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Anti-*Candida* activity and industrial properties of *Pediococcus pentosaceus* NOA-2142 isolate from traditional pickled gherkin

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Abstract

Antifungal activities of LAB have increased in many environments, especially in foods, due to the harms of chemical preservatives, as they are natural and capable of preventing both spoilage and infections. This antifungal activity is associated with metabolic compounds of LAB such as cyclic dipeptides, fatty acids, hydrogen peroxide, organic acids, and phenyl lactic acid (PLA) which are produced directly or indirectly. On the other hand, many *Candida* sp. such as *Candida albicans* is an opportunistic pathogen and can cause diseases ranging from superficial mucosal to life-threatening systemic infections, and spoilage in food. Therefore, the anti-candida activity of LAB is an important issue. In this study, it was aimed to reveal the anti-candida activity of *Pediococcus pentosaceus* NOA-2142 which isolated from a traditional pickled gherkin, and to investigate the industrial properties of this strain for widespread use. In the study, the NOA-2142 isolate was selected for its high anti-candida activity, and was determined to belong to *P. pentosaceus* species. Subsequently, the minimum inhibitory concentration (MIC) of the cell-free supernatant (CFS) of this isolate against pathogen strains of *Candida albicans* and *Candida tropicalis* was determined as 1/128 and 1/64, respectively. In addition, the D-3-phenyllactic acid content, which is the most likely cause of the anti-candida activity of the CFS, was determined as 163.21 mg/L. Moreover, the isolate were revealed to have the ability to grow at temperatures of 15°C and above, and in the range of 3–12% NaCl concentration and 3.0–9 pH value. The NOA-2142 isolate showed the highest susceptibility with 40.53 mm zone diameter to the clindamycin antibiotic disc. As a result, the *P. pentosaceus* NOA-2142 with antifungal potential could be a proper candidate as bio-preservative starter or adjunct culture, or the CFS of *P. pentosaceus* NOA-2142 could be used as a natural additive.

Keywords

Anti-*Candida* activity, *Pediococcus pentosaceus*, D-3-phenyllactic acid, Growth conditions, Antibiotic activity

Introduction

Pediococcus pentosaceus is one type of lactic acid bacteria (LAB), has a cocci shaped, gram-positive, and homofermentative LAB with facultative anaerobic and carbohydrate degradation features (Qiet al., 2021). It is known that strains belonging to this species isolated from many sources such as fermented foods, raw animal (unprocessed raw meat), plant products, human gastrointestinal tracts, and faces, have some functional properties. These functional properties can be expressed as improvement of organoleptic properties such as texture and sourness, promoting biological growth in plants and animals. In addition, they have anti-inflammatory, antimicrobial, antifungal, antioxidant effects, and cholesterol-lowering, and antagonizing effects on toxic substances. Even, some functional LAB strains are a possible probiotic candidate (Danielsenet al., 2007; Huanget al., 2020; Kuppusamy et al., 2020; Qiet al., 2021; Sellamaniet al., 2016; Shani et al., 2021).

Recently, the trend towards the antifungal activities of LAB has increased in many environments, especially in foods, due to the harms of chemical preservatives, as they are natural and capable of preventing both spoilage and infections. This antifungal activity is associated with metabolic compounds of LAB such as cyclic dipeptides,

fatty acids, hydrogen peroxide, organic acids, phenyl lactic acid (PLA), and diacetyl which are produced directly or indirectly (Aartiet al., 2018; Colorettiet al., 2007). The synthesis of PLA in LAB strains results from the catabolism of phenylalanine. This amino acid is transaminated to phenylpyruvic acid (PPA) and it further reduced to PLA. In addition to the fungal inhibitory activity of PLA has a broad spectrum against protogenic yeast (Mu et al., 2012a; Schnürer and Magnusson 2005; Schwenningeret al., 2008). Therefore, PLA can be used to increase the shelf life of food products by reducing the contamination of food-borne pathogens and food spoilage.

