Conversion of Inorganic Selenium to Organic Form(s) by *Lactobacillus acidophilus*

Zommara, M.A.[(1)] & Prokisch, J.[(2)]

(1) Department of Dairy Science, Fac. of Agric., Kafrelsheikh University, Kafr El-Sheikh, 33516, Egypt.

(2) Nanofood Laboratory, Fac.of Agric. and Food Sciences and Environmental Management, Debrecen University H-4032 Debrecen, Hungary.

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ABSTRACT

The bioconversion of 2 forms of inorganic selenium namely selenite (SeIV) and selenate (SeVI) to organic form(s) by *Lactobacillus acidophilus* strain was investigated. The cultured media (MRS) was supplemented with 1, 2, 5, 10, 20 ppm of Se in the form of sodium selenite (Na_2SeO_3, SeIV) or sodium selenate (Na_2SeO_4, SeVI) and incubated at 37°C up to 24 hr Both Se forms showed no marked effect on the bacterial growth indicating no cytotoxicity at these concentrations. However, the media supplemented with 5, 10 and 20 ppm of Se(IV), but not Se(VI), became reddish after 24 hr of incubation with increasing the red color intense with increasing the Se content in the media. The scanning electron microscope (SEM) investigation clarified the presence of Se-nano particles (SeNPs) in the media. Se speciation of the cultured media supernatant and its corresponding cell fractions using HPLC-ICP-MS technique indicated that the bioconversion rate of Se to organic form(s) was extremely higher in Se(IV) than Se(VI) in both fractions however, the cell fractions contained the highest content. The organic Se gradually increased in both fractions with increasing the media Se content. The inorganic Se was completely bio-converted to organic form(s) without any residual only in the medium contained 1 ppm Se(IV). Our results demonstrate the ability of *L. acidophilus* to convert Se(IV) but not Se(VI), at a limit concentration of 1 ppm and accumulate organic Se form(s) in the cell fraction. These results confirm the possible bio-production of organic Se enriched fermented dairy products.

Keywords: Bioremediation, organic selenium, inorganic selenium, L. acidophilus, nanoselenium

INTRODUCTION

The role of selenium for the maintenance of health and prevention of several diseases is probably more important than realized previously. Besides, its putative role as a protecting agent against heavy metal toxicity, cancer, and cardiovascular diseases selenium is now linked to a number of physiological functions. Young children have an increased selenium requirement because of their rapid growth and they could be, therefore, at greater risk in the case of inadequate intake. The recommended safe and adequate intake level of 50 µg/ day was defined by the National Research Council in the USA. On the other hand, several studies have been shown repeatedly that Se is more bioavailable to animals and humans in organic forms than in inorganic forms.

The selenium content of food depends upon the selenium content of the soil where the animal was raised or the plant grown. The Egyptian soil and plants content of Se is generally low and considered among the low-Se countries (Jansson and Sillanpää, 1992. Elsokkary (1980) determined the Se content of 55 soil samples collected from different parts in Egypt. The results vary from 0.18 to 0.85 ppm with an average of 0.45 ppm . The selenium intake in Egypt is sufficient, to some extent, because of the high-selenium wheat imported from North America. The estimated Se intake for the Egyptian adult is about 49µg/ day where bread supplies about 63.6% of the daily consumed Se (Hussein & Bruggeman, 1999). This situation could change because of more home-grown wheat production in recent years. Therefore, the search for other sources of selenium is very important to compensate for the expected shortage of selenium in the Egyptian diet.

Lactic acid bacteria (LAB) is an important part of diet in the form of fermented food products in different parts of the world and their biotransformation ability gives a cheap source of organic Se for human and animal nutrition (Zommara and

Prokisch, 2015, Pophaly *et al*., 2014). Different LAB strains were found to have the ability to accumulate organic selenium and elemental SeNPs in their bodies and media when cultivated with inorganic Se forms. This characteristic is very valuable as several LAB strains are in use for producing many fermented dairy products. Therefore, such ability gives a cheap source of organic Se for human and livestock nutrition.

