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Comparing fluidized bed spray-coating and spray-drying encapsulation of non-spore-forming Gram-negative bacteria

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Abstract

Microencapsulation of plant-beneficial bacteria for use as biopesticides is a growing area of interest in the search for alternatives to harmful chemical pesticides. In this study, we assessed the microencapsulation of the Gram-negative, non-spore-forming plant-beneficial bacterial strain *Collimonas arenae* Cal35 in novel cross-linked alginate microcapsules (CLAMs) formed by spray-drying, and in novel cross-linked alginate matrix shell (CLAMshell) particles formed by fluidized bed spray-coating. Survival of Cal35 was 10-fold greater in CLAMs than in CLAMshells. During individual stress tests, Cal35 was found to be more tolerant to high temperatures than to desiccation. This is consistent with the greater survival of Cal35 when encapsulated by spray-drying, which is a process that uses high inlet temperature and short exposure times to dry conditions. The particle diameter of CLAMs ranged from 5 to 20 µm, indicating potential for spray applications. CLAMshell particle diameters were between 250 and 300 µm, suggesting potential for seed coatings.

Keywords

spray-drying; fluidized bed spray-coating; microencapsulation; biocontrol; *Collimonas*

1. **Introduction**

Microencapsulation is a preservation technique wherein a sensitive cargo is protected within carrier material(s), often polymers such as polysaccharides, that can also feature additional functions, depending on the application[1]. For example, in the biomedical industry, drugs are encapsulated for targeted delivery and controlled release[2]. In the food industry, compounds are encapsulated to mask or retain certain flavors[3] and for enteric release to maximize bioavailability of bioactives. In the agricultural industry, pesticides are often encapsulated for greater stability and controlled release in cropland to combat a variety of pests[4,5]. It is of particular interest to encapsulate active ingredients in biopesticides, such as plant-beneficial biocontrol bacteria, as there is a growing need to replace traditional chemical pesticides that are harmful to humans and the environment.

The two primary means of applying plant-beneficial bacteria for crop utilization are by spraying in-furrow or in-field, and by coating onto seed. For spray applications, encapsulation of beneficial bacteria can help protect against other ingredients in the spray tank which may damage the bacteria, and against environmental effects in sprayed areas[6]. Microcapsule size is constrained by the nozzle size of the sprayer, on the scale of microns, as to limit blockages, settlement, and aggregation of particulates[6,7]. For coating applications, the seed coating itself serves as the protective encapsulation matrix for the bacteria. Rotary drum dryers are the most widely used method of coating seed; however, bacterial viability is typically diminished by other ingredients in the seed coating formulation (similar to spray tanks) and by the long drying process[8,9].

Encapsulation of plant-beneficial bacteria has been demonstrated using alginate. Alginate is a naturally derived polymer desirable as a microcapsule carrier material because it is a relatively low cost ingredient, is safe to handle, and has the ability to form a gel network[10,11]. Composed of alternating or repeating units of β-D-mannuronic acid and α-L-guluronic acid, the polyuronate backbone of alginate contains carboxylate groups allowing ion-mediated cross-linking to form a gel structure[12,13]. Alginate beads containing bacteria are typically formed by dripping a suspension of dissolved alginate and bacterial cells into a calcium chloride bath, where cross-linking occurs upon contact at the interface[14-16]. This gelling step is followed by a curing step, a washing step, and a drying step, before dry alginate beads are formed. The need for a separate extruder and calcium bath as well as many individual steps make producing alginate beads challenging to scale up[17].

Recently, methods for forming in situ cross-linked alginate microcapsules (CLAMs) by spray-drying[18], and in situ cross-linked alginate matrix shell (CLAMshell) particles by fluidized bed spray-coating[19] were developed at UC Davis. Encapsulation of bacteria[20,21], enzymes[22], polymers[23], and oils[24,25] in CLAMs have been demonstrated, as has encapsulation of enzymes in CLAMshell particles[26]. To produce these microcapsules, atomization at the nozzle evaporates a volatile base, lowering the pH of the droplet to solubilize the calcium salt, facilitating calcium-mediated cross-linking of alginate as the droplets dry into a particle (CLAM) or form a coating (CLAMshell). This single-step encapsulation process can be industrially scalable while still remaining relatively cheap[27]. In addition, both spray-drying and fluidized bed spray-coating are already widespread in industry, making the in situ cross-linking processes easier to implement.

