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Screening for contamination with genetically modified organisms in food, feed and their additives obtained in retail and online shops in Latvia

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Abstract

The aim of the study was to screen for contamination of food and feed products with genetically modified organisms (GMO) in several sample groups obtained from retail and online shops. The total number of samples was 171, including 67 of food, 26 feed, 27 additive, and 51 samples of dried pollen for human consumption. Detection and quantification of GMO in food and feed was performed according to the approved quantitative real-time PCR methods. Fermentation product samples were analyzed using screening for antimicrobial resistance genes, 16S RNA and ITS1 sequencing, isolation of bacterial cultures and whole genome sequencing. In total, GMO contamination was found in 12 samples (in 10.45% of food and in 19.23% of feed samples). A lower proportion of GMO containing samples were from the European Union (10.26 %) vs. samples from outside (17.02%), but none with ingredients of both origins. Non-compliant were three food and five feed samples. Of the 27 samples of food and feed additives and food supplements, 10 samples were possibly non-compliant, as they contained live bacteria with antimicrobial resistance genes, and seven of them contained possibly recombinant DNA. The study indicates presence of GMO contamination that emphasizes the need for further monitoring.

Key words: antimicrobial resistance genes, enzymes, fermentation products, genetically modified microorganism, genetically modified organism, live bacteria, vitamins.
Abbreviations: AMR, antimicrobial resistance; CGE, Center for Genomic Epidemiolgy; EU, European Union; GM, genetically modified; GMM, genetically modified microorganism; GMO, genetically modified organism; MALDI-TOF, matrix-assisted laser desorption ionization time of flight mass spectrometry; Patric, Pathosystems Resource Integration Center; qPCR, real time PCR; RASFF, Rapid Alert System for Food and Feed; WGS, whole genome sequencing.

Introduction

In the Rapid Alert System for Food and Feed (RASFF), there have been many notifications about genetically modified food or feed every year starting from 1998. Several authors have summarized these data for various time periods. There have been a total of 573 notifications about genetically modified food or feed within the time period till 2017 (Pigłowski 2019), 679 till 2018 (Rostoks et al. 2019), and 708 till 2020 (Pigłowski, Niewczas-Dobrowolska 2023). Within the time period of 2008 to 2014, the largest numbers of cases of border rejection by the European Union (EU) due to unauthorized genetically modified (GM) or novel food were from China (88 cases), the United States (46), Hong Kong (18), Thailand (12), and India (4) (Cuello et al. 2020). The novel food category is not separated from the GM containing products in the RASFF system. They represent a broad group of products that have not been consumed to a significant degree by humans in the EU before the 15 May 1997 (https://food.ec.europa.eu/safety/novel-food_en). On a global scale, the top 10 countries with the highest numbers of incidents recorded on the GM Contamination Register within the time period 1997 to 2013 were Germany, USA, France, Canada, The Netherlands, Australia, Austria, Italy, Sweden, and Japan (Price, Cotter 2014). GM crops are mostly grown outside the EU (Kleter et al. 2018; Turnbull et al. 2021). Examples of monitoring studies in some European and non-European countries are given in Table 1.

Possible transgenic contaminants can be plants, animals and microorganisms. In terms of the possibility to be detected as contaminated with GMO, the top crops are rice, maize, oilseed rape/canola, soybean, flax and cotton (Price, Cotter 2014; Bohanec et al. 2017). The AquAdvantage® Atlantic salmon (*Salmo salar*) developed by AquaBounty has not as yet been detected as an unauthorized GMO anywhere, but several laboratories have already developed appropriate detection methods for this GM salmon (Debode et al. 2018). In recent years the incidence of detection of GM microorganisms (GMMs) carrying antimicrobial resistance (AMR) genes in food and feed enzymes, additives and flavourings has increased (Fraiture et al. 2020). Several groups of scientists in Europe have found that food and feed additives (vitamins, enzymes, etc.) contain antibiotic resistance and other marker genes or even viable bacteria that are producers of these additives (Paracchini et al. 2017; Deckers et al. 2022). A similar group of products are food supplements (Sanchez et al. 2018).

A study carried out recently has gathered information on the probability that food and feed samples that are non-compliant with GMOs can be detected in official controls (Areal, Riesgo 2021). These are samples that contain authorized GMO events above the 0.9% threshold for food and feed, but are not labelled, or they contain EU unauthorized/withdrawn GMO events above the 0.1% threshold. The study covered official reports from individual EU Member States for the period 2000 to 2013. It was found that the probability of detecting non-compliant samples varies from country to country and year to year. In many countries, the probability of this non-detection decreased for food samples in the last years of the study, while in others (the Netherlands, Poland, Portugal, Romania, Slovenia and Sweden) the opposite trend was observed. The authors of the study used a term interception measure that was defined as the number of samples within a year where non-compliance was present. The probability of intercepting non-compliant imported foodstuffs above the 5 and 10% probability thresholds, established by the study authors, in Sweden in 2010 was 88.20 and 54.70%, respectively, but in many countries, this was mostly 0% during the period covered by the study, except in particular years. In all countries included in the study, a higher probability of detection was found for feed samples compared to food samples. The authors of the study conclude that the regulatory framework for food does not guarantee consumers' right to free choice of food and that consumers are likely to be misled as to whether food and feed contains GMOs. By increasing the number of inspections and analyzed samples, the probability of detecting non-compliant food and feed samples increases (Areal, Riesgo 2021).

The spectrum of genetically modified microorganisms (GMMs) used in the production of food additives is quite wide and includes bacteria, fungi and microalgae. Producers with a high titre of the produced product of 100 g L^{-1} and above are considered to be at the level III development stage, which means that such producers have available production methods that ensure economic profitability, and also available methods for purifying the produced product. Examples here include L-glutamate, riboflavin, L-ascorbic acid, succinic acid and lactic acid. The Level II development stage includes those microorganisms whose titre of the produced product is 10 to 100 g L^{-1}. They are close to economic viability and could soon be used in industry. Adipic acid and single-ring aromatic compounds belong to this group. The Level I stage of development includes producers whose product has a concentration measured in milligrams per litre (Kallscheuer 2018). Over

Table 1. Examples of monitoring studies in some European and non-European countries about GMO contamination in food, feed and food enzymes

Country	Year	Sample type	GMO presence	Reference
Hungary	2008	251 samples of soy-containing food, lectin gene confirmed in 208 samples	In 38% of samples presence of GTS40-3-2, 6% of those GTS40-3-2 > 0.9% m/m	Ujhelyi et al. 2008
Serbia	2009 – 2010	100 non-GMO labelled food samples from EU, USA, Argentina, Brazil, Thailand containing maize, soya, and/or rice	8 samples (8/32 (25 %) from soy-containing products) contained GTS40-3-2 < 0.9% m/m	Zdjelar et al. 2013
Czechia	2008-2013	1152 food samples containing soya, maize and rice	107 (9.3%) contained GMOs. GM maize 63 (21.9 %), GM rice 39 (13.5 %), GTS40-3-2 4 (1.4%)	Kyrova et al. 2018
Turkey	2015	75 soya containing food (*n* = 56) and feed (*n* = 19) samples with Turkish origin	20 samples in total (27%) contained GM soya (GTS40-3-2, MON89788, A2704-12) – 5 food and 15 feed samples	Turkec et al. 2016a
Turkey	2015	83 maize containing food and feed samples with Turkish origin	6% of food samples and 18.2% of feed samples tested positive	Turkec et al. 2016b
South Korea	2016	30 sausage samples containing soy	11 (36.67%) contained GTS40-3-2, A2704-12, and/or MON89788	Kim et al. 2016
Belgian and EU market	2022	51 samples of food enzymes	Presence of genetically modified microorganisms was proven in 56.5% of the samples	Deckers et al. 2022

300 food enzyme applications have been submitted to the European Commission for evaluation, where GMMs are in 87% of applications, but also animals or plants (13%) are indicated as producers. About half (50.5%) of all food enzyme-producing bacteria are genetically modified. The ten most frequently produced enzymes are α-amylase, triacylglycerol lipase, xylanase, β-galactosidase (lactase), glucoamylase, protease, endo-1,3(4)-β-glucanase, cellulase, cyclomaltodextrin glucanotransferase and polygalacturonase (Deckers et al. 2020a). The most often used bacteria as producers are from the genus *Bacillus*: *Bacillus subtilis, Bacillus licheniformis* and other species (Barbau-Piednoir et al. 2015a; Paracchini et al. 2017; Deckers et al. 2020a; Fraiture et al. 2021).

Amino acids such as L-arginine and L-lysine are often used in nutritional supplements used by athletes and other consumers. There are many publications in the scientific literature about the creation of genetically modified bacteria as producers for the production of these amino acids. L-arginine can be produced by *Corynebacterium glutamicum* (Park et al., 2014), *Escherichia coli* (Ginésy et al. 2017), *Bacillus* spp., *Serratia* spp. (Utagawa, 2004) etc. L-lysine can be produced by *C. glutamicum* (Vassilev et al. 2018), *E. coli* (Ma et al. 2015), etc. The same bacterial species are also used in L-leucine biosynthesis thanks to genetic modifications (Wang et al. 2019; Wang et al. 2020). *E. coli* and *Proteus* spp. are used for L-carnitine biosynthesis (Bernal et al. 2007).

The proposed detection strategy for GMMs includes screening for AMR genes (Fraiture et al. 2020a; Fraiture et al., 2020b; Fraiture et al. 2020c), application of 16S rRNA sequencing (Deckers et al. 2020), isolation of bacterial cultures and whole genome sequencing (Fraiture et al. 2020d).

In the present study, screening for GMO contamination in food, feed, their additives and food supplements obtained in retail and online shops in Latvia was performed. The objective of the study was to screen such products for the presence of genetically modified maize, soy, oilseed rape, other GM plants, salmon, and viable bacteria and its recombinant DNA. For possible genetically modified bacteria the focus was on AMR genes and other indications that these bacteria have been genetically modified.

Materials and methods

Samples

The total number of samples was 171, which included four sample sub-groups: 67 food samples (Table 2), 26 feed samples (Table 3), 27 food additives, food supplements, feed enzyme mixtures and animal feed additives (Table 4), and 51 samples of dried pollen for human consumption.

In the food and feed samples one sample was received from the Food and Veterinary Service Border Control

Table 2. List of food samples, their country of origin and GMO target organism. ID, sample identification

ID	Product	Country of origin	GMO target organism	Size (g)
1-1	Tofu pate with oyster mushrooms	Czechia	soy, canola	100
1-2	Tofu pate	Czechia	soy, canola	100
1-3	Fried tofu	Czechia	soy	210
1-4	Rice paper, gluten free	Cambodia	rice	110
1-5	Japanese-style tofu fillets	Germany	soy	160
1-6	Stewed tofu, canned	Great Britain	soy	225
1-7	Noodles, steamed, vacuum	South Korea	soy	200
1-8	Silken tofu	USA	soy	349
1-9	Breadcrumbs	China	wheat	120
1-10	Wheat noodles	China	wheat	1000
1-11	Gluten-free flour	Czechia	maize, rice	1000
1-12	Rice paper	Thailand	rice	454
1-13	Soybeans, whole	India	soy	2000
1-14	Wheat noodles	China	wheat	250
1-15	Dough mixture	South Korea	wheat, maize, soy, rice	1000
1-16	Light soybean paste	Japan	soy, rice	500
1-17	Soy dessert	Belgium	soy	125
1-18	Canned corn	Hungary	maize	150
1-19	Cornflakes	Poland	maize	500
1-20	Zucchini caviar	Ukraine	zucchini, tomatoes	500
1-21	tomato paste	Ukraine	tomatoes	70
1-23	Red caviar	USA	salmon	100

Continued

Table 2. *Continued*

ID	Product	Country of origin	GMO target organism	Size (g)
1-25	Ketchup	Ukraine	tomatoes, maize	450
1-26	Caramels	Ukraine	sugar beet, soy	250
1-27	Canned salmon	Norway	salmon	240
2-2	Soy milk, dry	Czechia	soy, maize	350
2-3	Maize grits	Poland	maize	1000
2-4	Soy noodles	Czechia	soy	200
2-5	Soybeans	Great Britain	soy	500
2-6	Soy and wheat chunks	India	soy	800
2-7	Quick soup	Japan	soy	92.5
2-8	Cheese soy silken tofu	USA	soy	349
2-9	Soy sauce	China	soy	150
2-10	Soybeans	Canada	soy	500
2-11	Soy sauce with reduced salt	Thailand	soy	250
2-12	Lemon-soy sauce	Philippines	soy	350
2-13	Soy sauce with mushroom flavor	Thailand	soy	300
2-20	Naturally fermented soy sauce	The Netherlands (for soybeans: USA, Canada, Brazil)	soy	1000
2-21	Seasoned soybean paste	South Korea	soy	500
2-25	Fish paste with salmon and trout	Belarus	salmon, soy, maize	140
2-26	White fish paste with salmon	Belarus	salmon	140
2-27	Moiva caviar cream with smoked salmon	Belarus	salmon	180
2-28	Salted Atlantic salmon fillet, with skin	Lithuania (salmon farmed in Norway)	salmon	150
2-29	Salmon pate	Sweden (salmon farmed in Norway)	salmon	145
2-30	Atlantic salmon fillet, cold smoked	Lithuania (salmon farmed in Norway)	salmon	100
2-31	Salmon fillets in spicy cream	Latvia (salmon farmed in Norway)	salmon, canola, maize	170
2-32	Salmon fillet in its own juice	Latvia (salmon farmed in Norway and in Scotland)	salmon	170
2-33	Root vegetable and salmon puree for children from 6 months	France	salmon, canola, maize	120
2-34	Salmon with greens for children from 12 months	Spain	salmon, canola, maize	235
2-35	Vegetable puree with salmon	Latvia	salmon	190
2-38	Green olives with salmon paste	Spain	salmon	300
2-39	Grilled corn flavored chips	South Korea	maize	117
2-40	Flavored cheese chips	USA	maize	482
2-41	Breakfast cereal, peanut butter puffs and crispy corn puffs	USA	maize	326
2-42	Chips with blue cheese flavor	USA	maize	198.5
2-43	Corn boiled 2nd class	India	maize	450
2-44	Dried papaya	Thailand	papaya	200
2-45	Cheese flavored popcorn	Lithuania (country of origin of maize grains: EU and non-EU)	maize, canola	150
2-46	Cheese flavored corn chips	Turkey	maize	100
2-47	Hamburger-flavored snacks	Poland	maize, canola	145
2-48	Wafer candies in dark chocolate with stevia	Russia	soy	150
2-49	Gluten-free sandwiches	Spain	maize	150
2-51	Pizza flavored corn sticks	Rumania	maize	180
2-52	Biscuits with milk chocolate flavor	Russia	soy, maize	175
2-53	Cake with cream flavored filling	Turkey	soy, maize	225
2-54	Cookies	Moldova	soy, maize	500
2-55	Waffles with plum ice cream flavor	Ukraine	soy, maize	300

Table 3. List of feed samples, their country of origin and GMO target organism. ID, sample identification

ID	Product	Country of origin	GMO target organism	Size (g)
1-22	Canned cat food	Hungary	salmon	100
1-24	Canned cat food	Spain	salmon, vegetables	340
1-28	Dog biscuits	Hungary	vegetables	200
1-29	Choline chloride, 60% corn	China	maize	4000
2-14	Delicacy with puffed rice and wheat, corn, oats and honey for rodents	Belgium	maize, rice	110
2-15	Granulated corn bait	Lithuania	maize	2000
2-16	Corn soy mixture (granulated) - fish feed	Lithuania	maize, soy, canola	2000
2-22	Fat balls, food for wild birds in winter	Poland	sunflowers, oil plants	3000
2-36	Canned food for cats, salmon, chicken pieces in sauce	France	salmon	85
2-37	Canned salmon for sterilized cats	Czechia	salmon	85
2-50	Dog food with chicken	Ukraine	maize	500
EZ-1	Fighting fish feed	Japan	soy, maize, rice	5
EZ-2	A treat for rodents - a grain stick with apples	Poland	maize	65
EZ-3	A treat for rodents - a grain stick with herbs	Poland	maize	65
EZ-4	Complete food for adult small breed dogs	France	soy, maize	50
EZ-5	Supplementary feed for rodents	Germany	canola	30
EZ-6	Complete food for dogs	France	soy, maize, rice	50
EZ-7	Dry cat food	Germany	maize, rice	650
EZ-8	Dry cat food	France	soy, maize, rice	400
EZ-9	Canned cat food	Switzerland	maize	100
EZ-10	Dry cat food	Czechia	maize, rice, sugar beet	300
EZ-11	Dry cat food	Slovenia	maize, canola	60
EZ-12	Dog treat	Belgium	canola	25
EZ-13	Dry dog food	Belgium	canola	50
EZ-14	Dry cat food	Great Britain	maize, canola	65
EZ-15	Feed for guinea pigs	Czechia	maize	300

Department in 2020. The rest of the samples were purchased in various markets and stores in Latvia or by ordering from companies' websites during the period 2020 – 2023. Four samples of those were purchased in stores of third countries goods in Riga. Within the food and feed samples the origin of 39 samples was the EU, the origin of 47 samples was non-EU countries, and for seven samples there was information on the label that they consist of ingredients with both origins (EU and non-EU).

Additionally, to the food sample group, 51 samples of dried pollen for human consumption were analyzed as well. The origin of 49 pollen samples was Latvia, but one sample was from Lithuania and one sample was from Germany.

Selection criteria for food and feed samples were the following: country of origin outside the European Union (primarily, but not an exclusive criterion), and contains soy, maize, canola, rice, wheat, tomatoes, sugar beet (sugar), or Atlantic salmon. There were a total of 18 products containing Atlantic salmon (*Salmo salar*). Some of the products had the origin of the salmon indicated on the label: North-East Atlantic, FAO 27, Norwegian Sea ($n = 1$); North-East Atlantic, FAO 27, aquaculture product ($n = 1$); or bred in Norway ($n = 5$). For the remaining samples, the origin of the salmon was not specified ($n = 11$).

Food and feed sample processing, GMO screening, detection and quantification

Different mills and blenders were used for sample homogenization, depending on the specifics of the sample (Grindomix GM 200 (Retsch), Laboratory blender (Waring), Profi I Cook), as well as a mortar and pestle. Samples with a liquid or powder consistency were not homogenized. Soy sauces were centrifuged (twice for 30 min,13000 × *g*) before DNA extraction to obtain a pellet, from which DNA was then isolated. DNA was primarily isolated from 200 mg of homogenized or original sample using a commercial reagent kit NucleoSpin Food, Mini kit (Macherey-Nagel). DNA was extracted from each sample in duplicate. DNA concentration and absorbance were determined with a NanoDrop ND-1000 at 230, 260, and 280 nm. The next DNA quality control step involved amplification of a plant chloroplast gene *trnL* (ISO 21569: 2005), which indicates whether the sample contains amplifiable plant DNA, and/or amplification of a plant taxon-specific gene: *hmg* (maize), *cruA* (canola), *lec* (soy), *pld* (rice), or *gs* (sugar beet) (JRC Compendium of Reference Methods for GMO Analysis). The GMO screening step was done using six screening elements: cauliflower mosaic virus (CaMV) 35S promoter (ISO 21569:2005, B.9.), *tNOS* (ISO

Table 4. List of analysed food and feed additives and samples of food supplements, their country of origin, sample size and information about obtained data sets from each sample. ID, sample identification

ID	Sample description	Country of origin	Size (g)	Obtained data		
				16S	ITS1	WGS
1-29	Choline chloride with 60% corn	China	4000	yes	yes	yes
2-1	Herbal enzyme mix for dogs	USA	75	yes	no	no
2-17	L-arginine in capsules	United Kingdom	15	yes	no	yes
2-18	L-lysine in tablets	United Kingdom	15	yes	no	no
2-19	Vitamin B2 in tablets	United Kingdom	3	yes	no	no
2-23	Additional mineral feed for cows	Poland	1000	yes	yes	yes
2-24	Complementary feed for dairy cows	Poland	2000	yes	no	no
2-56	L-lysine, powder	United Kingdom	100	yes	no	no
2-57	L-lysine, powder	Poland	200	yes	no	no
2-58	L-arginine with orange flavor	Poland	210	no	no	no
2-59	L-carnitine with chromium in capsules	Hungary	52	yes	no	no
2-60	L-leucine, L-isoleucine, L-valine, vitamin B6 in capsules	Hungary	380	yes	no	yes
2-61	Sorbitol	Spain	750	no	no	no
2-62	Citric acid	China	220	yes	no	no
1.1	Citric acid	China	100	no	no	no
1.2	Citric acid	China	220	no	no	no
1.3	Potassium sorbate	China	100	no	no	yes
1.4	Food coloring red (E120/E160a)	Not indicated	500	no	no	no
2.1	*Chlorella* powder	Latvia	100	no	no	yes
2.2	Supplementary mineral feed for horses	Poland	2000	no	no	yes
2.3	Supplementary feed for dogs	Poland	120	no	no	yes
2.4	Supplement for rabbits	United Kingdom	24	no	no	yes
2.5	Fish bone and fish oil powder for dogs and cats	Not indicated	58	no	no	yes
3.1	Bio Ca, K, Mg, Zn with vitamin D3	Israel	131	no	no	no
3.2	Vitamins B6, B12 with Zn	Israel	67	no	no	no
3.3	Calcium with vitamin D3	Germany	66	no	no	no
3.4	Food supplement – pancreatin enzyme	Latvia	12	no	no	yes

21569:2005, B.9.), *pat* gene, FMV 34S promoter, tE9, and cry1Ab/Ac (JRC Compendium of Reference Methods for GMO Analysis).