The aim of the present study is to investigate the ability of *Lactobacillus acidophilus* to bioconvert two inorganic selenium compounds to organic form(s) when grown on MRS medium supplemented with different doses of selenite (SeIV) or selenate (SeVI).

MATERIALS AND METHODS

Cultivation of *Lactobacillus acidophilus* with selenium

Pure culture of *Lactobacillus acidophilus* (*L. acidophilus*) NCAIM B 02085 strain was obtained from the National Collection of Agricultural and Industrial Microorganisms, Budapest, Hungary. The bacterial culture was cultivated in MRS broth medium as described by De-Man *et al*., (1960) amended with 0, 1, 2, 5, 10 and 20 ppm of filter sterilized (Sartorius AG, Germany) sodium selenite, Na_2SeO_3. $5H_2O$ [Se(IV)], or sodium selenate, Na_2SeO_4. 10 H_2O [Se(VI)] Sigma-Aldrich, Switzerland) and incubated at 37°C up to 24 hr.

Determination of bacterial growth

The bacterial growth was monitored at 2 hr intervals for 12 hr and after 24 hr of incubation the cultured media. The bacterial growth was determined by measuring the media absorbance at 650 nm (Loualeche *et al*., 1993) and changes in medium pH value (Radelkis Electrochemical Instruments, Hungary).

Analysis of selenium species in medium supernatant and cell fraction

Ten ml aliquots of media were removed after 24 hr of incubation. The media were centrifuged at 4500g (7000 rpm) for 20 min at 10°C to spin down the bacterial cells. The supernatant was carefully collected and kept under freezing for Se speciation analysis. The cell pellets were washed 2 times by Tris-HCl buffer (50 mM, pH 7.5) and finally with ultra-pure water. To the cell pellet 1 ml Tris-HCl buffer (10 mM, pH 8.0) was added followed by 100 µl of 10% lysozyme solution (Sigma-Aldrish, 100.000 U/ mg) and incubated overnight at room temperature. The hydrolyzed cell pellet was centrifuged at 5000g for 20 min and the supernatant was collected (cell fraction) for Se speciation analysis.

Media supernatant and the cell fractions were analyzed for Se species by inductively coupled plasma mass spectrometer (ICP-MS) (X series, Thermo Fisher Scientific, Germany) coupled to HPLC (Merk-Hitachi L06200A, Germany) equipped with an anion exchange chromatography column (Polyspher, IC-ANI, Merck, Germany) as previously described (Zommara *et al.*, 2007). Se standards namely, seleno-L-methionine (SeMet, Fluka Chemie, Switzerland), SeIV and SeIV were prepared in Milli-Q water.

Scanning Electron Microscopy (SEM) and SeNPs size determination

The SEM of the bacterial medium was carried out according to Nagy *et al*., (2016) using Hitachi S 4300 scanning electron microscope (Schaumburg, IL, USA). Size of the SeNPs was determined by particle size analyzer (Malvern, Mastersizer 2000) Malvern Instruments Ltd, UK.

RESULTS AND DISCUSSION

Data illustrated in Fig. (1) and Fig. (2) show the growth profile of *L. acidophilus* strain incubated in MRS media with different concentration of Se(IV). The bacterial growth rate was monitored during 24 hr of incubation using the progress of medium acidity and absorbance as viability indications. The pH data indicated no inhibition effect of Se(IV) on bacterial growth up to 5 ppm in the medium. However, addition of 10 and 20 ppm to the medium had a slight negative effect on bacterial growth. The media absorbance confirmed the pH data although the media amended with 10 and 20 ppm Se(IV) resulted in high absorbance after 4 hr of incubation compared to the media with less Se(IV) content. This increase may be explained by accumulation of red selenium nano-particles (SeNPs) in the media (Fig. 3). The scanning electron microscope (SEM) photo of the medium amended with 20 ppm Se(IV) showed the accumulation of SeNPs in the cultured medium and inside the bacterial cells (Fig. 4). In this respect, Alzate *et al*. (2010) stated that supplementation of milk with Se(IV) below 2 ppm had no negative effect in the growth of a mixed cultures

