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ORIGINAL PAPERS

Assessing of Morphological, Cultural, Biochemical Profile and Enzymatic Activity of a *Lactobacillus paracasei* CCM 1837 Strain

Mihaela DUMITRU[1,2], Ionuț SORESCU[1], Ștefana JURCOANE[2,3], Gheorghe CÂMPEANU[2], Cristina TABUC[1], Mihaela HĂBEANU[1]

[1]National Research Development Institute for Biology and Animal Nutrition (IBNA), Bucharest, No. 1, Balotesti, Ilfov, 077015, Romania

[2]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59, Marasti Blvd, District 1, Bucharest, Romania

[3]Academy of Romanian Scientists Bucharest, Romania

Coresponding author e-mail: mihaela.dumitru22@yahoo.com

Abstract

The purpose of this study was to examine a lactic acid bacteria (LAB) strain know as *Lactobacillus casei* CCM 1837. The strain derived from Microorganism Collection of Cehia. It was assayed morphologically, culturally, biochemically and enzymatically (amylolytic and cellulolytic activity). For current identification was effectuated three passages in MRS agar and broth Oxoid. The strain was identified and conserved as *Lactobacillus paracasei* spp. *paracasei* IBNA 04 in the Collection of INCDBNA. It is Gram positive cocobacilli, thin, non-spore forming, rounded ends, isolated, diplo form, in short chains in culture of 24 - 48 h in Oxoid MRS broth at 37°C incubation. The strain is facultative anaerobic with a slight preference for anaerobic. The identification and analysis of the biochemical characteristics was performed by catalase assay, API 50 CHL Biomerieux strips, apiweb API 50 CHL V 5.1 soft (good identification to the genus *Lactobacillus paracasei* spp. *paracasei* 1 or 3, 48-51% ID) and ABIS online (*Lactobacillus paracasei* spp. *paracasei*, ~ 90%). The enzymatic activity was determined byHostettler's method for amylase activity and the Petterson's and Porath's method for cellulolytic activity. The *L. paracasei* CCM 1837 was incubated at 37°C in aerobic and anaerobic atmosphere. An optimal growth was recorded in the MRS broth medium in aerobic conditions for 48-72 h. The strain had an amylase activity of 0.124 (UDNS/ml) to 24 h, compared with 0.158 (UDNS/ml) to 48 h, at 37°C. It record a cellulolytic activity 0.09 (UDNS/ml) at 24 h, compared with 0.04 (UDNS/ml) registered at 48 h, at 37°C. In conclusion, the results suggest that *L. paracasei* CCM 1837 strain had some probiotic characteristics and will be assessed to demonstrate its capacity to influence positively the gut animal ecosystem (must to survive the passage through the gastric juice in the stomach, to resist the bile acids and salts from pancreas etc.).

Key words*: Lactobacillus paracasei* CCM 1837, *API 50 CHL, enzymatic activity, pigs intestinal microbiota.*

Introduction

Microbiota stability and colonisation of the gastro intestinal tract (GIT) has a crucial role for preserving the host homeostasis and health animals (Wacklin et al., 2014). The intestinal microbiota is a complex ecosystem that is in a strong connection with the biology of their hosts (Clemente et al., 2014).

Understanding the variations of the commensal microbiota composition is crucial for a more efficient control of enteric infectious diseases and for reduction antibiotics use in animal production, which are the main points of interest for improved animal healthcare and welfare and for consumer health protection (Vincenzo Motta et al., 2017).

The pigs GIT microbiota has been studied to increase production efficiency, to improve productsquality and help attempt to reduce disease. During the developmental period from birth through weaning, the intestinal microbiota undergoes a rapid ecological succession (Simpson et al., 1999). Colonization of the intestinal tract (IT) in piglets is initiated at birth by microbiota from the maternal genital sow and their IT. After colonization of the IT of newborns is complemented by microbiota from the environment(Kubasova et al., 2016).Also, the pig's immune system at birth is fully detailed butnot so fully functional, maturing within 7–10 d; the pig is a good model of the human GIT with many similar traits (Quanshun Zhang et al., 2013).