In this study, we compared spray-drying and fluidized bed spray-coating to investigate encapsulation of the potential biocontrol bacterium *Collimonas arenae* Cal35 in CLAMs and CLAMshell particles. Cal35 is a non-spore-forming, Gram-negative soil isolate with demonstrated success as an antifungal agent in laboratory, greenhouse, and field settings[28-32].

Cal35 has also been shown to retain its antifungal properties after spray-drying and shelf storage[21]. Here, the survival of Cal35 in CLAMs and CLAMshells was assessed and compared, and correlated with the sensitivity of Cal35 to heat and desiccation stresses. Furthermore, spray-dried particles and fluidized bed spray-coated particles containing Cal35 were characterized by SEM, moisture content analysis, and water activity analysis.

2. Materials and Methods

2.1 Materials

Ammonium hydroxide, 28-30% (NH_4OH), dicalcium phosphate (Ca_2HPO_4), glycerol, magnesium sulfate heptahydrate ($MgSO_4 \cdot 7H_2O$), peptone, potassium phosphate dibasic (K_2PO_4), and succinic acid were purchased from Fisher (Waltham, MA). Low-viscosity alginate (A1112) and maltodextrin (dextrose equivalent 4.0-7.0) were acquired from Millipore-Sigma (Burlington, MA). Agar was purchased from BTS (Houston, TX). Concentrated (20x) phosphate-buffered saline (PBS) was purchased from VWR (Radnor, PA). HI-CAP 100 modified starch was purchased from Ingredion (Westchester, IL) and impact beads (glass cores) from Grainger (Lake Forest, IL). Milli-Q water from a Millipore Ultrapure Water Purification System was used to prepare all solutions. *C. arenae* Cal35 was originally isolated from forest soil in the Jug Handle State Natural Reserve in Mendocino County, CA[33].

2.2 Methods

2.2.1 Culture conditions for *Collimonas arenae* Cal35

Fresh cultures of *C. arenae* Cal35 were prepared and grown in King's medium B (KB) using the exact conditions described previously[21].

2.2.2 Testing sensitivity of Cal35 to temperature and desiccation

Cultures of Cal35 were harvested and collected by centrifugation at 5,000 rpm. Supernatant was removed and the remaining cell pellet was resuspended in 0.01 M phosphate buffered saline (1X PBS). Initial and final viable cell counts were measured using the serial dilution method by plating each dilution onto KB agar and counting colony forming units (CFUs).

To assess sensitivity towards temperature, Cal35 suspended in 1X PBS at a density of about 9.5 log[CFU/mL] was transferred to 15 mL conical tubes. The conical tubes (n = 3) were then placed into a heated water bath set to temperatures between 35 and 60°C and sampled periodically over three hours to determine viable cell counts.

To assess sensitivity towards desiccation, 3 mL of fresh Cal35 culture resuspended in 1X PBS at a density of about 9.5 log[CFU/mL] were transferred to the bottom of an empty plastic Petri dishes which were placed open in a biological safety cabinet at room temperature (~21°C), and dried for 24 hr using the airflow of the unit. Dishes containing the dehydrated Cal35 suspension were rehydrated with 3 mL of 1X PBS and plated to count CFUs.

2.2.3 Spray-drying formulation of Cal35 encapsulated in CLAMs

To prepare 50 g of feed for spray-drying, a 1.5 L KB culture of Cal35 was harvested after 24 h of growth and centrifuged at 4,200 rpm for 15 min to pellet the cells. The supernatant was removed and the cell pellet was resuspended in 12.5 g of 4% filter-sterilized succinic acid, which was previously adjusted with NH_4OH to pH 7. This cell suspension was added to an autoclaved preparation containing modified starch, maltodextrin, sodium alginate, and $CaHPO_4$. The final concentration of the feed for spray-drying was: 13% HI-CAP 100 modified starch, 2% maltodextrin, 2% sodium alginate, 1% succinic acid at pH 7, and 0.5% $CaHPO_4$.