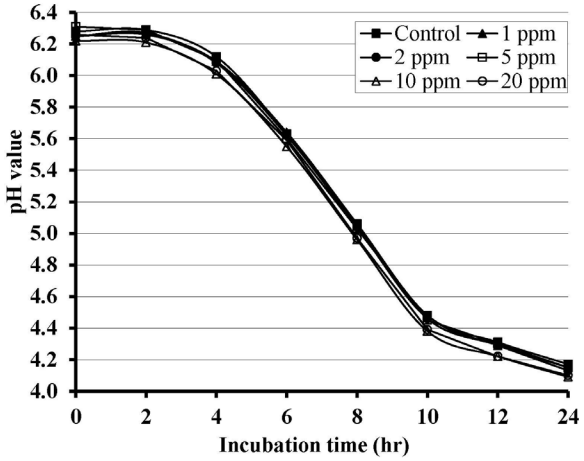

Fig. (1): Effect of selenium (Se IV) concentration on pH of MRS broth media incubated with *L. acidophilus* at 37 °C for 24 hrs.

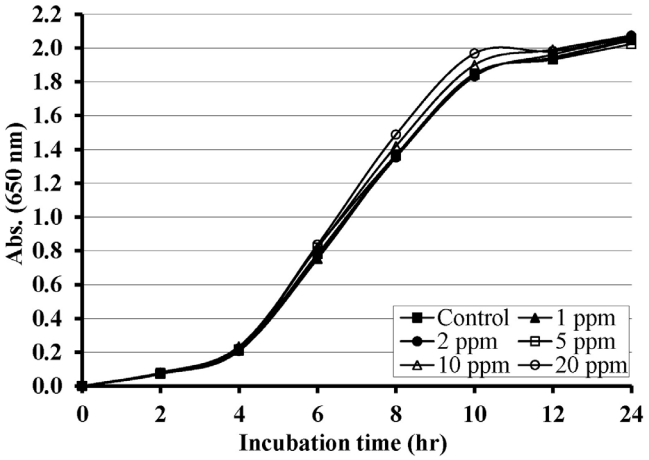

Fig. (2): Effect of selenium (Se IV) concentration on growth of *L. acidophilus* incubated in MRS broth media at 37 °C for 24 hrs.

Fig. (3): Accumulation of red SeNps in MRS media cultivated with *L. acidophilus* and 20 ppm of Se (IV) after 24 hr of incubation at 37°C

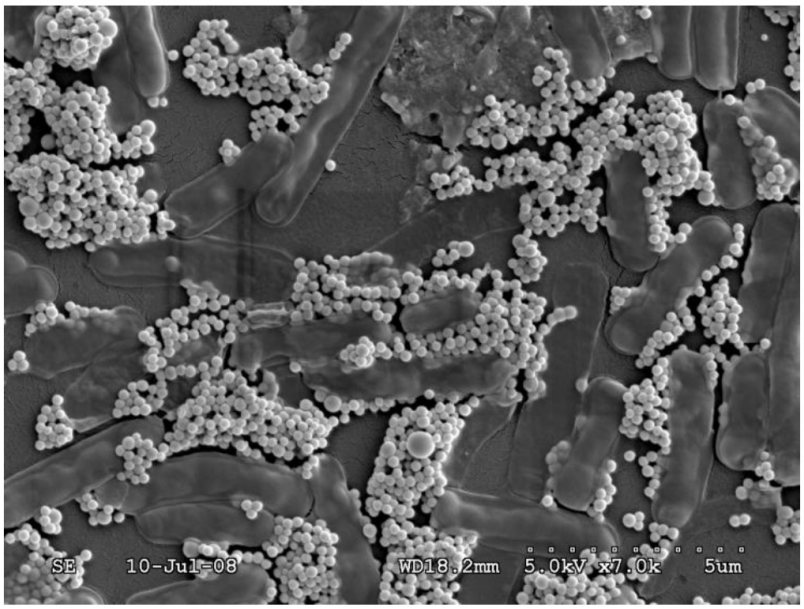

Fig. (4): Scanning electron microscope (SEM) photo of (SeNps) in MRS media cultivated with *L. acidophilus* and 20 ppm of Se(IV) after 24 hr of incubation at 37°C.