In general, among protogenic yeast, species of *Candida* are the most common, especially, *Candida albicans*, *Candida tropicalis*, and *Candida parapsilosis* (Paponet al., 2013). However, it is known that several types of *Candida* sp. are resistant to numerous non-natural antifungal agents, as well as the cost of available anti-*Candida* drugs is high (Aartiet al., 2018; Pemán et al., 2009; Ramageet al., 2005). Thus, this indicates the urgency of finding a new, cost-effective and harmless approach to the *Candida* sp.

In this study, , it was aimed to reveal the anti-*Candida* activity of *Pediococcus pentosaceus* NOA-2142 which isolated from a traditional pickled gherkin, and to investigate the industrial properties of this strain for widespread use.

Materials and Methods

Materials

A total of 162 possible LAB isolates which isolated from 12 traditional pickled gherkins samples were used as material. The isolates were activated using MRS-5C agar (Kaya and Şimşek 2020) at 30 ºC for 18 hours, added with a 30% glycerol solution and then stored at -80 ºC until they was analyzed. Besides, *C. albicans* ATCC 10131 and *C. tropicalis* ATCC 4563 strains (ATTC, American type culture collection, USA) were used as pathogen indicator microorganisms

Screening for anti-*Candida* activities of isolates

The cell-free supernatants (CFSs) of the isolates were tested for antifungal activity by agar well diffusion assays technique. Firstly, the 162 isolates were activated using MRS-5C agar at 30 ºC for 18 hours. After activation, the cells were separated from the active LAB cultures (6000 rpm, 15 min), the remaining supernatants were filtered by 0.22 µm pore size Millipore filters (Sigma-Aldrich, USA) to obtain CFCs. Later, these CFSs were neutralized to 7.0 pH using 1N NaOH and also subjected to catalase (Sigma, India; 1 mg/mL) treatment, incubated at 30°C for 2 h in order to avoid antifungal property of organic acids produced and hydrogen peroxide, respectively. Besides, fluconazole (10 µg/mL) was used as positive control solution. On the other hand, the *C. albicans* and *C tropicalis* strains as pathogen indicator microorganisms were activated in RPMI 1640 broth at at 28 °C. (Sigma-aldrich, USA). Following, yeast inoculums (5x 10^6 CFU/mL; 10^4 yeast/petri surface cm^2) were swabbed onto respective sterilized RPMI 1640 agar (Sigma-aldrich, USA) plates. Then, the control solution and the CFS solution were each individually impregnated with 10 µL on different discs with a diameter of 6.4 mm. These discs were placed on top of inoculated petri dishes. After an incubation at 28 °C for 48 h. The measurement of the growth inhibition zone (mm) surrounding the discs was taken (CLSI, 2008). Inhibition zone diameters were recorded as follows: (−) = no visible inhibition zone; (+) = weak inhibition (6-12 mm); (++) = medium inhibition (12-20 mm); or (+++) = strong inhibition (> 20 mm).

Identification of the isolate with the highest anti-*Candida* activity

Cell morphology was observed by light microscopy (Iolight, UK). The selected isolate was identified by sequence analysis of 16S rDNA. Bacterial DNA of the isolate was isolated from bacteria grown in MRS broth using DNA Mini Kit (Life Technologies). 16S rDNA was amplified by a thermocycler (Bio-Rad, California, USA) with a PCR program consisting of 1 cycle at 95 °C 15 min pre-denaturation, followed by 35 cycles at 95 ºC (1 min), 55 ºC (1 min), 72 ºC (3 min) and 72 ºC for 10 min stages, respectively. For this reaction, universal primers (forward primer-27F and reverse primer 1492R) were used. Then, the resulting PCR product was purified using the Qiagen PCR purification kit, and the purified fragment were partially sequenced using the Thermo Sequenase Dye Terminator Cycle Sequencing Pre-mix (Amersham Biosciences) and the automated sequence analyser ABI Prism 377XL (Perkin Elmer). Finally, 16S rRNA gene region nucleotide homology with the closest relative organism was determined by BLAST (Basic Local Alignment Search Tool) programme.

