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Enrichment of lactic acid silage bacteria with Selenium by growing cultures in modified MRS broth supplemented with sodium selenite

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Introduction

Selenium (Se) is a trace element essential for normal cellular function, which has been linked with reduced risk of cancer, cardiovascular disease, cognitive decline and thyroid disease in humans. Se deficiency in livestock is associated with white muscle disease, retained placenta, ill-thrift and mastitis. Where Se status or bioavailability from the soil for plants is poor, livestock rely on supplemental Se in their diets either as sodium selenite (inorganic form) or seleno-yeast (organic form). As lactic acid bacteria have been shown to incorporate Se as either organic or elemental (Nano-Se), (Eszenyi *et al.*, 2011) there may be potential to use silage inoculant bacteria to improve the Se status of feed to provide Se requirements to livestock. The aim of this experiment was to examine the growth, uptake of selenium as organic (selenocysteine and selenomethionine), inorganic (selenite and selenate) and Nano-Se by lactic acid bacterial isolates (LAB), which could then be used in future ensiling studies (Lee *et al.*, 2015) to produce high Se silage.

Materials and Methods

Each strain of Lactic acid bacteria from University of Bristol and Silage Solutions Ltd collections were grown in triplicate in 400 mL of either: MRS broth (Control, CMRS) or Selenium enriched MRS broth (SeMRS) (315 mg Na_2SeO_3 added to 400 mL MRS)

Cultures were inoculated with 0.1% v/v of overnight grown culture and incubated at 30^{o}C for 20 h. Each culture was grown in triplicate in both media (CMRS and SeMRS). At the end of the incubation period, each culture was subsampled to measure optical density (0.5 mL), pH (10 mL) and enumerate, a 1 mL aliquot of the culture was aseptically transferred to a sterile petri dish to which molten MRS agar was poured. The remaining sample was centrifuged at 12,000 rpm (2,800 *g*) for 20 minutes, the supernatant removed and the bacteria washed with saline and centrifuged for a further 20 minutes. Another saline wash performed followed by final wash in water to obtain a pure bacteria pellet. The weight of bacterial pellet was recorded and freeze-dried. An aliquot of LAB pellet (0.1 g) was accurately weighed into a 40 mL clean glass digestion vial followed by the addition of 3 mL concentrated nitric acid. The digestion vials were then heated at 120 °C for 1 h on a hot block before 1 ml H_2O_2 was added. The sample digests were then heated for an additional 1 h. An aliquot of the sample digest (0.2 mL) was transferred into a clean auto-sampler vessel and was diluted to the mark with deionised water. The solution was well mixed prior to the analysis by UV-HG-AFS for total Se (Nano, organic, inorganic). A one-step enzymatic extraction with protease XIV was applied for Se speciation. An aliquot of LAB sample (0.1 g) was accurately weighed into a 15 mL clean polypropylene centrifuge tube followed by the addition of 20 mg enzyme and 8 mL phosphate buffer (60 mM, pH 7.4). The samples were then capped tightly and put on an automatic shaker for 24 h at room temperature. After proteolysis, the samples were centrifuged for 20 min. The supernatants were filtered by 0.45 µm PTFE syringe filter and transferred into a clean vial. These solutions were then further diluted by deionised water and analysed by HPLC-UV-HG-AFS.

Results and Discussion

Growth of the bacteria in control MRS broth (CMRS) and MRS broth containing sodium selenite (SeMRS) are reported in Table 1. CMRS growth ranged from 9.6 – 10.9 Log^{10} CFU/mL whereas for SeMRS the range was 6.6 – 9.6 Log^{10} CFU/mL, with growth of all bacterium reduced as a response to the addition of sodium selenite. There was a wide range of ability to incorporate Se by the bacterium both between and within isolates, with the vast majority being in the form of Nano Se as previously reported (Eszenyi *et al.*, 2011). Total Se (Nano, Inorganic and Organic) ranged from 33.0 – 381 mgSe/g DM, with inorganic and organic ranging from 19.9 – 701 and 14.5 - 189 ugSe/mg DM, respectively. The organic form of Se was 97% Selenocysteine and 3% Selenomethionine.

