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IDENTIFICATION OF ENDOSPORE-FORMING BACTERIA ISOLATED FROM WEANING DRIED FOODS CONSUMED IN TAIZ CITY, REPUBLIC OF YEMEN

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ABSTRACT:

The present work was carried out on 64 random weaning dried food samples collected from local markets and pharmacies in Taiz city, Republic of Yemen. These samples included 24 commercial (CWF) and traditional (TWF) weaning food samples, in addition to 40 cereals and legumes samples comprised the ingredients of traditional weaning dried foods. The total bacterial isolates resulting from identification of mesophilic aerobic endospore-forming bacteria were 77 isolates. These isolates represent about 40.10% of studied samples comprised 15 isolates from the CWF (41.7%), 18 isolates from the TWF (50%) and 44 isolates (36.67%) from cereals and legumes samples. Ten *Bacillus* species and one species of *Brevibacillus*, *Paenibacillus* and *Virgibacillus* were identified.

Key words: Endospore-forming bacteria*, *Bacillus species*, *weaning food, cereals, legumes.

1. INTRODUCTION

Weaning foods include commercial and traditional weaning foods products. Commercial weaning foods are easy to prepare, hygienic provided it is packaged, but expensive and not available everywhere locally. However, traditional weaning foods are cheaper, always available locally (Castel & Wijngaart, 2005). Weaning foods made from ingredients do not differ from these for adult foods so that the same types and levels of microorganisms would occur on these ingredients naturally (ACMSF, 2006). Soil, water, air, dust, insects, rodents, birds, animals, humans, storage and shipping containers, and handling and processing equipment are the most important sources of the contaminated cereal grains (Bullerman & Bianchini, 2009). The most important factors associated with contamination of weaning foods are the preparation of food several hours prior of consumption, inadequate storage conditions, and insufficient cooking or reheating of stored food before feeding (WHO, 1984). Traditional weaning foods in Yemen are cereals-legumes blending, mostly composed of wheat, maize, barley, red and white sorghum, millet and rice blending in equal quantities, besides adequate amount of one this legumes: black or red lentil or both and sometimes fewer quantities of beans. Traditional weaning foods in Yemen are cereals-legumes blending, usually prepared as composite soft gruels and sometimes baked as cookies. Many literatures pointed out that the most commonly bacteria found surviving on weaning foods and cereals, the major components of weaning foods, and their flours are species of the spore-former *Bacillus* (Barrell & Rowland, 1980; Singh *et al.*, 1980; Michanie *et al.*, 1987; Becker *et al.*, 1994; Afifi *et al.*, 1998; Ikah *et al.*, 2001; Sheth & Arora, 2001; Berghofer *et al.*,

2003; Amusa *et al.*, 2005; Badau, 2006; Ifediora *et al.*, 2006; Aydin *et al.*, 2009; Abanno *et al.*, 2012; Adebayo-Tayo *et al.*, 2012; Nwogwugwu *et al.*, 2012; Bintu *et al.*, 2015; Degaga *et al.*, 2015 and Nworie *et al.*, 2016).

This work was carried out to identify *Bacillus* spp. isolated from tested samples in an attempt to shed light upon the microbial status of traditionally weaning dried foods consumed in Taiz City, Republic of Yemen.

2. MATERIALS AND METHODS

2.1. Samples collection

A total of 64 random weaning dried food samples (commercial, traditional and it's ingredients) were collected from local markets and pharmacies in Taiz city. These samples including 12 commercially weaning food samples, 12 traditionally weaning food samples and 40 cereal and legume samples (wheat, barley, corn, rice, millet, red sorghum, white sorghum, black lentil, red lentil and bean) as a total 4 samples from each kind of cereal and legume. Each sample was put in sterile polyethylene bag and transferred to the laboratory, where they were prepared for the microbiological examination.

2.2. Samples preparation

Twenty five grams of each sample were added to 225 ml of peptone water. Decimal dilution was prepared and spread on the appropriate plates (Harrigan & McCance, 1976).

2.3. Isolation and identification of mesophilic aerobic endospore forming bacteria:

Mesophilic aerobic endospore forming bacterial count were determined according to method described by Priest (1989) as follow: the suitable dilution was subjected to 85^{o}C at 10 min and cold at 45^{o}C. After that,1 ml of the dilution was transferred to a petri dish with tryptone glucose extract (TGE) agar and incubated for 48-72hr. The second step was subculture on slant agar for identification process as mentioned by Priest and Alexander (1988).