The small intestine of pigs is populated, generally, with anaerobic lactobacilli, but they can be found in high numbers in cecum and colon content. Intestinal content and feces can contain up to 10^{10} - 10^{12} bacterial cells g^{-1}(Richards et al., 2005). These numbers are consistent with the estimation that bacterial cells outnumber host cells by ten to one (Gaskins, 2001).

The aim of this study was conducted to assess a LAB strain known as *Lactobacillus paracasei*CCM 1837 and to describe her role andmorphologically, culturally, biochemically and enzymatically activity,as preliminary investigation of probiotic potential of this strain in order to use it in pigs nutrition.

Material and Methods

The identification of *L. paracasei* CCM 1837 strain was performed by biochemical tests (catalase assay,API 50 CHL Biomerieux strips), API 50 CHL V 5.1 and ABIS online soft.

The enzymatic activity strain was determined as folow: amylolytic activity by Hostettler's method and cellulase activityby Petterson's and Porath's method at 48 and 72 h incubation, at 37°C.

The research was carried out at Laboratory of Biotechnology of National Research Development Institute for Biology and Animal Nutrition Balotești (INCDBNA), Romania.

Morphological and cultural characters analysis

The culturing was realised in Oxoid MRS selective medium, in 24 - 72 h, at 37°C. The strain is anaerob facultative, with some preferences for anaerobic atmosphere(Oxoid Jar with Anaerogen 2.5 L).Morphologicaltraits were examined with a microscope.It was done threepassagesfor current identification, before conservation.

Biochemical characters

The catalase test

The test demonstrate the presence of catalase, an enzyme that catalyses the release of oxygen from hydrogen peroxide (H_2O_2). The concentration of H_2O_2 used was3% (Sagar, 2015). The catalase enzyme mediates the breakdown of H_2O_2 into oxygen and water ($2H_2O_2$ + Catalase → $2H_2O + O_2$). The presence of the enzyme in a bacterial isolate is obvious when a small inoculum is introduced in the H_2O_2 and the reaction forms bubbles by the rapid elaboration of oxygen (MacFaddin, 2000).

The API 50 CHL test

The isolate was identified using the API 50 CHL strips according to the manufacturer's protocol (BioMerieux). The test API 50 CHL put in evidence the enzymatic equipment of *L. paracasei* CCM 1837.

The API 50 CHL consists of 50 microtubes used to study fermentation of substrates belonging to the carbohydrate family and its derivatives. The fermentation tests are inoculated with bacterial strain suspension in API 50 CHL medium which rehydrates the substrates. During incubation, the ability of isolate to ferment carbohydrates is indicated by the colour change of the basal medium used, caused by the anaerobic production of acid and detected by the pH indicator present in the select medium.

The strips are read after 48 – 72 h of incubation. The results produced the biochemical profile which is used by strain's LAB identification with API 50 CHL V 5.1 and ABIS online software (Stoica and Sorescu, 2017).

Assessing the enzymatic activity

Enzymatic activity was determined at 48 and 72 h, at 37°C in aerobic atmosphere, by the following methods:

1. Hostettler's method for determination of amylolytic activity. It is based on the action of α-amylase on starch and determination of maltose released after the enzymatic hydrolysis process, witch the 3,5 - dinitrosalicylic (DNS), pH 6.9, at 30°C. Due to the reducing hemiacetal groups, consisting of maltose hydrolysate is reacted with 3,5 - DNS acid and form the nitroaminosalicilic acid, that can be measured colorimetrically and is proportional to the enzymatic activity value (Jurcoane et al., 2006).

The mixture of enzymatic solution (1 ml) and 1% starch was incubated in the buffer of pH 10, for 10 minutes at 30°C. The reaction was stopped with 2 ml of DNS. The analysed sample was kept for 5 minutes in the boiling water bath followed by cooling and dilution with distilled water to a 12 ml volume. After 20 minutes, the optical density of the sample was read at 546 nm, compared to the control. An amylase unit corresponds to a quantity of maltose (μmol) released under the action of 1 ml of enzyme preparation in one minute, at 30°C.