of *S. thermophilus*, *L. bulgaricus*, *L. acidophilus*, *L. paracasei* and *B. Lactis* until the 4th week of cold storage. They noticed segregated selenium as SeNPs in the fermented milk supplemented with 6, 10 and 20 ppm SeIV. Also, different LAB and bifidobacteria were found to accumulate SeNPs when cultivated in suitable media amended with different concentrations of SeIV (Prokisch & Zommara, 2011).

Our previous studies showed no inhibition effect of SeIV on the traditional yoghurt culture (*S. thermophilus* and *L. bulgaricus*) up to 20 ppm (Zommara & Prokisch, 2015). On the other hand, there was no inhibition effect of Se(VI) on the growth of *L. acidophilus* as indicated by following the media pH (Fig. 5) and absorbance (Fig. 6) although, the bacterial growth rate was slightly lower than that cultivated with Se(IV). Unlike Se(IV), use of Se(VI) did not led to accumulate the red SeNPs in the media (Fig. 3). The ability of lactic acid bacteria to bio-transform selenium Se (IV) to organic form(s) have been reported in plenty of research papers including different strains of lactobacilli and the traditional yoghurt starter culture (Prokisch *et al*., 2008, Pophaly *et al*., 2014, Zommara & Prokisch, 2015, Kurek, *et al*., 2016, Pescuma *et al*., 2017)

The formation of organic selenium takes place by replacing sulfur with Se in the sulfide amino acids in the proteins namely cysteine and methionine to form selenocysteine (Se-cys) and selenomethionine (Se-met) as the main organic selenium species found in plant and animal tissue (Alzate *et al*., 2007, Alzate *et al*., 2008, Galano *et al*., 2013, Palomo *et al*., 2014, Zommara & Prokisch, 2015, Pescuma *et al*., 2017). Also, the production of SeNPs by *L. acidophilus* cultivated in suitable medium supplemented with Se(IV) was repeatedly confirmed by many researchers (Eszenyi *et al* 2011, Rajasree & Gayathri, 2015, Nagy *et al*., 2016). Our results demonstrated that *Lactobacillus acidophilus* produce SeNPs with 100-200 nm dimeter when cultivated with MRS media amended with Se (IV). Diowksza *et al*., (1999) also observed a red color in *L. plantarum*, *L. brevis*, *L. sanfrancisco* biomass grown in MRS medium amended with Se(IV) exceeding 10 ppm. The formation of SeNPs by the bacterial cultures may be attributed to a detoxification mechanism (Prokisch *et al*., 2008).

In this respect, Zhang *et al*., (2004) studied the effect of different size of SeNPs (5-200 nm) on the induction of seleno-enzymes, namely, glutathione peroxidase (GPx), phospholipid hydroperoxide glutathione peroxidase (PhGPx) and thiredoxin reductase (TrxR) in mice and liver cell HepG2. They found no significant size effect and all nano-Se particles have equal capacity in the induction of the tested seleno-enzymes. However, Peng *et al*., (2007) suggested that Nano-Se should be most effective as a chemopreventive agent at smaller particle size. They stated that the SeNPs size effect may depend on the Se status in the cell and suggested that in Se deficiency, cells may up regulate different effective pathways for Se uptake, leading to no size effect of SeNPs on the synthesis of selenoenzymes.