The PIBWin (Probabilistic Identification of Bacteria for Windows) program software was applied to provides probabilistic identification of unknown bacterial isolates against identification matrices of known strains (Bryant, 2004) (Figure no.1), and data matrix described by Priest and Alexander (1988) (Figure no.2), were used for identification of mesophilic aerobic endospore-forming bacteria isolates.

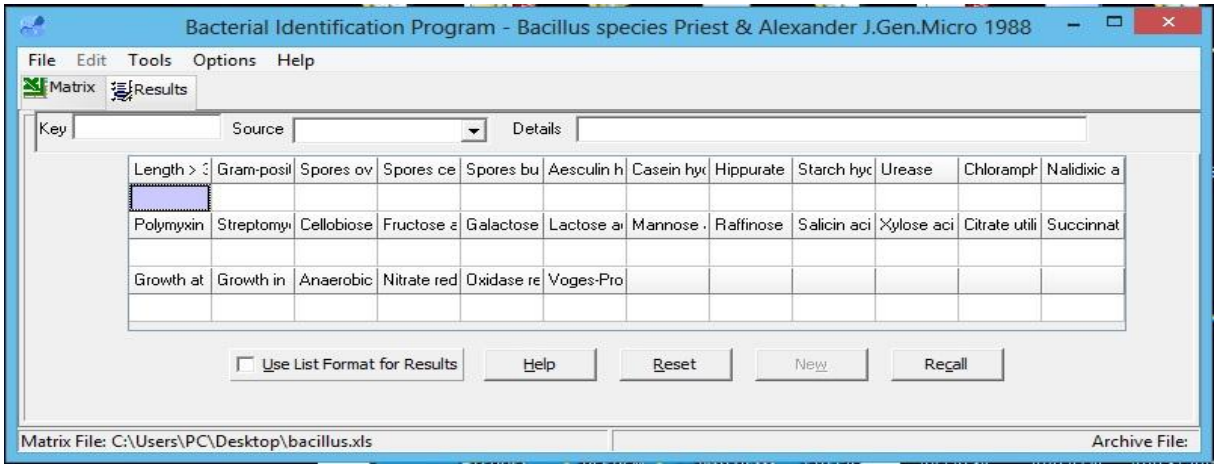
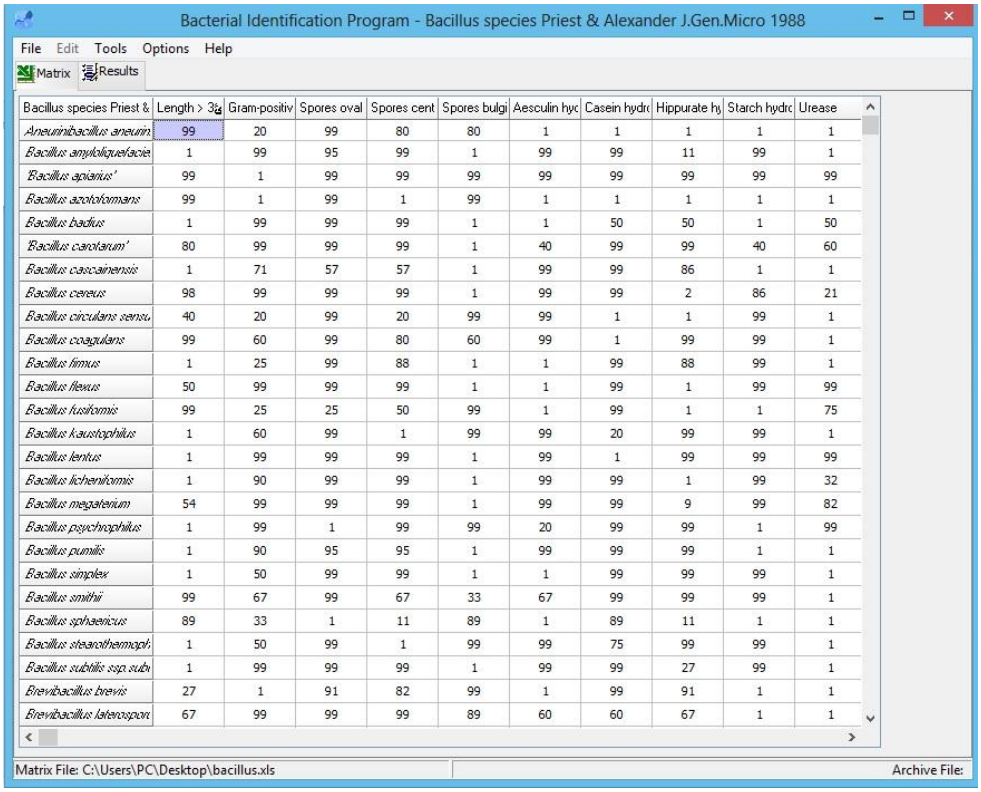


