100°C for 10 minutes. The vial contents

changed from pale yellow before reaction to colorless after reaction. The vial was cooled with water bath and 300 µl of methanol were added. Sample was filtered through membrane filter with 0.45 µm pore size and 13 mm. A 20 µl of derivative was injected onto the appropriate HPLC system. The mobile phase which was used on the fluorescent detection (FLD) system composed of a mixture of 1% glacial acetic acid, dionized water and HPLC grade 45% methanol. Mobile phase was filterd through Milipore 0.45 µm HV Durapore membrane filters (AGB, Dublin) and vacuum degassed prior to use. Compounds were eluted isocratically over a 7 min runtime at a flow rate 1.2 ml/min after 20 µl injection. The column was maintained at temperature of 30°C and samples/standards were kept at -20°C in blush freezer unless otherwise stated. The fluorescent detector was set at an excitation wavelength of 328 nm, an emission wavelength of 530 nm. Glutamic acid was identified on its characteristic retention times as determined by standard injections which were run at regular intervals during sample analysis. Sample peak heights were measured and compared with standard injections in order to quantify the glutamic acid.

3. Discussion and Analysis

3.1 Screening of Lactic Acid Bacteria

"*Tapaipulut*" was used as a source of lactic acid bacteria. Twenty five bacterial isolates were obtained and screened for lactic acid bacteria via catalase test and Gram's stain. From the results shown 8 from 25 isolates were found to be catalase — negative and Gram's positive, and all were rod-shaped. Catalase test were performed as a first line screening for lactic acid bacteria. In this procedure, 3% H_2O_2 was dropped on lactic acid bacteria streaked on glass microscope slide. Result showed that, lactic acid bacteria did not produce bubbles (indication of O_2 release). It was proved that lactic acid bacteria are all aero – tolerant anaerobes that grow readily on the surface of solid media exposed to air. However, they are unable to syntheses ATP which reflection of their failure to synthesis cytochromes and other hem containing enzymes. One consequence of the inability to synthesis hem proteins is that the lactic acid bacteria are catalase negative, and hence cannot mediate the decomposition of H_2O_2. Then, the isolates were Gram stained. According manual on determination of bacteriology, lactic acid bacteria are gram positive. From the experiment, it showed that, eight were found to be gram positive, rod shaped and chain, except for the rest was found to be coccus and yeast. Gram-positive bacteria have a thick mesh-like cell wall made from peptidoglycan (90% of cell wall), which were stain purple. While Gram-negative bacteria have a thinner layer (10% of cell wall), which were stain pink. Gram-negative bacteria also have an additional outer membrane which contains lipids, and it is separated from the cell wall by periplasmic space.

3.2 Quantification of Glutamic Acid Production Using High Performance Liquid Chromatography (HPLC)

The productions of glutamic acid were measured by comparing between peak areas of samples with the glutamic acid standard. The purpose of these tests was to quantify the capability of lactic acid bacteria isolates from "*tapaipulut*" to biosynthesis the glutamic acid. Fig. 1 shows the growth pattern and concentration of glutamic acid by lactic acid bacteria over a period of 4 days fermentation. The MRS media was used as glucose was the primary carbon source and ammonium nitrate as the nitrogen source.

From the result shown, the maximum production of glutamic acid was obtained after 48 hours fermentation strain TUS 20 (18.000 µg/ml). While the concentration of glutamic acid after 96 hours was very low (0.405 µg/ml) in some strains likes TUS 11 (4.894 µg/ml), TUS 2 (0.0245 µg/ml). According to Jyothi et al. (2005) [4], glutamic acid production in the medium could increase with time of incubation. Glutamate was detectable in the medium after 12 hours of fermentation and attained a steady state after 48 hours. The maximum yield for strains TUS 25 (18.409 µg/ml),

TUS 17 (18.437 µg/ml), TUS 7 (17.228 µg/ml), TUS 8 (14.033 µg/ml) and TUS 3 (7.810 µg/ml) were recorded after 72 hours of fermentation. Meanwhile, strains TUS 25, TUS 17, TUS 7, TUS 8 and TUS 3 showed some potential to produce glutamic acid production after 72 hours of fermentation and strains TUS 20, TUS 11 and TUS 2 were after 48 hours of fermentation.