Table 1. Growth and Se uptake of lactic acid bacteria

Bacteria	**CFU Log^{10}/mL (CMRS)**	**CFU Log^{10}/mL (SeMRS)**	**Total Se (mg/g DM)**	**Total Inorganic Se (ug/g DM)**	**Total Organic Se (ug/g DM)**
L. plantarum LF1	10.2 (±0.03)	9.3 (±0.02)	97.9 (±5.67)	132 (±21.1)	91.4 (±15.2)
*L. plantarum*L 54	9.7 (±0.01)	9.6 (±0.03)	211 (±37.2)	203 (±13.9)	72.9 (±9.81)
L. plantarum MTD1	9.7 (±0.02)	8.5 (±0.03)	73.8 (±14.64)	37.3 (±0.41)	29.7 (±1.55)
P. acidilactici CNCM I-3237	9.8 (±0.02)	8.4 (±0.01)	33.0 (±6.95)	19.9 (±1.49)	14.5 (±1.79)
L. salivarius CNCM I-3238	9.6 (±0.03)	7.6 (±0.02)	381 (±139.5)	460 (±84.9)	122 (±33.2)
L. brevis LB1 L1529	10.1 (±0.02)	7.7 (±0.03)	238 (±76.0)	619 (±22.2)	44.9 (±11.16)
L. planatrum Lp3 A0905	10.0 (±0.04)	7.7 (±0.01)	264 (±174.3)	520 (±104.1)	142 (±81.1)
L. planatrum MA16/4U	10.2 (±0.02)	9.2(±0.03)	292 (±78.7)	63.8 (±4.73)	36.6 (±3.15)
P.acidilactici MA18/5M	10.9 (±0.03)	9.1 (±0.02)	267 (±106.6)	95.5 (±1.27)	66.0 (±8.49)
P. pentocaseous NCIMB 12455	10.1 (±0.01)	8.7 (±0.03)	263 (±6.4)	547 (±78.7)	104 (±14.6)
L. plantarum DSMZ 16627	9.9 (±0.05)	7.4 (±0.02)	131 (±48.7)	450 (±35.7)	115 (±5.2)
L. paracasei NCIMB 30151	9.9 (±0.02)	7.4 (±0.01)	107 (±18.5)	433 (±15.1)	123 (±4.0)
P. acidilactici NCIMB 30005	9.7 (±0.02)	7.8 (±0.02)	319 (±30.6)	361 (±19.8)	45.7 (±7.07)
P. pentocaseous NCIMB 30044	9.6 (±0.04)	6.6 (±0.05)	347 (±129.6)	378 (±173.2)	189 (±84.1)
L. brevis DSMZ 16680	10.6 (±0.03)	8.2 (±0.02)	210 (±22.3)	613 (±67.2)	148 (±14.5)
L. fermentum NCIMB 30169	10.5(±0.03)	8.4 (±0.03)	174 (±281.2)	102 (±20.1)	49.3 (±20.1)
L. plantarum	10.5 (±0.01)	8.1 (±0.01)	212 (±54.8)	701 (±38.2)	149 (±3.3)
L. casei JB008	10.3 (±0.02)	8.3 (±0.03)	180 (±33.7)	33.3 (±15.4)	30.9 (±15.8)
L. acidophilus ASF 360	10.5 (±0.03)	8.7 (±0.02)	302 (±32.4)	52.9 (±6.51)	25.9 (±2.03)

Conclusion

Sodium selenite addition into the growth medium of lactic acid bacteria (LAB) reduced growth rates but also resulted in the conversion of the inorganic sodium selenite into predominately Nano (elemental) Se and organic Selenocysteine. Based on a rank analysis of growth in SeMRS and ability to take up (total Se content) and convert inorganic Se (Nano and organic Se content), three bacterium were selected for further investigation for their ability to act as silage inoculants in the presence of sodium selenite to provide a more bioavailable form of Se in silage (Lee *et al*., 2015). The three bacterium selected were: *L. Brevis* DSMZ, *L. plantarum* LF1, and *L. plantarum.*

References

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