Figure 1: The PIBWin program software that provides probabilistic identification of unknown bacterial isolates against identification matrices of known strains.



Bacillus species Priest &	Length > 3µ	Gram-positiv	Spores oval	Spores cent	Spores bulgi	Aesculin hyc	Casein hydr	Hippurate hy	Starch hydr	Urease
Aneurinibacillus aneurin	99	20	99	80	80	1	1	1	1	1
Bacillus amyloliquefacie	1	99	95	99	1	99	99	11	99	1
'Bacillus apiarius'	99	1	99	99	99	99	99	99	99	99
Bacillus azotoformans	99	1	99	1	99	1	1	1	1	1
Bacillus badius	1	99	99	99	1	1	50	50	1	50
'Bacillus canotarum'	80	99	99	99	1	40	99	99	40	60
Bacillus cascainensis	1	71	57	57	1	99	99	86	1	1
Bacillus cereus	98	99	99	99	1	99	99	2	86	21
Bacillus circulans sensu	40	20	99	20	99	99	1	1	99	1
Bacillus coagulans	99	60	99	80	60	99	1	99	99	1
Bacillus firmus	1	25	99	88	1	1	99	88	99	1
Bacillus flexus	50	99	99	99	1	1	99	1	99	99
Bacillus fusiformis	99	25	25	50	99	1	99	1	1	75
Bacillus kaustophilus	1	60	99	1	99	99	20	99	99	1
Bacillus lentus	1	99	99	99	1	99	1	99	99	99
Bacillus licheniformis	1	90	99	99	1	99	99	1	99	32
Bacillus megaterium	54	99	99	99	1	99	99	9	99	82
Bacillus psychrophilus	1	99	1	99	99	20	99	99	1	99
Bacillus pumilis	1	90	95	95	1	99	99	99	1	1
Bacillus simplex	1	50	99	99	1	1	99	99	99	1
Bacillus smithii	99	67	99	67	33	67	99	99	99	1
Bacillus sphaericus	89	33	1	11	89	1	89	11	1	1
Bacillus stearothermoph	1	50	99	1	99	99	75	99	99	1
Bacillus subtilis ssp.subt	1	99	99	99	1	99	99	27	99	1
Brevibacillus brevis	27	1	91	82	99	1	99	91	1	1
Brevibacillus laterosporu	67	99	99	99	89	60	60	67	1	1

Figure 2: The data matrix described by Priest and Alexander (1988) used for identification of mesophilic aerobic endospore-forming bacteria isolates.

3. RESULTS AND DISCUSSION

The average of mesophilic aerobic endospore forming bacterial counts were 2.44 ± 0.57 and 2.35 ± 0.46 $\log_{10}$ CFU g^{-1} for TWF and CWF, respectively. Such results are in general coinciding with those reported by Ikah *et al.* (2001); Ifediora *et al.* (2006) and Bintu *et al.* (2015).

