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The Use of *Frankia* Spores As Inocula For *Casuarina equisetifolia* Plants

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ABSTRACT

Spores of four *Frankia-Casuarina* strains were tested for their ability to infect and fix atmospheric nitrogen in combination with *Casuarina equisetifolia* plants, after being stored for 3 and 6 months in different media. The media used were liquid cultures (BAP) and wet or dry polyacrylamide. The *Frankia* strains used were: UGL020601, UGL020604, HFPCcI3 and ORS021001. Inoculation with stored spore inocula showed reduction in total nitrogen content. The reduction ranged between 33 and 88% of freshly prepared spores-inoculated plants, depending on strain and the inoculum type. The wet gel-incorporated *Frankia* strains scored the best values within all treatments. In general, the reduction in plant total nitrogen was lower after 3 months than after 6 months of storage at 7°C for all treatments indicating better performance after short storage. The number of nodules decreased gradually with the increase in storage time for all strains and treatments. For all the tested strains, spores scored better values for root/shoot and nodules/plant ratios (i.e. lower and higher ratios) for all strains stored for 3 months, as wet gel, than for other treatments. Dry powdered gel may have an advantage of long "shelf- life" than the other treatments and may be used also as a preservation medium for large-scale inoculation with *Frankia*. In general, it is recommended to store dry or wet gel-immobilized *Frankia* spores in the refrigerator for up to 3 months for commercial purposes.
Key words: *Casuarina equisetifolia*, *Frankia,* Polyacrylamide gel, spores.

INTRODUCTION

Frankia, the nitrogen-fixing endosymbiont of many actinorhizal plants, is difficult to preserve and requires special nutrient formulations and culture maintenance conditions (Fontaine *et al.,* 1986; Sayed *et al.,* 2000; Hahn *et al.,* 2003). Infective and effective *Frankia* is often low or may be absent within the total population of some rhizospheric soils (Smolander and Sundman, 1987; Dawson *et al.,* 1989; Visser *et al.,* 1990; Kohls *et al.,* 1994). In such soils, especially those with low N-content, inoculation with a compatible *Frankia* strain is necessary for successful plant growth (Sprent and Parsons, 2000). Several types of *Frankia* inocula and inoculation procedures were tested in many studies including immobilization in polyacrylamide gel or alginate beads (Martin *et al.,* 1991; Wheeler *et al.,* 1991; Kohls *et al.,* 1999; Sayed *et al.,* 2002; Salem, 2003). Inoculation with pure culture carries the advantage of introducing the more beneficial organism, although this requires special techniques and is a time consuming process (Diem and Dommergues, 1990; Sayed *et al.,* 2002). Traping infective *Frankia* in special carriers including polyacrylamide was also tested. Alginate and k-carrageenan are among the specific carriers for entrapping viable cells (El-Komy, 2001). Alginate was an appropriate carrier for *Frankia* in many studies (Frioni *et al.,* 1994; Sougoufara *et al.,* 1989; Borthakur *et al.,* 1996; Sayed *et al.,* 1997). Positive results were also obtained for using polyacrylamide gel (PAG), as it was the appropriate carrier for immobilization and storage of different *Frankia* strains (Sayed *et al.,* 2002).

The present study was carried out to investigate the possibility to use freshly prepared and dried PAG, mixed with spore suspension, as carriers after being stored for 3 and 6 months at 7 ºC. Storage conditions of *Frankia* inoculants should maintain the viability and improve the quality of *Frankia* inoculation process and consequently the plant growth and overall performance.