Spray-drying was performed using a BUCHI B-290 benchtop spray-dryer (BUCHI Corporation, New Castle, DE) (**Figure 1**). The control parameters used were: 90°C inlet temperature, 40% spray rate (~12 g/min), 40 mm nozzle pressure (0.5 bar), 100% aspirator. The average outlet temperature was 42 ± 1°C. After the 50 g of feed was sprayed, particles were removed and collected from the product chamber.

2.2.4 Fluidized bed spray-coating formulation of Cal35 encapsulated in CLAMshells

To prepare 200 g of feed for spray-coating, 6 L of culture was harvested and prepared in the same manner as spray-drying, described in the previous section. The final concentration of the feed for spray-coating was the same as the feed for spray-drying: 13% HI-CAP 100 modified starch, 2% maltodextrin, 2% sodium alginate, 1% succinic acid at pH 7, and 0.5% $CaHPO_4$.

Spray-coating was performed using a UniGlatt 2 L capacity fluidized bed coater in Wurster configuration (Glatt Air Techniques, Inc., Ramsey, NJ) (**Figure 2**). For the core material, 600 g of 250-300 μm glass beads were inserted into the product chamber of the unit. The control parameters used were: 55-60°C inlet temperature (lower and upper limit), 32% spray rate (~10 g/min), 3.5 bar nozzle pressure, 25 deg airflow flap angle. The average outlet temperature was 40 ± 0.6°C. In-process samples were taken using the sample port to measure viability during the coating process. Once all 200 g of the feed was applied, fluidization continued for two minutes to allow more drying of the coatings before collection.

2.2.5 Characterization of spray-dried and spray-coated Cal35

Viability of Cal35 before encapsulation was measured by sampling from each feed suspension and using the dilution method to plate onto KB agar and count CFUs. Viability of Cal35 after encapsulation was measured by dissolving 0.03 ± 0.001 g of spray-dried particles and

2.0 ± 0.1 g spray-coated particles in 12.5 mL 1X PBS. Samples were mixed by inversion at room temperature for one hour to fully dissolve particles and release all bacteria before being plated onto KB agar to count CFUs.

Product yield was calculated by taking the percent of the collected solids to sprayed solids, either as the total spray-dried solids or total coated solids.

Particle morphology was visualized by scanning electron microscopy (SEM) using a Thermo Scientific Quattro SEM (Thermo Fisher Scientific, Waltham, MA). Samples of spray-dried and spray-coated particles were mounted onto carbon tape and sputter-coated in gold to a thickness of 120 angstrom. A beam voltage of 10 kV and a spot size of 3.0 were used for imaging.

Moisture content was measured gravimetrically by oven drying for three days at 60-70°C. Water activity was measured using an Aqualab Series 3TE water activity meter (METER, Pullman, WA).

2.2.6 Statistical analysis

Data was analyzed using the statistical software JMP (Statistical Discovery from SAS, Cary, NC). One way ANOVA and post hoc multiple comparison tests were used to determine significance of factors and differences between means. P-values less than 0.05 indicated a significant difference.

3. Results and Discussion

Spray-drying and fluidized bed spray-coating are two common industrial high throughput drying processes that can be used for microencapsulation. Spray-drying (**Figure 1**) relies on significantly hotter air currents to quickly dry atomized droplets into particles, whereas spray-coating (**Figure 2**) exploits long drying times of continuously fluidizing particles being coated to require cooler drying temperatures (**Table 1**).

Table 1 Process parameters for spray-drying and fluidized bed spray-coating in this study.

