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Molecular detection of *Weissella* isolated from fermented food

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Abstract
Weissella is a member of Lactic acid bacteria; most of the *Weissella* are considered non pathogens for people and other animals and have potential probiotic properties.

Oligonucleotide primers for the polymerase chain reaction (PCR) that enable genus-specific detection of members of the genus *Weissella* were tested. The primers amplify a 725-bp genetic sequence of members of the genus *Weissella.* Seventy three isolates from fermented food were analyzed by conventional PCR to rapid molecular screening of *Weissella*. 14 (19.2%) strains produced specific PCR fragments with specific primers. Using an initial screening of Lactic acid bacteria, the results of the 73 selected strains as belonging to lactic acid bacteria. The strains that had positive results were proved by testing the morphological and biochemical characteristics.

Introduction

Probiotics are live microorganisms that are administered to human beings and farm animals for the purpose of improving the general health conditions of hosts [1, 2, 3, and 4]. Lactic acid bacteria (LAB) are the most important probiotics employed because of their long history of uses as starters for various fermented foods with few adverse effects on the health [5 and 6]. Importance of LAB has been increased because use of antibiotics for farm animals has been tightly controlled or banned in many countries [7and 8].

Weissella species are members of LAB. Its genus status was proposed in 1993 [9] and 14 species are officially recognized [10]. All *Weissella* species are nonspore formers, all Grams positive, catalase negative and they ferment glucose via heterolactic fermentation pathway, producing lactic acid and carbon dioxide . *Weissella* species are distributed among various environments including fermented vegetables, fermented foods, sugar cane, and gastrointestinal tracts of human and animals [11]. *Weissella* strains with *B*-Glu activities were previously isolated from human faeces and used as starters for fermented soymilk enriched in isoflavonoid aglycones [12].

Before the reclassification, the *Weissella* species have been classified as *Lactobacillus* or *Leuconostoc* species. This genus comprised seven members, namely *Weissella confusa, Weissella minor, Weissella halotolerans, Weissella viridescens, Weissella kandleri, Weissella paramesenteroides, and Weissella hellenica* [9]. *Weissella thailandensis, Weissella cibaria and Weissella kimchii* [13, 14 and 15], and *Weissella soli* and *Weissella hanii*. However, it is difficult to identify these *Weissella* by classical phenotypic methods [16] Therefore; alternative molecular biological approaches have been developed therefore, in this study; we used PCR methods using genus specific primer to identify and screening of *Weissella* rapidly.

Materials and Methods

Bacterial isolation: The lactic acid bacteria isolates were obtained from fermented food.
Ten grams of each sample were aseptically added into 90 ml of sterile 0.9% NaCl solution. Homogenized and serially diluted, 1 ml of the diluents was pour-plated on de Man, Rogosa and Sharpe (MRS) agar. Plates were incubated for 24 hrs at 30 °C under anaerobic condition (anaerobic generating Kit, Merck). Total of 73 representative colonies were randomly picked and sub-cultured to obtained pure culture. The isolates were maintained on MRS agar plates kept at 4°C. The stock cultures were stored at -20°C in MRS broth supplemented with 15% of glycerol for subsequent use.
The bacteria were characterized by microscopic morphological examination and by conventional biochemical and physiological tests. Gram staining, catalase activity, gas production from

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glucose,growth in NaCl (2-6.5%), growth at different temperature (10-45°C), and production of amino acid from arginine and the identification work were done [17 and 18].

Extraction of bacterial DNA: Total DNA was extracted from colonies grown on agar plates by boiling method according to [19] with some modifications .one bacterial colony was scraped using sterile toothpick from surface of agar plates and suspended in 40 µl Tris–EDTA buffer. The suspension was heated for 15min at 100°c followed by 5 min on ice rapidly. The suspension containing DNA was stored at-20 C until used as template for PCR.

Some Bacterial strains were obtained from Department of Microbiology, Mahidol University, Thailand, used as standard strains, to demonstrate the specificity of the PCR primers for the genus *Weissella*, and were included in the study.

PCR amplifications. Oligonucleotide primers amplified region of a 725 bp segment of 16S rDNA sequences of *Weissella* species and not producing fragment from other related bacteria including *Leuconostoc* species and *Lactobacillus* species. The primer sequences for the upper and lower oligonucleotides were as follows: forward primer is CGTGGGAAACCTACCTCTTA and the reverse primer is CCCTCAAACATCTAGCAC [20 and 21].