MATERIALS AND METHODS

Preparation of spore suspensions

Four strains of *Frankia* were used: UGL020601 (Sayed *et al.*, 1998), UGL020604 (Sempavalan *et al.,* 1996), HFPCcI3 (Zhang *et al.,* 1984), and ORS021001 (Diem *et al.,* 1983). These strains were subcultured in liquid spore-inducing BAP medium (modified from Murry *et al.*, 1984). After four weeks of incubation at 28°C, spores were released from sporangia and collected by filtration through Whatman No.1 filter paper (Mansour and Torrey, 1991). The filtrates were centrifuged at 2500 rpm for 1h, the supernatant was discarded and the collected spores were washed twice in distilled water. After microscopic examination, spores were re-suspended in distilled water and parts of the pure spore suspensions were sonicated (Sayed and Wheeler, 1999). Protein concentration was determined (Bradford, 1976) and then adjusted to 2.5µgml^{-1} (Sayed *et al*., 2005).

Polyacrylamide-entrapped *Frankia* (PEF)

Spore suspensions of different strains were immobilized in polyacrylamide gel (Dommergues *et al.*, 1979). Acrylamide, bisacrylamide and other solutions were prepared according to Sayed *et al.* (2002). Gelation was completed in about 20 minutes and the solidified culture blocks were cut into smaller blocks

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(0.5-1 cm^3) and washed overnight under running water. The gel blocks were stored in 0.2 M phosphate buffer, pH: 7.0 (wet PEF). Since the entrapping procedure was not carried out aseptically, fungal contaminants were avoided by the addition of cycloheximide (150 µg/ml, Jung *et al.,* 1982). Two sets of the wet PEF were prepared, one was stored for one day only and the other was stored in sealed polyethylene bags (50g each) in a refrigerator (5-7 ºC) for three and six months. A third set was spread out on a sheet of polyethylene, dried in a desiccator at a relative humidity 40%. After dryness, the dried gel was ground in a tissue grinder and stored as dried powder **(**dry PEF**,** Fig. 1), in the refrigerator for three and six months. Inoculation of plants with different treatments was carried out in order to compare dried and wet PEF inocula at different storage times to those applied to *Casuarina* plants immediately after preparation (control). Plants inoculated with fresh prepared spores of the same *Frankia* strains were used as reference treatments after being treated as the others.

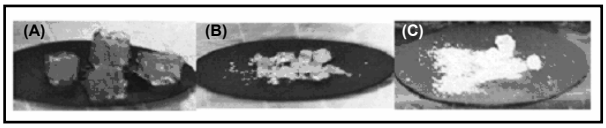

Figure (1): Polyacrylamide gel containing spore suspensions. (A) wet PEF, (B) dried blocks and (C) powdered blocks or dry PEF.

Survival of *Frankia* in wet and dry polyacrylamide

The viability of *Frankia* spores, entrapped in polyacrylamide gel, were tested by measuring the protein concentration, as mentioned above, after rehydration with liquid PAB medium immediately and after 4 weeks. Growth and emergence of hyphae was also examined microscopically.

Infectivity and effectiveness of *Frankia* spores

Six-week old *C. equisetifolia* seedlings were inoculated with liquid cultures, wet PEF, and dry PEF after 1-day (zero time, control treatments), 3- and 6-month of storage at 7 ºC. For liquid cultures, inoculation was carried out according to Baker (1987), and the inoculation was repeated after one week. For both wet and dry PEF the contents of each bag were ground using methanol-sterilized mortar and pestle, and the homogenates were mixed with 1 kg of sterilized 2:1 (w/w) sand/clay mixture (Sayed *et al.,* 2002). Seed germination and plant growth conditions were carried out according to Sayed (1995). After inoculation, plants were grown in green house and harvested 3 months after inoculation. Plant dry mass and nodulation were determined. Plant total nitrogen content was also measured using Kjeldahl micro-technique (Nelson and Sommers, 1973). For the used instrumentation and calculation, methods described by Sayed (2003) were followed.

Statistical analysis

The data were subjected to analysis statistically using the least significant differences test (L. S. D) (PC-STAT program version 1A, University of Georgia).

RESULTS

Effect of spore-preserved media and storage time on nodulation and plant performance.