The ability of *L. acidophilus* to convert different concentrations of Se(IV) to organic form in MRS media is shown in Fig. (7) and Fig. (8). The data in Fig. (7) clearly show that most of Se(IV)

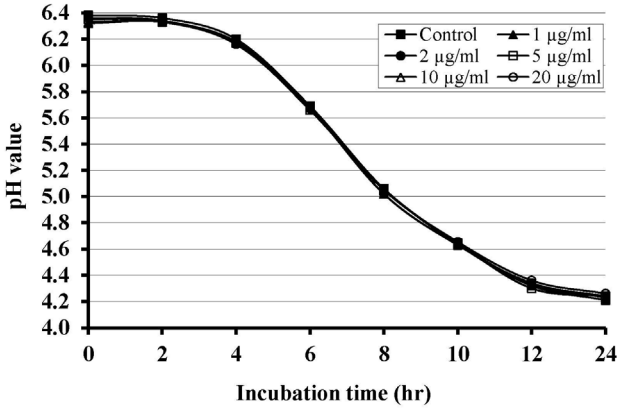

Fig. 5: Effect of selenium Se (VI) concentration on pH of MRS broth media incubated with *L. acidophilus* at 37 °C for 24 hrs.

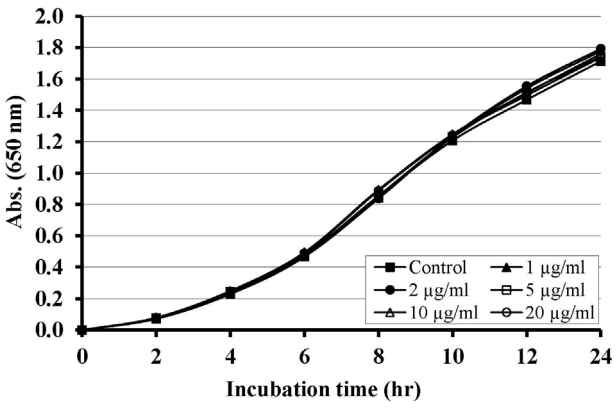

Fig. 6: Effect of selenium Se (VI) concentration on growth of *L. acidophilus* incubated in MRS broth media at 37°C for 24 hrs

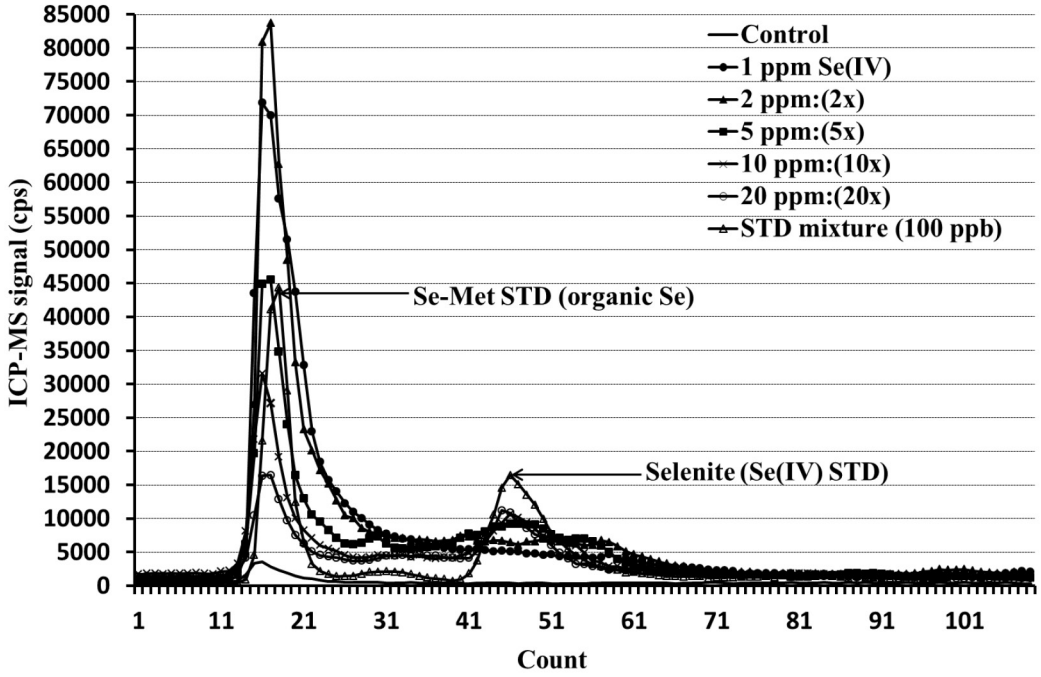

Fig. 7: Conversion of different concentrations of sodium selenite [Se(IV)] to organic form in MRS media supernatant incubated with *L. acidophilus* at 37°C for 24 hrs.