The total mesophilic aerobic endospore forming bacterial counts in cereals and legumes were 2.26 ± 0.48 $\log_{10}$ CFU g^{-1} that varied from 2.73 ± 0.92 $\log_{10}$ CFU g^{-1} in rice to 1.87 ± 0.27 $\log_{10}$ CFU g^{-1} in red sorghum samples. Berghofer *et al.* (2003) reported that the mesophilic aerobic spore counts were 10 and 1 CFU/g for Australian wheat and flour, respectively. These differences can be attributed to the differences in the storage conditions of these crops. The data presented in Tables No.1 & No.2 showed the results of mesophilic aerobic endospore-forming bacteria isolates identification by the PIBWin program software. Ten *Bacillus* species and one species of *Brevibacillus*, *Paenibacillus* and *Virgibacillus* in addition to three unnamed taxa *i.e.* Taxon 18, Taxon 27 and Taxon 28 were recorded from these investigation samples. A total of 77 isolates, 38 isolates of *Bacillus* spp., 10 isolates of *Brevibacillus laterosporus* (formerly *Bacillus laterosporus)*,7 isolates of *Virgibacillus pantothenticus* (formerly *Bacillus pantothenticus)*, 2 isolates of *Paenibacillus alvei* (formerly *Bacillus alvei)* and 20 isolates of unnamed taxa (3 of Taxon 18, 15 of Taxon 27 and 2 of Taxon 28) were identified in our study in 40.10% of studied samples comprising 15 isolates from CWF (41.7% of CWF total), 18 isolate from TWF (50% of TWF total) and 44 isolate (36.67%) from cereal & legume samples. Similar trend of results reported for isolation of *Bacillus* from different weaning food samples as these reported by Ikah *et al.* (2001); Ifediora *et*

_al_. (2006); Abanno _et al_. (2012); Nwogwugwu _et al_. (2012); Bintu _et al_. (2015); Degaga _et al_. (2015) and Nworie _et al_. (2016).

In depending on the number of isolate cases, _B. coagulans_ was the most frequently species, it was isolated from 21.9% of the total samples comprising 41.7% of the TWF samples (in samples No.T2a, T2b, T3, T4a, $T4_b$, T6, T12) and 22.5% of cereal samples in samples No.2 (wheat), No.10 and 12 (barley), No.15 (millet), No.26 (rice), No.29 (red lentil), No.33a, 33b, 34 and 35 (black lentil). This species was not reported from CWF samples. Such results are in good accord with Badau (2006) who isolated _Bacillus coagulans_ from pearl millet. The second rank in the number of isolation cases was occupied by Taxon 27 that occurred in 18.8% of samples contributing 16.7% of CWF samples (samples No.C8 & C9), 41.7% of TWF samples (samples No. T4a, $T4_b$, T7, T8, T9 & T11) and 12.5% of cereal samples in samples No.7 (maize), No.12 (barley), No.17a,17b,18a and 18b (white sorghum) and No.26 (red sorghum).

***Bacillus cereus* and *Brevibacillus laterosporus* came behind Taxon 27 in the number of cases of isolation. *B. cereus* was recorded in samples Nos. C4, C9, C10, C11 and C12 from the CWF samples (41.7%), also in samples No.4 (wheat), No.11 (barley), No.20 (white sorghum), No.21b (red sorghum) and No.29 (red lentil) in 12.5% of cereal samples but was not recorded in the TWF samples. Some studies mentioned to isolation of *B. cereus* such as those reported by Barrell & Rowland (1980); Singh _et al_. (1980); Michanie _et al_. (1987); Sheth & Arora (2001) and Amusa _et al_. (2005) from the weaning food samples, as well as Berghofer _et al_. (2003) and Aydin _et al_. (2009) from wheat; Badau (2006) from pearl millet.**

***Brevibacillus laterosporus* was isolated from one sample (8.3%) of the CWF samples, 16.7% of the TWF samples in samples Nos.T6a, T6b & T12 and from 15% of cereal samples in samples No.2 (wheat), No.15 (millet), No.18 (white sorghum), No.30 (red lentil), No.34 (black lentil) and No.37 (bean). The next species was *Virgibacillus pantothenticus* that isolated from 10.9% of samples comprising 16.7% of CWF samples in samples Nos.C9 and C11, also from 12.5% of cereal samples in samples No.1 (wheat), 14 (millet), 18 (white sorghum), 28 (rice), and 35 (black lentil). This kind of *Bacillus* species was not recorded in the TWF samples.**

***Bacillus amyloliquefaciens* was isolated from 9.4% of samples comprising 16.7% of the CWF samples in samples Nos.C1 & C2 and from 10% of the cereal samples in samples No.5 (maize), 11 (barley), 26 (rice) and 37 (bean).**

Taxon 18 was isolated from 4.5% of the three types of samples in sample No.C10 (CWF), T8 (TWF) and 13 (millet).