(1) Nodulation

Nodulation was reduced for plants inoculated with *Frankia* stored at 7 °C for three and six months in all the three treatments (Table 1). However, inoculation with strain UGL020604 stored in liquid medium for three and six months showed total inhibition of nodule formations. Same results were obtained with the same strain in dry PAG after six month, and with strain UGL020601 in dry PAG after storage for three and six months (Table 1). At zero-time-prepared inocula, the wet PEF showed better performance with the *Casuarina* seedlings plant than the other treatments. Nodule number, and nodule dry weights per plant, were all significantly higher in the wet PAG-immobilized

Table (1): Effect of spore-preserved media and storage time on nodule formation, nodule number and nodule dry weight of *Casuarina equisetifolia* seedlings.

Preserved media	Nodule formation (+, -)			Nodule number plant^{-1}			Nodule dry weight (mg plant^{-1})		
	Storage time (month)								
Liquid BAB	Zero	3	6	Zero	3	6	Zero	3	6
ORS021001	+	+	+	0.6 ±	0.4 ± 0.0	0.2 ± 0.0	0.8 ± 0.5	0.4 ± 0.0	0.2 ± 0.0
UGL020604	+	-	-	0.8 ±	0.0*	0.0*	1± 0.5	0.0**	0.0**
HFPCcI3	+	+	+	0.4± 0.0	0.6± 0.0	0.2 ± 0.0	0.8 ± 0.0	0.6 ± 0.0	0.2 ± 0.0
UGL020601	+	+	+	1 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	1.6 ± 0.2	0.6** ± 0.0	0.4** ± 0.0
Wet PAG									
ORS021001	+	+	+	1.2 ±	0.6 ± 0.0	0.4 ± 0.0	2.2 ± 0.4	0.8** ± 0.6	0.4** ± 0.0
UGL020604	+	+	+	1.4 ±	0.4* ± 0.0	0.2* ± 0.0	2.6 ± 0.5	0.4** ± 0.0	0.2** ± 0.0
HFPCcI3	+	+	+	0.6 ±	0.8 ± 0.0	0.6 ± 0.0	2.8 ± 0.8	1** ± 0.0	0.6** ± 0.0
UGL020601	+	+	+	0.8 ±	0.6 ± 0.0	0.4 ± 0.0	1 ± 0.0	0.8 ± 0.0	0.52 ± 0.0
Dry PAG									
ORS021001	+	+	+	0.6 ±	0.4 ± 0.0	0.2 ± 0.0	0.6 ± 0.0	0.4 ± 0.0	0.18 ± 0.0
UGL020604	+	+	-	0.4 ±	0.2 ± 0.0	0.0*	0.4 ± 0.0	0.2 ± 0.0	0.0
HFPCcI3	+	+	+	0.8 ±	0.4 ± 0.0	0.2 ± 0.0	1 ± 0.0	0.4 ± 0.0	0.2 ± 0.0
UGL020601	+	-	-	0.2 ±	0.0*	0.0*	0.2± 0.0	0.0	0.0

* = Significantly different, ** = Highly significant.

cultures than the two other treatments (Table 1). Nodulation as a percentage of whole plant dry weight decreased gradually with the increase in storage time for all treatments (data not shown).

(2) Plant performance

Shoot dry weights and whole plant dry weights were significantly greater for the wet PAG-immobilized cultures than that of the two other treatments. There was also highly significant reduction in these two parameters for all treatments compared to its control. Consequently, root/shoot ratios were higher for all treatments than their controls (Table 3).

Significant reductions occurred in shoot height with strain UGL020601 for all treatments and strain ORS021001 only for the wet PAG cultures after six months and dry PAG cultures after both three and six months (Table 2).

Total nitrogen content in all plant treatments, after three and six months, was reduced significantly when compared to control plants (zero-time inoculation) except for the liquid cultures of strain HFPCcI3 (Table 2).