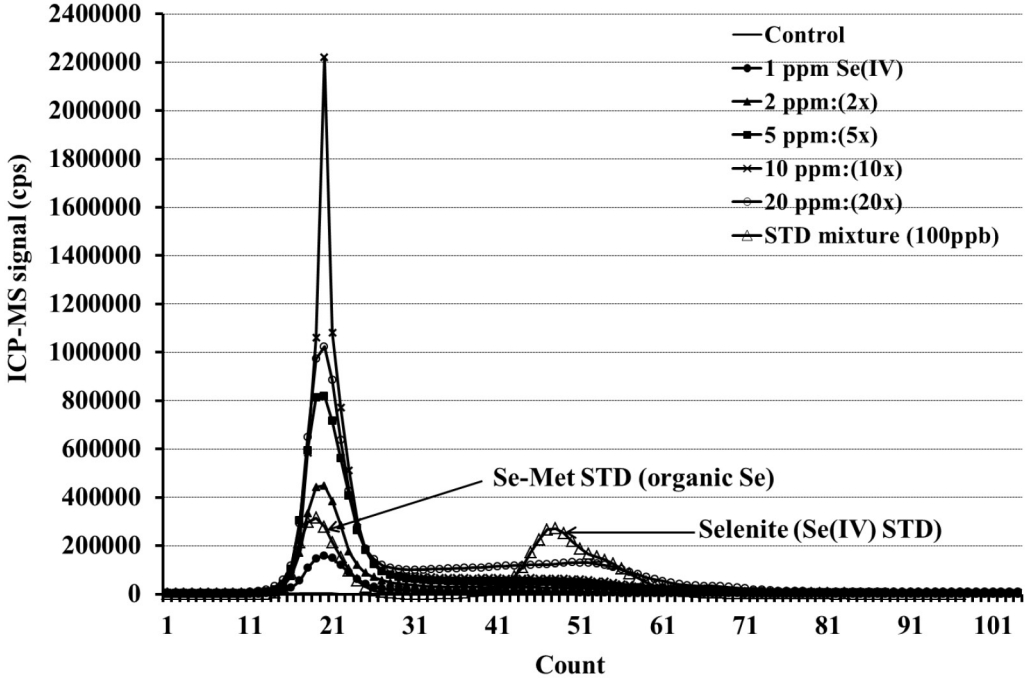

Fig. 8: Conversion of different concentrations of sodium selenite [Se(IV)] to organic form in cell fraction of *L. acidophilus* incubated in MRS media at 37°C for 24 hrs.

in the cultured media was converted to an organic form during the incubation period (24hr). However, The medium supplemented with 1 ppm almost depleted from the inorganic Se (IV). On the other side, the bacterial cell fractions had higher concentration of organic Se (Fig. 8) compared to its corresponding cultured medium supernatant (Fig. 7). Except for the medium supplemented with 20 ppm Se (IV), the selenium was almost detected inside the bacterial cell in an organic form.

The bacterial cell fraction of the medium fortified with 1 ppm Se (IV) contained no inorganic selenium which indicate a complete conversation of Se (IV) to organic form at that concentration or less. In

this respect, Prokisch *et al*., (2008) stated that whey media cultivated with yoghurt culture (*S. thermophilus* and *L. bulgaricus*) and different concentrations of Se(IV) markedly increased the organic form of selenium in the supernatant. The bacterial culture consumed the inorganic Se and completely converted it to an organic form at concentration of 1 ppm in the cultured media.