Growth testing at the different temperature, pH, and salt values of the selected isolate

The isolate with the highest anti-*Candida* activity was activated in MRS-5 broth for 18 hours. The active isolate was inoculated in the tubes containing MRS-5 broth media for growth testing at 4, 10, 15, 40 ºC, and 40 °C, in the tubes containing MRS-5 broth media containing 3.0, 5.0, 6.5, 8.0, 9.0, 10.0, and 12.0% NaCl for growth testing at different salt concentrations, and in the tubes containing MRS-5 broth with pH values adjusted to 2.0, 3.0, 3.5, 4.0, 4.5 and 9.5 pH for growth testing at different pH values, by 1%. After, the tubes were incubated for 7 days for the temperature test, and for 3 days for the other tests but at 30ºC. The growth in the tubes showing an increase of 0.3 units in the OD_{600} value was evaluated as positive (Akoğluet al., 2016; G-Alegríaet al., 2004).

Antibiotic susceptibility test of the selected isolate

The susceptibilities of the isolate to clindamycin, metronidazole, vancomycin and tetracycline antibiotic discs were tested. The active culture of the isolate were inoculated to be equal to 0.5 McFarland (~10^8 CFU/mL) on Mueller-Hinton agar. Then, the discs were placed onto inoculated plates. After 24 hours incubation at 30°C, the diameters of the inhibition zones around the discs were measured. The results were evaluated according to NCLS standards (Plessaset al., 2017).

Determination of the minimum inhibition concentration (MIC) of the CFS of the selected isolate

The antimicrobial activity of the CFS of the selected isolate were determined as minimum inhibitory concentration (MIC) by the microdilution technique (96-well microplates technique) using the National Committee for Clinical Laboratory Standards (NCCLS) recommendations (CLSI, 2002). Only the dilution part was modified. In this assay, the analysing CFS to be tested was prepared the serial two-fold dilutions (from 1/1 to 1/256 CFS solution) in in RPMI 1640 broth (Merck)

medium, for the pathogen yeasts (the above-mentioned *C. albicans* and *C tropicalis* strains). They were added as 100 mL in each well of microplate. On the other hand, for inoculum, the 24 h cultures of the yeast strains were used. They were adjusted to a turbidity equivalent to 10^8 CFU/ mL, and diluted in broth media to give a final concentration of 0.5×10^3 to 2.5×10^3 CFU/mL for yeast. Then the microplates were incubated at 28 ºC for 48 h. The lowest concentration that inhibited no growth was determined as the MIC value.

Determination of D-3-phenyllactic acid amount in the CFS of the selected isolate

D-3-Phenyllactic acid was determined using HPLC using a column (Capcell Pak C18; 4.6× 250 mm, 5 µm, Shiseido Co. Japan). Firstly, the CFS was adjusted to pH 2.0, and D-3-Phenyllactic acid was extracted with 20 mL of ethyl acetate from the CFS. This extract was dried using Na_2SO_4 and concentrated in a rotary evaporator. The dried residue was added with 5 mL of 2.5 mM H_3PO4. It was applied a linear gradient program using mobile phases; 5% H3PO4 (v/v) (mobile phase-A) and 0.5% H3PO4-CH3CN (V/V) (mobile phase-B) at 1 mL /min, at 30 ºC. The program was run at the A/B ratios of mobile phases of 80:20 (0-14 min), 80:20 (14-16 min), 0:100 (16-18 min), and 10:90 (25-32 min) (v/v). D-PLA was detected at 210 nm (Li et al., 2007; Yoo et al., 2016).

Statistical Analysis

The experiment was carried out in duplicates. The results are expressed as means ± standard error (SE) of three experiments done in triplicate. Analysis of statistical significance was performed with Duncan test and t-student ($p<0.05$) using SPSS program (SPSS 22.0, USA).

