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Cite This: Int. J. of. Endocri. Diab. and Metaba 2021, 1-1, 11-14

Sciforce

International Journal of Endocrinology: Diabetes and Metabolism
Journal homepage: www.sciforce.org

Natural Competence for Transformation in Lactobacili

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ARTICLE INFO

Article history:
Received: 20210924
Received in revised form: 20211005
Accepted: 20211005
Available online: 20211015

Keywords:
Lactobacilli;
Lactobacillus buchneri NRRLB1837;
Lactobacillus casei MT205;
the natural competence for the foreign plasmid DNA uptake and the genetic transformation;
lyophilization medium composition.

ABSTRACT

We have detected the changes in the molecular biologic properties of the lascobacilus strain Lactibacillus buchneri NRRLB1837 after its storage in the lyophilized state using some patented lyophilization media. The change noticed was the 3 orders of magnitude decrease of the natural competence for the genetic transformation capability of said lac-tobacillus strain. Using noted difference we have transformed L. buchneri NRRLB1837 with the largest plasmid of Lactobacillus casei MT205, possessing strong antibacterial activity against gram-positive, gram-negative bacteria and Candida albicans. The trans formants became resistant to Erythromycin (30 mcg/ml) and also had strong antibacterial activity, just like L. casei MT205, which was extractable from the concentrated by the evaporation cultural fluid of the respective transformants, The role of the lyophilization medium on the molecular biological properties of the lactobacilli stored lyophilized is dis-cussed.

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Introduction

We have noticed about three orders in magnitude difference in the natural transfroma-tion capability in the strain of Lactobacillus buchneri NRRLB1837 depending on the par-ticular conditions of its lyophilization. Said difference is esssential, since sometimes the natural capability to uptake the foreign plasmid DNA gets below the threshold of de-tection and may not be noticed. We have detected, that for the strain of Lactobacillus buchneri NRRLB1837 such activity is the highest, if the lyophilization was performed using standard lyophilization medium comprising only sucrose and gelatin, but not the 0.1 M Trtis (pH8.0) and 10% adonit [1]. Routinely, deep freezers (-80oC) are used for the prolonged storage of the microbial cultures under the normal room temperatures. However, most of the shipments are performed with the microbial cultures, lyophilized for the long-term storage. We have noticed significant difference in the natural transforma-tion capability of Lactobacillus buchneri NRRLB1837 depending on the composition of the medium for its freeze drying. In particular, we noticed significant decrease of the natural genetic transformation capability in Lactobacillus buchneri NRRLB1837 depend-ing on the compositon of the medium for its freeze drying. The decrease of that capabil-ity by three orders of magnitude was observed for the freeze dryed culture of said lacto-bacilli freeze dryed in our patented medium for the freeze drying [1]. The core of our patent was the neutralization of the organic acids produced enormously by the intestinal lactobacilli by the 0.1 M Tris buffer (pH8.0) and the lyophilization viability was substan-tially (by 90%) increased when we added some cryoprotective sugar up to 10 vol % to said 0.1 M Tris buffer (autoclaved at 120oC for 30 min). Today we have faced the fact, that the natural competence of the plasmid DNA uptake reported by us earlier [2] was accompanied with the decreased frequency of the genetic transfer for the plasmid DNA pCB20 [3]. Said natural comptence for the transfer of the foreign plasmid DNA has been decreased by the three orders in its magnitude from about 10-5 to 10-8 per the recipient cell concentrations [2]. Originally we got the Lactobacillus burchneri NRRLB1837 in the lyofilized state from the Russian Collection of the International Microorganisms of the RAS (Russian Academy of Sciences, Moscow, the Former USSR). The standard lyophi-lization procedure there was used: the lyophilization medium composed of 10% sucrose and 1% gelatin only, the sucrose solution was autoclaved separately, then the sterile gelatin was added to 1% aseptically. Said strain of Lactobacillus buichneri NRRLB1837

helped us a lot for the plasmid DNA transfer to investigate the antibiotic resistance Markers and other properties given by

plasmids in said various plasmids from the isolate Lactobacillus casei MT205, when we have detected at least 8 plasmid DNA molecules in the human intestinal isolate of Lactobacillus casei isolated from the intestinal content of the individual working under the heavily contaminated by produced antibiotics conditions (said individual did not follow the mandatory safety precautions before he got fired from said antibiotic producing plant) [4]. We have brought hereby the notice of the change in the biological properties of the certain microbial strains due to the fact how such strains have been lyophilized before the investigation [5]. The role of the lyophilization on the physiological properties of certain microbial strains is discussed, since the lyophilization is one of the main methods of the pure culture maintenance for the long-term storage and shipping of the microbial strains. Further we used only the strain of L. buchneri NRRLB1837, stored lyophilized in sucrose/gelatin lyophoilization medium for the genetic transformation with the largest of the plasmids, about 120 kb in size [4]. In respective transformants of L. bucheri NRRLB1837, said plasmid immediately was giving the set f plasmid DNA molecules ranging in size of 4 kb, 8 kb, 12 kb, 24 kb, but not the original size of the used for the transformation plasmid of L. casei MT205 with the size of about 120 kb [4]. The role of the lyophilization medium composition on the molecular biologi-cal properties of the lactobacilli stored lyophilized is discussed.