2. Petterson's and Porath's method for cellulolytic activity. It's based on the enzymatic hydrolysis of carboxymethylcellulose (CMC) and the dosing of reductant groups released with the tetrosalicylic reagent. A cellulolytic activity unit represents the amount of enzyme which releases from a CMC solution, an amount of reducing carbohydrates, which form with the DNS reagent, the same optical density, similar to a milligram of glucose (Jurcoane et al., 2006).

Results and discussion

Characterization of Lactobacillus paracasei CCM 1837 strain

The strain was from Microorganism Collection of Cehia. It appears as Gram-positive rods, cocobacilli, square ends, nonmotile, isolated, diplo or in short – chained arranged. A slow growth was observed on agar nutritiv medium.In the first 24 h, the strain determine the formation of small colonies, punctiform (~ 2 mm). In 48 – 72 h, in medium inclined tubes appear colonies, especially tip S, rou°nd end with regular forms, opaque, creamy white-yellowish pigment. In MRS broth, in 24 – 48 h, an intense turbidity with abundant deposits was observed, without surface formations. The 72 h culture on MRS agar is sufficiently aged and the passage will be done with difficulty, but the MRS broth agar culture is more viable. It is necessary to use a young culture (24, maximum 48 h) for performing a solid passage.

The strain was catalase negative.

In this study, the *Lactobacillus paracasei*CCM 1837strain was identified biochemically by API 50 CHL V5.1 (*Lactobacillus paracasei spp. paracasei*1 and

3, good identification to the genus 48-51% ID) and ABIS online (*Lactobacillus paracasei spp. paracasei,* ~ 90%). The capability of isolate in fermenting carbohydrate was observed by the discoloration of basal medium, where medium from purple to yellow (Figure 1).

Figure 1. API 50 CHL strips inoculated with *Lactobacillus paracasei*CCM 1837

The results by API 50 CHL was interpreted after 2 – 6 days, at 37°C (Table 1). The *Lactobacillus paracasei*CCM 1837fermented D-ribose, D-galactose, D-glucose, D-fructose, D-mannose, L-sorbose, D-mannitol, D-sorbitol (3-6 days), N-acetylglucosamine, arbutin, esculin, salicin, D-celobiose, D-lactose, D-trehalose, D-melezitose, D-turanose, D-tagatose. The *Lactobacillus paracasei* CCM 1837strain was negative for fermentation of glycerol, erythritol, D-arabinose, L-arabinose, D-xilose, L-xilose, D-adonitol, methyl-βD-xylopyranoside, L-rhamnose, dulcitol, inositol, methyl-αD-mannopyranoside, methyl-αD-glucopyranoside, amygdalin, D-melibiose, inulin, D-saccharose (sucrose), D-raffinose, amidon (starch), glycogen, xylitol, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium 2-ketogluconate, potassium 5-ketogluconate.

D-maltose, gentibiose and potassium gluconate was weak fermented.

Table 1. The results obtained with API 50 CHL for *Lactobacillus paracasei* CCM 1837

Glycerol –	Salicin +
Erythritol –	D-cellobiose +
D-arabinose –	D-maltose?
L-arabinose –	D-lactose +
D-ribose +	D-melibiose –
D-xylose –	D-saccharose (sucrose) -
L-xylose –	D-trehalose +
D-adonitol –	Inulin –
Methyl-βD-xylopyranoside –	D-melezitose +
D-galactose +	D-raffinose -
D-glucose +	Sarch) -
D-fructose +	Glycogen –
D-mannose +	Xylitol –
L-sorbose +	Gentibiose ?
L-rhamnose –	D-turanose +
Dulcitol –	D-lyxose –
Inositol –	D-tagatose +
D-mannitol +	D-fucose –
D-sorbitol +	L-fucose –
Methyl-αD-mannopyranoside –	D-arabitol –
Methyl-αD-glucopyranoside –	L-arabitol –
N-acetylglucosamine +	Potassium gluconate?
Amygdalin –	Potassium 2-ketogluconate –
Arbutin +	Potassium 5-ketogluconate –
Esculin +	

„-" Negative test; „+ " Positive test; „?" Weakly positive.