A PCR mixture was prepared consisting of 1.25 ul (10µM) of upper and lower primers, 12.5 ul of 2X KAPA2G Robust HotStart ReadyMix (KAPA2G Robust HotStart DNA Polymerase 1 U per 25 µl reaction in a proprietary reaction buffer containing dNTPs ,0.2 mM of each dNTP at 1X, $MgCl_2$ 2 mM at 1X) and 10ul of bacterial DNA per reaction. The reaction was run for initial denaturation at 94 °C for 5 min followed by 35 cycles of 45 sec at 94 °C (denaturation), 45 sec at 60 °C (annealing), and 1min at 72 °C (extension) and 5min at 72°C(final extension) in a thermocycler . Products of the PCR were electrophoresed on a 1% agarose gel containing ethidium bromide and photographed. Positive results were indicated by the presence of a 725 bp band seen on the gel with an Ultraviolet transilluminator.

Results and Discussion

Seventy three Strains were analyzed by PCR to rapid screening of *Weissella* .The results of the specific PCR reactions are shown in Fig (1) .14 (19.2%) strains produced PCR fragments with specific primers.

The amplified target sequence of the PCR was identified for all species of *Weissella* that were tested. The predicted amplification product of 725 bp was seen as a band on 1% agarose gels. This band was not identified for any other genus of bacteria studied, including other members of the lactic acid bacteria.

Most of the *Weissella* are considered non pathogens for people and other animals. Genus-specific detection of *Weissella* has application to probiotic situations in which detection of *Weissella* would support or confirm probiotic investigations [22]. A genus-specific diagnosis of *Weissella* would provide important information regarding, detection of many species of *Weissella* that is importment in treatment, control and prevention of many disease.

Determination of the species associated with *Weissella* isolates has important probiotic implications; a highly sensitive and specific test to identify members of this genus might augment probiotic investigations. Examples of application of the technique described in this paper to probiotic investigations include determining prevalence in a population where the species of *Weissella* has been determined from a sample of the population and providing rapid, highly sensitive identification of materials with *Weissella*. Amplification of DNA using PCR can be accomplished rapidly and is of particular value when concentrations of viruses or bacteria are low, when bacteria that are shed are nonviable, or when isolation of an organism is difficult.[23,24,25 and 26].The PCR can be used as a highly sensitive and specific test for the presence of bacteria in clinical specimens[24 and 25].Although other molecular genetic techniques require careful handling of specimens to provide good quality DNA for analysis, [27] sheared or degraded DNA can be used for the PCR.

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Further studies are needed to evaluate the effectiveness of these synthetic primers and PCR for detecting *Weissella* species in human specimens and environmental samples. Amplification of DNA using the PCR may enable detection of microorganisms below the level that can be detected by microbiologic culture [23, 24, 25, and 26] Identification of *Weissella* species by microbiologic culture frequently entails submission of multiple samples because of variability in the number of organisms being shed. Because detection of specific regions of the bacterial genome by the PCR is more sensitive than microbiologic culture, only a single sample might be needed for detection of *Weissella* species by the PCR. Compared to methods of gene amplification that do not utilize thermostable DNA polymerases, the PCR is simple and rapid and increases the specificity, yield, sensitivity, and length of targets that can be amplified [25 and 28]