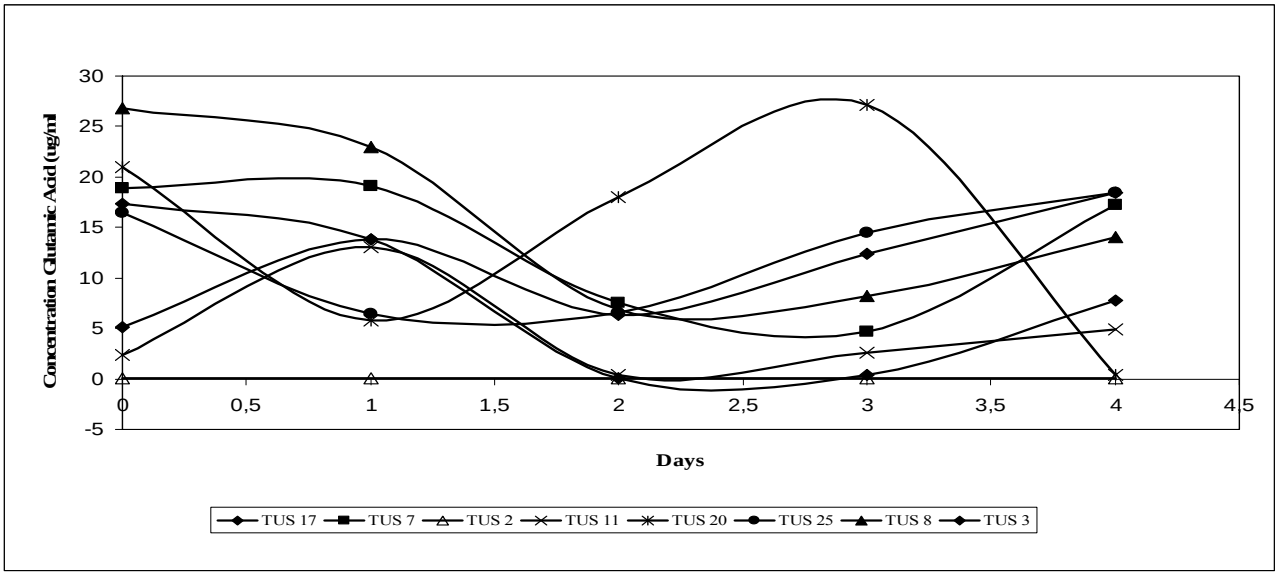

Fig. 1 Concentration of glutamic acid production from potential lactic acid bacteria.

4. Conclusion

Indigenous lactic acid bacteria could be isolated from local foodstuffs. The choice of isolation media plays a crucial role in isolating these lactic acid bacteria. Lactic acid bacteria are fastidious by nature as they require substantial amount polypeptides, amino acid, B vitamins and some essential minerals for growth. MRS media used in the isolation of these lactic acid bacteria contains these nutrients, and with a slight acidic pH, 25 bacterial isolates were isolated from local fermented food sources as "*tapaipulut*".

The isolates were put through catalase test and gram staining to differentiate the lactic acid bacteria. Cut of 25 strains, 8 were identified as lactic acid bacteria, i.e., they were gram-positive and catalase-negative. Before these lactic acid bacteria isolates can be considered for potential glutamic acid production, they need to be quantified. All of the 8 strains were do quantification using High Performance Liquid Chromatography (Shidmazu Fluorescent Detector RF-10AXL) to see whether the strain is potential to produce high glutamic acid compare to each other. The five of strains (TUS 25, TUS 17, TUS 7, TUS 8, and TUS 3) showed the capability of producing high glutamic acid after 96 hours' fermentation. While the other three strains (TUS 11, TUS 2, TUS 20) were before 48 hours and 72 hours' fermentation.

From the studies, it shown that lactic acid bacteria from "*tapaipulut*" have a capability of producing highest glutamic acid at certain time of incubation. It has good characteristics such as rod and chain, a high glutamic acid production profile at higher temperatures (37°C to 45°C). It also displayed good lactic acid bacteria from "*tapaipulut*" also shows potential produce high glutamic acid and ability to grow at low initial pH, i.e., at pH 4.5.

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