The calibration curve was used to determine and calculate the enzymatic activity value (Figures 2 and 3). The strain had an amylase activity of 0.124 (UDNS/ml) to 24 h, compared with 0.158 (UDNS/ml) to 48 h, at 37°C (Figure 4). It record a cellulolytic activity 0.09 (UDNS/ml) at 24 h, compared with 0.04 (UDNS/ml) registered at 48 h, at 37°C (Figure 5).

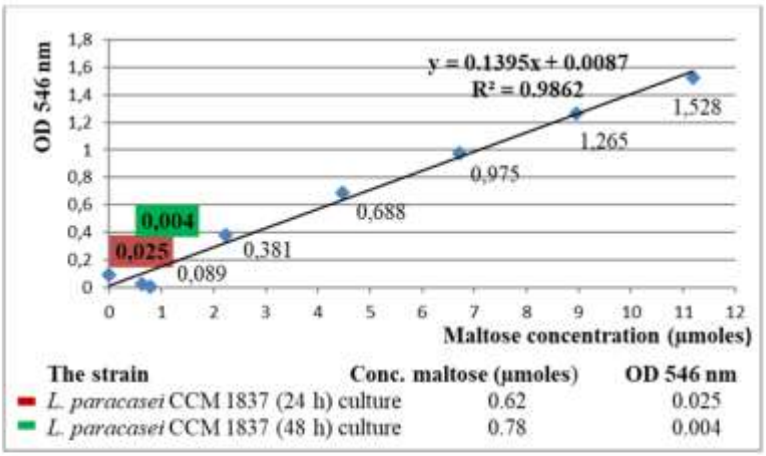

Figure 2. The calibration curve for determine the amylase activity of *L. paracasei*CCM 1837 by Hostettler's method

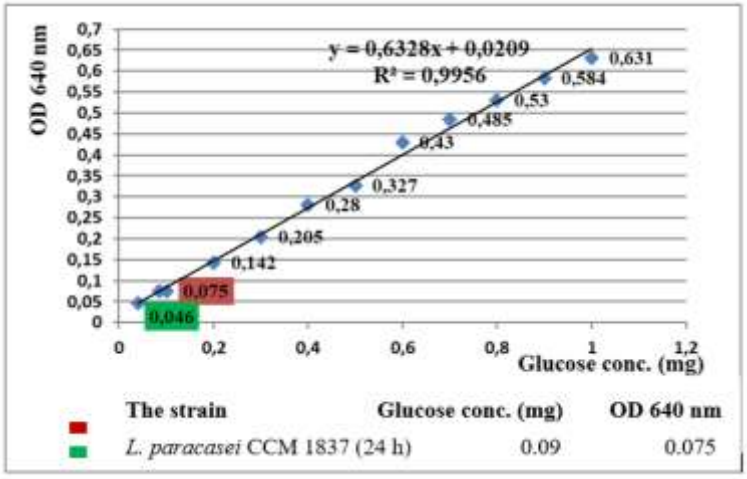

Figure 3. The calibration curve for determine the cellulolytic activity by Petterson's and Porah's method of *L. paracasei*CCM 1837