In general, better plant performance was obtained for cultures stored as wet PAG cultures for 3 and 6 months. On the other hand, the dried inoculants, stored for 3 months, recorded approximately the same values (or better) as for liquid cultures of strains ORS021001 and UGL020604 (Table 2).

(3) Survival of Frankia in wet and dry polyacrylamide

Gel-incorporated *Frankia* spores (wet and dry) were examined after 30 days of rehydration with liquid BAP medium incubated at 28 ºC. These cultures showed the emergence of dense *Frankia* hyphae under the microscope. The increase in their protein content indicated growth and viability of these dried spores (data not shown).

DISCUSSION

There are different processes for bacterial invasion of plant roots with symbiotic nitrogen-fixing bacteria, that result in successful relationship with the host plant. Bacteria should reach the root system, soil particles should contain bacteria in great numbers, and bacterial compatibility with its host (Bashan, 1986). Furthermore, bacteria should remain viable in the dry soil for long time, and should proliferate rapidly and immediately to colonize the root system of the seedlings (Bashan and Levanony, 1985). All these difficulties may explain the limited commercial use of bacterial inoculation with *Frankia*, but not for the legume-*Rhizobium* system (Thompson, 1980). To date, only a few different methods of inoculation are used. The simplest inoculation method is the application of bacteria in liquid broth (Bashan, 1986; Salem, 2003; Abdel-Karim, 2004) or dried bacterial cultures on seeds (Wilkinson *et al.,* 1982; Kohls *et al.,* 1999).

In the present study, wet and dry polyacrylamide gel-containing *Frankia* spores, were evaluated for their performance with *Casuarina* plants. These inoculants, along with alginate beads, are synthetic, simple to use, and biodegradable by soil microorganisms. They contain a large uniform bacterial population, slowly releasing the bacteria for long periods. Furthermore, these inoculants can be stored for long periods up to six months without any apparent effect on its bacterial population (Sayed *et. al.*, 2002; Abdel-Karim, 2004). Metabolic activities of the cells and their efficiencies may undergo changes during immobilization and the immobilized microbial cells increase their biocatalytic capacity due to increased densities and stabilized enzymatic activities (Borthakur *et al.,* 1996). Therefore, we performed immobilization experiments on *Frankia* spore cultures to investigate the difference between freshly prepared and dried PAG inoculants after storage for 3 and 6 months at 7 ºC.

The results obtained in our study coincide with the previous studies (Sayed *et al.*, 2002, 2005). Nodule formation was completely inhibited on plants inoculated with strain UGL020604 in liquid medium stored for three and six months, and in dried PAG after six month. Similary, inhibition occurred for plants inoculated with strain UGL020601 in dried PAG cultures stored for three and six months at 7°C. These strains may be stimulated for their nodulation capacity by changing the storage temperature (Sayed *et. al*., 2002). Stimulation may also be achieved by increasing the spore protein titers as some spores showed lower or no DNA content in a study by Krumholz *et. al*. (2003). The freshly prepared inoculants were the highest between all treatments. Only strain HFPCcI3, that was stored in liquid medium, was the same or better than its control in some criteria. Some variations occurred between strains in the results obtained for wet and dry PAG treatments. Similar variations at optimum temperature were reported by Tisa *et. al*. (1983). Also, at lower temperature, variations were recorded for maximum growth of different strains in culture medium leading to variations in the N_2-fixing ability (Sayed *et al.*, 2002).

In the current study, higher nodulation of *Casuarina* plants was obtained with spores incorporated in wet PAG than those of liquid cultures but significantly lower where stored for either 3 or 6 months. The dried gel gave approximately similar results for plant performance and nodulation as for the liquid cultures (Table 1 and 2).