Parameter	**Spray-drying**	**Spray-coating**
Inlet Temperature	90°C	55-60°C
Outlet Temperature	42±1°C	40±0.6°C
Residence Time	<1-1.5 sec	20-25 min

3.1 Bacterial isolate Cal35 showed greater survival with spray-drying than with fluidized bed spray-coating

To produce a collectable product, a blend of maltodextrin and modified starch was used as excipients in the CLAMs and CLAMshell formulations. Previously, these excipients enhanced the survival of Cal35 during spray-drying and shelf-storage in CLAMs[21].

Prior to encapsulation, the viable cell counts of Cal35 in both feed suspensions were above 10 log[CFU/g solids] (**Figure 3A**). Cal35 experienced approximately 3 log reduction during spray-drying compared to a 4 log reduction during fluidized bed spray-coating (**Figure 3B**). Even though outlet temperatures for both processes were around 40°C, the inlet temperature of spray-drying was considerably higher than that of fluidized bed spray-coating (90 versus 55-60°C). Another key difference between these encapsulation processes was residence time (**Table 1**). For the Buchi B-290 benchtop spray-dryer, the residence time can span in the milliseconds to seconds range, as droplets quickly pass through the evaporation chamber and fall into the collection chamber (**Figure 1**). The resulting CLAMs contained more viable bacteria (7 log[CFU/g solids]) than CLAMshells that were produced by spray-coating (6 log[CFU/g coated solids]). In the UniGlatt fluidized bed spray-coater, the residence time ranges from minutes to hours, depending on the amount of coating being applied to the fluidized particles (**Figure 2**). In the present study, the residence time for spray-coating was about 22 minutes; 20 minutes for the coating suspension to be applied and two minutes of extra drying time (**Figure 4**). During the coating process, the viability of Cal35 consistently measured about 7.5 log[CFU/g coated solids]. However, the end product of spray-coating was lower in viability compared to spray-drying per gram of sprayed solids due to the final drying step. Although the viability of Cal35 was sustained in-process for the short coating duration, it is possible that thicker coatings may be applied to increase the total CFU in the coating. And although the total CFU count in spray-dried CLAMs can be increased by simply spraying a greater volume of feed, the particle size of CLAMs would not change. Perhaps a thicker CLAMshell coating thus larger particle size would confer greater protection and stability to the encapsulated bacteria over time.

Here, spray-drying encapsulation, which operates with high temperatures and a short residence time was less damaging to Cal35, yet there is still room to minimize the 3 log reduction in the viable cell count (**Figure 3B**). Cellular accumulation of osmoprotectants, mainly in the form of disaccharides, has been attributed to enhancing desiccation stress resistance[34]. For example, when grown in media supplemented with trehalose, survival of *Raoultella terrigena* dried in alginate beads was improved[16]. Similarly, cellular accumulation of sucrose by *Pseudomonas chlororaphis* enhanced survival during freeze-drying[35]. Osmoprotectants are beneficial because they help maintain osmotic balance, prevent unfolding of proteins, and preserve membrane fluidity of the cell[36]. This strategy of introducing osmoprotectants is especially useful because it occurs during the culturing phase of bacteria, prior to formulation, meaning it can be easily implemented.

3.2 Temperature and desiccation sensitivity of Cal35

Cal35 was very sensitive to a temperature of 60°C, with no viable cells countable after five minutes of exposure (**Figure 5**). The number of viable bacteria declined more rapidly with increasing temperature. The viability of Cal35 incubated at 35°C did not change over the three hour period of the experiment. After one hour, Cal35 held at 40°C still showed no decrease in viability; however, raising the temperature to 45°C reduced viability by more than 5 log units, and up to 50°C resulted in colony counts above the limit of detection. In the three hour duration, there was a 2.5 log reduction at 40°C and a 5.5 log reduction at 45°C. At 45 and 50°C, the decline in log CFU begins to flatten, indicative of tolerance to higher temperatures[37].

Cal35 did not handle desiccation very well. With an initial cell count of ~9.5 log CFU/mL, no viable bacteria were recovered after a culture aliquot was left to dry in a dish for 24 hours at room temperature (21°C)

For bacteria that are tolerant of heat but sensitive to drying, it would seem to make sense that a process like fluidized bed spray-coating where very low moisture environments exist for extended periods of time be more detrimental to Cal35 than spray-drying. In fact, the moisture content of spray-coated particles was 0.28%, over ten times less than for spray-dried particles (**Figure 3D)**, with no significant difference in the overall product yield (**Figure 3C**). Water activity was also significantly lower in spray-coated particles (**Figure 3E**).