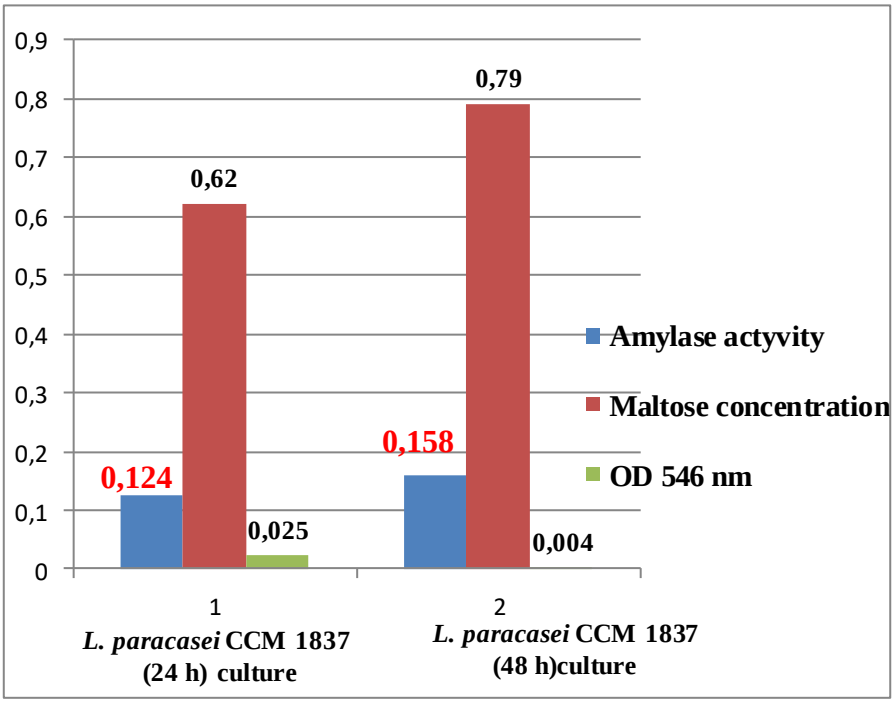

Figure 4. The amylase activity of *L. paracasei*CCM 1837

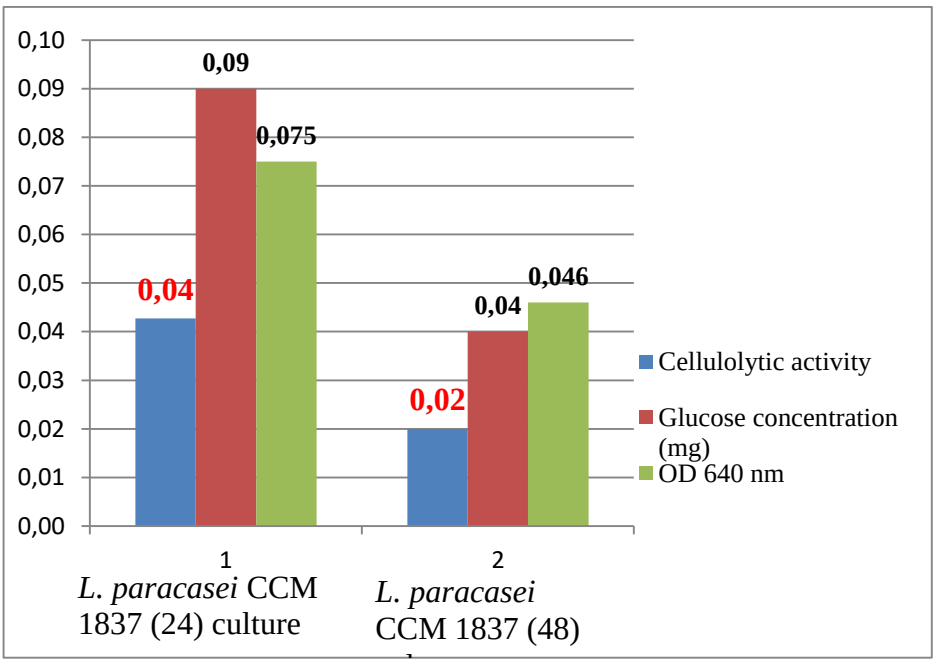

Figure 5. The cellulolytic activity of *L. paracasei CCM 1837*

Conclusions

The results suggest that *Lactobacillus paracasei*CCM 1837 strain presents some probiotic traits and can be further assessed for other characteristics as resistance to pH 2.0, resistance to bile acids and salts, antibacterial activity, induction of local immune response etc., in order to evaluate its probiotic utility in nutrition's pigs.The production of amylase's strain is 0.124UDNS/ml at 24 h, respectively 0.158 UDNS/ml at 48 h incubation.
The *Lactobacillus paracasei* CCM 1837 present the capacity to produce cellulase as 0.04 (UDNS/ml) at 24 h compared with 0.02 (UDNS/ml) at 24 h.

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