On contrast, *L. acidophilus* had weak ability to convert Se(VI) to an organic form compared to Se(IV). The data illustrated in Fig. (9) and Fig. (10) obviously show the remaining of high residual content of Se(VI) in the cultured medium supernatant (Fig. 9), however, all the Se detected in the cell fraction was in the organic form (Fig. 10). In conclusion, The obtained results indicate that *L.*

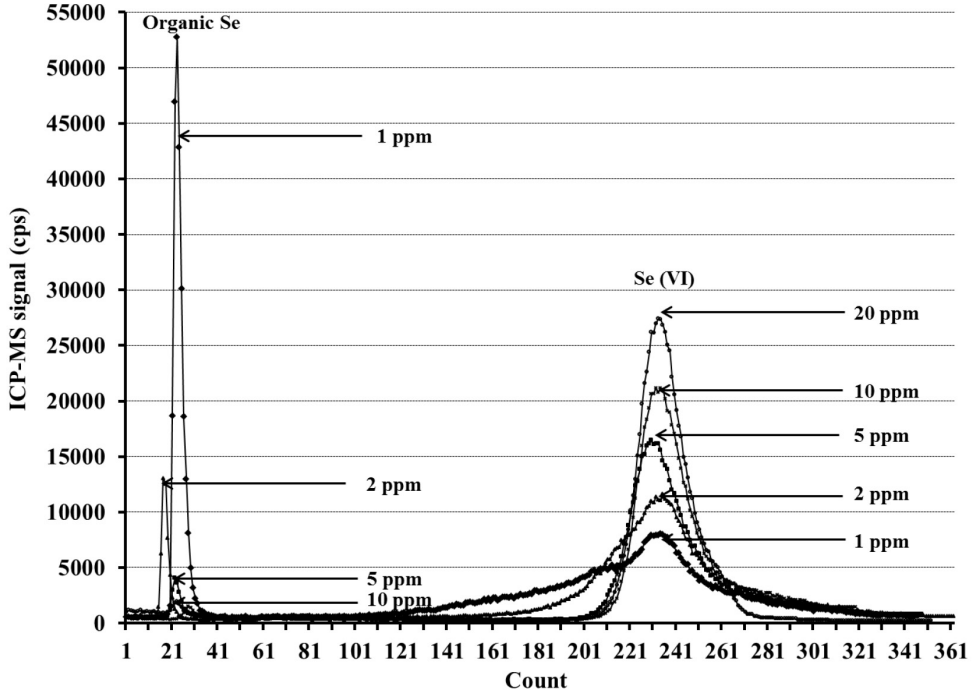

Fig. 9: Conversion of different concentrations of sodium selenate [Se(VI)] to organic form in MRS media supernatant incubated with *L. acidophilus* at 37°C for 24 hrs.