On the basis of dry mass and plant total nitrogen, the wet PAG-inoculated plants showed substantial differences from dried PAG and liquid cultures at zero time (control). It is also obvious that storage for shorter times, for these types of inoculants, is better for successful nitrogen-fixing capability of the immobilized *Frankia* spores (i. e. 3 months better than 6, see Table 1). The used titer in this study was 2.5 µg ml^{-1} spore protein that recorded the highest nodulation and plant performance in another study by Sayed *et. al*. (2005). The use of lower protein titers may create the appropriate conditions for spores to grow inside the

Table (2): Effect of spore-preserved media and storage time on performance of *Casuarina equisetifolia* seedlings after 6 months of inoculation.

Preserved media	Plant growth (mm)						Plant dry weight (mg)						Plant total N content (mg/g)		
	Shoot height			Root length			Shoot			Root					
	Time of storage														
Liquid	**Zero**	**3**	**6**	**Zero**	**3**	**6**	**Zero**	**3**	**6**	**Zero**	**3**	**6**	**Zero**	**3**	**6**
ORS021001	149 ± 2.2	148 ± 4	148 ± 4	47 ± 10	48 ± 2.4	49 ± 2	12 ± 4.4	10.4 ± 0.8	9.6 ± 0.5	6 ± 1.4	6.4 ± 0.5	6.2 ± 0.4	9 ± 0.3	3.8** ± 0.0	1.7** ± 0.0
UGL020604	134 ± 5	130 ± 6.3	126 ± 5	57 ± 7	52 ± 2.4	55 ± 4.5	17.5 ± 0.9	10.2** ± 0.4	9.4** ± 0.5	5.6 ± 0.5	4.8 ± 0.4	5.4 ± 0.5	4.5 ± 0.0	2.1** ± 0.3	1.4** ± 0.3
HFPCcI3	148 ± 8	154 ± 4.9	148 ± 4	50 ± 10	59 ± 2	52 ± 4	15.2 ± 5	12 ± 1.4	10.4 ± 0.5	8.4 ± 1.5	6.8* ± 1	6** ± 0.0	1.7 ± 0.0	4.7** ± 0.3	3.1** ± 0.0
UGL020601	162 ± 4	150** ± 6	148** ± 4	55 ± 4.5	59 ± 2	59 ± 2	16.6 ± 0.8	12** ± 0.9	11** ± 0.9	9 ± 0.6	7.8* ± 0.4	7** ± 0.6	8.4 ± 0.9	4** ± 0.3	3.1** ± 0.6
Wet PAG															
ORS021001	152 ± 8	152 ± 4	124** ± 10	50 ± 8	49 ± 2	45 ± 4	28 ± 4.4	13.2** ± 1.9	9.4** ± 0.8	2.2 ± 4.4	0.8** ± 0.6	0.4** ± 0.0	16.6 ± 0.9	6.6** ± 0.6	2.8** ± 0.3
UGL020604	159 ± 9	154 ± 5	154 ± 5	43 ± 10	53** ± 4	55** ± 4.5	24 ± 5.4	14.4** ± 1.6	11.6** ± 2.3	7.8 ± 0.8	7.2 ± 0.4	7 ± 0.4	14.7 ± 1.2	6.1** ± 0.3	1.7** ± 0.0
HFPCcI3	162 ± 8	154 ± 4.9	152 ± 4	54 ± 11	59 ± 2	53 ± 6	22.2 ± 3	14.4** ± 3.6	12** ± 2.5	13.2 ± 4.3	6.8** ± 0.7	7.8** ± 0.7	12 ± 1.4	6.6** ± 0.0	3.3** ± 0.3
UGL020601	140** ± 6	152** ± 7	158 ± 4	51 ± 2	47 ± 2.4	54 ± 5	15.2 ± 0.4	9.8** ± 0.4	13.2* ± 1.7	8.8 ± 0.4	6.6** ± 0.5	8* ± 0.6	8 ± 0.6	5** ± 0.6	2.8** ± 0.3
Dry PAG															
ORS021001	158 ± 4	128** ± 4	117** ± 16	59 ± 2	51** ± 2	44** ± 3.7	12.4 ± 8	9.4** ± 0.8	7.2** ± 0.4	7.2 ± 0.7	5** ± 0.6	4.6** ± 0.5	6.8 ± 0.3	4.2** ± 0.6	1.4** ± 0.3
UGL020604	154 ± 5	148 ± 4	138** ± 4	59 ± 2	66** ± 5	51** ± 2	15.4 ± 0.5	10.8** ± 1.1	8.8** ± 0.4	8.2 ± 0.4	7.8 ± 0.7	5.2** ± 0.4	6 ± 0.6	4** ± 0.3	1** ± 0.6
HFPCcI3	164 ± 10	156 ± 8	150 ± 10	55 ± 4.5	57 ± 4	59 ± 10	15.6 ± 0.5	11.4** ± 1.7	9.2** ± 0.7	8 ± 0.0	5.8** ± 0.4	7.8 ± 0.4	7 ± 0.3	2.8** ± 0.3	1.2** ± 0.3
UGL020601	158 ± 4	148* ± 4	140** ± 6	51 ± 2	50 ± 3.2	53 ± 4	10 ± 0.0	9.8 ± 1.6	9.4 ± 0.5	7.8 ± 0.4	7.8 ± 0.4	7.6 ± 0.5	2.4 ± 0.0	1.2** ± 0.3	1** ± 0.0