Results and Discussion

Isolates with anti-*Candida* activity

It was determined that a total of 14 of 162 LAB isolate CFS isolated from the samples showed anti-candida activity against at least one of the *C. albicans* ATCC 10131 and *C. tropicalis* ATCC 4563 pathogen strains (Table 1). The CFSs of the NOA-2047, NOA-2142 and NOA-2027 isolates showed a strong inhibition activity against the *C. albicans* strain, while the CFSs of the NOA-2142 and NOA-2007 isolates showed a strong inhibition activity against the *C. tropicalis* strain. Among the others, the CFSs of the NOA-2011 NOA-2098 NOA-2324 NOA-2072 NOA-2058 showed a medium inhibition against the *C. albicans* strain, but they showed either a weaker effect or no inhibition against the *C. tropicalis* strain. On the other hand, only the CFS of the NOA-2252 isolate showed a higher inhibition against the *C. tropicalis* strain than the *C. albicans* strain, despite a moderate activity. According to all these results, the CFS of NOA-2142-strain which had 25.31 mm and 23.57 mm zone diameters for the *C. albicans* and *C. tropicalis* strains respectively, was chosen because it showed greater activity than the others against both pathogen strains. The fluconazole compounds that used as a positive control, showed similar activity to the CFS. This showed that the CFS had a strong activity. In the literature, some study on anti-candidate activities of LABs were found. For example, in a study, a culture of *Lacticaseibacillus paracasei* subsp. *paracasei* was found to retard growth of various *Candida* species in an in situ yoghurt model as well as on cheese surface (Schwenninger and Meile 2004). In another study, *Pediococcus acidilactici* KTU05-7, and *Pediococcus pentosaceous* KTU05-8 and KTU05-10 strains provided protection against *Candida parapsilosis* C.7.2 on the surface of bread (Cizeikieneet al., 2013). Similarly, in another study, probiotic *Lacticaseibacillus rhamnosus* GR-1 and *Limosilactobacillus reuteri* RC-14 strains provided protection against pathogen *C. albicans* strains (Köhler et al., 2012). In a study (Bulgasemet al., 2016), the CFS of *Pediococcus pentosaceus* HM strain which had 13.31 mm and 12.20 mm zone diameters for the *C. albicans* and *C. tropicalis* strains respectively, This strain had lower activity than the CFS of NOA-2142-strain. Besides, in a study (Lu et al., 2011), it was determined that garlic extarct had antimicrobial activity against *C. albicans* with 34.0 ± 0.30 mm zone diameter and *C. tropicalis* with 30.0 ± 0.30 mm zone diameter, pathogen strains. Garlic contains compounds, such as diallyl sulfides, phenolic compounds and steroid saponins, and as most other antifungals, these compounds penetrate the cell membranes and cause a leakage of cellular components, leading to cell death.

Table 1. Anti-*Candida* Activities of LAB-CFSs against *C. albicans* and *C. Tropicalis*

CFS* codes	Indicator strains		CFS* codes	Indicator strains	
	C. albicans **	*C. tropicalis***		*C. albicans* **	*C. tropicalis***
NOA-2047cfs*	+++	+	NOA-2011cfs	++	—
NOA-0043cfs	—	+	NOA-2134cfs	+	—
NOA-2324cfs	++	—	NOA-2027cfs	+++	—
NOA-2252cfs	+	++	NOA-2098cfs	++	+
NOA-2142cfs	+++	+++	NOA-2116cfs	+	—
NOA-2072cfs	++	—	NOA-2007cfs	++	+++
NOA-2058cfs	++	+	NOA-2083cfs	+	—

*; CFS or cfs was steriled and neutralized supernatant from the LAB isolates. Inhibition zone diameters were recorded as follows: (−) = no visible inhibition zone; (+) = weak inhibition (6-12 mm); (++) = medium inhibition (12-20 mm); or (+++) = strong inhibition (> 20 mm).