An additional screening gene was used for pollen samples, bar (ISO 21569:2005, B.8.). In addition to these screening genes, all pollen samples were tested for the presence of genetic material specific to the GM canola event DP-073496-4 (Jacchia et al. 2014), as there is no screening element for this event. All samples that were positive for the presence of any of the screened genetic elements were further examined. Detection of GM soy and maize events was done using event-specific pre-spotted plates produced by the Joint Research Centre.

Quantification of specific GMO events in food and feed samples was done according to the official methods approved in EU (https://gmo-crl.jrc.ec.europa.eu/gmomethods/). GMO detection in food and feed samples was conducted at the Institute of Food Safety, Animal Health and Environment "BIOR" is accredited according to the LVS EN ISO/IEC 17025:2017.

Samples containing salmon were tested for GM AquAdvantage™ salmon using a commercial reagent kit, GMIOdent RT IPC Accelerated Growth Salmon (Eurofins).

Processing and analysis of food and feed additives and food supplement samples

Homogenization and DNA extraction was done as described in the previous chapter. Since in several cases the manufacturer had indicated that the product in question was of plant origin, these samples were also amplified for *trnL* and plant taxon-specific genes. If plant material was found to be present, GMO screening was performed with the six screening genes mentioned above, and amplification to the *nptII* gene with conventional PCR was additionally performed using ISO 21569:2005, Annex B4 "Screening method for DNA detection of GM plants (*nptII* gene)".

Amplification of GMM marker genes nptII, 558, cat, tetL, aadD

Amplification of GMM markers *nptII, 558, cat, tetL* and *aadD* was performed on DNA extracts obtained from samples of food and animal feed additives and food supplements in two replications, as well as DNA extracts from isolated *Bacillus* spp. cultures in at least two replicates. The primer and probe sequences used are given in Table 5. Plasmid pENGL-00-EM-01/18-01 received from EURL GMFF was used as a positive control for the amplification of marker 558. The conventional PCR for *nptII* gene was carried out using the method described above. For the other markers, the reaction conditions were optimized until amplification was obtained for one of the samples. The first positive sample was used as positive control for subsequent reactions. Amplification of antibiotic resistance genes was performed first by real time PCR (qPCR) and then by conventional PCR. Amplification products obtained by conventional PCR were sequenced by Sanger method. Multiple sequence alignment was performed with an open access program T-Coffee https://www.ebi.ac.uk/Tools/msa/tcoffee/.

Bacterial isolates from food and feed additives and food supplement samples

Bacterial isolates from the samples were obtained using standard microbiology techniques. Isolation of viable cultures of *Bacillus subtilis* and *Bacillus licheniformis* were attempted from samples of food and feed additives and food supplements, as these bacteria are the dominant species among recombinant producers. Isolation of bacteria was performed using classical microbiological methods. Each sample was diluted 1:10 with Maximum recovery dilution solution and the appropriate amount of suspension was inoculated in microbiological diagnostic media: Blood agar, Tryptone soy agar and Nutrient broth, and incubated at 37 °C. From the prepared Maximum recovery dilution the next steps were as follows: (1) direct inoculation with a sterile 10 µL loop on the surface of each agar in Petri plates, incubation temperature 37 °C, incubation time from 24 to 72 h; (2) enrichment of bacteria in the Nutrient broth (1 mL of suspension from a 1:10 dilution was inoculated in 9 mL of the Nutrient broth), incubating 24 h at 37 °C and performing further transfer on Blood agar and Tryptone soy agar (incubation at 37 °C, 24 h). *Bacillus* spp. colonies were identified using matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF, Bruker, Germany). Colony morphology was identified as described in the handbook (Markey et al. 2013). The isolated bacterial cultures were preserved in the Institute of Food Safety, Animal Health and Environment "BIOR" Collection of Microorganism Cultures.

16S/ITS1 rRNA amplicon sequencing of bacterial isolates from food/feed additive and food supplement samples

The presence of bacterial DNA in samples of food and feed additives and food supplements was tested by amplifying

Table 5. Oligonucleotides and fluorescent probes used in the study

Gene	Sequence	Reference
tetL-F	ATA AAA CGA GCC ATA ATA AGT AAG GAA AAG	Fraiture et al. 2020c
tetL-R	TCA ATT AGG CAT CAA AAG GTT ACT CC	Fraiture et al. 2020c
tetL-F1	TTG CAA TAC CTG TTC CCT CTG	Fraiture et al. 2020c
tetL-R1	ACT GGG TGA ACA CAG CCT TT	Fraiture et al. 2020c
aadD-F1	GAA TAT TGG ATA AAT ATG GGG ATG A	Fraiture et al. 2020b
aadD-R1	TAT CCG TGT CGT TCT GTC CA	Fraiture et al. 2020b
aadD-F2	ATG GCT CTC TTG GTC GTC AG	Fraiture et al. 2020b
aadD-R2	CCT GAA TCC CAT TCC AGA AA	Fraiture et al. 2020b
aadD-F	ATC AGA TTG GCC GCT TAC AC	Fraiture et al. 2020b
aadD-R	ATA AGG GCA CAA ATC GCA TC	Fraiture et al. 2020b
cat-F1	TTT GAA CCA ACA AAC GAC TTT	Fraiture et al. 2020a
cat-R1	GGC CTA TCT GAC AAT TCC TGA	Fraiture et al. 2020a
cat-F2	CCA ACA AAC GAC TTT TAG TAT AAC C	Fraiture et al. 2020a
cat-R2	TCC TGC ATG ATA ACC ATC AC	Fraiture et al. 2020a
cat-F	GTG ACA AGG GTG ATA AAC TCA AAT AC	Fraiture et al. 2020a
cat-R	TGT ATA AAG TGG CTC TAA CTT ATC CC	Fraiture et al. 2020a
558-F	CgA gCT TTT gCg CgT ATA	Paracchini et al. 2017
558-R	gCC ATT CCA ATA CAA AAC CAC ATA	Paracchini et al. 2017
tetL-P	FAM-CCAACAAACCCAATTACCGACCCGAAACAA-TAMRA	Fraiture et al. 2020c
aadD-P	FAM-CGGTAGAAGCCCAAACGTTCCAC-TAMRA	Fraiture et al. 2020b
cat-P	FAM-ACC TAA CTC TCC GTC GCT ATT GTA ACC AGT-TAMRA	Fraiture et al. 2020a
558-Tm	FAM-Cgg ATC TAA CgC ATg CTC CgC A-BBQ	Paracchini et al. 2017

the V4-V5 region of the 16S rRNA gene, while the presence of fungal DNA was tested by amplifying the ITS1 region of the genome. To elucidate the taxonomic composition of bacteria and fungi in DNA obtained from food and feed additives and nutritional supplements, 16S rRNA and ITS1 PCR products were sequenced with a 600-cycle v3 reagent kit using a MiSeq sequencer (Illumina). Sequencing libraries were prepared following Illumina protocols. Reads were extracted from each sample and subjected to quality control, filtering and taxonomic classification in the QIIME2 data analysis environment (Bolyen et al. 2019). The SILVA 138 database was used for 16S classification (Quast et al. 2012). The database UNITE version 8.3 was used for the taxonomic classification of fungal sequences with the dynamic species hypothesis threshold (Nilsson et al. 2018). By using an artificial microorganism community (Zymo Research Catalog No. D6300) containing *B. subtilis* as a positive control, it was possible to verify that the 16S V4-V5 region sequencing method allows for the detection of the presence of this bacterial species.

Whole-genome sequencing of bacterial isolates

DNA extracts from bacterial isolates were subjected to whole-genome sequencing on an Illumina MiSeq sequencer using the 600-cycle v3 reagent kit. Sequencing libraries were prepared following Illumina protocols. Reads were obtained from each sample and subjected to quality control and de novo genome assembly using various programs such as SPAdes and SPAdes with Patric 3.6.12 [Pathosystems Resource Integration Center (at present, Bacterial and Viral Bioinformatics Resource Center, bv-brc.org)]. Genome annotation and Comprehensive Genome Analysis was done using Patric 3.6.12. Bacterial species identification was done by PubMLST.org and KmerFinder-3.2 of the Center for Genomic Epidemiolgy (CGE, http://www.genomicepidemiology.org). Searching for antibiotic resistance genes was done using ResFinder 4.1 (CGE), Card, and Patric 3.6.12. Search for plasmids and mobile genetic elements was done using PlasmidFinder-2.0 and MGEFinder v1.0.3. of CGE, and Patric 3.6.12. MyDbFinder 2.0. (CGE) was used to check the genomes of the bacterial isolates for plasmids from Fraiture et al. (2020) and Berbers et al. (2020) publications: M19465.1 (pUB110, pWB980, pP43NMK, pMA5), LT622641.1 (pGMBsub01, pGMBsub02), LT622642.1 (pGMBsub03), LT622643.1 (pGMBsub04, pMX45), AY357120 (pSM19035), M77789.2 (pUC19), AJ277764.1 (pHY300PLK), J01749.1 (pBR322, pBR325), NC_002013.1 (pC194, pHT01, pJH101, pBGSC6), LO8855.1 (pBR325), V01278.1 (pE1278.1), X06402.1 (pACYC177), and DQ264732.1 (pHT01, pP43NMK, pUC57, pUC19, pBGSC6).

Detection and identification of GMMs was done according to the methods published in recent studies (Paracchini et al. 2017; Berbers et al. 2020; Fraiture et al. 2020; etc.).

The results of the analysis of the whole-genome sequencing of bacterial isolates have to be considered as preliminary because no long reads and hybrid genome assembly were used. Sequences are available upon request from the corresponding author.

Results and discussion

GM plant presence in food and feed samples

None of the pollen samples showed amplification of any of the seven screening genes and canola event DP-073496-4. GM plant sequences were found in 12 food and feed samples. A higher proportion of GM plant-contaminated food and feed samples was found for samples from outside the EU ($n = 8$, 17.02%) compared to samples from the EU ($n = 4$, 10.26%), but none of the samples with both origins contained GM plant admixtures ($n = 0$). Comparing food samples with animal feed samples, it was detected that 10.45 % of food samples ($n = 7$) and 19.23% of animal feed samples ($n = 5$) had GM plant contamination. From these samples non-compliant to GMO regulations were three food and five feed samples (Table 6). The percentage of positive food samples is similar to the study in Czech Republic (Kyrova et al. 2018), but the percentage of positive feed samples was similar to the study in Turkey (Turkec et al. 2016b). Therefore, we can conclude that the situation with accidental GMO contamination in the Latvian market is similar to that in other EU and non-EU countries.

Presence of microorganisms and their DNA in food and feed additives and food supplement samples: analysis of antibiotic resistance genes

Several total sample DNA extracts showed amplification of AMR genes. Amplification of the *nptII* gene was detected in 11 samples, the *cat* gene was detected in two samples, the *tetL* gene was detected in four samples, and the *aadD* gene was detected in seven samples (Table 7).

In Table 8 the results of the amplification of AMR genes are given of DNA extracts from isolated bacterial cultures, where it can be seen that conventional PCR for the tetL gene showed amplification for several cultures that did not amplify by qPCR. Such a situation is also described by the method developers for several wild-type isolates from species such as *B. subtilis, Bacillus velezensis and Bacillus amyloliquefaciens*. Their recommendation is to sequence only the conventional PCR products of samples that have also been amplified by qPCR (Fraiture et al. 2020c). Of the analyzed samples, only the *B. subtilis* culture of sample 2-17 had this case (Table 8). However, qPCR amplification was obtained for only one colony from the culture of sample 2-17 with a C_t of 40.22, which can also be considered as non-specific amplification. The second sample was a choline chloride sample (1-29) from which a culture of *Bacillus cereus* was isolated with amplification of the *tetL* gene by conventional PCR only. Amplification

Table 6. Food and feed samples containing GM plant impurities. ID, sample identificatio; LOQ, limit of quantification. *, this sample contained several other GM maize and soy events as well that were not quantified: maize BT11, DAS59122, GA21, MON87460, MON88017, MON89034, NK603, MIR604, MON810, MON87427, soy 12704, A5547, DAS44406, A2704, FG72, GTS40-3-2, MON87701, MON87708

ID	Detected GMO events (% m/m ± SD)	Compliance with GMO labelling requirements
1-8	Soy GTS40-3-2 (0.15 ± 0.11), A2704 (0.13 ± 0.01), MON89788 (0.03 ± 0.01)	Compliant because GM soybean impurities are < 0.9 % m/m
1-15	Soy MON89788 (74.75 ± 20.94)	Non-compliant, as GM soybean impurities are > 0.9 % m/m and it is not indicated on the label that product contains GMOs
1-24	Soy MON89788 (78.74 ± 9.70)	Non-compliant, as GM soybean impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
2-8	Soy A2704 (< LOQ)	Compliant because GM soybean impurities are < 0.9 % m/m
2-15	Soy - A2704 (< LOQ), GTS40-3-2 (6.74 ± 2.41), MON87701 (0.25 ± 0.07), MON87708 (3.40 ± 0.11), MON89788 (< LOQ). Maize MON863 (< LOQ)	Non-compliant, as GM soybean impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
2-16	Soy GTS40-3-2 (1.28 ± 0.05), MON87701 (101.41 ± 6.67), MON87708 (0.01 ± 0.00), MON89788 (1.02 ± 0.20). Maize MON863 (< LOQ), 89034 (< LOQ)	Non-compliant, as GM soybean impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
2-39	Maize TC1507 (0.01 ± 0.00)	Compliant because GM soybean impurities are < 0.9 % m/m
2-40	Maize MON810 (15.72 ± 5.84), DAS59122 (0.63 ± 0.05), MIR604 (99.80 ± 9.30)	Non-compliant, as GM maize impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
2-41	Maize MON810 (28.04 ± 2.64)	Compliant because on the label it is stated that it contains bioengineered food ingredients
2-42	Maize DAS59122 (71.36 ± 13.27), MIR604 (7.70 ± 2.26)	Non-compliant, as GM maize impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
EZ-1	Soy MON89788 (24.12 ± 0.50), maize TC1507 (13.96 ± 6.48)*	Non-compliant, as GM soy and maize impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs
EZ-2	Soy GTS40-3-2 (24.28 ± 5.11)	Non-compliant, as GM soy impurities are > 0.9% m/m and it is not indicated on the label that product contains GMOs

products obtained by conventional PCR were sequenced by Sanger sequencing and showed low similarity with the reference gene. It can be concluded that the obtained tetL gene sequences show random similarity to the reference sequence (Fig. 1).

The aadD1 gene sequences obtained from the choline chloride sample (1-29) or the bacterial cultures (*Bacillus laterosporus* and *Bacillus pumilus*) isolated from it matched 100% with the reference sequence from the publication of the method developers (Fig. 2). The authors of the method believe that the *aadD1* gene does not naturally occur in wild-type bacteria known to be GM producers, with the exception of *E. coli* (Fraiture et al. 2020b).

PCR amplification with nptII-specific PCR primers was obtained from DNA extracts of 11 samples, but the amplification products were mostly shorter than the amplification product of the positive control (215 bp). Using reference sequences of the *nptII* gene available in the NCBI database (MF521566.1, AB264038.1 and KY964325.1) and the Center for Genomic Epidemiology tool MyDbFinder 2.0, the *nptII* gene was not found in any of the genomes of bacterial isolates obtained from these samples. It can be concluded that it was most likely non-specific amplification.

Whole genome sequencing (WGS) of bacterial isolates

Results of species determination of bacterial isolates using MALDI-TOF and two bioinformatic tools are given in Table 9. Table 10 mainly provides information about the presence of those antibiotic genes whose existence was expected according to other studies of GMM detection in enzymes and vitamins: *tetL, Erm(D), Erm(K), cat86*, and *aadK*. They were also given primary attention in the analysis.

Plasmid search with PlasmidFinder-2.0, MGEFinder and Patric 3.6.12. did not show the presence of plasmids in any of the isolates. Searching for mobile genetic elements with MGEFinder detected insertion elements with low sequence identity (ISBpu1, ISBsu1, and ISDha13), mini-inverted repeats (MITEBth3) and composite transposons (Table S1) that are related to antimicrobial resistance genes and virulence factors (Johansson et al. 2021).

Further analysis revealed that of the 27 samples of food and feed additives and food supplements, 10 samples were possibly non-compliant, i.e., containing live bacteria with various AMR genes (Table 8 and Table 10), and seven of them possibly contained recombinant DNA (Table 11).

Using MyDbFinder in the WGS sequence of *B. cereus*/B. *subtilis* isolate from a choline chloride sample 1-29 we found part of three recombinant plasmids pGMBsub01, pGMBsub03, and pGMBsub04 (Table 11), that have been found in other studies as well (Paracchini et al. 2017; Berbers et al. 2020). According to the literature, they

Table 7. Characterization of food and feed additives and food supplement samples. ID, sample identification. *Amplification products have also been obtained for the sample by conventional PCR; for the +/– sample, amplification was detected in only one of the two replicates; neg, negative; pos, positive

ID	**Marker genes of GMMs in total sample DNA extracts by qPCR**					**Abundance of target bacterial and fungal species and genera according to 16S and ITS1 sequencing (% of all sequences)**	**Bacterial isolate**
	***nptII* (conv. PCR)**	***558***	***cat***	***tetL***	***aadD***		
1-29	neg	neg	pos	pos*	pos	*Bacillus* spp. (0.45)	*Bacillus cereus, Bacillus pumilus, Brevibacillus laterosporus*
2-1	neg	neg	neg	neg	neg	*Lactobacillus plantarum* (16.87), *Bacillus* (0.71)	None
2-17	neg	neg	neg	neg	pos +/–	*Tepidiphilus* (19.94), *Anoxybacillus* (10.48), *Bacillus* sp. (9.81)	*Bacillus subtilis*
2-18	neg	neg	neg	neg	pos+/–	*Lactobacillus* sp. (9.12), *Anaerobacillus* sp. (4.00), *Corynebacterium* sp. (0.02)	None
2-19	neg	neg	neg	neg	neg	*Anaerobacillus* sp. (5.68), *Bacillus* sp. (0.14)	None
2-23	neg	neg	neg	neg	neg	*Bacillus* spp. (4.15, including *B. licheniformis* (3.64), *Saccharomyces* sp. (99.14)	*Bacillus licheniformis*
2-24	neg	neg	neg	neg	neg	*Staphylococcus* sp. (5.02), *Bacillus* sp. (0.21)	None
2-56	neg	neg	neg	neg	neg	*Bacillus* sp. (2.79)	None
2-57	neg	neg	neg	neg	neg	*Bacillus* sp. (1.65)	None
2-58	neg	neg	neg	neg	pos +/–	No amplification of 16S, ITS1	None
2-59	neg	neg	neg	neg	neg	*Bacillus* sp. (2.62)	None
2-60	neg	neg	neg	neg	neg	*Bacillus* sp. (3.96)	*Bacillus pumilus*
2-61	neg	neg	neg	neg	neg	No amplification of 16S, ITS1	None
2-62	neg	neg	neg	neg	neg	*Bacillus* sp. (1.02)	None
1.1	183/190	neg	neg	neg	neg	Not performed	None
1.2	169	neg	neg	neg	neg	Not performed	None
1.3	188	neg	neg	neg	neg	Not performed	*Bacillus subtilis*
1.4	162/175	neg	neg	pos	pos +–	Not performed	None
2.1	300/304	neg	neg	neg	neg	Not performed	*Bacillus cereus*
2.2	neg	neg	pos+/-	pos	neg	Not performed	*Bacillus subtilis, Bacillus licheniformis*
2.3	178/187	neg	neg	neg	neg	Not performed	*Bacillus subtilis, Bacillus licheniformis, Solibacillus silvestris*
2.4	181	neg	neg	neg	neg	Not performed	*Saccharomyces cerevisiae, Enterococcus faecium*
2.5	175/178	neg	neg	neg	pos	Not performed	*Bacillus licheniformis*
3.1	179/182	neg	neg	neg	neg	Not performed	None
3.2	169	neg	neg	neg	neg	Not performed	None
3.3	neg	neg	neg	neg	neg	Not performed	None
3.4	168/172	neg	neg	pos	pos +/–	Not performed	*Bacillus licheniformis*

contain ampicillin, kanamycin, bleomycin, erythromycin *erm(B)* and tetracycline resistance genes (Paracchini et al. 2017), and this isolate contained the *tetL* gene according to PCR results in our study. None of the other six isolates from this sample carried these plasmids. These plasmids have been found in vitamin B2 producers in other studies (Paracchini et al. 2017). Later studies found that at least part of the identified plasmids are integrated into the genome (Berbers et al. 2020). There is little scientific literature data on the production of choline using microorganisms as producers (Fujio et al. 1997; Fujio, Maruyama 1997), and only one patent (https://patents.google.com/patent/US20170211103A1/en).