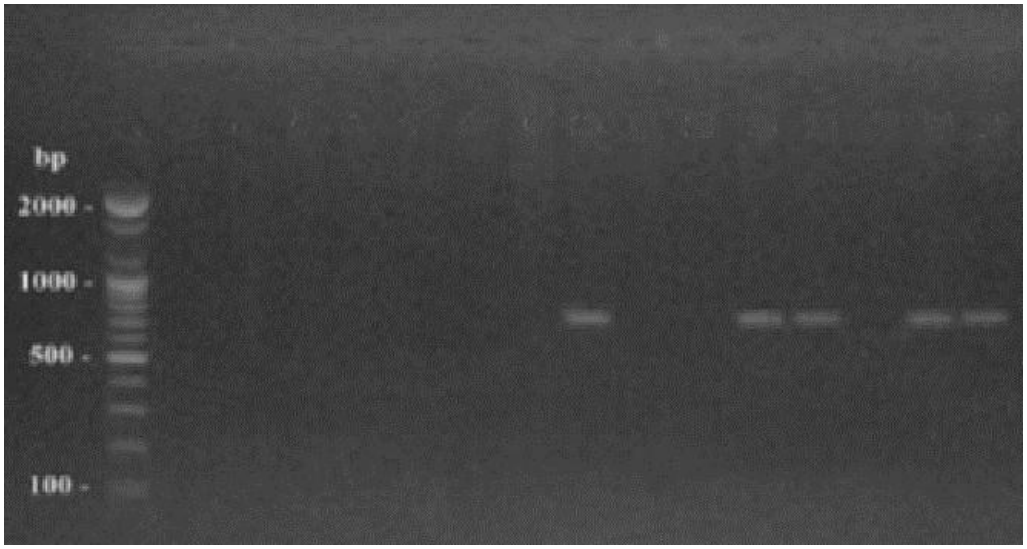

Figure (1) Agarose gel electrophoresis of PCR products of 725 bp using of *Weissella* specific primer, Lane L, DNA ladder.

A B

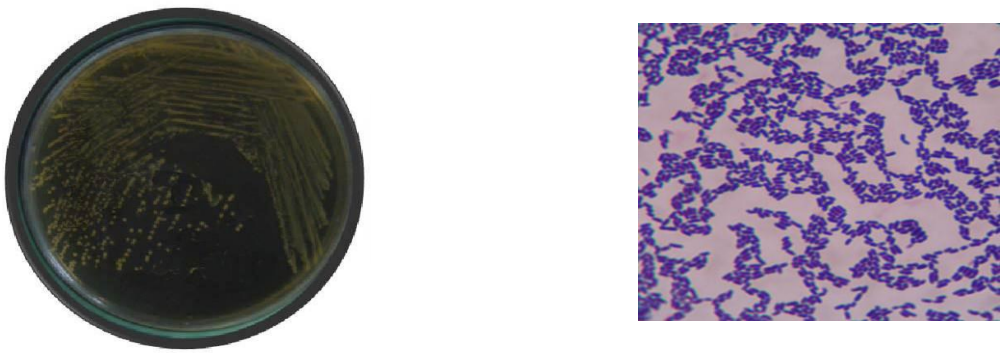

Figure (2): **A** The isolate was grown on MRS agar medium, 30°C, 18h, and anaerobic conditions. Colonies appears small, grayish white, circular, low convex with entire margin, and non-pigmented **B**: Morphology of the isolate stained with Gram stain under light microscope .Cells showed Gram positive rods.

Characteristics of the selected isolate are summarized in figures (2). Colonies of the isolates on MRS agar plates were small, grayish white, circular, low convex with entire margin, and non-pigmented. Cells were Gram positive, non-motile, nonspore former, short rods in shape and appeared either singly or in pairs. Growth occured at pH 4.4 and 8.0 but not at pH 9.6. The isolate

could not grow in presence of 8% NaCl but grew in 6.5% NaCl .Growth occured at 15, 37 and 45 °C, but not at 4°C. Catalase negative, Arginine is hydrolysed. CO_2 is produced from glucose.

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التشخيص الجزيئي لبكتريا *Weissella* المعزولة من الاغذية المتخمرة

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الخلاصة :
تنتمي *Weissella* الى بكتريا حامض اللاكتيك. معظم بكتريا *Weissella* غير ممرضة للانسان والحيوانات الاخرى كما تمتلك صفات كامنة لاستعمالها ك probiotic. تم اختبار البادئات التي تستعمل للكشف النوعي عن جنس *Weissella* باستعمال تقنية تفاعل البلمرة المتسلسل .البادئات النوعية تؤدي الى تضخيم تسلسل طوله 725 زوج قاعدي في جينوم جنس *Weissella*. اجريت غربلة جزيئية سريعة لثلاثة وسبعون عزلة من الاغذية المتخمرة باستعمال تقنية تفاعل البلمرة المتسلسل للكشف عن جنس *Weissella*.لوحظ ان اربعة عشر (19.2 %) من العزلات اظهرت حزم نوعية باستعمال البادئات النوعية.تمت غربلة اولية للعزلات المدروسة ووجد انها تعود الى بكتريا حامض اللاكتيك.السلالات التي اظهرت حزم (نتائج موجبة) تم تاكيد عودتها الى جنس *Weissella* باستعمال الاختبارات المظهرية والكيموحيوية.