Materials and Methods

Growth, media, selective antibiotics

Lactobacilli strains were maintained and grew up in the Lactobacillus MRS Medium [4]. For plates the 1.25% agar agar was added to the MRS medium before autoclaving at ^{120oC} for 30 min. For the selection of the transformants with the plasmid DNA pCB20 we added Erythromycin (Em) and Lincomycin (Lm) by 20mcg/ml each to the MRS agar, after that was autoclaved and chilled down to 45 o C before pouring into the100 mm Petri dishes [6]. The antibiotic powders were aseptically added to the sterile Eppendorf tubes [7] by 100 mg each and the sterile ddH_2O was added to each sterile Eppendorf tube by 1 ml. Said poured Petri dishes were dried at the laminar flow cabinet [8] and inoculated with the natural transformants, prepared as described [3]. Forspreading of the transformants on the surface of said Petri dishes only sterile plastic loops were used [9]. For the easy the calculations of the transformation efficiencies only seventh, sixth and fifth decimal dilutions of the natural transformation samples were used. To prepared said decimal dilutions of said natural transformants samples we used the DB [10].

The DB composition: 0.1 M Tris, 0.15 M NaCl, 1.0 M urea, 10 mM $CaCl_2$, 0.1 M citric acid monohydrate, 5 g BSA and 1.0 g cysteine-HCL (pH 6.00). The DB was sterilized by autoclaving at 1 atm for 30 min. The DB was distributed into the sterile 1.5 ml Eppendorf

tubes by 900 microliters [10]. The 0.1 ml of the resulting the said natural transformants samples were transferred to the second Eppendorf tube with 900 microliters of DB (the 2nd dilution of said samples), and so on, to make the necessary for the plating decimal dilutions of the volunteer's feces. The procedure to dilute feces was, as follows. The 100 microliter aliquiotes of the first dilution of the natural transformation samples were added [11] to the second 1.5ml Eppendorf tube with 900 microliters of DB, etc, to make the second, the 3rd, 4th, 5^{t}h, 6th and 7th, etc. dilutions of said natural transformants samples. The inoculation was performed as described starting from the last dilution of the recipient cells/natural transformants to save the amount of the plastic microbiological loops used.

The concentration of the recipient cells was determined in the five repeats of said experiments (for the purposes of the proper experimental statistics). For that purpose the decimal dilutions of the recipient cells were prepared as above, additionally adding the seventh, the eighth and the ninth dilutions of the recipient cells. The inoculation was performed as described starting from the last dilution of the recipient cells to save the amount of the microbiological loops used.

Lyophilization was repeated as described [1].We have repeated the lyophilization as the culture of Lactobacillus buchneri NRRLB 1837 was maintained at the Collection of the Russian Academy of Sciences five times for the proper statistical analysis of our data and then lyophilizaed the same lactobacillus culture as it was written in our patent. The strain was recovered from its lyophilized state and was used for the experiments to reveal the frequency of the natural transformation capability after the lyophilization under the conditions of the Russian Academy of Sciences and according to our Patent # 1652336 [1].

Concentration of the 72h old cultural fluid of the transformant of L. buchneri NRRLB1837 with the largest plasmid DNA (about 120 kb), extracted from the gel before the genetic transformation

We grew the transformant in 100 ml of MRS broth for 72 hours at 37^{o}C; the cultural fluid was subjected to the evaporation under vacuum in the Vacuum Evaporator [16].

Results

The concentration of the recipient cells was determined in the five independent ex-periments for the proper statistical analysis of data. So, the concentration varied from 9 x 109 cells to 7 x 109 in all our experiments.

The evaluation of the natural transformation efficiency using genetic transformation of L. buchneri NRRLB1837 with the standard plasmid DNA pCB20

As we have reported earlier, the recipient cells of L. buchneri NRRLB 1837 with the standard plasmid DNA pCB20 resulted in the revealing of EmLm-resistant transformants of L. buchneri NRRLB1837 with the frequency of 9x10-5 EmLmR-transformants per the num-

ber of the recipient cells used for such experiments (data of five independent genetic transformsation experiments for the proper statistical evaluation purposes).

Genetic transformation of the cells of L. buchneri NRRLB1837 with the largest plasmid DNA found in L. casei MT205 (size of about 120 kb).