From L-arginine sample 2-17, *B. velezensis* was isolated containing part of the cloning vector pCCR9 and plasmid pGMBsub01. The cloning vector pCCR9 according to the literature should contain the *tetL* gene (Randegger et al. 2000), and isolate 17098-2021-B contained this gene in the

Table 8. Characterization of bacterial cultures isolated from food and feed additives and food supplement samples. ID, sample identification. *week amplification

No.	**ID**	**Culture**	**Real time PCR (Ct)**				**Conventional PCR (bp)**		
			tetL	***aadD1***	***558***	***cat1***	***tetL***	***aadD1***	***cat1***
1	1-29	*B. cereus*	neg	neg	neg	neg	959	neg	neg
2	1-29	*B. cereus*	neg	neg	neg	neg	986	neg	neg
3	1-29	*B. laterosporus*	neg	38.81	neg	neg	neg	750	neg
4	1-29	*B. laterosporus*	neg	neg	neg	neg	neg	neg	neg
5	1-29	*B. laterosporus*	neg	neg	neg	neg	1087*	neg	neg
6	1-29	*B. laterosporus*	38.79	38.31	neg	neg	neg	757	neg
7	1-29	*B. pumilus*	neg	neg	neg	neg	1067, 1843*	758	neg
8	1-29	*B. pumilus*	40.04	neg	neg	neg	1314*	1358*	neg
9	1-29	*B. pumilus*	neg	neg	neg	neg	909, 1451*	1370*	neg
10	1-29	*B. pumilus*	neg	38.94	neg	neg	1460*	neg	neg
11	1-29	*B. pumilus*	neg	38.76	neg	neg	neg	neg	neg
12	1-29	*B. pumilus*	neg	neg	neg	neg	neg	neg	neg
13	2-17	*B. subtilis*	40.22	neg	neg	neg	899	neg	neg
14	2-17	*B. subtilis*	neg	neg	neg	neg	916	neg	neg
15	2-23	*B. licheniformis*	neg	neg	neg	neg	neg	neg	neg
16	2-23	*B. licheniformis*	neg	neg	neg	neg	1072*	neg	neg
17	2-60	*B. pumilus*	neg	neg	neg	neg	neg	neg	neg
18	2-60	*B. pumilus*	neg	neg	neg	neg	neg	neg	neg

```
Consensus_17_tetL          -------ATTAAGCAGACTCATTTCCGGCGCCGGCTTCCTTTTGTTTCAA
Consensus_17x_tetL         --GTAAAATTAAGCAGACTCAT-TCCGGCGCCGGCTTCCTTTTGTTTCAA
Consensus_H_B_C_tetL       --GTGAAATTAAGCAAACTCAT-TCCAGCGCCCGCTTCCTTTTCTTTCAA
Consensus_H_B_C_x_tetL     --GTGAAATTAAGCAAACTCAT-TCCAGCGCCCGCTTCCTTTTCTTTCAA
tet-L_GenBank_D00946.1     TGGTAAAGTTAAGCAAACTCAT-TCCAGCACCAGCTTCCTGCTGTTTCAA
                                   ******* ****** *** ** ** *******  * ******

Consensus_17_tetL          ACTGCCAGACACAATTGTAGATATAACC-GTTTTTTGTGAAGGAGAGCCC
Consensus_17x_tetL         ACTGCCAGACACAATTGTAGATATAACC-GT-TTTTGTGAAGGAGAGCCC
Consensus_H_B_C_tetL       ACCGCTAGACACAACTGTAGATATAACTKT--TTTTGTGAAAGATAGCCC
Consensus_H_B_C_x_tetL     ACCGCTAGACACAACTGTAGATATAACTCGT-TTTTGTGAAAGATAGCCC
tet-L_GenBank_D00946.1     GCTACTTGAAACAATTGTTGATATAACT-GT-TTTGGTGAACGAAAGCCC
                            *  *  ** **** *** ********     *** ***** ** *****

Consensus_17_tetL          CCCCAAAAACAAAAAACAATTATAACGGTCATGAGCCAAGGTGTCGCATC
Consensus_17x_tetL         CCCAAAAAACAAA-AACAATTATAACGGTCATGAGCCAAGGTGTCGCATC
Consensus_H_B_C_tetL       TCCAAAAAACAAA-AATTACTATAATTGTCATGATCCATGGTGCTGCATC
Consensus_H_B_C_x_tetL     TCCAA-AAACAAA-AATTACTATAATTGTCATGATCCATGGTGCTGCATC
tet-L_GenBank_D00946.1     ACCTA-AAACAAA-TACGATTATAATTGTCATGAACCATGATGTTGTTTC
                            ** * *******  *  * *****  ******* *** * **  *  **

Consensus_17_tetL          TATAAAAAAGGCAGCGATTAAAAAGCTGGAAGACATCAATACAATCCCGA
Consensus_17x_tetL         TATAAAAAAGGCAGCGATTAAAAAGCTGGAAGACATCAATACAATCCCGA
Consensus_H_B_C_tetL       TATAAAAAAGGCAGCGATTAAAAAGCCCGAAGAAAGCAATGCACTTCCAA
Consensus_H_B_C_x_tetL     TATAAAAAAGGCAGCGATTAAAAAGCCCGAAGAAAGCAATGCACTTCCAA
tet-L_GenBank_D00946.1     TAAAAGAAAGGAAGCAGTTAAAAAGCTAACAGAAAGAAATGTAACTCCGA
                           ** ** ***** ***  *********    *** *  ***  *   ** *

Consensus_17_tetL          TGGTGAGCACATATAAAGAACCTTTCCGATCAACAAGCAATCCTCCAATG
Consensus_17x_tetL         TGGTGAGCACATATAAAGAACCTTTCCGATCAACAAGCAATCCTCCAATG
Consensus_H_B_C_tetL       TAGTTAGTACATACAAGGAACCTTTCCGATCAACAAGCAATCCCCCAATG
Consensus_H_B_C_x_tetL     TAGTTAGTACATACAAGGAACCTTTCCGATCAACAAGCAATCCCCCAATG
tet-L_GenBank_D00946.1     TGTTTAACACGTATAAAGGACCTCTTCTATCAACAAGTATCCCACCAATG
                           *  * *  ** ** ** * **** * * ********* *  ** ******

Consensus_17_tetL          TAACCGAAGATGATGACGCTCATCGTCCCGGGGAACATAATCCCGCTTCC
Consensus_17x_tetL         TAACCGAAGATGATGACGCTCATCGTCCCGGGGAACATAATCCCGCTTCC
Consensus_H_B_C_tetL       TAACCAAAGATGATGACACTCATTGTTCCGGGGAAAATAATGCCGCTTCC
Consensus_H_B_C_x_tetL     TAACCAAAGATGATGACACTCATTGTTCCGGGGAAAATAATGCCGCTTCC
tet-L_GenBank_D00946.1     TAGCCGAAAATAATGACACTCATTGTTCCAGGGAAAATAATTACACTTCC
                           ** ** ** ** ***** ***** ** ** ***** *****  * *****

Consensus_17_tetL          AATTGCCGCAGTACTTAAATGGTGAACATCTTTCATCATATAAGGAACCA
Consensus_17x_tetL         AATTGCCGCAGTACTTAAATGGTGAACATCTTTCATCATATAAGGAACCA
Consensus_H_B_C_tetL       AATTGCTGTAGTACTTAAATGGTGAACATCTTTCATCATGTAAGGAACCA
Consensus_H_B_C_x_tetL     AATTGCTGTAGTACTTAAATGGTGAACATCTTTCATCATGTAAGGAACCA
tet-L_GenBank_D00946.1     GATTTCGGCAGTACTTAGCTGGTGAACATCTTTCATCATATAAGGAACCA
                            *** * * ********  ******************** **********

Consensus_17_tetL          TAGACACAAATCCTGCAGCAGTGCCGAATATCAGGCCTCCGCAAAAGACG
Consensus_17x_tetL         TAGACACAAATCCTGCAGCAGTGCCGAATATCAGGCCTCCGCAAAAGACG
Consensus_H_B_C_tetL       TAGAGACAAATCCTGCTACTGTACCAAATATTAGTCCTCCGCAAAGGGTT
Consensus_H_B_C_x_tetL     TAGAGACAAATCCTGCTACTGTACCAAATATTAGTCCTCCGCAAAGGGTT
tet-L_GenBank_D00946.1     TAGAGACAAACCCTGCTACTGTTCCAAATATAATTCCCCCACAAAGAACT
                           **** ***** *****  * ** ** ***** *  ** ** ****

Consensus_17_tetL          CCCATCATAAACAGGACATTTTTCCCCAGTGCAGGGTCAACAAAGGGATC
Consensus_17x_tetL         CCCATCATAAACAGGACATTTTTCCCCAGTGCAGGGTCAACAAAGGGATC
Consensus_H_B_C_tetL       CCAATCACAAAAAAGACGTTTTTCCCTAATTCAGGGTCAACAAAAGGGTC
Consensus_H_B_C_x_tetL     CCAATCACAAAAAAGACGTTTTTCCCTAATTCAGGGTCAACAAAAGGGTC
tet-L_GenBank_D00946.1     CCAATCATAAAAGGTATATTTTTCCCTAATCCGGGATCAACAAAAGGATC
                           ** **** ***    *  ******** * * * ** ******** ** **

Consensus_17_tetL          TTGAGTTTTCCTAATATGTTTCACAAATATCAGGAAAGCTAGAATACTGA
Consensus_17x_tetL         TTGAGTTTTCCTAATATGTTTCACAAATATCAGGAAAGCTAGAATACTGA
Consensus_H_B_C_tetL       CTGAGCTTTCCTAATGTGTTTTACAAATATGAAGAAAGCAAGAATACTGA
Consensus_H_B_C_x_tetL     CTGAGCTTTCCTAATGTGTTTTACAAATATGAAGAAAGCAAGAATACTGA
tet-L_GenBank_D00946.1     TGTTACTTTCCTGATATGTTTTACAAATATCAGGAATGACAGCACGCTAA
                                 ****** ** ***** ******** * *** *  ** *  ** *

Consensus_17_tetL          TGGTCAGAAAAGACATTCTATATGACGTTGTAAACAGCAGAAAAAATACG
Consensus_17x_tetL         TGGTCAGAAAAGACATTCTATATGACGTTGTAAACAGCAGAAAAAATACG
Consensus_H_B_C_tetL       TGATCAGAAAAGAAAATCTATAAGATGTTGTAAACAGCATAAAAAATACG
Consensus_H_B_C_x_tetL     TGATCAGAAAAGAAAATCTATAAGATGTTGTAAACAGCATAAAAAATACG
tet-L_GenBank_D00946.1     CGATAAGAAAAGAAATGCTATATGATGTTGTAAACAACATAAAAAATACA
                            * * ******** *  ***** ** ********** ** *********

Consensus_17_tetL          ATGCCTGCAGACATTAATATAATCCCCGCCATATCAAAGCGTCCGCTGAT
Consensus_17x_tetL         ATGCCTGCAGACATTAATATAATCCCCGCCATATCAAAGCGTCCGCTGAT
Consensus_H_B_C_tetL       ATACCTGCAGACATCAATATAATCCCTTCCATATCAATGTGTCCTCTTAT
Consensus_H_B_C_x_tetL     ATACCTGCAGACATCAATATAATCCCTTCCATATCAATGTGTCCTCTTAT
tet-L_GenBank_D00946.1     ATGCCTACAGACATTAGTATAATTCCTTTGATATCAAAATGACCTTTTAT
                           ** *** ******* * ****** **    *******   * **  * **

Consensus_17_tetL          TCTTTCTTCCTTTTCAAGCAGTTTTATCAGGAATGGAACGGTGATAACTG
Consensus_17x_tetL         TCTTTCTTCCTTTTCAAGCAGTTTTATCAGGAATGGAACGGTGATAACTG
Consensus_H_B_C_tetL       TCTCTCTTCCTTTTTCAACAATTTTATAAGGAATGGAACGGTGATAATTG
Consensus_H_B_C_x_tetL     TCTCTCTTCCTTTTTCAACAATTTTATAAGGAATGGAACGGTGATAATTG
tet-L_GenBank_D00946.1     CCTTACTTCTTTCTTTAATAATTTCATAAGAAACGGAACAGTGATAATTG
                            **  **** ** *  *  * *** ** ** ** ***** ******* **

Consensus_17_tetL          TTGCTGTTGGAATAAGCAGCAAATATGACCAGTGAATATAATGAGCGATC
Consensus_17x_tetL         TTGCTGTTGGAATAAGCAGCAAATATGACCAGTGAATATAATGAGCGATC
Consensus_H_B_C_tetL       TTGCAGTTGGAATAAGCAGCAAATACGACCAATGGATATAATGAGCAACC
Consensus_H_B_C_x_tetL     TTGCAGTTGGAATAAGCAGCAAATACGACCAATGGATATAATGAGCAACC
tet-L_GenBank_D00946.1     TTATCATAGGAATGAGTAGAAGATAGGACCAATGAATATAATGGGCTATC
                           **    * ***** ** ** * *** ***** ** ******** ** * *

Consensus_17_tetL          ATTCCGCCAATAGCTGGTCCGACACCTTCCCCCATTGCCACCAGAGACCC
Consensus_17x_tetL         ATTCCGCCAATAGCTGGTCCGACACCTTCCCCCATTGCCACCAGAGACCC
Consensus_H_B_C_tetL       ATTCCGCCAATAGCTGGCCCAACACCTTCTCCCATTGCTACCAGGGACCC
Consensus_H_B_C_x_tetL     ATTCCGCCAATAGCTGGCCCAACACCTTCTCCCATTGCTACCAGGGACCC
tet-L_GenBank_D00946.1     ATTCCACCAATCGCTGGACCGACTCCTTCTCCCATGGCTACTATCGATCC
                           ***** ***** ***** ** ** ***** ***** ** ** *  ** **

Consensus_17_tetL          GATCAGACCAAATGCTTTACCCCTGTTTTCTTTCGGAATATAGCGTACGA
Consensus_17x_tetL         GATCAGACCAAATGCTTTACCCCTGTTTTCTTTCGGAATATAGCGTACGA
Consensus_H_B_C_tetL       GATCAGGCCAAATGCTTTCCCCCTGTTCTCTTTTGGAATATAGCGCGCAA
Consensus_H_B_C_x_tetL     GATCAGGCCAAATGCTTTCCCCCTGTTCTCTTTTGGAATATAGCGCGCAA
tet-L_GenBank_D00946.1     AATAAGACCAAATGCTTTACCCCTATTTTCCTTTGGAATATAGCGCGCAA
                            ** ** *********** ***** ** ** ** ***********  * *

Consensus_17_tetL          CTGCAACCATTACAAGTGCCGGGAATGCGGCAGCACCAGCCCCTTGAATA
Consensus_17x_tetL         CTGCAACCATTACAAGTGCCGGGAATGCGGCAGCACCAGCCCCTTGAATA
Consensus_H_B_C_tetL       CGACAACCATCACAAGAGCTGGGAATGCGGCTGCACCAATTCCTTGAATA
Consensus_H_B_C_x_tetL     CGACAACCATCACAAGAGCTGGGAATGCGGCTGCACCAATTCCTTGAATA
tet-L_GenBank_D00946.1     CTACAACCATTACGAGTGCTGGAAATGCAGCTGCACCAGCCCCTTGAATA
                           *  ******* ** ** ** ** ***** ** ******   *********

Consensus_17_tetL          AATCGGGCCAGAATGAGTAAAGGAAAGAAAGAGTGTCCGACAAATCCAAT
Consensus_17x_tetL         AATCGGGCCAGAATGAGTAAAGGAAAGAAAGAGTGTCCGACAAATCCAAT
Consensus_H_B_C_tetL       AATCGGGCTAGAATGAGAATAGGAAAGAAAGAGTGTCCTACAAATCCAAT
Consensus_H_B_C_x_tetL     AATCGGGCTAGAATGAGAATAGGAAAGAAAGAGTGTCCTACAAATCCAAT
tet-L_GenBank_D00946.1     AAACGAGCCATAATAAGTAAGGAAAAGAAAGAATGGCCAACAAACCCAAT
                           ** ** ** * *** ** *  * ********* ** ** ***** *****

Consensus_17_tetL          GATTGATCCGAAGCCATTGACCATAATCCCAAA-CAG-GAGCAGCTTTTT
Consensus_17x_tetL         GATTGATCCGAAGCCATTGACCATAATCCCAAA-CAGGGAGCAGCTTTTT
Consensus_H_B_C_tetL       GATCGACCCTAAGCCATTTACCATAATCCCAAA-TAG-AAGCAGATTTTT
Consensus_H_B_C_x_tetL     GATCGACCCTAAGCCATTTACCATAATCCCAAAATAG-AAGCAGATTTTT
tet-L_GenBank_D00946.1     TACCGACCCGAAACAATTTATTATAATTCCAAA-TAG-GAGTAACCT-TT
                            *  ** ** ** * *** *  ***** *****  **  ** *   * **

Consensus_17_tetL          TGATGCCCAGTTGATCCGATAACTTCCCATACAGAGCAGTTCCGATGGAA
Consensus_17x_tetL         TGATGCCCAGTTGATCCGATAACTTCCCATACAGAGCAGTTCCGATGGAA
Consensus_H_B_C_tetL       TGATGCCCATCTGGTCTGATAGCTTTCCATATAGAGCTGTTCCAATGGAG
Consensus_H_B_C_x_tetL     -KATGC--------------------------------------------
tet-L_GenBank_D00946.1     TGATGCCTAATTGATCAGATAGCTTTCCATATACAGCTGTTCCAATGGAA
                             ****
```