3.3 Outlook and applications

Whether bacterial biopesticides are applied on seed or in-field will largely impact the method of encapsulation. Spray-drying of CLAMs produced particles with a diameter between 5 and 20 µm (**Figure 6A**). Particles in this size range can be readily incorporated into spray-tanks for application in-furrow or on foliage. Fluidized bed spray-coating of CLAMshells onto cores produced coated particles between 250-300 µm (**Figure 6B**). Despite resulting in slightly greater losses in viability of Cal35 compared to spray-drying (**Figure 6B)**, fluidized bed spray-coating could be an enticing method for coating beneficial bacteria directly onto seeds. Seed coatings are conventionally applied by rotary pan or drum drying, where nutrients, pesticides, and other active compounds that benefit the seed and root are sprayed onto seeds as they are mixing or tumbling in the drum[38]. Because drying is completed at ambient conditions, the process is long, resulting in bacteria losing viability due to desiccation[8]. However, in addition to the use of osmoprotectants, formulation with polymers such as gum acacia, methylcellulose, polyvinyl alcohol, and polyvinyl pyrrolidone have improved survival of rhizobial legume inoculants in the seed coating process[9,39]. With bacteria such as Cal35 that are less tolerant to drying, introducing some heat to speed up the coating process, similar to what is happening during fluidized bed spray-coating, may potentially be a more favorable encapsulation method.

Continuous fluidized bed spray-coating may also offer a unique solution to address issues of long drying times. While the Wurster configuration of spray-coating used this study is a batch process, moving towards a continuous process where seeds are fluidized, directly coated, and collected can greatly lower the residence time while maintaining tolerable drying temperatures. Issues of agglomeration and proper coating formation when spraying with polymers can be addressed with optimization experiments of process and formulation parameters[26,40,41], however, when coating onto seed specifically, it is important to understand the limits of the drying temperature so as to not negatively impact seed germination efficiency. Seeing that desiccation stress was the more detrimental to Cal35, perhaps the ideal conditions for encapsulating similar bacterial inoculants calls for shorter residence times by elevating drying temperatures, similar to the drying conditions seen in spray-drying where survival of Cal35 was greatest.

Ultimately the survival of bacteria during any encapsulation method may mostly be influenced by their structure and mechanisms to tolerate various environmental stresses. Gram-positive probiotic bacteria such as *Bifidobacteria* and *Lactobacillus* lose little viability during spray-drying and can have survival rates greater than 90% due to their more protective membrane structure[42]. Spore-formers such as *Bacillus* are easily encapsulated and are already used in agriculture as biopesticides because of the robust nature of spores[43]. The ability of a non-spore-forming Gram-negative *Methylobacterium* strain to remain viable during spray-drying may be a consequence of being acclimated to the extreme conditions that characterize the natural habitat of this bacterium, namely the plant leaf surface[20]. Future work in encapsulation of plant beneficial bacteria and biopesticides will be to consider how these underlying cellular mechanisms to tolerate or adapt to stresses can help design microcapsules for improving the protected bacteria's long-term stability for crop applications.

4. Conclusions

Encapsulation of the non-spore-forming plant-beneficial bacterial strain *Collimonas arenae* Cal35 in CLAMs by spray-drying or in CLAMshells by fluidized bed spray-coating was investigated. Spray-drying encapsulation of Cal35 had greater survival compared to fluidized bed spray-coating, which is consistent with the observation that Cal35 was much more tolerant to heat than to desiccation. However, if the coating process would continue for longer periods of time to create thicker coated CLAMshells, it is possible that the total CFUs per sprayed solids would be comparable or better than spray-drying. During spray-drying, high inlet temperatures allow a very short residence time of bacteria being encapsulated, while during fluidized bed spray-coating, low inlet temperatures rely on a long residence time of continuously fluidizing particles. CLAMs produced by spray-drying were smaller, ranging between 5-20 µm, while CLAMshell particles ranged between 250-300 µm. Both methods of encapsulation are relevant for encapsulation of bacterial biopesticides; CLAMs were sized appropriately for spray applications, while CLAMshells may be more useful for coating onto seed.