Identity of the ısolate with the highest anti-*Candida* activity

In the study, it was determined that the selected NOA-2142 isolate was similar to *Pediococcus pentosaceus* DSM 20336 with 99.93% (1,454 bp) (Figure 1). In the literature, strains of this species are known to have various activities such as antifungal, antimicrobial, antioxidant. For example, in a study conducted by Cortés-Zavaletaet al., (2014), the anti-candida activity of *Limosilactobacillus fermentum*, *Latilactobacillus sakei*, *L. rhamnosus*, *L. casei*, *L. reuteri* strains was determined. In another study conducted by Diguțăet al., (2020), it was determined that *P. pentosaceus* (L3) and *Pediococcus acidiliactici* (L5) strains showed the anti-candida activity

against *Candida albicans* ATCC 10231 strain. In a study conducted by Bamideleet al., (2019), *P. pentosaceus* BTA 51 from cucumber showed anti-candida activity at neutral pH (12 mm diameter).

Growth ability of the selected NOA-2142 at the different temperature, pH, and salt values

Growth properties of the isolate were analyzed at 3.0–9.5 pH, 4.0–45 ºC, and 3.0–12% NaCl (Table 2). According to the results, it was observed that the isolate had the ability to grow at temperatures of 15 ºC and above. On the other hand, it was determined that it could grow in the range of 3.0-9.5 pH values, and showed poor growth only at 2.0 pH values. As for the growth test at different salt concentrations, it was determined that this strain could grow at all concentrations in the range of 3.0-9.0%. It could not grow at concentrations in the 10.0% and 12.0% NaCl. These results showed that the NOA-2142 isolate had a wide spectrum of use as a natural preservative in food products with different environments or different processes. In other words, the usage area may not be limited to pickles only. In the literature, growth characteristics of the isolates that were similar to *Pediococcus pentosaceus* strains such as the NOA-2142 isolate were determined. For example, in a study (Diguțăet al., 2020), it was determined that *P. pentosaceus* L3 strain could grow at concentrations in 2.5%, 5.0%, and 7.5% NaCl, but no grow at concentrations in 9.5%, and 11.5% NaCl. This result was similar to the presented study.

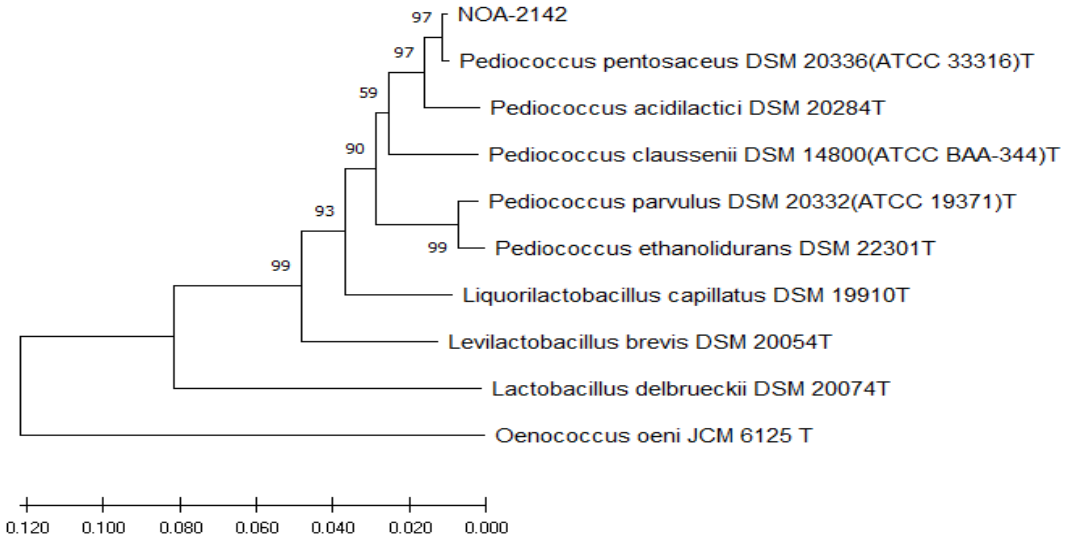

Figure 1. Neighbor-joining tree based on 16S rDNA sequences showing genetic relatedness between *Pediococcus pentosaceus* NOA-2142 and related species. The optimal tree with the sum of branch length = 0.45301296 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. Evolutionary analyses were conducted in MEGA X.