Fig. 1. Multiple sequence alignment of *tetL* gene consensus sequences obtained from sample 2-17 (indicated here as 17), and bacterial culture *B. cereus* from sample 1-29 (indicated here as H) in two replications (indicated here as x) and the reference sequence D00946.1.

```
Consensus_H_B_L_2_aadD1    ------------------TTGGTCGTCAGACTGATGGGCCCTATTCGGAT
Consensus_H_B_L_2x_aadD1   ----------------TCTTGGTCGTCAGACTGATGGGCCCTATTCGGAT
Consensus_H_B_L_aadD1      -------------------TGGTCGTCAGACTGATGGGCCCTATTCGGAT
Consensus_H_B_L_x_aadD1    ---------------TTCTTGGTCGTCAGACTGATGGGCCCTATTCGGAT
Consensus_H_B_P_3_aadD1    ---------------------GTCGTCAGACTGATGGGCCCTATTCGGAT
Consensus_H_aadD1          ------------------------GTCAGACTGATGGGCCCTATTCGGAT
aadD_gene_GenBank_M19465.1 TTGGTGTTTATGGCTCTCTTGGTCGTCAGACTGATGGGCCCTATTCGGAT
                                                   **************************

Consensus_H_B_L_2_aadD1    ATTGAGATGATGTGTGTCATGTCAACAGAGGAAGCAGAGTTCAGCCATGA
Consensus_H_B_L_2x_aadD1   ATTGAGATGATGTGTGTCATGTCAACAGAGGAAGCAGAGTTCAGCCATGA
Consensus_H_B_L_aadD1      ATTGAGATGATGTGTGTCATGTCAACAGAGGAAGCAGAGTTCAGCCATGA
Consensus_H_B_L_x_aadD1    ATTGAGATGATGTGTGTCATGTCAACAGAGGAAGCAGAGTTCAGCCATGA
Consensus_H_B_P_3_aadD1    ATTGAGATGATGTGTGTCATGTCAACAGAGGAAGCAGAGTTCAGCCATGA
Consensus_H_aadD1          ATTGAGATGATGTGTGTCATGTCAACAGAGGAAGCAGAGTTCAGCCATGA
aadD_gene_GenBank_M19465.1 ATTGAGATGATGTGTGTCATGTCAACAGAGGAAGCAGAGTTCAGCCATGA
                           **************************************************

Consensus_H_B_L_2_aadD1    ATGGACAACCGGTGAGTGGAAGGTGGAAGTGAATTTTGATAGCGAAGAGA
Consensus_H_B_L_2x_aadD1   ATGGACAACCGGTGAGTGGAAGGTGGAAGTGAATTTTGATAGCGAAGAGA
Consensus_H_B_L_aadD1      ATGGACAACCGGTGAGTGGAAGGTGGAAGTGAATTTTGATAGCGAAGAGA
Consensus_H_B_L_x_aadD1    ATGGACAACCGGTGAGTGGAAGGTGGAAGTGAATTTTGATAGCGAAGAGA
Consensus_H_B_P_3_aadD1    ATGGACAACCGGTGAGTGGAAGGTGGAAGTGAATTTTGATAGCGAAGAGA
Consensus_H_aadD1          ATGGACAACCGGTGAGTGGAAGGTGGAAGTGAATTTTGATAGCGAAGAGA
aadD_gene_GenBank_M19465.1 ATGGACAACCGGTGAGTGGAAGGTGGAAGTGAATTTTGATAGCGAAGAGA
                           **************************************************

Consensus_H_B_L_2_aadD1    TTCTACTAGATTATGCATCTCAGGTGGAATCAGATTGGCCGCTTACACAT
Consensus_H_B_L_2x_aadD1   TTCTACTAGATTATGCATCTCAGGTGGAATCAGATTGGCCGCTTACACAT
Consensus_H_B_L_aadD1      TTCTACTAGATTATGCATCTCAGGTGGAATCAGATTGGCCGCTTACACAT
Consensus_H_B_L_x_aadD1    TTCTACTAGATTATGCATCTCAGGTGGAATCAGATTGGCCGCTTACACAT
Consensus_H_B_P_3_aadD1    TTCTACTAGATTATGCATCTCAGGTGGAATCAGATTGGCCGCTTACACAT
Consensus_H_aadD1          TTCTACTAGATTATGCATCTCAGGTGGAATCAGATTGGCCGCTTACACAT
aadD_gene_GenBank_M19465.1 TTCTACTAGATTATGCATCTCAGGTGGAATCAGATTGGCCGCTTACACAT
                           **************************************************

Consensus_H_B_L_2_aadD1    GGTCAATTTTTCTCTATTTTGCCGATTTATGATTCAGGTGGATACTTAGA
Consensus_H_B_L_2x_aadD1   GGTCAATTTTTCTCTATTTTGCCGATTTATGATTCAGGTGGATACTTAGA
Consensus_H_B_L_aadD1      GGTCAATTTTTCTCTATTTTGCCGATTTATGATTCAGGTGGATACTTAGA
Consensus_H_B_L_x_aadD1    GGTCAATTTTTCTCTATTTTGCCGATTTATGATTCAGGTGGATACTTAGA
Consensus_H_B_P_3_aadD1    GGTCAATTTTTCTCTATTTTGCCGATTTATGATTCAGGTGGATACTTAGA
Consensus_H_aadD1          GGTCAATTTTTCTCTATTTTGCCGATTTATGATTCAGGTGGATACTTAGA
aadD_gene_GenBank_M19465.1 GGTCAATTTTTCTCTATTTTGCCGATTTATGATTCAGGTGGATACTTAGA
                           **************************************************

Consensus_H_B_L_2_aadD1    GAAAGTGTATCAAACTGCTAAATCGGTAGAAGCCCAAACGTTCCACGATG
Consensus_H_B_L_2x_aadD1   GAAAGTGTATCAAACTGCTAAATCGGTAGAAGCCCAAACGTTCCACGATG
Consensus_H_B_L_aadD1      GAAAGTGTATCAAACTGCTAAATCGGTAGAAGCCCAAACGTTCCACGATG
Consensus_H_B_L_x_aadD1    GAAAGTGTATCAAACTGCTAAATCGGTAGAAGCCCAAACGTTCCACGATG
Consensus_H_B_P_3_aadD1    GAAAGTGTATCAAACTGCTAAATCGGTAGAAGCCCAAACGTTCCACGATG
Consensus_H_aadD1          GAAAGTGTATCAAACTGCTAAATCGGTAGAAGCCCAAAAGTTCCACGATG
aadD_gene_GenBank_M19465.1 GAAAGTGTATCAAACTGCTAAATCGGTAGAAGCCCAAACGTTCCACGATG
                           ************************************** ***********

Consensus_H_B_L_2_aadD1    CGATTTGTGCCCTTATCGTAGAAGAGCTGTTTGAATATGCAGGCAAATGG
Consensus_H_B_L_2x_aadD1   CGATTTGTGCCCTTATCGTAGAAGAGCTGTTTGAATATGCAGGCAAATGG
Consensus_H_B_L_aadD1      CGATTTGTGCCCTTATCGTAGAAGAGCTGTTTGAATATGCAGGCAAATGG
Consensus_H_B_L_x_aadD1    CGATTTGTGCCCTTATCGTAGAAGAGCTGTTTGAATATGCAGGCAAATGG
Consensus_H_B_P_3_aadD1    CGATTTGTGCCCTTATCGTAGAAGAGCTGTTTGAATATGCAGGCAAATGG
Consensus_H_aadD1          CGATTTGTGCCCTTATCGTAGAAGAGCTGTTTGAATATGCAGGCAAATGG
aadD_gene_GenBank_M19465.1 CGATTTGTGCCCTTATCGTAGAAGAGCTGTTTGAATATGCAGGCAAATGG
                           **************************************************

Consensus_H_B_L_2_aadD1    CGTAATATTCGTGTGCAAGGACCGACAACATTTCTACCATCCTTGACTGT
Consensus_H_B_L_2x_aadD1   CGTAATATTCGTGTGCAAGGACCGACAACATTTCTACCATCCTTGACTGT
Consensus_H_B_L_aadD1      CGTAATATTCGTGTGCAAGGACCGACAACATTTCTACCATCCTTGACTGT
Consensus_H_B_L_x_aadD1    CGTAATATTCGTGTGCAAGGACCGACAACATTTCTACCATCCTTGACTGT
Consensus_H_B_P_3_aadD1    CGTAATATTCGTGTGCAAGGACCGACAACATTTCTACCATCCTTGACTGT
Consensus_H_aadD1          CGTAATATTCGTGTGCAAGGACCGACAACATTTCTACCATCCTTGACTGT
aadD_gene_GenBank_M19465.1 CGTAATATTCGTGTGCAAGGACCGACAACATTTCTACCATCCTTGACTGT
                           **************************************************

Consensus_H_B_L_2_aadD1    ACAGGTAGCAATGGCAGGTGCCATGTTGATTGGTCTGCATCATCGCATCT
Consensus_H_B_L_2x_aadD1   ACAGGTAGCAATGGCAGGTGCCATGTTGATTGGTCTGCATCATCGCATCT
Consensus_H_B_L_aadD1      ACAGGTAGCAATGGCAGGTGCCATGTTGATTGGTCTGCATCATCGCATCT
Consensus_H_B_L_x_aadD1    ACAGGTAGCAATGGCAGGTGCCATGTTGATTGGTCTGCATCATCGCATCT
Consensus_H_B_P_3_aadD1    ACAGGTAGCAATGGCAGGTGCCATGTTGATTGGTCTGCATCATCGCATCT
Consensus_H_aadD1          ACAGGTAGCAATGGCAGGTGCCATGTTGATTGGTCTGCATCATCGCATCT
aadD_gene_GenBank_M19465.1 ACAGGTAGCAATGGCAGGTGCCATGTTGATTGGTCTGCATCATCGCATCT
                           **************************************************

Consensus_H_B_L_2_aadD1    GTTATACGACGAGCGCTTCGGTCTTAACTGAAGCAGTTAAGCAATCAGAT
Consensus_H_B_L_2x_aadD1   GTTATACGACGAGCGCTTCGGTCTTAACTGAAGCAGTTAAGCAATCAGAT
Consensus_H_B_L_aadD1      GTTATACGACGAGCGCTTCGGTCTTAACTGAAGCAGTTAAGCAATCAGAT
Consensus_H_B_L_x_aadD1    GTTATACGACGAGCGCTTCGGTCTTAACTGAAGCAGTTAAGCAATCAGAT
Consensus_H_B_P_3_aadD1    GTTATACGACGAGCGCTTCGGTCTTAACTGAAGCAGTTAAGCAATCAGAT
Consensus_H_aadD1          GTTATACGACGAGCGCTTCGGTCTTAACTGAAGCAGTTAAGCAATCAGAT
aadD_gene_GenBank_M19465.1 GTTATACGACGAGCGCTTCGGTCTTAACTGAAGCAGTTAAGCAATCAGAT
                           **************************************************

Consensus_H_B_L_2_aadD1    CTTCCTTCAGGTTATGACCATCTGTGCCAGTTCGTAATGTCTGGTCAACT
Consensus_H_B_L_2x_aadD1   CTTCCTTCAGGTTATGACCATCTGTGCCAGTTCGTAATGTCTGGTCAACT
Consensus_H_B_L_aadD1      CTTCCTTCAGGTTATGACCATCTGTGCCAGTTCGTAATGTCTGGTCAACT
Consensus_H_B_L_x_aadD1    CTTCCTTCAGGTTATGACCATCTGTGCCAGTTCGTAATGTCTGGTCAACT
Consensus_H_B_P_3_aadD1    CTTCCTTCAGGTTATGACCATCTGTGCCAGTTCGTAATGTCTGGTCAACT
Consensus_H_aadD1          CTTCCTTCAGGTTATGACCATCTGTGCCAGTTCGTAATGTCTGGTCAACT
aadD_gene_GenBank_M19465.1 CTTCCTTCAGGTTATGACCATCTGTGCCAGTTCGTAATGTCTGGTCAACT
                           **************************************************

Consensus_H_B_L_2_aadD1    TTCCGACTCTGAGAAACTTCTGGAATCGCTAGAGAATTTCTGGAATGGGA
Consensus_H_B_L_2x_aadD1   TTCCGACTCTGAGAAACTTCTGGAATCGCTAGAGAATTTCTGGAATGGGA
Consensus_H_B_L_aadD1      TTCCGACTCTGAGAAACTTCTGGAATCGCTAGAGAATTTCTGGAATGGGA
Consensus_H_B_L_x_aadD1    TTCCGACTCTGAGAAACTTCTGGAATCGCTAGAGAATTTCTGGAATGGGA
Consensus_H_B_P_3_aadD1    TTCCGACTCTGAGAAACTTCTGGAATCGCTAGAGAATTTCTGGAATGGGA
Consensus_H_aadD1          TTCCGACTCTGAGAAACTTCTGGAATCGCTAGAGAATTTCTGGAATGGGA
aadD_gene_GenBank_M19465.1 TTCCGACTCTGAGAAACTTCTGGAATCGCTAGAGAATTTCTGGAATGGGA
                           **************************************************
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Fig. 2. Multiple sequence alignment of *aadD1* gene sequences obtained from sample 1-29 (indicated here as H) and cultures of *B. laterosporus* (indicated here as B_L) and *B. pumilus* (indicated here as B_P) in several replications (indicated here with x and numbers), and the reference sequence M19465.1.

Table 9. Results of species determination of bacterial isolates using MALDI-TOF and two bioinformatic tools. *This bacterial isolate has not been preserved in the Institute BIOR Collection of Microorganism Cultures

Sample	Isolate No.	Species according to MALDI-TOF	PubMLST.org, support %	KmerFinder-3.2, query coverage %
2-17	17098-2021-B	*B. subtilis*	*Bacillus* 100 % (*B. amyloliquefaciens / B. velezensis*)	*B. velezensis* 99.60%
2-23	17079-2021-B	*B. licheniformis / B. sonorensis*	*B. paralicheniformis* 100%	*B. paralicheniformis* 99.45%
2-60	17128-2021-B	*B. pumilus*	*B. safensis* 98%	*B. safensis* 79.85%
1-29	17080-2021-B	*B. laterosporus*	*B. laterosporus* 57%, *B. halotolerans* 42%	*B. laterosporus* 52.26%
1-29	17081-2021-B	*B. pumilus*	*B. safensis* 98%	*B. safensis* 81.90%
1-29	17082-2021-B	*B. pumilus*	*B. safensis* 98%	*B. safensis* 82.77%
1-29	17129-2021-B	*B. laterosporus*	*B. laterosporus* 57%, *B. halotolerans* 42%	*B. laterosporus* 50.58%
1-29	17130-2021-B	*B. pumilus*	*B. safensis* 98%	*B. safensis* 80.45%
1-29	B. cereus*	*B. cereus*	*B. subtilis* 66%, *P. lactis* 32 %	*B. subtilis* 34.14%
1.3	20313-2023-B	*B. subtilis*	*B. subtilis* 96 %	*B. subtilis* 82.63%
2.1	20308-2023-B	*B. cereus*	*Bacillus* 100 %	*B. pacificus* 90.59%
2.2	20310-2023-B	*B. licheniformis*	*B. paralicheniformis* 100%	*B. paralicheniformis* 99.02%
2.3	20307-2023-B	*Solibacillus silvestris*	*S. silvestris* 78%; *Solibacillus isronensis* 19%	–
2.4	20345-2023-B	*B. licheniformis*	*B. paralicheniformis* 100 %	*B. paralicheniformis* 99.02%
2.5	20312-2023-B	*B. licheniformis*	*B. licheniformis* 100%	*B. licheniformis* 96.24%

Table 10. Detection of antibiotic resistance genes in WGS sequences of bacterial isolates

Sample	Isolate No.	ResFinder 4.1, identity %, resitance	Card, identity %, resistance	Patric 3.6.12., resistance
2-17	17098-2021-B	*Cfr(B)* 88.61, chloramphenicol; *tetL* 86.87, tetracycline; *Cfr(B)* 88.61, chloramphenicol	*clbA* 99.43, lincosamide antibiotic; *mdtA* 90.45, aminocoumarin antibiotic; *lmrB* 88.58, lincosamide antibiotic	*Tet(L)*, tetracycline; *Cfr*, phenicol antibiotics; *Lmr(B)*, lincomycin resistance; and other 47 genes
2-23	17079-2021-B	*Erm(D)* 99.77, macrolide resistance	*bcrA, bcrB, bcrC*, > 98, peptide antibiotics; *Erm(D)* 99.65, macrolide resistance; *aadK* 50.57, aminoglycoside antibiotic	*Erm(D)*/*Erm(K)*, macrolide resistance; *bcrA, bcrB, bcrC*, peptide antibiotics; class A beta-lactamase; and other 44 genes
2-60	17128-2021-B	*cat86* 93.31, chloramphenicol resistance	*mphK* 97.47, macrolide antibiotic; *cat86* 93.31, chloramphenicol resistance	*CatA6* family, chloramphenicol resistance; and other 41 genes
1-29	17080-2021-B	*VanHAX* 95.98, vancomycin, teicoplanin	*vanXA* 90.1, *vanRA* 93.51, *vanA* 85.13 glycopeptide antibiotic	*VanF/M*-type and other nine genes associated with vancomycin resistance; and other 37 genes
1-29	17081-2021-B	*cat86* 94.56, chloramphenicol resistance	*cat86* 93.85, chloramphenicol resistance	*CatA6* family, chloramphenicol resistance; and other 42 genes
1-29	17082-2021-B	*cat86* 94.78, chloramphenicol resistance	*cat86* 95.49, chloramphenicol resistance	*CatA6* family, chloramphenicol resistance; and other 41 gene
1-29	17129-2021-B	*VanHAX* 95.98, vancomycin, teicoplanin	*vanRA* 93.51, *vanXA* 90.10, glycopeptide antibiotic	*VanF/M*-type and other nine genes associated with vancomycin resistance; and other 39 genes
1-29	17130-2021-B	*cat86* 94.56, chloramphenicol resistance	*Y56* 98.61, *BlaA* beta-lactamase; *cat86* 95.24, chloramphenicol resistance	*CatA6* family, chloramphenicol resistance; and other 41 gene
1-29	B. cereus	*tetL* 99.13, tetracycline resistance; *aadK* 98.72, aminoglycoside resistance; *Mph(K)* 97.50, macrolide spiramycin	*ykkC* 99.11, aminoglycoside antibiotic; *ykkD* 98.10, aminoglycoside antibiotic; *tet(45)* 75.16, tetracycline antibiotic	*tetL*, tetracycline resistance; *YkkCD*, broad-specificity multidrug efflux pump; and other 104 genes
1.3	20313-2023-B	*AadK* 99.29, streptomycin; *mph(K)* 99.23, spiramycin, telithromycin; *tetL* 99.06, doxycycline, tetracycline	*aadK*, aminoglycoside antibiotic	*ANT(6)-I,* aminoglycoside 6-nucleotidyltransferase; *tetL*, tetracycline resistance; and other 44 genes
2.1	20308-2023-B	*FosB1* 87.41, fosfomycin; *FosB* 98.80, fosfomycin	*dfrE* 80.00, thymidylate synthase; *BcII* 98.00, subclass B1 beta-lactamase; *FosB* 88.00, fosfomycin resistance; *BLA1* 89.00, class A beta-lactamase	*FosB* 88,00, fosfomycin resistance; class A beta-lactamase gene; and other 41 gene
2.2	20310-2023-B	*erm(D)* 99.77, erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin, virginiamycin	*ErmD*, macrolide, lincosamide, streptogramin, streptogramin A, streptogramin B	*Erm(D)*/*Erm(K)*, lincosamides, streptogramins, macrolides; *CatA* family, chloramphenicol; and other 45 genes
2.3	20307-2023-B	none	none	*BceA, BceB*, bacitracin; and other 33 genes
2.4	20345-2023-B	*erm(D)* 99.77, erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin, virginiamycin	*ErmD*, macrolide antibiotic, lincosamide antibiotic, streptogramin antibiotic, streptogramin A antibiotic, streptogramin B antibiotic	*CatA* family, chloramphenicol; *BceA, BceR, BcrC*, bacitracin; and other 12 genes
2.5	20312-2023-B	*erm(D)* 95.68, erythromycin, lincomycin, clindamycin, quinupristin, pristinamycin, virginiamycin	*ErmD*, macrolide antibiotic, lincosamide antibiotic, streptogramin antibiotic, streptogramin A antibiotic, streptogramin B antibiotic	*Erm(D)*/*Erm(K)*, lincosamides, streptogramins, macrolides; and other 45 genes

Table 11. Presence of plasmids and other genetic elements in the WGS sequences of bacterial isolates

Isolate and sample	Genetic element	Identity (%)	Query / template length	Contig, description	Position in contig
B. cereus / *B. subtilis* isolate from choline chloride sample 1-29	LT622641.1 *B. subtilis* pGMBsub01 recombinant plasmid	77.95	4935 / 11378	Contig 1, length 772282, coverage 23.8, normalized coverage 0.46	635610…640488
	LT622642.1 *B. subtilis* pGMBsub03 recombinant plasmid	99.1	2221 / 8544	Contig 1, length 772282, coverage 23.8, normalized coverage 0.46	638305…640525
	LT622643.1 *B. subtilis* pGMBsub04 recombinant plasmid	97.24	8079 / 29760	Contig 1, length 772282, coverage 23.8, normalized coverage 0.46	632478…640525
	AJ277764.1 Cloning vector pCCR9	79.72	1489 / 2568	Contig 3, length 940757, coverage 307.9, normalized coverage 0.82	373419…374895
B. subtilis isolate 17098-2021-B from L-arginine sample 2-17	LT622641.1 *B. subtilis* pGMBsub01 recombinant plasmid	95.24	6895 / 11378	Contig 3, length 940757, coverage 307.9, normalized coverage 0.82	675658…682548
B. paralicheniformis isolate 17079-2021-B from vitamine mix sample 2-23	LT622641.1 *B. subtilis* pGMBsub01 recombinant plasmid	72.79	2944 / 11378	Contig 2, length 1112839, coverage 145.8, normalized coverage 0.82	787379…790272
B. subtilis isolate 20313-2023-B from potassium sorbate sample 1.3	LT622642.1 *B. subtilis* pGMBsub03 recombinant plasmid	99.03	3622 / 8544	Contig 6, length 359932, coverage 175.2, normalized coverage 0.84	299575...303196
B. licheniformis isolate from supplementary mineral feed sample 2.2.1	LT622641.1 *B. subtilis* pGMBsub01 recombinant plasmid	72.79	2944 / 11378	Contig 2	787068...789961
B. licheniformis isolate from supplementary feed for dogs sample 2.3.2	LT622641.1 *B. subtilis* pGMBsub01 recombinant plasmid	72.79	2944 / 11378	Contig 1, length 1112431, coverage 172.0, normalized coverage 0.88	787291...790184
B. licheniformis isolate from fish bone and fish oil powder sample 2.5	LT622641.1 *B. subtilis* pGMBsub01 recombinant plasmid	72.63	2948 / 11378	Contig 1	607457...610350