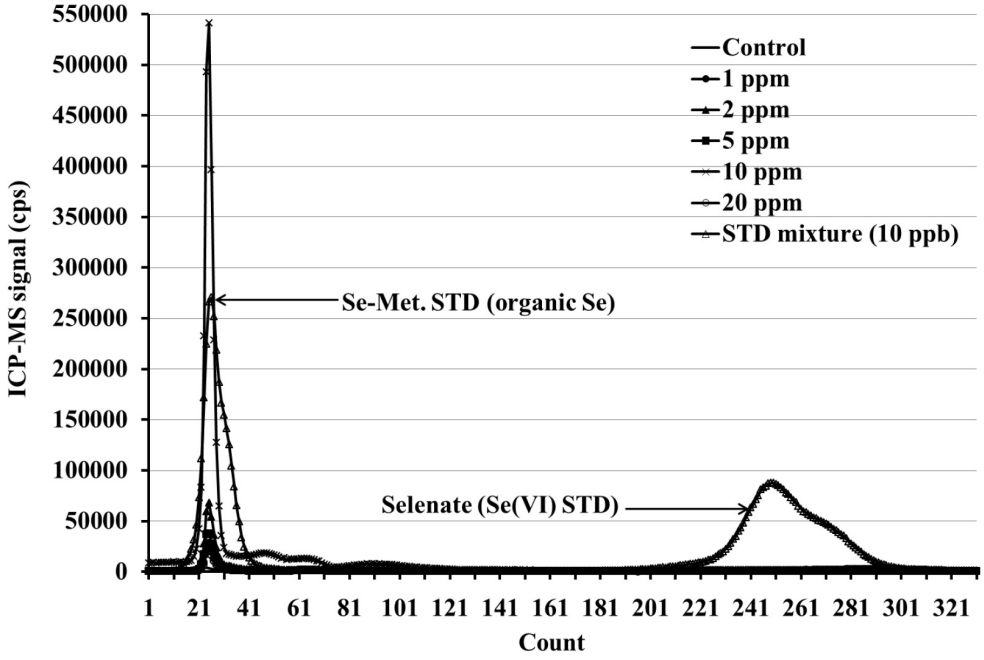

Fig. 10: Conversion of different concentrations of sodium selenate [Se(VI)] to organic form in cell fraction of *L. acidophilus* incubated in MRS media at 37°C for 24 hrs.

acidophilus could be used as Se-enriched probiotic using Se(IV) or Se(VI) up to 20 ppm with superior to Se(IV), or as a starter culture for producing Se rich fermented dairy foods using Se(IV) at a limit concentration of 1 ppm. Also, in higher SeIV concentration *L. acidophilus* produces SeNPs which may be applied in different applications, however still need more adequate research in this regard.

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تحويل السيلنيوم غير العضوى إلى عضوى باستخدام مزارع *Lactobacillus acidophilus*

محسن عبد العزيز زماره[١]، جوزيف بروكش[٢]

[١]قسم علوم الألبان - كلية الزراعة - جامعة كفر الشيخ – كفر الشيخ رقم بريدى ٣٣٥١٦ – ج م ع.

[٢]معمل النانوفود – كلية الزراعة وعلوم الأغذية وإدارة البيئة- جامعة دبرتسن رقم بريدى ٤٠٣٢- دبرتسن - المجر

لقد تم دراسة التحويل البيولوجى للسيلنيوم فى نوعين من أملاح السيلنيوم غير العضوى هما صوديوم سيلنيت وصوديوم سالينات إلى الصورة العضوية بواسطة بكتريا *Lactobacillus acidophilus*. لهذا الغرض تم مقارنة إضافة السيلنيوم بتركيزات ١، ٢، ٥، ١٠، ٢٠ جزء فى المليون لبيئة MRS والتحضين على درجة ٣٧°م لمدة ٢٤ ساعة. كما أوضحت النتائج أن كلا النوعين من السيلنيوم (سيلنيت أو سالينات) لم يكن لهما تأثير ملحوظ على نمو البكتريا المستخدمة مما يؤكد عدم وجود سمية لكلا النوعين من السيلنيوم عند التركيزات المستخدمة. كذلك لوحظ تلون البيئة المضاف لها السيلنيت بتركيز٥، ١٠، ٢٠ جزء فى المليون – وليس السالينات– بلون أحمر فى نهاية مدة التحضين مع زيادة تركيز اللون بزيادة تركيز السيلنيوم فى البيئة. أوضحت صور الميكروسكوب الإلكترونى الماسح لعينات من البيئة الحمراء وجود جزيئات (حبيبات) من النانوسيلنيوم فى البيئة. وأظهرت نتائج تحليل عينات من راشح بيئة الزرع بعد الطرد المركزى وكذلك الراسب المحتوى على الخلايا البكتيرية بواسطة جهاز HPLC–ICP–MS أن معدلات تحويل السيلنيوم غير العضوى للصورة العضوية كان كبيراً بشكل ملحوظ باستخدام السيلنيت عن السالينات على الرغم من إحتواء الخلايا البكتيرية على التركيز الأعلى من السيلنيوم العضوى. لقد تم التحويل الكامل للسيلنيوم غير العضوى إلى صورة عضوية باستخدام تركيز ١ جزء فى المليون من ملح السيلنيت.

النتائج المتحصل عليها توضح إمكانية إنتاج منتجات لبنية متخمرة بواسطة بكتريا *Lactobacillus acidophilus* عند تدعيم اللبن الخام بتركيز ١ جزء فى المليون من السيلنيوم على هيئة صوديوم سيلنيت.