It is known [4] that identification of the exact phenotype, which the plasmid DNA offers to the strain is possible, when said plasmid is extracted from the gel and used for the genetic transformation of the sensitive to the specific antibiotics strain of the same genus. So, we used the naturally competent for the genetic transformation strain of L. buchneri NRRLB1837 to study the changes in its phenotype after the genetic transformation with the largest plasmid of L. casei MT205 (about 120 kb), excised from the agarose gel. The excision of the plasmid DNA band from the agarose gel was successfully performed as described [12]. The purified plasmid DNA, excised from the agarose gel, gave us the genetic transformants resistant to 128 mcg/ml of Em and Lm, each added to the selective medium MRC prior to said genetic transformation. In addition to the resistance to the two MLS antibiotics Em and Lm. In addition to said antibiotic resistance, we have noticed, that the transformants of L. buchneri NRRLB1837 started expressing the antimicrobial activity on said transformants again the majority of Gram-positive and Gram-negative bacteria and also against the eukaryotes Candida albicans (we used strain C. albicans 537 for said testing [4]).

Extraction of the revealed antoginistic activity from the cultural fluid of the transformant L. buchneri NRRLB1837 plasmid DNA of about 120 kb from L. casei MT205.

We grew up this transformant in 100 ml of the standard liquid MRS medium for 72 hours and then substantially decreased the volume of said 72hcell-free culture (cells were collected by centrifugation for 15 min at 12,000g). Said cell-free medium was con-centrated using the vacuum evaporator [ ] as described earlier [4]. The final volume was about 2 ml. That amount was exactly transferred to two identical glass tubes [15]. Said 1 ml of the concentrated in the vacuum evaporator cultured fluid was extracted with the 2 ml of the chloroform [13], and the other 1 ml was extracted with the ether [14]. The extracts were dried from the chloroform and from the ether in the chemical hood to allow the exhaust of the toxic chloroform or ether vapors out of the laboratory, and then the extracts were re-suspended each in 500 mcl of the sterile bi-distilled H_2O for the sto-rage at +4 ^oC. The antimicrobial properties of said water dissolved extracts were tested as before [4], including also the Candida albicans 537 [4]. Said extracted with either chloroform or the diethyl ester fractions of the 72h cultural fluid of the genetic transformant of L. buchneri NRRLB1837 with the plasmid of about 120 kb from the L. casei MT205 . The chloroform extract had no any antibacterial activity when tested as described [4]. The ether extract, in the opposite to that, had strong antibacterial activity against both Gram-positive and Gram-negative bacteria tested and also against the eu-karyotic organisms Candida albicans 327 when tested as described [4].

Effect of lyophilization in sucrose/gelatin cryoprotective medium.

As we have found before, said lyophilization procedure did not change our previous data on the fre-quency of the natural transfromation of Lactobacillus buchneri NRRLB1837 with the plasmid DNA of pCB20. The EmLm-resistant natural transformants have been revealed with the frequency of about 2-9 x 10-5 transformants per the amount of the recipient cells/mcg of pCB20 DNA [3] We further used the L. buchneri NRRLB1837, lyophilized as above, to genetically transform the largest plasmid DNA molecule (about 120 kb in size) extracted from gel and further purified as described [4,12].

Effect of lyophilization in 0.1 M Tris (pH8.0) and 10% of adonitol added for the cryo-protective properties of the freeze drying medium. We have noticed herein that the nat-ural transformation capability of the freeze dried strain of Lactobacillus buchneri NRRLB1837 has dropped to 1-3 x 10-8 transformants per the amount of the recipient cells of L. buchneri NRRLB1837/mcg of the plasmid DNA pCB20 just as we have no-ticed before in [3].

Discussion

We detected the phenotipical properties the plasmid DNA of the largest size of about 120 kb has encoded in Lactobacillus casei MT205 [4]. The genetic transformants with said plasmid DNA extracted from the agarose gel prior to the genetic transformation experiments carried out with the capable of the spontaneous genetic transformation strain of Lactobacillus buchneri NRRLB1837 showed, that said plasmid DNA encodes the antibiltic resistance to the bouth MLS group antibiptics Em and Lm, and also encodes the storng antiba cterial activity against a wide lrange of the tested Garm-positive and Gram-negative microorgamiss, and also has the antagonistic activity against Candida albicans 327 as tested according to [4]. This is the first ether phenoltypical characteristic of the largest plasmid DNA originally isolated from the strain of Lac-tobacillus casei MT205 (described in [4]).

The changes in the physiological properties of microbial cultures due to the conditions of culture storage have been noticed before (see for instance [5]. Said physiological changes happen due to the changes happening to the cell membranes under said vari-ous conditions of lyophilization. We may conclude, that the noted difference in the fre-quency of the revealed before natural compentence for the foreign plasmid DNA uptake in the strain of Lactobacillus buchneri NRRLB1837 is affected by the compositoon of the medium for the freeze drying of said strain of lactobacilli.

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