same contig as part of the plasmid pGMBsub01. The *tetL* gene sequence of this isolate is similar to *tetL* gene of *B. subtilis* subsp. *subtilis* str. 168 (according to Comprehensive Genome Analysis results by Patric). It would be quite difficult to prove that this isolate is genetically modified because the L-arginine biosynthesis pathway is quite large, involving many enzymes and therefore many genes (Park et al., 2014). Microorganisms have two types of metabolic control mechanisms for L-arginine biosynthesis pathway: Type 1, found in *E. coli* and *B. subtilis*; and Type 2, found in *Corynebacterium* (Utagawa, 2004). In Type 1, N-acetylglutamate is synthesized from L-glutamate with the help of N-acetylglutamate synthase, but this enzyme is strongly inhibited by L-arginine. This isolate has a DNA-directed RNA polymerase beta subunit that confers resistance to rifampicin, as well as translation elongation factor Tu on the same contig.

The next sample with possibly recombinant DNA was supplementary mineral feed for cows (sample 2-23) containing several vitamins: beta-carotene (provitamin A), vitamin A, vitamin D3, vitamin E, vitamin B6, vitamin B12, biotin (B7), tocopherol (vitamin E), micro and macro elements, and *Saccharomyces cerevisiae* MUCL 39885 – 1.5 × 10^{10} CFU kg^{-1}). From this sample *Bacillus paralicheniformis* was isolated containing part of the plasmid pGMBsub01, which according to the literature should contain ampicillin, kanamycin and neomycin resistance genes (Paracchini et al. 2017; Berbers et al. 2020). This isolate has a class A beta-lactamase gene that confers ampicillin resistance. The comprehensive genome analysis by Patric shows that this genome contains pathways for the biosynthesis of several vitamins. To prove that one of these is GM, one can start by looking at the antibiotic resistance gene *erm(D)*, which MGEFinder showed to be in the insertion sequence. Theoretically, the identified erythromycin resistance gene should be present in plasmid pGMBsub04, but this plasmid was not found in this isolate.

The above-mentioned plasmid pGMBsub03 was detected in the *B. subtilis* isolate 20313-2023-B from potassium sorbate (sample 1.3) as well, but the plasmid pGMBsub01 was detected in three more samples: *B. licheniformis* isolate from supplementary mineral feed (sample 2.2), *B. licheniformis* isolate from supplementary feed for dogs (sample 2.3), and *B. licheniformis* isolate from fish bone and fish oil powder (sample 2.5).

One sample (2-60, mixture of L-leucine, L-isoleucine, L-valine and vitamin B6) was considered as suspicious, meaning that *Bacillus safensis* culture was isolated, and the marker gene *cat86* of chloramphenicol resistance in isolated culture was detected. The chloramphenicol acetyltransferase gene *cat86* (K00544) has been cited in publications from the 1980s as "highly promising for use in biotechnology" because the *cat86* gene sequence contains four restriction sites that can accommodate heterologous coding sequences in each of the three reading frames. The product is expressed as fusion protein (Ambulos et al. 1985). The *cat* gene has been found in plasmid pC194 (GenBank: NC_002013.1) in other studies, but this plasmid was not detected in this isolate.

Last three samples containing live bacteria with various AMR genes were 2.2 (supplementary mineral feed for horses), 2.5 (fish bone and fish oil powder for dogs and cats) and 3.4 (food supplement – pancreatin enzyme). These samples were considered as suspicious as well.

In conclusion, the study indicates presence of unintended GMO contamination in several samples of food, feed, their additives and food supplements, which emphasizes the need for monitoring of such goods in order to protect consumer rights to be informed about the content of the products.

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Table S1. List of mobile genetic elements found by MGEFinder in WGS sequences of bacterial isolates

Isolate No	mge_no	name	prediction method	type	allele_ len	depth	e_value	identity	coverage	gaps	sub-stitu-tion	contig	start	stop	cigar
17079-2021-B	2	ISBpu1	alignment to reference	Insertion sequence	179	38.211068	1.16109e-34	0.8277777777777777	0.10122878876535986	4	27	NODE_1_length_1183245_cov_38.211068	389782	389960	M63 D1 M60 I1 M3 D1 M15 D1 M35
17079-2021-B	3	ISBsu1	alignment to reference	Insertion sequence	364	37.294995	4.70284e-88	0.843915343915344	0.2808378588052754	15	44	NODE_2_length_1113463_cov_37.294995	546733	547096	M50 I3 M1 I1 M2 I2 M1 I4 M198 I2 M26 D1 M37 I2 M48
17079-2021-B	4	ISBpu1	alignment to reference	Insertion sequence	179	37.294995	5.08344e-33	0.8212290502793296	0.1023990637799883	2	30	NODE_2_length_1113463_cov_37.294995	891663	891841	M26 D1 M88 D1 M63
17079-2021-B	5	ISBpu1	alignment to reference	Insertion sequence	179	37.294995	3.95764e-29	0.8111111111111111	0.10005851375073142	5	29	NODE_2_length_1113463_cov_37.294995	555492	555670	M59 I1 M3 D1 M63 D1 M1 D1 M21 D1 M28
17079-2021-B	7	ISBpu1	alignment to reference	Insertion sequence	178	54.570839	3.78641e-29	0.8100558659217877	0.10064365125804564	4	30	NODE_3_length_1065292_cov_54.570839	720606	720783	M63 D1 M60 I1 M3 D1 M21 D1 M28
17079-2021-B	8	ISBpu1	alignment to reference	Insertion sequence	177	54.570839	6.29134e-32	0.8202247191011236	0.10005851375073142	4	28	NODE_3_length_1065292_cov_54.570839	693444	693620	M63 D1 M60 I1 M3 D1 M21 D1 M27
17079-2021-B	9	ISBpu1	alignment to reference	Insertion sequence	178	50.08977	2.70198e-31	0.8156424581005587	0.10064365125804564	4	29	NODE_5_length_353662_cov_50.089770	276389	276566	M63 D1 M60 I1 M3 D1 M21 D1 M28
17079-2021-B	10	ISBpu1	alignment to reference	Insertion sequence	178	50.08977	2.70198e-31	0.8156424581005587	0.10064365125804564	4	29	NODE_5_length_353662_cov_50.089770	210964	211141	M63 D1 M60 I1 M3 D1 M24 D1 M25
17079-2021-B	11	ISBpu1	alignment to reference	Insertion sequence	178	50.08977	1.24825e-34	0.8268156424581007	0.10064365125804564	4	27	NODE_5_length_353662_cov_50.089770	288874	289051	M63 D1 M60 I1 M3 D1 M15 D1 M34
17079-2021-B	9_7_8	cn_27340_ISBpu1	inferred	Composite transposon	27340	54.570839	6.29134e-32	0.8202247191011236	0.10005851375073142	4	28	NODE_3_length_1065292_cov_54.570839	693443	720783	-
17079-2021-B	12_10	cn_5241_ISBpu1	inferred	Composite transposon	5241	50.08977	2.70198e-31	0.8156424581005587	0.10064365125804564	4	29	NODE_5_length_353662_cov_50.089770	210963	216204	-
17079-2021-B	13_11_9	cn_12663_ISBpu1	inferred	Composite transposon	12663	50.08977	2.70198e-31	0.8156424581005587	0.10064365125804564	4	29	NODE_5_length_353662_cov_50.089770	276388	289051	-
17080-2021-B	6	MITEBth3	alignment to reference	Miniature Inverted Repeat	52	14.815897	1.68535e-11	0.9230769230769231	0.16938110749185667	0	4	NODE_43_length_16596_cov_14.815897	316	367	M52
17081-2021-B	1	ISBpu1	alignment to reference	Insertion sequence	176	27.678818	2.07549e-59	0.9261363636363636	0.10298420128730251	0	13	NODE_1_length_609264_cov_27.678818	395017	395192	M176
17081-2021-B	2	ISBpu1	alignment to reference	Insertion sequence	177	27.678818	3.42423e-67	0.9378531073446328	0.1023990637799883	1	10	NODE_1_length_609264_cov_27.678818	391961	392137	M50 D1 M126
17081-2021-B	3	ISBpu1	alignment to reference	Insertion sequence	175	27.678818	5.8116e-55	0.8971428571428571	0.1023990637799883	0	18	NODE_1_length_609264_cov_27.678818	520916	521090	M175
17081-2021-B	4	ISBpu1	alignment to reference	Insertion sequence	174	27.678818	3.47303e-57	0.9080459770114944	0.10064365125804564	1	15	NODE_1_length_609264_cov_27.678818	529324	529497	M79 D1 M94
17081-2021-B	5	ISBpu1	alignment to reference	Insertion sequence	180	31.036793	7.8794e-53	0.8839779005524863	0.10415447630193095	2	19	NODE_2_length_493593_cov_31.036793	311671	311850	M3 D1 M30 I1 M146
17081-2021-B	6	ISBpu1	alignment to reference	Insertion sequence	177	31.036793	1.658e-69	0.943502824858757	0.10356933879461673	0	10	NODE_2_length_493593_cov_31.036793	450018	450194	M177
17081-2021-B	7	ISBpu1	alignment to reference	Insertion sequence	176	31.036793	1.29998e-60	0.9147727272727273	0.10298420128730251	0	15	NODE_2_length_493593_cov_31.036793	490134	490309	M176
17081-2021-B	8	ISBpu1	alignment to reference	Insertion sequence	176	31.036793	4.74218e-50	0.8806818181818182	0.10181392627267408	1	20	NODE_2_length_493593_cov_31.036793	292133	292308	M116 D1 M59
17081-2021-B	9	ISBpu1	alignment to reference	Insertion sequence	175	31.036793	9.90826e-72	0.9542857142857143	0.1023990637799883	0	8	NODE_2_length_493593_cov_31.036793	176541	176715	M175
17081-2021-B	10	ISBpu1	alignment to reference	Insertion sequence	173	31.036793	6.1344e-49	0.8786127167630058	0.10122878876535986	0	21	NODE_2_length_493593_cov_31.036793	234759	234931	M173
17081-2021-B	11	ISBpu1	alignment to reference	Insertion sequence	183	58.415617	5.14274e-64	0.9180327868852459	0.10590988882387362	1	14	NODE_3_length_422682_cov_58.415617	369759	369941	M168 D1 M14
17081-2021-B	12	ISBpu1	alignment to reference	Insertion sequence	176	52.176354	2.32936e-67	0.9375	0.10298420128730251	0	11	NODE_4_length_414418_cov_52.176354	247008	247183	M176
17081-2021-B	13	ISBpu1	alignment to reference	Insertion sequence	175	52.176354	8.43736e-62	0.92	0.1023990637799883	0	14	NODE_4_length_414418_cov_52.176354	309846	310020	M175
17081-2021-B	14	ISBpu1	alignment to reference	Insertion sequence	184	38.191225	2.74584e-51	0.875	0.1053247513165594	2	21	NODE_5_length_369717_cov_38.191225	331279	331462	M83 D1 M39 D1 M60
17081-2021-B	15	ISBpu1	alignment to reference	Insertion sequence	178	38.191225	1.61787e-63	0.9213483146067416	0.10415447630193095	0	14	NODE_5_length_369717_cov_38.191225	143680	143857	M178
17081-2021-B	16	ISBpu1	alignment to reference	Insertion sequence	178	38.191225	2.74584e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_5_length_369717_cov_38.191225	81410	81587	M67 D1 M110
17081-2021-B	17	ISBpu1	alignment to reference	Insertion sequence	175	38.191225	1.62935e-58	0.9090909090909091	0.1023990637799883	1	15	NODE_5_length_369717_cov_38.191225	199795	199969	M3 I1 M172
17081-2021-B	18	ISBpu1	alignment to reference	Insertion sequence	176	38.191225	9.73704e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_5_length_369717_cov_38.191225	45468	45643	M176
17081-2021-B	19	ISBpu1	alignment to reference	Insertion sequence	179	38.191225	5.77786e-68	0.9385474860335195	0.10122878876535986	3	8	NODE_5_length_369717_cov_38.191225	129014	129192	M126 D3 M50
17081-2021-B	20	ISBpu1	alignment to reference	Insertion sequence	175	38.191225	2.68818e-66	0.9371428571428572	0.10122878876535986	1	10	NODE_5_length_369717_cov_38.191225	336555	336729	M80 D1 M94
17081-2021-B	22	ISBpu1	alignment to reference	Insertion sequence	176	25.050512	4.35167e-64	0.9265536723163842	0.10298420128730251	1	12	NODE_6_length_357669_cov_25.050512	290530	290705	M61 I1 M115
17081-2021-B	23	ISBpu1	alignment to reference	Insertion sequence	176	25.050512	2.03901e-57	0.9034090909090909	0.10298420128730251	0	17	NODE_6_length_357669_cov_25.050512	254792	254967	M176
17081-2021-B	24	ISBpu1	alignment to reference	Insertion sequence	177	25.050512	9.41971e-61	0.9152542372881356	0.1023990637799883	1	14	NODE_6_length_357669_cov_25.050512	303294	303470	M96 D1 M80
17081-2021-B	25	ISBpu1	alignment to reference	Insertion sequence	174	25.050512	1.23588e-49	0.8793103448275862	0.10181392627267408	0	21	NODE_6_length_357669_cov_25.050512	130704	130877	M174
17081-2021-B	26	ISBpu1	alignment to reference	Insertion sequence	176	25.050512	7.28189e-62	0.9204545454545454	0.10064365125804564	2	12	NODE_6_length_357669_cov_25.050512	147472	147647	M94 D1 M77 D1 M3
17081-2021-B	27	ISBpu1	alignment to reference	Insertion sequence	173	25.050512	9.35331e-66	0.9364161849710982	0.10122878876535986	0	11	NODE_6_length_357669_cov_25.050512	195703	195875	M173
17081-2021-B	28	ISBpu1	alignment to reference	Insertion sequence	171	25.050512	1.23588e-49	0.8837209302325582	0.10005851375073142	1	19	NODE_6_length_357669_cov_25.050512	107079	107249	M162 I1 M9
17081-2021-B	29	ISBpu1	alignment to reference	Insertion sequence	173	40.959398	6.03374e-52	0.8876404494382022	0.10122878876535986	5	15	NODE_7_length_292205_cov_40.959398	128366	128538	M4 I1 M84 I4 M85
17081-2021-B	30	ISBpu1	alignment to reference	Insertion sequence	177	40.959398	4.63148e-58	0.903954802259887	0.10356933879461673	0	17	NODE_7_length_292205_cov_40.959398	21908	22084	M177
17081-2021-B	31	ISBpu1	alignment to reference	Insertion sequence	176	40.959398	7.69547e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_7_length_292205_cov_40.959398	84841	85016	M176
17081-2021-B	32	ISBpu1	alignment to reference	Insertion sequence	181	33.136387	9.23179e-54	0.8895027624309392	0.10005851375073142	5	15	NODE_8_length_207991_cov_33.136387	36853	37033	M60 D5 M116
17081-2021-B	33	ISBpu1	alignment to reference	Insertion sequence	176	33.136387	1.1527e-77	0.9715909090909091	0.10298420128730251	0	5	NODE_8_length_207991_cov_33.136387	30914	31089	M176
17081-2021-B	34	ISBpu1	alignment to reference	Insertion sequence	172	33.136387	5.47805e-61	0.92	0.10064365125804564	3	11	NODE_8_length_207991_cov_33.136387	33026	33197	M49 I3 M123

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Table S1. *Continued*

Isolate No	mge_no	name	prediction method	type	allele_ len	depth	e_value	identity	coverage	gaps	sub- stitu- tion	contig	start	stop	cigar
17081-2021-B	35	ISBpu1	alignment to reference	Insertion sequence	194	47.626045	1.29774e-71	0.9238578680203046	0.11351667641895846	3	12	NODE_9_length_179745_cov_47.626045	135309	135502	M12 I1 M5 I1 M1 I1 M176
17081-2021-B	36	ISBpu1	alignment to reference	Insertion sequence	177	47.626045	1.02473e-57	0.9039548022598871	0.1023990637799883	1	16	NODE_9_length_179745_cov_47.626045	89958	90134	M94 D1 M82
17081-2021-B	37	ISBpu1	alignment to reference	Insertion sequence	174	47.626045	1.33498e-51	0.8850574712643678	0.10181392627267408	0	20	NODE_9_length_179745_cov_47.626045	53872	54045	M174
17081-2021-B	38	ISBpu1	alignment to reference	Insertion sequence	177	36.270366	1.21627e-64	0.9269662921348316	0.10122878876535986	3	10	NODE_10_length_99977_cov_36.270366	9830	10006	M50 D1 M64 I1 M2 D1 M59
17081-2021-B	39	ISBpu1	alignment to reference	Insertion sequence	183	56.428051	1.05392e-58	0.8972972972972973	0.10356933879461673	5	14	NODE_15_length_18508_cov_56.428051	8375	8557	M3 D1 M3 I1 M76 D1 M54 I1 M3 D1 M41
17081-2021-B	5_1_2	cn_3232_ISBpu1	inferred	Composite transposon	3232	27.678818	3.42423e-67	0.9378531073446328	0.1023990637799883	1	10	NODE_1_length_609264_cov_27.678818	391960	395192	-
17081-2021-B	6_1	cn_47065_ISBpu1	inferred	Composite transposon	47065	27.678818	2.07549e-59	0.9261363636363636	0.10298420128730251	0	13	NODE_1_length_609264_cov_27.678818	395016	442081	-
17081-2021-B	7_3	cn_2420_ISBpu1	inferred	Composite transposon	2420	27.678818	5.8116e-55	0.8971428571428571	0.1023990637799883	0	18	NODE_1_length_609264_cov_27.678818	518670	521090	-
17081-2021-B	8_3_4	cn_8582_ISBpu1	inferred	Composite transposon	8582	27.678818	5.8116e-55	0.8971428571428571	0.1023990637799883	0	18	NODE_1_length_609264_cov_27.678818	520915	529497	-
17081-2021-B	9_4	cn_17875_ISBpu1	inferred	Composite transposon	17875	27.678818	3.47303e-57	0.9080459770114944	0.10064365125804564	1	15	NODE_1_length_609264_cov_27.678818	529323	547198	-
17081-2021-B	11_10	cn_13458_ISBpu1	inferred	Composite transposon	13458	31.036793	6.1344e-49	0.8786127167630058	0.10122878876535986	0	21	NODE_2_length_493593_cov_31.036793	234758	248216	-
17081-2021-B	12_8	cn_44209_ISBpu1	inferred	Composite transposon	44209	31.036793	4.74218e-50	0.8806818181818182	0.10181392627267408	1	20	NODE_2_length_493593_cov_31.036793	248099	292308	-
17081-2021-B	13_5_8	cn_19718_ISBpu1	inferred	Composite transposon	19718	31.036793	4.74218e-50	0.8806818181818182	0.10181392627267408	1	20	NODE_2_length_493593_cov_31.036793	292132	311850	-
17081-2021-B	14_6_7	cn_40292_ISBpu1	inferred	Composite transposon	40292	31.036793	1.658e-69	0.943502824858757	0.10356933879461673	0	10	NODE_2_length_493593_cov_31.036793	450017	490309	-
17081-2021-B	14_13	cn_13721_ISBpu1	inferred	Composite transposon	13721	52.176354	8.43736e-62	0.92	0.1023990637799883	0	14	NODE_4_length_414418_cov_52.176354	309845	323566	-
17081-2021-B	21_18	cn_24546_ISBpu1	inferred	Composite transposon	24546	38.191225	9.73704e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_5_length_369717_cov_38.191225	45467	70013	-
17081-2021-B	22_16	cn_11743_ISBpu1	inferred	Composite transposon	11743	38.191225	2.74584e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_5_length_369717_cov_38.191225	69844	81587	-