***Bacillus baduis*, *Paenibacillus alvei*, and Taxon 28 were isolated from 3.1% of samples. *B. baduis* was isolated from one sample of the CWF (sample No.C2) and one sample of cereal samples (sample No.26 in rice), also *Paenibacillus alvei* was isolated from one sample of CWF (sample No.C7) and one sample from cereal samples (sample No. 1 in wheat). Whereas, Taxon 28 was isolated from two samples of cereal (samples No.13 & No.21a in millet and red sorghum, respectively).**

The remaining species were isolated one time from one sample, *B. pumils* was recorded from TWF in sample No.T12, *B. subtilis* ssp. *Subtilis* from wheat in sample No.4, and *B. stearothermophilus* from red lentil in sample No.30. Amusa _et al_. (2005); Adebayo-Tayo _et al_. (2012) and Bintu _et al_. (2015) reported isolation of *B. subtilis* from weaning foods samples. As well as millet (Badau, 2006).

Table 1: Mesophilic Aerobic Endospore-forming Bacteria Isolates recorded from the Investigated Samples Identified by PIBWin program software.

Isolate code	Identification	ID score
BC1	*Bacillus amyloliquefaciens*	93.13%
BC2a	*B. baduis*	99.95%
BC2b	*B. amyloliquefaciens*	99.49%
BC4	*B. cereus*	97.73%
BC7a	*Paenibacillus alvei*	98.03%
BC7b	*Brevibacillus laterosporus*	99.98%
BC8	Taxon 27	99.90%
BC9a	*Virgibacillus pantothenticus*	99.49%
BC9b	*B. cereus*	96.48%
BC9c	Taxon 27	90.62%
BC10a	Taxon 18	99.03%
BC10b	*B. cereus*	99.23%
BC11a	*Virgibacillus pantothenticus*	99.94%
BC11b	*B. cereus*	97.92%
BC12	*B. cereus*	92.91%
BT2a	*B. coagulans*	92.80%
BT2b	*B. coagulans*	99.50%
BT3	*B. coagulans*	65.36%
BT4a	Taxon 27	95.72%
BT4b	Taxon 27	95.72%
BT5a	*B. coagulans*	99.91%
BT5b	*B. coagulans*	99.10%
BT6a	*Brevibacillus laterosporus*	90.44%
BT6b	*Brevibacillus laterosporus*	94.82%
BT6c	*B. coagulans*	99.87%
BT7	Taxon 27	97.08%
BT8a	Taxon 18	98.33%
BT8b	Taxon 27	97.12%
BT9	Taxon 27	97.81%
BT11	Taxon 27	97.08%
BT12a	*B.pumils*	96.75%
BT12b	*B. coagulans*	99.95%
BT12c	*Brevibacillus laterosporus*	92.72%
B1a	*Paenibacillus alvei*	99.65%
B1b	*Virgibacillus pantothenticus*	99.84%
B2a	*Brevibacillus laterosporus*	99.92%
B2b	*B. coagulans*	99.32%
B4a	*B. subtilis* ssp.*Subtilis*	97.25%
B4b	*B. cereus*	98.58%
B5	*B. amyloliquefaciens*	99.78%
B7	Taxon 27	98.90%
B10	*B. coagulans*	99.62%
B11a	*B. amyloliquefaciens*	96.30%
B11b	*B. cereus*	99.58%
B12a	*B. coagulans*	92.43%
B12b	Taxon 27	98.09%
B13a	Taxon 18	46.34%
B13b	Taxon 28	43.04%
B14	*Virgibacillus pantothenticus*	99.20%
B15a	*Brevibacillus laterosporus*	98.16%

B15b	***B. coagulans***	**98.04%**

Table 1: Continued:

Isolate code	Identification	ID score
B17a	**Taxon 27**	**97.02%**
B17b	**Taxon 27**	**94.92%**
B18a	***Brevibacillus laterosporus***	**92.24%**
B18b	***Virgibacillus pantothenticus***	**90.10%**
B18c	**Taxon 27**	**95.93%**
B18d	**Taxon 27**	**96.07%**
B20	***B. cereus***	**91.10%**
B21a	**Taxon 28**	**50.77%**
B21b	***B. cereus***	**97.73%**
B23	**Taxon 27**	**96.07%**
B26a	***B. amyloliquefaciens***	**98.94%**
B26b	***B. coagulans***	**99.91%**
B26c	***B. baduis***	**98.24%**
B28	***Virgibacillus pantothenticus***	**90.10%**
B29a	***B. coagulans***	**97.27%**
B29b	***B. cereus***	**96.77%**
B30a	***Brevibacillus laterosporus***	**93.09%**
B30b	***B. stearothermophilus***	**98.86%**
B33a	***B. coagulans***	**97.87%**
B33b	***B. coagulans***	**96.65%**
B34a	***B. coagulans***	**96.52%**
B34b	***Brevibacillus laterosporus***	**93.06%**
B35a	***Virgibacillus pantothenticus***	**99.98%**
B35b	***B. coagulans***	**98.03%**
B37a	***Brevibacillus laterosporus***	**90.58%**
B37b	***B. amyloliquefaciens***	**99.51%**