[a] Means of 5 experiments ± SD; control = zero-time inoculation, [b] Media: **PAG** = polyacrylamide gel, **BAP** = liquid spore-inducing medium, * Significant differences (P = 0.05) and ** highly significant differences (P = 0.01) as compared with control.

Table (3): Effectiveness of stored *Frankia* spores in symbiosis with *Casuarina equisetifolia*.

Frankia strains		ORS021001		HFP CcI3		UGL020604		UGL020601	
Medium	Month	R:S	N:P	R:S	N:P	R:S	N:P	R:S	N:P
	Control	52 ± 11	4.7 ± 4.4	62.5 ± 28.4	3.8 ± 0.0	31.8 ± 2	4.1 ± 2.5	54 ± 2.4	6 ± 0.8
Liquid BAP	3	62 ± 4	2.1 ± 2	58 ± 13	2.3 ± 0.3	47** ± 4	0.0**	65* ± 6	2.8 ± 2.3
	6	65 ± 7	1.2 ± 1	58 ± 2.6	1.1 ± 0.0	58** ± 7.5	0.0**	64* ± 8	2.2 ± 0.0
	Control	31.3 ± 10	5.8 ± 1.6	60 ± 20.6	7.4 ± 2.2	33.6 ± 6	7.7 ± 1.7	58 ± 2.7	4 ± 2.5
Wet PAG	3	58* ± 12	3.7 ± 3.6	52 ± 19	4.4 ± 3	50* ± 5	1.6** ± 0.0	67 ± 4	4.5 ± 0.0
	6	81** ± 15	2.4 ± 2	67 ± 12	3 ± 2.5	63** ± 14	1** ± 0.0	61 ± 8	2.5 ± 0.0
	Control	58 ± 5	2.9 ± 2.4	51 ± 1.6	4 ± 2.4	53 ± 2	1.5 ± 0.0	78 ± 4	1 ± 0.0
Dry PAG	3	53 ± 7	2.7 ± 2	52 ± 10	2.3 ± 0.0	73** ± 7	1 ± 0.0	82 ± 14	0.0
	6	64 ± 6	1.5 ± 1	85** ± 10	1.1 ± 0.0	59 ± 8	0.0*	81 ± 9	0.0

[a] **Ratios**: **R:S** = root/shoot, **N:P** = nodule/plant; means of 5 experiments ± SD, * Significant differences (P = 0.05) and ** highly significant differences (P =0.01) as compared with control.