17081-2021-B	23_16	cn_30875_ISBpu1	inferred	Composite transposon	30875	38.191225	2.74584e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_5_length_369717_cov_38.191225	81409	112284	-
17081-2021-B	24_19	cn_17037_ISBpu1	inferred	Composite transposon	17037	38.191225	5.77786e-68	0.9385474860335195	0.10122878876535986	3	8	NODE_5_length_369717_cov_38.191225	112155	129192	-
17081-2021-B	25_19	cn_12567_ISBpu1	inferred	Composite transposon	12567	38.191225	5.77786e-68	0.9385474860335195	0.10122878876535986	3	8	NODE_5_length_369717_cov_38.191225	129013	141580	-
17081-2021-B	26_15	cn_2419_ISBpu1	inferred	Composite transposon	2419	38.191225	1.61787e-63	0.9213483146067416	0.10415447630193095	0	14	NODE_5_length_369717_cov_38.191225	141438	143857	-
17081-2021-B	27_14_20	cn_5451_ISBpu1	inferred	Composite transposon	5451	38.191225	2.74584e-51	0.875	0.1053247513165594	2	21	NODE_5_length_369717_cov_38.191225	331278	336729	-
17081-2021-B	29_28	cn_52212_ISBpu1	inferred	Composite transposon	52212	25.050512	1.23588e-49	0.8837209302325582	0.10005851375073142	1	19	NODE_6_length_357669_cov_25.050512	55037	107249	-
17081-2021-B	30_25_28	cn_23799_ISBpu1	inferred	Composite transposon	23799	25.050512	1.23588e-49	0.8837209302325582	0.10005851375073142	1	19	NODE_6_length_357669_cov_25.050512	107078	130877	-
17081-2021-B	31_25_26	cn_16944_ISBpu1	inferred	Composite transposon	16944	25.050512	1.23588e-49	0.8793103448275862	0.10181392627267408	0	21	NODE_6_length_357669_cov_25.050512	130703	147647	-
17081-2021-B	32_26_27	cn_48404_ISBpu1	inferred	Composite transposon	48404	25.050512	7.28189e-62	0.9204545454545454	0.10064365125804564	2	12	NODE_6_length_357669_cov_25.050512	147471	195875	-
17081-2021-B	21	ISBpu1	alignment to reference	Insertion sequence	1683	25.050512	1.6446e-28	0.7955732371040425	0.1860737273259216	17	52	NODE_6_length_357669_cov_25.050512	217266	218948	M33 D1 M49 D1 M5 N1359 M8 I1 M88 D1 M7 I2 M3 I2 M2 I3 M1 I5 M7 I1 M118
17081-2021-B	33_21_27	cn_23246_ISBpu1	inferred	Composite transposon	23246	25.050512	9.35331e-66	0.9364161849710982	0.10122878876535986	0	11	NODE_6_length_357669_cov_25.050512	195702	218948	-
17081-2021-B	34_21_23	cn_37702_ISBpu1	inferred	Composite transposon	37702	25.050512	1.6446e-28	0.7955732371040425	0.1860737273259216	17	52	NODE_6_length_357669_cov_25.050512	217265	254967	-
17081-2021-B	35_22_23	cn_35914_ISBpu1	inferred	Composite transposon	35914	25.050512	2.03901e-57	0.9034090909090909	0.10298420128730251	0	17	NODE_6_length_357669_cov_25.050512	254791	290705	-
17081-2021-B	36_22_24	cn_12941_ISBpu1	inferred	Composite transposon	12941	25.050512	4.35167e-64	0.9265536723163842	0.10298420128730251	1	12	NODE_6_length_357669_cov_25.050512	290529	303470	-
17081-2021-B	32_31	cn_10680_ISBpu1	inferred	Composite transposon	10680	40.959398	7.69547e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_7_length_292205_cov_40.959398	84840	95520	-
17081-2021-B	33_29	cn_33164_ISBpu1	inferred	Composite transposon	33164	40.959398	6.03374e-52	0.8876404494382022	0.10122878876535986	5	15	NODE_7_length_292205_cov_40.959398	95374	128538	-
17081-2021-B	35_33_34	cn_2284_ISBpu1	inferred	Composite transposon	2284	33.136387	1.1527e-77	0.9715909090909091	0.10298420128730251	0	5	NODE_8_length_207991_cov_33.136387	30913	33197	-
17081-2021-B	36_32_34	cn_4008_ISBpu1	inferred	Composite transposon	4008	33.136387	5.47805e-61	0.92	0.10064365125804564	3	11	NODE_8_length_207991_cov_33.136387	33025	37033	-
17081-2021-B	38_36_37	cn_36263_ISBpu1	inferred	Composite transposon	36263	47.626045	1.33498e-51	0.8850574712643678	0.10181392627267408	0	20	NODE_9_length_179745_cov_47.626045	53871	90134	-
17081-2021-B	39_35_36	cn_45545_ISBpu1	inferred	Composite transposon	45545	47.626045	1.02473e-57	0.9039548022598871	0.1023990637799883	1	16	NODE_9_length_179745_cov_47.626045	89957	135502	-
17081-2021-B	39_38	cn_27682_ISBpu1	inferred	Composite transposon	27682	36.270366	1.21627e-64	0.9269662921348316	0.10122878876535986	3	10	NODE_10_length_99977_cov_36.270366	9829	37511	-
17082-2021-B	1	ISBpu1	alignment to reference	Insertion sequence	180	16.398517	5.9238e-53	0.8839779005524863	0.10415447630193095	2	19	NODE_1_length_371095_cov_16.398517	147224	147403	M3 D1 M30 I1 M146
17082-2021-B	2	ISBpu1	alignment to reference	Insertion sequence	177	16.398517	1.2465e-69	0.943502824858757	0.10356933879461673	0	10	NODE_1_length_371095_cov_16.398517	285571	285747	M177
17082-2021-B	3	ISBpu1	alignment to reference	Insertion sequence	176	16.398517	9.77334e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_1_length_371095_cov_16.398517	325687	325862	M176
17082-2021-B	4	ISBpu1	alignment to reference	Insertion sequence	176	16.398517	3.56521e-50	0.8806818181818182	0.10181392627267408	1	20	NODE_1_length_371095_cov_16.398517	127686	127861	M116 D1 M59
17082-2021-B	5	ISBpu1	alignment to reference	Insertion sequence	175	16.398517	7.44911e-72	0.9542857142857143	0.1023990637799883	0	8	NODE_1_length_371095_cov_16.398517	12094	12268	M175
17082-2021-B	6	ISBpu1	alignment to reference	Insertion sequence	173	16.398517	4.61189e-49	0.8786127167630058	0.10122878876535986	0	21	NODE_1_length_371095_cov_16.398517	70312	70484	M173
17082-2021-B	7	ISBpu1	alignment to reference	Insertion sequence	173	21.024452	6.47718e-52	0.8876404494382022	0.10122878876535986	5	15	NODE_2_length_313678_cov_21.024452	163668	163840	M85 I4 M84 I1 M4
17082-2021-B	8	ISBpu1	alignment to reference	Insertion sequence	177	21.024452	4.97187e-58	0.903954802259887	0.10356933879461673	0	17	NODE_2_length_313678_cov_21.024452	270122	270298	M177
17082-2021-B	9	ISBpu1	alignment to reference	Insertion sequence	176	21.024452	8.26105e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_2_length_313678_cov_21.024452	207190	207365	M176
17082-2021-B	10	ISBpu1	alignment to reference	Insertion sequence	176	15.724335	7.19793e-60	0.9261363636363636	0.10298420128730251	0	13	NODE_3_length_211267_cov_15.724335	132	307	M176

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Table S1. *Continued*

Isolate No	mge_no	name	prediction method	type	allele_len	depth	e_value	identity	coverage	gaps	sub-stitu-tion	contig	start	stop	cigar
17082-2021-B	11	ISBpu1	alignment to reference	Insertion sequence	175	15.724335	2.0155e-55	0.8971428571428571	0.1023990637799883	0	18	NODE_3_length_211267_cov_15.724335	126031	126205	M175
17082-2021-B	12	ISBpu1	alignment to reference	Insertion sequence	174	15.724335	1.20447e-57	0.9080459770114944	0.10064365125804564	1	15	NODE_3_length_211267_cov_15.724335	134439	134612	M79 D1 M94
17082-2021-B	13	ISBpu1	alignment to reference	Insertion sequence	194	23.616038	1.43878e-71	0.9238578680203046	0.11351667641895846	3	12	NODE_4_length_199277_cov_23.616038	139342	139535	M12 I1 M5 I1 M1 I1 M176
17082-2021-B	14	ISBpu1	alignment to reference	Insertion sequence	177	23.616038	1.1361e-57	0.9039548022598871	0.1023990637799883	1	16	NODE_4_length_199277_cov_23.616038	93991	94167	M94 D1 M82
17082-2021-B	15	ISBpu1	alignment to reference	Insertion sequence	174	23.616038	1.48007e-51	0.8850574712643678	0.10181392627267408	0	20	NODE_4_length_199277_cov_23.616038	57905	58078	M174
17082-2021-B	16	ISBpu1	alignment to reference	Insertion sequence	184	20.653077	1.31975e-51	0.875	0.1053247513165594	2	21	NODE_5_length_177694_cov_20.653077	22757	22940	M60 D1 M39 D1 M83
17082-2021-B	17	ISBpu1	alignment to reference	Insertion sequence	175	20.653077	7.83125e-59	0.9090909090909091	0.1023990637799883	1	15	NODE_5_length_177694_cov_20.653077	154250	154424	M172 I1 M3
17082-2021-B	18	ISBpu1	alignment to reference	Insertion sequence	175	20.653077	1.29203e-66	0.9371428571428572	0.10122878876535986	1	10	NODE_5_length_177694_cov_20.653077	17490	17664	M94 D1 M80
17082-2021-B	19	ISBpu1	alignment to reference	Insertion sequence	174	13.455277	6.12415e-50	0.8793103448275862	0.10181392627267408	0	21	NODE_6_length_177230_cov_13.455277	36421	36594	M174
17082-2021-B	20	ISBpu1	alignment to reference	Insertion sequence	176	13.455277	3.60839e-62	0.9204545454545454	0.10064365125804564	2	12	NODE_6_length_177230_cov_13.455277	19651	19826	M3 D1 M77 D1 M94
17082-2021-B	21	ISBpu1	alignment to reference	Insertion sequence	171	13.455277	6.12415e-50	0.8837209302325582	0.10005851375073142	1	19	NODE_6_length_177230_cov_13.455277	60049	60219	M9 I1 M162
17082-2021-B	22	ISBpu1	alignment to reference	Insertion sequence	178	18.887264	7.73096e-64	0.9213483146067416	0.10415447630193095	0	14	NODE_7_length_176664_cov_18.887264	143693	143870	M178
17082-2021-B	23	ISBpu1	alignment to reference	Insertion sequence	178	18.887264	1.3121e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_7_length_176664_cov_18.887264	81423	81600	M67 D1 M110
17082-2021-B	24	ISBpu1	alignment to reference	Insertion sequence	176	18.887264	4.65284e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_7_length_176664_cov_18.887264	45481	45656	M176
17082-2021-B	25	ISBpu1	alignment to reference	Insertion sequence	179	18.887264	2.76095e-68	0.9385474860335195	0.10122878876535986	3	8	NODE_7_length_176664_cov_18.887264	129027	129205	M126 D3 M50
17082-2021-B	26	ISBpu1	alignment to reference	Insertion sequence	183	24.87255	1.95779e-64	0.9180327868852459	0.10590988882387362	1	14	NODE_8_length_160911_cov_24.872550	42161	42343	M14 D1 M168
17082-2021-B	27	ISBpu1	alignment to reference	Insertion sequence	181	18.463812	6.77318e-54	0.8895027624309392	0.10005851375073142	5	15	NODE_10_length_152607_cov_18.463812	15725	15905	M116 D5 M60
17082-2021-B	28	ISBpu1	alignment to reference	Insertion sequence	177	18.463812	1.85674e-64	0.9269662921348316	0.10122878876535986	3	10	NODE_10_length_152607_cov_18.463812	62460	62636	M50 D1 M64 I1 M2 D1 M59
17082-2021-B	29	ISBpu1	alignment to reference	Insertion sequence	176	18.463812	8.45715e-78	0.9715909090909091	0.10298420128730251	0	5	NODE_10_length_152607_cov_18.463812	21669	21844	M176
17082-2021-B	30	ISBpu1	alignment to reference	Insertion sequence	172	18.463812	4.01913e-61	0.92	0.10064365125804564	3	11	NODE_10_length_152607_cov_18.463812	19561	19732	M123 I3 M49
17082-2021-B	31	ISBpu1	alignment to reference	Insertion sequence	177	14.627488	7.02372e-68	0.9378531073446328	0.1023990637799883	1	10	NODE_14_length_124966_cov_14.627488	2993	3169	M126 D1 M50
17082-2021-B	32	ISBpu1	alignment to reference	Insertion sequence	175	26.010305	2.21117e-62	0.92	0.1023990637799883	0	14	NODE_15_length_108616_cov_26.010305	104265	104439	M175
17082-2021-B	33	ISBpu1	alignment to reference	Insertion sequence	177	11.779049	2.74245e-61	0.9152542372881356	0.1023990637799883	1	14	NODE_16_length_104141_cov_11.779049	5220	5396	M96 D1 M80
17082-2021-B	35	ISBpu1	alignment to reference	Insertion sequence	176	12.390997	5.69032e-58	0.9034090909090909	0.10298420128730251	0	17	NODE_17_length_99826_cov_12.390997	22892	23067	M176
17082-2021-B	36	ISBpu1	alignment to reference	Insertion sequence	173	12.390997	2.61025e-66	0.9364161849710982	0.10122878876535986	0	11	NODE_17_length_99826_cov_12.390997	81984	82156	M173
17082-2021-B	37	ISBpu1	alignment to reference	Insertion sequence	176	23.967607	5.21604e-68	0.9375	0.10298420128730251	0	11	NODE_18_length_92800_cov_23.967607	52447	52622	M176
17082-2021-B	38	ISBpu1	alignment to reference	Insertion sequence	176	11.346262	2.4881e-65	0.9265536723163842	0.10298420128730251	1	12	NODE_29_length_20470_cov_11.346262	7497	7672	M115 I1 M61
17082-2021-B	39	ISBpu1	alignment to reference	Insertion sequence	183	25.880039	1.05392e-58	0.8972972972972973	0.10356933879461673	5	14	NODE_31_length_18508_cov_25.880039	8375	8557	M3 D1 M3 I1 M76 D1 M54 I1 M3 D1 M41
17082-2021-B	7_6	cn_13458_ISBpu1	inferred	Composite transposon	13458	16.398517	4.61189e-49	0.8786127167630058	0.10122878876535986	0	21	NODE_1_length_371095_cov_16.398517	70311	83769	-
17082-2021-B	8_4	cn_44209_ISBpu1	inferred	Composite transposon	44209	16.398517	3.56521e-50	0.8806818181818182	0.10181392627267408	1	20	NODE_1_length_371095_cov_16.398517	83652	127861	-
17082-2021-B	9_1_4	cn_19718_ISBpu1	inferred	Composite transposon	19718	16.398517	3.56521e-50	0.8806818181818182	0.10181392627267408	1	20	NODE_1_length_371095_cov_16.398517	127685	147403	-
17082-2021-B	10_2_3	cn_40292_ISBpu1	inferred	Composite transposon	40292	16.398517	1.2465e-69	0.943502824858757	0.10356933879461673	0	10	NODE_1_length_371095_cov_16.398517	285570	325862	-
17082-2021-B	11_3	cn_7758_ISBpu1	inferred	Composite transposon	7758	16.398517	9.77334e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_1_length_371095_cov_16.398517	325686	333444	-
17082-2021-B	10_7	cn_33164_ISBpu1	inferred	Composite transposon	33164	21.024452	6.47718e-52	0.8876404494382022	0.10122878876535986	5	15	NODE_2_length_313678_cov_21.024452	163667	196831	-
17082-2021-B	11_9	cn_10680_ISBpu1	inferred	Composite transposon	10680	21.024452	8.26105e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_2_length_313678_cov_21.024452	196685	207365	-
17082-2021-B	12_8	cn_37832_ISBpu1	inferred	Composite transposon	37832	21.024452	4.97187e-58	0.903954802259887	0.10356933879461673	0	17	NODE_2_length_313678_cov_21.024452	270121	307953	-
17082-2021-B	13_10	cn_47065_ISBpu1	inferred	Composite transposon	47065	15.724335	7.19793e-60	0.9261363636363636	0.10298420128730251	0	13	NODE_3_length_211267_cov_15.724335	131	47196	-
17082-2021-B	14_11	cn_2420_ISBpu1	inferred	Composite transposon	2420	15.724335	2.0155e-55	0.8971428571428571	0.1023990637799883	0	18	NODE_3_length_211267_cov_15.724335	123785	126205	-
17082-2021-B	15_11_12	cn_8582_ISBpu1	inferred	Composite transposon	8582	15.724335	2.0155e-55	0.8971428571428571	0.1023990637799883	0	18	NODE_3_length_211267_cov_15.724335	126030	134612	-
17082-2021-B	16_12	cn_17875_ISBpu1	inferred	Composite transposon	17875	15.724335	1.20447e-57	0.9080459770114944	0.10064365125804564	1	15	NODE_3_length_211267_cov_15.724335	134438	152313	-
17082-2021-B	16_14_15	cn_36263_ISBpu1	inferred	Composite transposon	36263	23.616038	1.48007e-51	0.8850574712643678	0.10181392627267408	0	20	NODE_4_length_199277_cov_23.616038	57904	94167	-
17082-2021-B	17_13_14	cn_45545_ISBpu1	inferred	Composite transposon	45545	23.616038	1.1361e-57	0.9039548022598871	0.1023990637799883	1	16	NODE_4_length_199277_cov_23.616038	93990	139535	-
17082-2021-B	19_16_18	cn_5451_ISBpu1	inferred	Composite transposon	5451	20.653077	1.29203e-66	0.9371428571428572	0.10122878876535986	1	10	NODE_5_length_177694_cov_20.653077	17489	22940	-
17082-2021-B	22_19_20	cn_16944_ISBpu1	inferred	Composite transposon	16944	13.455277	3.60839e-62	0.9204545454545454	0.10064365125804564	2	12	NODE_6_length_177230_cov_13.455277	19650	36594	-
17082-2021-B	23_19_21	cn_23799_ISBpu1	inferred	Composite transposon	23799	13.455277	6.12415e-50	0.8793103448275862	0.10181392627267408	0	21	NODE_6_length_177230_cov_13.455277	36420	60219	-
17082-2021-B	24_21	cn_52212_ISBpu1	inferred	Composite transposon	52212	13.455277	6.12415e-50	0.8837209302325582	0.10005851375073142	1	19	NODE_6_length_177230_cov_13.455277	60048	112260	-
17082-2021-B	26_24	cn_24546_ISBpu1	inferred	Composite transposon	24546	18.887264	4.65284e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_7_length_176664_cov_18.887264	45480	70026	-
17082-2021-B	27_23	cn_11743_ISBpu1	inferred	Composite transposon	11743	18.887264	1.3121e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_7_length_176664_cov_18.887264	69857	81600	-
17082-2021-B	28_23	cn_30875_ISBpu1	inferred	Composite transposon	30875	18.887264	1.3121e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_7_length_176664_cov_18.887264	81422	112297	-

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Table S1. *Continued*

Isolate No	mge_no	name	prediction method	type	allele_len	depth	e_value	identity	coverage	gaps	sub-stitu-tion	contig	start	stop	cigar
17082-2021-B	29_25	cn_17037_ISBpu1	inferred	Composite transposon	17037	18.887264	2.76095e-68	0.9385474860335195	0.10122878876535986	3	8	NODE_7_length_176664_cov_18.887264	112168	129205	-
17082-2021-B	30_25	cn_12567_ISBpu1	inferred	Composite transposon	12567	18.887264	2.76095e-68	0.9385474860335195	0.10122878876535986	3	8	NODE_7_length_176664_cov_18.887264	129026	141593	-
17082-2021-B	31_22	cn_2419_ISBpu1	inferred	Composite transposon	2419	18.887264	7.73096e-64	0.9213483146067416	0.10415447630193095	0	14	NODE_7_length_176664_cov_18.887264	141451	143870	-
17082-2021-B	31_27_30	cn_4008_ISBpu1	inferred	Composite transposon	4008	18.463812	6.77318e-54	0.8895027624309392	0.10005851375073142	5	15	NODE_10_length_152607_cov_18.463812	15724	19732	-