Table 2: Number of Cases of Isolation (NCI) of the Mesophilic Aerobic Endospore-forming Bacteria Recovered from Investigated Samples.

Bacterial species	NIC	Keys of samples contained that isolates
Bacillus amyloliquefaciens	**6**	**C1,C2, 5, 11,26,37**
B. baduis	**2**	**C2, 26**
B. cereus	**10**	**C4, C9, C10, C11, C12, 4, 11, 20, 21b, 29**
B. coagulans	**17**	**T2a, T2b,T3,T4a,T4b,T6,T12, 2,10, 12, 15, 26, 29, 33a, 33b, 34, 35**
B. pumils	**1**	**T12**
B. stearothermophilus	**1**	**30**
B. subtilis* ssp.*Subtilis	**1**	**4**
Brevibacillus laterosporus	**10**	**C7, T6a, T6b, T12, 2, 15, 10, 30, 34, 37**
Paenibacillus alvei	**2**	**C7, 1a**
Virgibacillus pantothenticus	**7**	**C9, C11, 1b, 14, 18, 28, 35**
Taxon 18	**3**	**C10, T8, 13**
Taxon 27	**15**	**C8, C9, T4a, T4b, T19, T8, T9, T11, 7, 12, 17a, 17b, 18a, 18b, 26**
Taxon 28	**2**	**13, 21a**
Total= 13	**77**	

4. CONCLUSION:

From these results, it could be concluded that the total bacterial isolates of mesophilic aerobic endospore-forming bacteria from investigated samples were 77 isolates, and that were occurred in 40.10% of studied samples comprised 15 isolates from CWF (41.7%), 18 isolates from TWF (50%) and 44 isolates (36.67%) from cereals and legumes samples. Ten Bacillus species and one species of *Brevibacillus*, *Paenibacillus* and *Virgibacillus* were identified. Consequently we recommended that the sufficient cooking for these foods is a critical point to reduce the microbial load to safe limit. As well as the important of preparation this kind of food under hygienic condition that include, hand washing, cleaned utensils, and pure water.

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تعريف البكتيريا المكونة للأبواغ الداخلية المعزولة من أغذية الفطام الجافة المستهلكة في مدينة تعز، الجمهورية اليمنية

فهد عبد الحميد الشرجبي وأماني مصطفى القدسي

قسم الميكروبيولوجي التطبيقي، كلية العلوم التطبيقية، جامعة تعز، تعز، الجمهورية اليمنية

الملخص العربي:

أجريت الدراسة على ٦٤ عينة من أغذية الفطام الجافة تم تجميعها من الأسواق المحلية والصيدليات في مدينة تعز بالجمهورية اليمنية. شملت تلك العينات ٢٤ عينة من أغذية الفطام التجارية وأغذية الفطام المحضرة بالطريقة التقليدية، أضافة إلى ٤٠ عينة من الحبوب والبقوليات التي تشكل المكونات الداخلة في إعداد أغذية الفطام المحضرة بالطريقة التقليدية. تم تعريف ٧٧ عزلة بكتيرية ناتجة من عزلات البكتيريا الهوائية المحبة لدرجة الحرارة المتوسطة المكونة للجراثيم الداخلية والتي ظهرت في ٤٠,١٠% من العينات المدروسة حيث شملت ١٥ عزلة من أغذية الفطام التجارية (٤١,٧% من إجمالي تلك العينات) و ١٨ عزلة من أغذية الفطام المحضرة تقليديا (٥٠% من إجمالي تلك العينات) و ٤٤عزلة من عينات الحبوب والبقوليات (٣٦,٦٧% من إجمالي تلك العينات). ولقد توزعت هذه الأنواع كالتالي: ١٠ أنواع تابعة لجنس *Bacillus* ونوع واحد من كلا من *Virgibacillus* و *Paenibacillus* و *Brevibacillus*.