Antibiotic susceptibility ability of the selected NOA-2142

The NOA-2142 isolate showed the highest susceptibility with 40.53 mm zone diameter to the clindamycin antibiotic disc, followed by the amoxicillin, ampicillin, and tetracycline antibiotic discs (Figure 2A). It was determined that this isolate was resistant to the metronidazole, erythromycin and vancomycin antibiotic discs. This has demonstrated its reliability in its use as a preservative. There are some studies examining the antibiotic activities of the isolates that were similar to *Pediococcus pentosaceus* strains such as the NOA-2142 isolate. For example, in a study (Ilavenilet al., 2016), antibiotic activty of *P. pentosaceus* KCC-23 strain determined as sensitive against tetracycline (100 µg) and ampicillin (10 µg) discs, while it resisted antibiotic discs including gentamicin (10 µg). In another study (Diguțăet al., 2020), *P. pentosaceus* L3 resisted antibiotic discs including tetracycline (30 µg), and vancomycin (10 µg), while it showed sensitivity against erythromycin (10 µg).

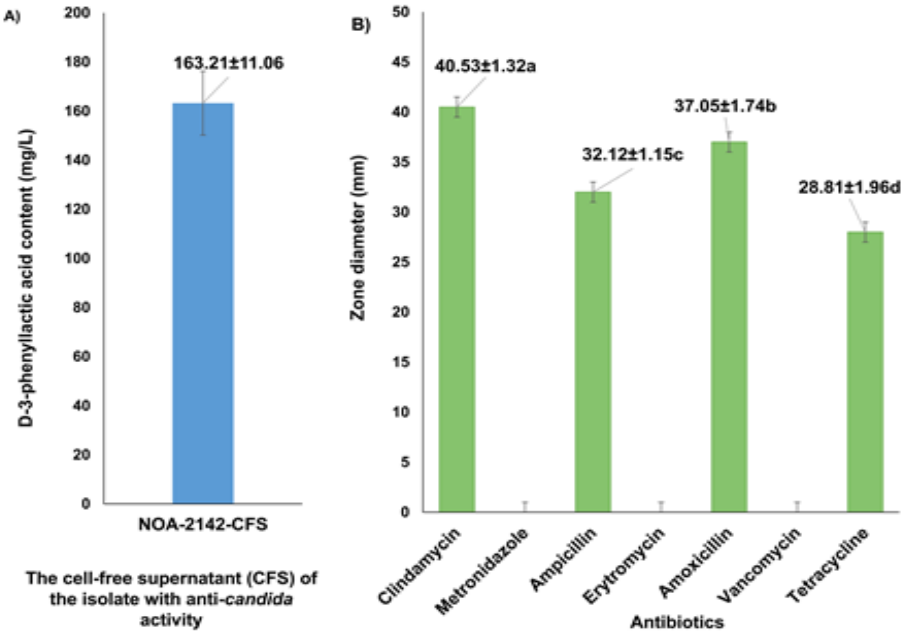

Figure 2. **(**A) D-3-phenyllactic acid content (mg/L) in the cell-free supernatant (CFS) of the NOA-2142 isolate with anti-*Candida* activity. (B) Antibiotic susceptibility ability of the selected NOA-2142

Minimum inhibition concentration (MIC) of the NOA-2142-CFS

In the study, the MIC values of the NOA-2142-CFS were determined as 1/128 and 1/64 concentrations of the NOA-2142-CFS against the *C. albicans* and *C tropicalis* pathogen strains, respectively (Table 2). Here, the lower concentration equaled MIC value showed that the NOA-2142-CFS was more effective against the *C. albicans* pathogen strain, according to the *C tropicalis* pathogen strain. In fact, these values indicated quite strong activity. In the literature, in a study (Salari et al., 2020), the CFSs of *Lactiplantibacillus plantarum* and *Lactobacillus acidophilus* strains had MIC values in the range of 50-200 µL/mL, against the *Candida* sp. pathogen strain.