17082-2021-B	32_29_30	cn_2284_ISBpu1	inferred	Composite transposon	2284	18.463812	4.01913e-61	0.92	0.10064365125804564	3	11	NODE_10_length_152607_cov_18.463812	19560	21844	-
17082-2021-B	33_28_29	cn_40968_ISBpu1	inferred	Composite transposon	40968	18.463812	8.45715e-78	0.9715909090909091	0.10298420128730251	0	5	NODE_10_length_152607_cov_18.463812	21668	62636	-
17082-2021-B	34_28	cn_27682_ISBpu1	inferred	Composite transposon	27682	18.463812	1.85674e-64	0.9269662921348316	0.10122878876535986	3	10	NODE_10_length_152607_cov_18.463812	62459	90141	-
17082-2021-B	32_31	cn_3169_ISBpu1	inferred	Composite transposon	3169	14.627488	7.02372e-68	0.9378531073446328	0.1023990637799883	1	10	NODE_14_length_124966_cov_14.627488	0	3169	-
17082-2021-B	33_32	cn_13721_ISBpu1	inferred	Composite transposon	13721	26.010305	2.21117e-62	0.92	0.1023990637799883	0	14	NODE_15_length_108616_cov_26.010305	90718	104439	-
17082-2021-B	34	ISBpu1	alignment to reference	Insertion sequence	1683	12.390997	4.58963e-29	0.7955732371040425	0.1860737273259216	17	52	NODE_17_length_99826_cov_12.390997	58911	60593	M118 I1 M7 I5 M1 I3 M2 I2 M3 I2 M7 D1 M88 I1 M8 N1359 M5 D1 M49 D1 M33
17082-2021-B	37_34_35	cn_37702_ISBpu1	inferred	Composite transposon	37702	12.390997	5.69032e-58	0.9034090909090909	0.10298420128730251	0	17	NODE_17_length_99826_cov_12.390997	22891	60593	-
17082-2021-B	38_34_36	cn_23246_ISBpu1	inferred	Composite transposon	23246	12.390997	4.58963e-29	0.7955732371040425	0.1860737273259216	17	52	NODE_17_length_99826_cov_12.390997	58910	82156	-
17098-2021-B	none														
17128-2021-B	1	ISBpu1	alignment to reference	Insertion sequence	183	46.872735	1.61366e-60	0.908108108108108	0.10356933879461673	5	12	NODE_1_length_612762_cov_46.872735	546414	546596	M34 D1 M2 D1 M4 I2 M137 D1 M3
17128-2021-B	2	ISBpu1	alignment to reference	Insertion sequence	181	46.872735	5.84497e-55	0.8950276243093923	0.10005851375073142	5	14	NODE_1_length_612762_cov_46.872735	122363	122543	M60 D5 M116
17128-2021-B	3	ISBpu1	alignment to reference	Insertion sequence	177	46.872735	1.60229e-65	0.9325842696629213	0.10122878876535986	3	9	NODE_1_length_612762_cov_46.872735	75632	75808	M59 D1 M2 I1 M64 D1 M50
17128-2021-B	4	ISBpu1	alignment to reference	Insertion sequence	177	46.872735	3.44389e-67	0.9378531073446328	0.1023990637799883	1	10	NODE_1_length_612762_cov_46.872735	510315	510491	M126 D1 M50
17128-2021-B	5	ISBpu1	alignment to reference	Insertion sequence	176	46.872735	3.39551e-77	0.9715909090909091	0.10298420128730251	0	5	NODE_1_length_612762_cov_46.872735	116420	116595	M176
17128-2021-B	6	ISBpu1	alignment to reference	Insertion sequence	176	46.872735	1.26521e-51	0.8863636363636365	0.10181392627267408	1	19	NODE_1_length_612762_cov_46.872735	381496	381671	M94 D1 M81
17128-2021-B	7	ISBpu1	alignment to reference	Insertion sequence	175	46.872735	3.46834e-62	0.9371428571428572	0.1023990637799883	0	11	NODE_1_length_612762_cov_46.872735	118532	118706	M175
17128-2021-B	8	ISBpu1	alignment to reference	Insertion sequence	174	46.872735	2.08741e-59	0.9142857142857143	0.10064365125804564	2	13	NODE_1_length_612762_cov_46.872735	142316	142489	M112 I1 M2 D1 M59
17128-2021-B	9	ISBpu1	alignment to reference	Insertion sequence	173	46.872735	3.49297e-57	0.9075144508670521	0.10122878876535986	0	16	NODE_1_length_612762_cov_46.872735	48711	48883	M173
17128-2021-B	10	ISBpu1	alignment to reference	Insertion sequence	180	42.28149	1.75516e-59	0.9060773480662984	0.10415447630193095	2	15	NODE_2_length_515175_cov_42.281490	23542	23721	M146 I1 M30 D1 M3
17128-2021-B	11	ISBpu1	alignment to reference	Insertion sequence	178	42.28149	4.81144e-70	0.9438202247191011	0.10415447630193095	0	10	NODE_2_length_515175_cov_42.281490	113862	114039	M178
17128-2021-B	12	ISBpu1	alignment to reference	Insertion sequence	177	42.28149	1.37616e-50	0.8813559322033898	0.10122878876535986	2	19	NODE_2_length_515175_cov_42.281490	42894	43070	M59 D1 M39 D1 M77
17128-2021-B	13	ISBpu1	alignment to reference	Insertion sequence	175	42.28149	1.03415e-71	0.9542857142857143	0.1023990637799883	0	8	NODE_2_length_515175_cov_42.281490	156436	156610	M175
17128-2021-B	14	ISBpu1	alignment to reference	Insertion sequence	174	42.28149	8.22394e-53	0.8908045977011494	0.10181392627267408	0	19	NODE_2_length_515175_cov_42.281490	434858	435031	M174
17128-2021-B	15	ISBpu1	alignment to reference	Insertion sequence	172	42.28149	1.06383e-51	0.8895348837209303	0.10064365125804564	0	19	NODE_2_length_515175_cov_42.281490	411237	411408	M172
17128-2021-B	16	ISBsu1	alignment to reference	Insertion sequence	357	42.28149	1.08665e-36	0.7590027700831025	0.24747866563227308	23	64	NODE_2_length_515175_cov_42.281490	514811	515167	M42 D1 M1 D1 M115 D1 M1 D1 M5 I1 M4 D1 M6 I1 M1 I1 M64 D3 M1 D2 M1 D3 M1 D1 M2 D3 M2 D1 M34 D1 M7 I1 M51
17128-2021-B	17	ISBpu1	alignment to reference	Insertion sequence	176	37.093035	6.16862e-59	0.9096045197740112	0.10298420128730251	1	15	NODE_3_length_503419_cov_37.093035	424129	424304	M115 I1 M61
17128-2021-B	18	ISBpu1	alignment to reference	Insertion sequence	176	37.093035	2.86998e-57	0.9034090909090909	0.10298420128730251	0	17	NODE_3_length_503419_cov_37.093035	459865	460040	M176
17128-2021-B	19	ISBpu1	alignment to reference	Insertion sequence	178	58.893172	1.80739e-63	0.9213483146067416	0.10415447630193095	0	14	NODE_4_length_413023_cov_58.893172	274058	274235	M178
17128-2021-B	20	ISBpu1	alignment to reference	Insertion sequence	178	58.893172	3.06749e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_4_length_413023_cov_58.893172	336260	336437	M110 D1 M67
17128-2021-B	21	ISBpu1	alignment to reference	Insertion sequence	175	58.893172	8.46865e-57	0.9034090909090909	0.1023990637799883	1	16	NODE_4_length_413023_cov_58.893172	214841	215015	M172 I1 M3
17128-2021-B	22	ISBpu1	alignment to reference	Insertion sequence	179	58.893172	1.38735e-69	0.9441340782122905	0.10122878876535986	3	7	NODE_4_length_413023_cov_58.893172	288723	288901	M50 D3 M126
17128-2021-B	23	ISBpu1	alignment to reference	Insertion sequence	175	58.893172	6.54667e-58	0.9085714285714286	0.10122878876535986	1	15	NODE_4_length_413023_cov_58.893172	365612	365786	M99 D1 M75
17128-2021-B	24	ISBpu1	alignment to reference	Insertion sequence	175	58.893172	6.45469e-68	0.9428571428571428	0.10122878876535986	1	9	NODE_4_length_413023_cov_58.893172	75277	75451	M99 D1 M75
17128-2021-B	25	ISBpu1	alignment to reference	Insertion sequence	172	58.893172	3.88473e-65	0.936046511627907	0.10064365125804564	0	11	NODE_4_length_413023_cov_58.893172	60381	60552	M172
17128-2021-B	26	ISBpu1	alignment to reference	Insertion sequence	183	75.634849	1.00946e-65	0.9234972677595629	0.10590988882387362	1	13	NODE_5_length_386014_cov_75.634849	53187	53369	M14 D1 M168
17128-2021-B	27	ISBsu1	alignment to reference	Insertion sequence	191	69.696406	4.61848e-71	0.9267015706806283	0.14817688130333592	0	14	NODE_6_length_177858_cov_69.696406	1	191	M191
17128-2021-B	28	ISBsu1	alignment to reference	Insertion sequence	191	69.696406	4.61848e-71	0.9267015706806283	0.14817688130333592	0	14	NODE_6_length_177858_cov_69.696406	177668	177858	M191
17128-2021-B	29	ISBpu1	alignment to reference	Insertion sequence	177	69.696406	2.21044e-49	0.8764044943820225	0.10122878876535986	3	19	NODE_6_length_177858_cov_69.696406	49143	49319	M91 I1 M7 D1 M48 D1 M29
17128-2021-B	30	ISBpu1	alignment to reference	Insertion sequence	174	69.696406	1.31165e-56	0.9022988505747126	0.10181392627267408	0	17	NODE_6_length_177858_cov_69.696406	70932	71105	M174
17128-2021-B	31	ISBpu1	alignment to reference	Insertion sequence	176	61.595351	4.33916e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_7_length_164756_cov_61.595351	160619	160794	M176
17128-2021-B	32	ISBpu1	alignment to reference	Insertion sequence	173	61.595351	9.45931e-53	0.8908045977011494	0.10005851375073142	2	17	NODE_7_length_164756_cov_61.595351	117894	118066	M81 D1 M2 I1 M89
17128-2021-B	33	ISBsu1	alignment to reference	Insertion sequence	903	66.711348	0	0.8993362831858407	0.7005430566330488	1	90	NODE_8_length_149930_cov_66.711348	1	903	M861 I1 M42
17128-2021-B	34	ISBpu1	alignment to reference	Insertion sequence	181	50.227924	1.18236e-55	0.8950276243093923	0.10005851375073142	5	14	NODE_10_length_123949_cov_50.227924	23651	23831	M74 D3 M1 D2 M101

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Table S1. *Continued*

Isolate No	mge_no	name	prediction method	type	allele_len	depth	e_value	identity	coverage	gaps	sub-stitu-tion	contig	start	stop	cigar
17128-2021-B	35	ISBpu1	alignment to reference	Insertion sequence	176	50.227924	5.57937e-44	0.8579545454545454	0.10181392627267408	1	24	NODE_10_length_123949_cov_50.227924	116220	116395	M99 D1 M76
17128-2021-B	36	ISBpu1	alignment to reference	Insertion sequence	179	67.371739	4.71008e-53	0.88268156424581	0.10473961380924517	0	21	NODE_12_length_82042_cov_67.371739	59293	59471	M179
17128-2021-B	37	ISBpu1	alignment to reference	Insertion sequence	174	67.371739	6.09287e-52	0.8850574712643678	0.10181392627267408	0	20	NODE_12_length_82042_cov_67.371739	28119	28292	M174
17128-2021-B	38	ISBpu1	alignment to reference	Insertion sequence	194	64.800189	2.24687e-70	0.9187817258883249	0.11351667641895846	3	13	NODE_13_length_66900_cov_64.800189	2300	2493	M176 I1 M1 I1 M5 I1 M12
17128-2021-B	39	ISBsu1	alignment to reference	Insertion sequence	384	48.049603	1.64963e-140	0.9036458333333334	0.2979053529868115	0	37	NODE_16_length_54237_cov_48.049603	53854	54237	M384
17128-2021-B	40	ISBpu1	alignment to reference	Insertion sequence	182	48.724653	4.91654e-62	0.907608695652174	0.10415447630193095	4	13	NODE_19_length_18689_cov_48.724653	8557	8738	M3 D1 M3 I1 M130 I1 M3 D1 M41
17128-2021-B	41	ISBsu1	alignment to reference	Insertion sequence	1059	315.510182	1.32439e-31	0.7344464451355922	0.5135764158262219	31	157	NODE_22_length_1060_cov_315.510182	1	1059	M33 I1 M6 I1 M11 D2 M6 I1 M4 D1 M12 I1 M46 I1 M1 I1 M10 D2 M55 D1 M3 I1 M43 I1 M7 D1 M63 I1 M3 I3 M7 D2 M2 D1 M3 D1 M72 N367 M77 D1 M2 D1 M12 I1 M4 I1 M28 I1 M7 D1 M94 I1 M7 D1 M59
17128-2021-B	10_3_9	cn_27098_ISBpu1	inferred	Composite transposon	27098	46.872735	3.49297e-57	0.9075144508670521	0.10122878876535986	0	16	NODE_1_length_612762_cov_46.872735	48710	75808	-
17128-2021-B	11_3_5	cn_40964_ISBpu1	inferred	Composite transposon	40964	46.872735	1.60229e-65	0.9325842696629213	0.10122878876535986	3	9	NODE_1_length_612762_cov_46.872735	75631	116595	-
17128-2021-B	12_5_7	cn_2287_ISBpu1	inferred	Composite transposon	2287	46.872735	3.39551e-77	0.9715909090909091	0.10298420128730251	0	5	NODE_1_length_612762_cov_46.872735	116419	118706	-
17128-2021-B	13_2_7	cn_4012_ISBpu1	inferred	Composite transposon	4012	46.872735	3.46834e-62	0.9371428571428572	0.1023990637799883	0	11	NODE_1_length_612762_cov_46.872735	118531	122543	-
17128-2021-B	14_2_8	cn_20127_ISBpu1	inferred	Composite transposon	20127	46.872735	5.84497e-55	0.8950276243093923	0.10005851375073142	5	14	NODE_1_length_612762_cov_46.872735	122362	142489	-
17128-2021-B	15_6	cn_26153_ISBpu1	inferred	Composite transposon	26153	46.872735	1.26521e-51	0.8863636363636365	0.10181392627267408	1	19	NODE_1_length_612762_cov_46.872735	355518	381671	-
17128-2021-B	16_6	cn_2421_ISBpu1	inferred	Composite transposon	2421	46.872735	1.26521e-51	0.8863636363636365	0.10181392627267408	1	19	NODE_1_length_612762_cov_46.872735	381495	383916	-
17128-2021-B	17_4	cn_49924_ISBpu1	inferred	Composite transposon	49924	46.872735	3.44389e-67	0.9378531073446328	0.1023990637799883	1	10	NODE_1_length_612762_cov_46.872735	460567	510491	-
17128-2021-B	18_1_4	cn_36282_ISBpu1	inferred	Composite transposon	36282	46.872735	3.44389e-67	0.9378531073446328	0.1023990637799883	1	10	NODE_1_length_612762_cov_46.872735	510314	546596	-
17128-2021-B	16_10_12	cn_19529_ISBpu1	inferred	Composite transposon	19529	42.28149	1.75516e-59	0.9060773480662984	0.10415447630193095	2	15	NODE_2_length_515175_cov_42.281490	23541	43070	-
17128-2021-B	17_12	cn_20198_ISBpu1	inferred	Composite transposon	20198	42.28149	1.37616e-50	0.8813559322033898	0.10122878876535986	2	19	NODE_2_length_515175_cov_42.281490	42893	63091	-
17128-2021-B	18_11	cn_29073_ISBpu1	inferred	Composite transposon	29073	42.28149	4.81144e-70	0.9438202247191011	0.10415447630193095	0	10	NODE_2_length_515175_cov_42.281490	84966	114039	-
17128-2021-B	19_11_13	cn_42749_ISBpu1	inferred	Composite transposon	42749	42.28149	4.81144e-70	0.9438202247191011	0.10415447630193095	0	10	NODE_2_length_515175_cov_42.281490	113861	156610	-
17128-2021-B	20_15	cn_49166_ISBpu1	inferred	Composite transposon	49166	42.28149	1.06383e-51	0.8895348837209303	0.10064365125804564	0	19	NODE_2_length_515175_cov_42.281490	362242	411408	-
17128-2021-B	21_14_15	cn_23795_ISBpu1	inferred	Composite transposon	23795	42.28149	1.06383e-51	0.8895348837209303	0.10064365125804564	0	19	NODE_2_length_515175_cov_42.281490	411236	435031	-
17128-2021-B	19_17_18	cn_35912_ISBpu1	inferred	Composite transposon	35912	37.093035	6.16862e-59	0.9096045197740112	0.10298420128730251	1	15	NODE_3_length_503419_cov_37.093035	424128	460040	-
17128-2021-B	26_24_25	cn_15071_ISBpu1	inferred	Composite transposon	15071	58.893172	3.88473e-65	0.936046511627907	0.10064365125804564	0	11	NODE_4_length_413023_cov_58.893172	60380	75451	-
17128-2021-B	27_19	cn_2419_ISBpu1	inferred	Composite transposon	2419	58.893172	1.80739e-63	0.9213483146067416	0.10415447630193095	0	14	NODE_4_length_413023_cov_58.893172	274057	276476	-
17128-2021-B	28_22	cn_12567_ISBpu1	inferred	Composite transposon	12567	58.893172	1.38735e-69	0.9441340782122905	0.10122878876535986	3	7	NODE_4_length_413023_cov_58.893172	276334	288901	-
17128-2021-B	29_20_22	cn_47715_ISBpu1	inferred	Composite transposon	47715	58.893172	1.38735e-69	0.9441340782122905	0.10122878876535986	3	7	NODE_4_length_413023_cov_58.893172	288722	336437	-
17128-2021-B	30_20	cn_11749_ISBpu1	inferred	Composite transposon	11749	58.893172	3.06749e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_4_length_413023_cov_58.893172	336259	348008	-
17128-2021-B	31_23	cn_17954_ISBpu1	inferred	Composite transposon	17954	58.893172	6.54667e-58	0.9085714285714286	0.10122878876535986	1	15	NODE_4_length_413023_cov_58.893172	347832	365786	-
17128-2021-B	31_29_30	cn_21963_ISBpu1	inferred	Composite transposon	21963	69.696406	2.21044e-49	0.8764044943820225	0.10122878876535986	3	19	NODE_6_length_177858_cov_69.696406	49142	71105	-
17128-2021-B	33_32	cn_32362_ISBpu1	inferred	Composite transposon	32362	61.595351	9.45931e-53	0.8908045977011494	0.10005851375073142	2	17	NODE_7_length_164756_cov_61.595351	117893	150255	-
17128-2021-B	34_31	cn_10684_ISBpu1	inferred	Composite transposon	10684	61.595351	4.33916e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_7_length_164756_cov_61.595351	150110	160794	-
17128-2021-B	36_34	cn_7753_ISBpu1	inferred	Composite transposon	7753	50.227924	1.18236e-55	0.8950276243093923	0.10005851375073142	5	14	NODE_10_length_123949_cov_50.227924	16078	23831	-
17128-2021-B	38_36_37	cn_31353_ISBpu1	inferred	Composite transposon	31353	67.371739	6.09287e-52	0.8850574712643678	0.10181392627267408	0	20	NODE_12_length_82042_cov_67.371739	28118	59471	-
17129-2021-B	10	MITEBth3	alignment to reference	Miniature Inverted Repeat	52	15.436213	1.68535e-11	0.9230769230769231	0.16938110749185667	0	4	NODE_64_length_16596_cov_15.436213	316	367	M52
17129-2021-B	14	MITEPlu5	alignment to reference	Miniature Inverted Repeat	123	0.722222	3.38321e-9	0.7560975609756098	1	0	30	NODE_274_length_451_cov_0.722222	62	184	M123
17129-2021-B	3	ISBpu1	alignment to reference	Insertion sequence	1265	19.476282	2.28801e-31	0.7496261748988641	0.3727325921591574	10	153	NODE_17_length_83269_cov_19.476282	48660	49924	M92 D1 M3 I1 M212 N431 M72 D1 M4 I1 M47 D1 M6 I1 M50 N189 M3 I1 M4 D1 M4 I1 M136 I1 M8
17129-2021-B	4	ISBpu1	alignment to reference	Insertion sequence	1265	19.476282	2.28801e-31	0.7496261748988641	0.3727325921591574	10	153	NODE_17_length_83269_cov_19.476282	33346	34610	M8 I1 M136 I1 M4 D1 M4 I1 M3 N189 M50 I1 M6 D1 M47 I1 M4 D1 M72 N431 M212 I1 M3 D1 M92
17129-2021-B	5_3_4	cn_16579_ISBpu1	inferred	Composite transposon	16579	19.476282	2.28801e-31	0.7496261748988641	0.3727325921591574	10	153	NODE_17_length_83269_cov_19.476282	33345	49924	-