polymer for longer periods. This may indicate the possibility of using more lower titers (i. e. ≤ 2.5 µg ml^{-1}) for storage periods longer than 3 or 6 months or higher titers for those strains that did not nodulate. Moreover, the number of nodules per plant is the best measure of the infection process (Kohls *et al.,* 1999). The data obtained here indicate the superiority of wet gel-incorporated *Frankia* for storage and subsequently, successful nodulation than the other used media (Tables 1 and 2). Our data are consistent also with the concept that overall nodule weight corresponds to plant productivity in nitrogen-limited environments (Hielman and Ekuan, 1982). Higher nodule dry mass and lower root to shoot ratios, for plants inoculated with the polymerized *Frankia* indicates a shift in dry weight allocation towards shoot growth and plant productivity (Table 2). Moisture deficits can adversely affect *Frankia* growth (Shipton and Burggraaff, 1982). This suggests that the polymer itself may facilitate the uptake of water and associated nutrients and the rhizosphere moisture conditions are ameliorated by the water retaining capacity of the polymer (Table 2; Hielman and Ekuan, 1982; Arnone *et al.,* 1994; Kohls *et al.,* 1999). Furthermore, we have observed highly significant differences between the effectiveness of the varied three treatments, when stored for three and six months, and its control (zero-time). In the present, the gel-incorporated *Frankia* spores can also be stored in the refrigerator (i.e. at 7 ºC) for more than 3 months. The stored dried gel-containing *Frankia* spores can also be used for large-scale inoculation procedures in nurseries and field, as well as the wet gel, but the inoculum nodulation capacity should be improved first as discussed above.

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إستخدام جراثيم الفرانكيا كلقاحات لنباتات الكازوارينا إكويستيفوليا

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الملخص العربـــى

تم إختبار قدرة جراثيم أربعة سلالات من الفرانكيا- كازوارينا على تكوين العقد الجذرية وتثبيت النيتروجين مع نباتات الكازوارينا إكويستيفوليا وذلك بعد تخزينها لمدة ثلاثة وستة أشهر فى أوساط مختلفة. والأوساط المستخدمة هى الوسط السائل BAP و جل البولى أكريلاميد الرطب والجاف. أما سلالات الفرانكيا المستخدمة فهى UGL020601, UGL020604, HFPCcI3, ORS021001. وقد حدث إنخفاض فى محتوى النيتروجين الكلى بعد التلقيح بلقاحات الجراثيم المخزنة وتراوح الانخفاض بين 33 إلى 88% بالمقارنة بالنباتات الملقحة بجراثيم محضرة حديثا (قبل التجربة مباشرة) وذلك التفاوت بإختلاف السلالة ونوع اللقاح المستخدم.

وقد سجلت سلالات الفرانكيا المدمجة فى لقاح الجل الرطب أفضل القيم بين جميع المعاملات وبوجه عام فإن الإنخفاض فى محتوى النيتروجين الكلى كان أقل بعد 3 أشهر من الإنخفاض بعد 6 أشهر من التخزين عند 7 درجات مئوية لجميع المعاملات مما يدل على أداء أفضل بعد التخزين لمدة قصيرة. وقد إنخفض عدد العُقد تدريجيا بإطالة مدة التخزين لجميع السلالات والمعاملات. وقد سجلت جراثيم جميع السلالات المختبرة والمخزنة لمدة 3 شهور كجل رطب قيما أفضل لمعدلات الجذر/ساق والعُقد/نبات (أي نسب أقل وأعلى على التوالى) من المعاملات الأخرى (الجل الجاف والمزارع السائلة). أما الجل المجفف كمسحوق فقد يكون له مميزات أفضل من ناحية مدة الصلاحية من المعاملات الأخرى وربما يكون أفضل كوسط للحفظ فى حالات التلقيح على نطاق واسع كما فى المشاتل الكبيرة. وبوجه عام فإنه يوصى بتخزين جراثيم الفرانكيا المدمجة فى الجل الرطب أو الجاف لمدة تصل إلى ثلاثة اشهر للأغراض التجارية.