It is known that the anti-yeast activities of LABs are dependent on many compounds such as their metabolites organic acids such as lactic acid and propionic acid, fatty acids such as butyric acid and caproic acid, peptides and cyclic peptides such as cyclo (LPhe-L-Pro) and cis-cyclo (L-Leu-L-Pro), low-molecular-mass compounds such as methylhydantoin, mevalonolactonan, benzoic acid and reuterin, and phenyllactic acid (PLA) derivatives (Crowley et al., 2013; Nasrollahzadehet al., 2022). The most common of these is compound D-3-phenyllactic acid for antifungal activity (Aartiet al., 2018; Colorettiet al., 2007).

Table 2. Growth Conditions and MIC Values of the NOA-2142 Isolate with Anti-*Candida* Activity

Temperature (°C)*					pH values*					
4	10	15	40	45	2.0	3.0	3.5	4.0	4.5	9.5
—	—	+	+	+	—	+	+	+	+	+
Salt (NaCl) (g/100 g)*										
3.0	4.0	5.0	6.5	8.0	9.0	10.0	12.0			
+	+	+	+	+	+	—	—			
Minimum inhibition concentration (MIC) of the NOA-2142-CFS										
Candida albicans strain				*Candida tropicalis* strain						
1/128 µL/mL CFS				1/64 µL/mL CFS						

*+; There is growth, — ;There is not growth ** n.d.; not detected

D-3-phenyllactic acid content of the NOA-2142-CFS

The D-3-phenyllactic acid content of the NOA-2142-CFS was determined quantitatively by HPLC instrument. According to the result (Figure 2A), it was determined that the D-3-phenyllactic acid contained 163.21 mg/L of the CFS. It is known that D-Lactate dehydrogenase (D-LDH) from *P. pentosaceus* ATCC 25745 produced D-3-phenyllactic acid from phenylpyruvate (Yuet al., 2012). In the literature, in a study (Yuet al., 2015), the CFS of *P. pentosaceus* SK25 contained D-3- phenyllactic acid at 135 mg/L. In other study (Mu et al., 2012b), the CFS of *P. acidilactici* DSM 20284 contained D-3- phenyllactic acid contained D-3- phenyllactic acid at 108 mg/L. While these amounts was lower than that in the presented study, in another study (Bustos et al., 2018), D-3- phenyllactic acid which the CFS of *P. acidilactici* CRL 1753 contained, had a higher concentrate with 186.50 mg/L than that in the presented study. Besides, in a study (Cortés-Zavaletaet al., 2014), it was determined that the CFSs of the *L. casei, L. fermentum, L. rhamnosus, L. sakei, L. reuteri* strains contained D-3- phenyllactic acid in the concentration

range of 3.49-45.70 mg/L. According to these, it was observed that the CFS of the NOA-2142 isolate in the presented study had a quite effective D-3-phenyllactic acid content.

Conclusion

The *P. pentosaceus* NOA-2142 isolated originally from pickle are probably the best candidates as bio-preservatives, because it is well adapted to the non-aseptic conditions in the production of fermented food such as pickle and sourdough. Also, its antifungal activity in neutral pH indicated this isolate produced some antifungal compounds. Anti-*candida* activity of CFS of the NOA-2142 isolate was demonstrated by its high D-3-phenyllactic acid content. Therefore, *P. pentosaceus* NOA-2142 with antifungal potential could be a proper candidate as bio-preservative starter or adjunct culture, or the CFS of *P. pentosaceus* NOA-2142 could be used as a natural additive.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the author(s) to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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