17130-2021-B	2	ISBpu1	alignment to reference	Insertion sequence	180	57.562104	1.67206e-52	0.8839779005524863	0.10415447630193095	2	19	NODE_1_length_1047524_cov_57.562104	223690	223869	M146 I1 M30 D1 M3
17130-2021-B	3	ISBpu1	alignment to reference	Insertion sequence	176	57.562104	1.27442e-63	0.9265536723163842	0.10298420128730251	1	12	NODE_1_length_1047524_cov_57.562104	825942	826117	M61 I1 M115
17130-2021-B	4	ISBpu1	alignment to reference	Insertion sequence	177	57.562104	3.51839e-69	0.943502824858757	0.10356933879461673	0	10	NODE_1_length_1047524_cov_57.562104	85346	85522	M177
17130-2021-B	5	ISBpu1	alignment to reference	Insertion sequence	176	57.562104	5.9714e-57	0.9034090909090909	0.10298420128730251	0	17	NODE_1_length_1047524_cov_57.562104	790204	790379	M176
17130-2021-B	6	ISBpu1	alignment to reference	Insertion sequence	177	57.562104	2.75864e-60	0.9152542372881356	0.1023990637799883	1	14	NODE_1_length_1047524_cov_57.562104	838706	838882	M96 D1 M80

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Table S1. *Continued*

Isolate No	mge_no	name	prediction method	type	allele_ len	depth	e_value	identity	coverage	gaps	sub-stitu-tion	contig	start	stop	cigar
17130-2021-B	7	ISBpu1	alignment to reference	Insertion sequence	176	57.562104	2.75864e-60	0.9147727272727273	0.10298420128730251	0	15	NODE_1_length_1047524_cov_57.562104	45231	45406	M176
17130-2021-B	8	ISBpu1	alignment to reference	Insertion sequence	176	57.562104	1.00632e-49	0.8806818181818182	0.10181392627267408	1	20	NODE_1_length_1047524_cov_57.562104	243232	243407	M59 D1 M116
17130-2021-B	9	ISBpu1	alignment to reference	Insertion sequence	175	57.562104	2.1026e-71	0.9542857142857143	0.1023990637799883	0	8	NODE_1_length_1047524_cov_57.562104	358825	358999	M175
17130-2021-B	10	ISBpu1	alignment to reference	Insertion sequence	174	57.562104	3.61937e-49	0.8793103448275862	0.10181392627267408	0	21	NODE_1_length_1047524_cov_57.562104	666116	666289	M174
17130-2021-B	11	ISBpu1	alignment to reference	Insertion sequence	176	57.562104	2.13256e-61	0.9204545454545454	0.10064365125804564	2	12	NODE_1_length_1047524_cov_57.562104	682884	683059	M94 D1 M77 D1 M3
17130-2021-B	12	ISBpu1	alignment to reference	Insertion sequence	173	57.562104	1.30176e-48	0.8786127167630058	0.10122878876535986	0	21	NODE_1_length_1047524_cov_57.562104	300609	300781	M173
17130-2021-B	13	ISBpu1	alignment to reference	Insertion sequence	173	57.562104	2.73919e-65	0.9364161849710982	0.10122878876535986	0	11	NODE_1_length_1047524_cov_57.562104	731115	731287	M173
17130-2021-B	14	ISBpu1	alignment to reference	Insertion sequence	171	57.562104	3.61937e-49	0.8837209302325582	0.10005851375073142	1	19	NODE_1_length_1047524_cov_57.562104	642491	642661	M162 I1 M9
17130-2021-B	15	ISBpu1	alignment to reference	Insertion sequence	194	94.445261	7.01538e-71	0.9238578680203046	0.11351667641895846	3	12	NODE_2_length_971770_cov_94.445261	413834	414027	M176 I1 M1 I1 M5 I1 M12
17130-2021-B	16	ISBpu1	alignment to reference	Insertion sequence	184	94.445261	7.21674e-51	0.875	0.1053247513165594	2	21	NODE_2_length_971770_cov_94.445261	331279	331462	M83 D1 M39 D1 M60
17130-2021-B	17	ISBpu1	alignment to reference	Insertion sequence	183	94.445261	1.18226e-63	0.9180327868852459	0.10590988882387362	1	14	NODE_2_length_971770_cov_94.445261	601950	602132	M14 D1 M168
17130-2021-B	18	ISBpu1	alignment to reference	Insertion sequence	178	94.445261	4.25215e-63	0.9213483146067416	0.10415447630193095	0	14	NODE_2_length_971770_cov_94.445261	143680	143857	M178
17130-2021-B	19	ISBpu1	alignment to reference	Insertion sequence	178	94.445261	7.21674e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_2_length_971770_cov_94.445261	81410	81587	M67 D1 M110
17130-2021-B	20	ISBpu1	alignment to reference	Insertion sequence	177	94.445261	5.53955e-57	0.9039548022598871	0.1023990637799883	1	16	NODE_2_length_971770_cov_94.445261	459202	459378	M82 D1 M94
17130-2021-B	21	ISBpu1	alignment to reference	Insertion sequence	175	94.445261	4.28234e-58	0.9090909090909091	0.1023990637799883	1	15	NODE_2_length_971770_cov_94.445261	199795	199969	M3 I1 M172
17130-2021-B	22	ISBpu1	alignment to reference	Insertion sequence	176	94.445261	2.55914e-60	0.9147727272727273	0.10298420128730251	0	15	NODE_2_length_971770_cov_94.445261	45468	45643	M176
17130-2021-B	23	ISBpu1	alignment to reference	Insertion sequence	179	94.445261	1.51856e-67	0.9385474860335195	0.10122878876535986	3	8	NODE_2_length_971770_cov_94.445261	129014	129192	M126 D3 M50
17130-2021-B	24	ISBpu1	alignment to reference	Insertion sequence	174	94.445261	7.21674e-51	0.8850574712643678	0.10181392627267408	0	20	NODE_2_length_971770_cov_94.445261	495291	495464	M174
17130-2021-B	25	ISBpu1	alignment to reference	Insertion sequence	175	94.445261	7.06519e-66	0.9371428571428572	0.10122878876535986	1	10	NODE_2_length_971770_cov_94.445261	336555	336729	M80 D1 M94
17130-2021-B	26	ISBpu1	alignment to reference	Insertion sequence	176	58.424843	1.75353e-59	0.9261363636363636	0.10298420128730251	0	13	NODE_3_length_514696_cov_58.424843	300449	300624	M176
17130-2021-B	27	ISBpu1	alignment to reference	Insertion sequence	177	58.424843	2.89305e-67	0.9378531073446328	0.1023990637799883	1	10	NODE_3_length_514696_cov_58.424843	297393	297569	M50 D1 M126
17130-2021-B	28	ISBpu1	alignment to reference	Insertion sequence	175	58.424843	4.91008e-55	0.8971428571428571	0.1023990637799883	0	18	NODE_3_length_514696_cov_58.424843	426348	426522	M175
17130-2021-B	29	ISBpu1	alignment to reference	Insertion sequence	174	58.424843	2.93427e-57	0.9080459770114944	0.10064365125804564	1	15	NODE_3_length_514696_cov_58.424843	434756	434929	M79 D1 M94
17130-2021-B	30	ISBpu1	alignment to reference	Insertion sequence	176	98.995226	2.32936e-67	0.9375	0.10298420128730251	0	11	NODE_4_length_414418_cov_98.995226	167236	167411	M176
17130-2021-B	31	ISBpu1	alignment to reference	Insertion sequence	175	98.995226	8.43736e-62	0.92	0.1023990637799883	0	14	NODE_4_length_414418_cov_98.995226	104399	104573	M175
17130-2021-B	32	ISBpu1	alignment to reference	Insertion sequence	173	82.107606	6.47505e-52	0.8876404494382022	0.10122878876535986	5	15	NODE_5_length_313575_cov_82.107606	149736	149908	M4 I1 M84 I4 M85
17130-2021-B	33	ISBpu1	alignment to reference	Insertion sequence	177	82.107606	4.97024e-58	0.903954802259887	0.10356933879461673	0	17	NODE_5_length_313575_cov_82.107606	43278	43454	M177
17130-2021-B	34	ISBpu1	alignment to reference	Insertion sequence	176	82.107606	8.25833e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_5_length_313575_cov_82.107606	106211	106386	M176
17130-2021-B	35	ISBpu1	alignment to reference	Insertion sequence	181	69.028068	9.22651e-54	0.8895027624309392	0.10005851375073142	5	15	NODE_6_length_207872_cov_69.028068	170840	171020	M116 D5 M60
17130-2021-B	36	ISBpu1	alignment to reference	Insertion sequence	176	69.028068	1.15204e-77	0.9715909090909091	0.10298420128730251	0	5	NODE_6_length_207872_cov_69.028068	176784	176959	M176
17130-2021-B	37	ISBpu1	alignment to reference	Insertion sequence	172	69.028068	5.47491e-61	0.92	0.10064365125804564	3	11	NODE_6_length_207872_cov_69.028068	174676	174847	M123 I3 M49
17130-2021-B	38	ISBpu1	alignment to reference	Insertion sequence	177	76.222994	1.21627e-64	0.9269662921348316	0.10122878876535986	3	10	NODE_7_length_99977_cov_76.222994	9830	10006	M50 D1 M64 I1 M2 D1 M59
17130-2021-B	39	ISBpu1	alignment to reference	Insertion sequence	183	113.192046	1.05392e-58	0.8972972972972973	0.10356933879461673	5	14	NODE_10_length_18508_cov_113.192046	8375	8557	M3 D1 M3 I1 M76 D1 M54 I1 M3 D1 M41
17130-2021-B	15_7	cn_7758_ISBpu1	inferred	Composite transposon	7758	57.562104	2.75864e-60	0.9147727272727273	0.10298420128730251	0	15	NODE_1_length_1047524_cov_57.562104	37648	45406	-
17130-2021-B	16_4_7	cn_40292_ISBpu1	inferred	Composite transposon	40292	57.562104	2.75864e-60	0.9147727272727273	0.10298420128730251	0	15	NODE_1_length_1047524_cov_57.562104	45230	85522	-
17130-2021-B	17_2_8	cn_19718_ISBpu1	inferred	Composite transposon	19718	57.562104	1.67206e-52	0.8839779005524863	0.10415447630193095	2	19	NODE_1_length_1047524_cov_57.562104	223689	243407	-
17130-2021-B	18_8	cn_44209_ISBpu1	inferred	Composite transposon	44209	57.562104	1.00632e-49	0.8806818181818182	0.10181392627267408	1	20	NODE_1_length_1047524_cov_57.562104	243231	287440	-
17130-2021-B	19_12	cn_13458_ISBpu1	inferred	Composite transposon	13458	57.562104	1.30176e-48	0.8786127167630058	0.10122878876535986	0	21	NODE_1_length_1047524_cov_57.562104	287323	300781	-
17130-2021-B	20_14	cn_52212_ISBpu1	inferred	Composite transposon	52212	57.562104	3.61937e-49	0.8837209302325582	0.10005851375073142	1	19	NODE_1_length_1047524_cov_57.562104	590449	642661	-
17130-2021-B	21_10_14	cn_23799_ISBpu1	inferred	Composite transposon	23799	57.562104	3.61937e-49	0.8837209302325582	0.10005851375073142	1	19	NODE_1_length_1047524_cov_57.562104	642490	666289	-
17130-2021-B	22_10_11	cn_16944_ISBpu1	inferred	Composite transposon	16944	57.562104	3.61937e-49	0.8793103448275862	0.10181392627267408	0	21	NODE_1_length_1047524_cov_57.562104	666115	683059	-
17130-2021-B	23_11_13	cn_48404_ISBpu1	inferred	Composite transposon	48404	57.562104	2.13256e-61	0.9204545454545454	0.10064365125804564	2	12	NODE_1_length_1047524_cov_57.562104	682883	731287	-
17130-2021-B	1	ISBpu1	alignment to reference	Insertion sequence	1683	57.562104	4.81634e-28	0.7955732371040425	0.1860737273259216	17	52	NODE_1_length_1047524_cov_57.562104	752678	754360	M33 D1 M49 D1 M5 N1359 M8 I1 M88 D1 M7 I2 M3 I2 M2 I3 M1 I5 M7 I1 M118
17130-2021-B	24_1_13	cn_23246_ISBpu1	inferred	Composite transposon	23246	57.562104	2.73919e-65	0.9364161849710982	0.10122878876535986	0	11	NODE_1_length_1047524_cov_57.562104	731114	754360	-
17130-2021-B	25_1_5	cn_37702_ISBpu1	inferred	Composite transposon	37702	57.562104	4.81634e-28	0.7955732371040425	0.1860737273259216	17	52	NODE_1_length_1047524_cov_57.562104	752677	790379	-
17130-2021-B	26_3_5	cn_35914_ISBpu1	inferred	Composite transposon	35914	57.562104	5.9714e-57	0.9034090909090909	0.10298420128730251	0	17	NODE_1_length_1047524_cov_57.562104	790203	826117	-
17130-2021-B	27_3_6	cn_12941_ISBpu1	inferred	Composite transposon	12941	57.562104	1.27442e-63	0.9265536723163842	0.10298420128730251	1	12	NODE_1_length_1047524_cov_57.562104	825941	838882	-
17130-2021-B	26_22	cn_24546_ISBpu1	inferred	Composite transposon	24546	94.445261	2.55914e-60	0.9147727272727273	0.10298420128730251	0	15	NODE_2_length_971770_cov_94.445261	45467	70013	-

Continued

Table S1. *Continued*

Isolate No	mge_no	name	prediction method	type	allele_ len	depth	e_value	identity	coverage	gaps	sub-stitu-tion	contig	start	stop	cigar
17130-2021-B	27_19	cn_11743_ISBpu1	inferred	Composite transposon	11743	94.445261	7.21674e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_2_length_971770_cov_94.445261	69844	81587	-
17130-2021-B	28_19	cn_30875_ISBpu1	inferred	Composite transposon	30875	94.445261	7.21674e-51	0.8820224719101125	0.10298420128730251	1	20	NODE_2_length_971770_cov_94.445261	81409	112284	-
17130-2021-B	29_23	cn_17037_ISBpu1	inferred	Composite transposon	17037	94.445261	1.51856e-67	0.9385474860335195	0.10122878876535986	3	8	NODE_2_length_971770_cov_94.445261	112155	129192	-
17130-2021-B	30_23	cn_12567_ISBpu1	inferred	Composite transposon	12567	94.445261	1.51856e-67	0.9385474860335195	0.10122878876535986	3	8	NODE_2_length_971770_cov_94.445261	129013	141580	-
17130-2021-B	31_18	cn_2419_ISBpu1	inferred	Composite transposon	2419	94.445261	4.25215e-63	0.9213483146067416	0.10415447630193095	0	14	NODE_2_length_971770_cov_94.445261	141438	143857	-
17130-2021-B	32_16_25	cn_5451_ISBpu1	inferred	Composite transposon	5451	94.445261	7.21674e-51	0.875	0.1053247513165594	2	21	NODE_2_length_971770_cov_94.445261	331278	336729	-
17130-2021-B	33_15_20	cn_45545_ISBpu1	inferred	Composite transposon	45545	94.445261	7.01538e-71	0.9238578680203046	0.11351667641895846	3	12	NODE_2_length_971770_cov_94.445261	413833	459378	-
17130-2021-B	34_20_24	cn_36263_ISBpu1	inferred	Composite transposon	36263	94.445261	5.53955e-57	0.9039548022598871	0.1023990637799883	1	16	NODE_2_length_971770_cov_94.445261	459201	495464	-
17130-2021-B	30_26_27	cn_3232_ISBpu1	inferred	Composite transposon	3232	58.424843	2.89305e-67	0.9378531073446328	0.1023990637799883	1	10	NODE_3_length_514696_cov_58.424843	297392	300624	-
17130-2021-B	31_26	cn_47065_ISBpu1	inferred	Composite transposon	47065	58.424843	1.75353e-59	0.9261363636363636	0.10298420128730251	0	13	NODE_3_length_514696_cov_58.424843	300448	347513	-
17130-2021-B	32_28	cn_2420_ISBpu1	inferred	Composite transposon	2420	58.424843	4.91008e-55	0.8971428571428571	0.1023990637799883	0	18	NODE_3_length_514696_cov_58.424843	424102	426522	-
17130-2021-B	33_28_29	cn_8582_ISBpu1	inferred	Composite transposon	8582	58.424843	4.91008e-55	0.8971428571428571	0.1023990637799883	0	18	NODE_3_length_514696_cov_58.424843	426347	434929	-
17130-2021-B	34_29	cn_17875_ISBpu1	inferred	Composite transposon	17875	58.424843	2.93427e-57	0.9080459770114944	0.10064365125804564	1	15	NODE_3_length_514696_cov_58.424843	434755	452630	-
17130-2021-B	32_31	cn_13721_ISBpu1	inferred	Composite transposon	13721	98.995226	8.43736e-62	0.92	0.1023990637799883	0	14	NODE_4_length_414418_cov_98.995226	90852	104573	-
17130-2021-B	35_33	cn_37832_ISBpu1	inferred	Composite transposon	37832	82.107606	4.97024e-58	0.903954802259887	0.10356933879461673	0	17	NODE_5_length_313575_cov_82.107606	5622	43454	-
17130-2021-B	36_34	cn_10680_ISBpu1	inferred	Composite transposon	10680	82.107606	8.25833e-61	0.9147727272727273	0.10298420128730251	0	15	NODE_5_length_313575_cov_82.107606	106210	116890	-
17130-2021-B	37_32	cn_33164_ISBpu1	inferred	Composite transposon	33164	82.107606	6.47505e-52	0.8876404494382022	0.10122878876535986	5	15	NODE_5_length_313575_cov_82.107606	116744	149908	-
17130-2021-B	38_35_37	cn_4008_ISBpu1	inferred	Composite transposon	4008	69.028068	9.22651e-54	0.8895027624309392	0.10005851375073142	5	15	NODE_6_length_207872_cov_69.028068	170839	174847	-
17130-2021-B	39_36_37	cn_2284_ISBpu1	inferred	Composite transposon	2284	69.028068	5.47491e-61	0.92	0.10064365125804564	3	11	NODE_6_length_207872_cov_69.028068	174675	176959	-
17130-2021-B	39_38	cn_27682_ISBpu1	inferred	Composite transposon	27682	76.222994	1.21627e-64	0.9269662921348316	0.10122878876535986	3	10	NODE_7_length_99977_cov_76.222994	9829	37511	-
Sample 1-29, B. cereus	2	ISDha13	alignment to reference	Insertion sequence	76	16.909556	2.42635e-7	0.8157894736842105	0.10201342281879194	0	14	NODE_20_length_110262_cov_16.909556	110041	110116	M76
Sample 1-29, B. cereus	3	ISBpu1	alignment to reference	Insertion sequence	181	7.898012	7.5933e-42	0.846153846153846	0.10356933879461673	3	25	NODE_23_length_100815_cov_7.898012	33705	33885	M117 D1 M18 I1 M3 D1 M41
Sample 1-29, B. cereus	4	ISBpu1	alignment to reference	Insertion sequence	177	13.700656	9.06505e-40	0.8426966292134831	0.10122878876535986	3	25	NODE_43_length_71924_cov_13.700656	51224	51400	M113 D1 M18 I1 M3 D1 M41
Sample 1-29, B. cereus	7	ISDha13	alignment to reference	Insertion sequence	76	15.766531	4.78289e-8	0.8157894736842105	0.10201342281879194	0	14	NODE_153_length_21753_cov_15.766531	21628	21703	M76
Sample 1-29, B. cereus	8	ISDha13	alignment to reference	Insertion sequence	76	18.542686	3.57856e-8	0.8157894736842105	0.10201342281879194	0	14	NODE_176_length_16280_cov_18.542686	49	124	M76
Sample 1-29, B. cereus	9	ISDha13	alignment to reference	Insertion sequence	76	17.308718	2.6815e-8	0.8157894736842105	0.10201342281879194	0	14	NODE_199_length_12206_cov_17.308718	12083	12158	M76
Sample 1-29, B. cereus	13	ISBpu1	alignment to reference	Insertion sequence	178	0.875887	2.86227e-54	0.8820224719101125	0.10298420128730251	1	20	NODE_376_length_409_cov_0.875887	78	255	M110 D1 M67
20313-2023-B	none														
20308-2023-B	8	ISBce19	alignment to reference	Insertion sequence	1433		0	0.9502074688796681	0.9903114186851211	14	58	assembly_contig_17 length 125534 coverage 146.2 normalized_cov 1.07	89737	91169	M1125 I1 M175 I4 M1 I8 M91 D1 M40
20308-2023-B	25	ISBce3	alignment to reference	Insertion sequence	1805		0	0.9905817174515236	1	0	17	assembly_contig_82 length 1811 coverage 142.5 normalized_cov 1.04	2	1806	M1805
20310-2023-B	none														
20307-2023-B	none														
20345-2023-B	none														
20312-2023-B	none														