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6. Author Disclosure Statement

Author Tina Jeoh is an inventor of the CLAMs process (United States Patent 9,700,519). Authors Tina Jeoh and Ryan Kawakita are inventors of the CLAMshell process (United States Patent Application 63085669).

7. References

1 Jyothi, NVN, Prasanna, PM, Sakarkar, SN *et al.* Microencapsulation techniques, factors influencing encapsulation efficiency. *Journal of Microencapsulation* 2010; 27(3): 187-197.

2 Ma, G. Microencapsulation of protein drugs for drug delivery: strategy, preparation, and applications. *Journal of Controlled Release* 2014; 193324-340.

3 Nazzaro, F, Orlando, P, Fratianni, F *et al.* Microencapsulation in food science and biotechnology. *Current Opinion in Biotechnology* 2012; 23(2): 182-186.

4 Alonso, ML, Laza, JM, Alonso, RM *et al.* Pesticides microencapsulation. A safe and sustainable industrial process. *Journal of Chemical Technology & Biotechnology* 2014; 89(7): 1077-1085.

5 Tsuji, K. Microencapsulation of pesticides and their improved handling safety. *Journal of Microencapsulation* 2001; 18(2): 137-147.

6 Preininger, C, Sauer, U, Bejarano, A *et al.* Concepts and applications of foliar spray for microbial inoculants. *Applied Microbiology and Biotechnology* 2018; 102(17): 7265-7282.

7 Jambhulkar, PP, Sharma, P & Yadav, R. in *Microbial Inoculants in Sustainable Agricultural Productivity: Vol. 2: Functional Applications* (eds Dhananjaya Pratap Singh, Harikesh Bahadur Singh, & Ratna Prabha) 199-218 (Springer India, 2016).

8 Hartley, EJ, Gemell, LG & Deaker, R. Some factors that contribute to poor survival of rhizobia on preinoculated legume seed. *Crop and Pasture Science* 2012; 63(9): 858-865.

9 Deaker, R, Roughley, RJ & Kennedy, IR. Legume seed inoculation technology - a review. *Soil Biology & Biochemistry* 2004; 36(8): 1275-1288.

10 Lee, KY & Mooney, DJ. Alginate: properties and biomedical applications. *Prog Polym Sci* 2012; 37(1): 106-126.

11 Draget, KI & Taylor, C. Chemical, physical and biological properties of alginates and their biomedical implications. *Food Hydrocolloids* 2011; 25(2): 251-256.

12 Hecht, H & Srebnik, S. Structural characterization of sodium alginate and calcium alginate. *Biomacromolecules* 2016; 17(6): 2160-2167.

13 Bruchet, M & Melman, A. Fabrication of patterned calcium cross-linked alginate hydrogel films and coatings through reductive cation exchange. *Carbohydrate Polymers* 2015; 13157-64.

14 Rohman, S, Kaewtatip, K, Kantachote, D *et al.* Encapsulation of *Rhodopseudomonas palustris* KTSSR54 using beads from alginate/starch blends. *Journal of Applied Polymer Science* 2021; 138(12): 50084.

15 Wu, ZS, He, YH, Chen, LJ *et al.* Characterization of *Raoultella planticola* Rs-2 microcapsule prepared with a blend of alginate and starch and its release behavior. *Carbohydrate Polymers* 2014; 110259-267.

16 Schoebitz, M, Simonin, H & Poncelet, D. Starch filler and osmoprotectants improve the survival of rhizobacteria in dried alginate beads. *Journal of Microencapsulation* 2012; 29(6): 532-538.

17 Ching, SH, Bansal, N & Bhandari, B. Alginate gel particles–a review of production techniques and physical properties. *Critical Reviews in Food Science and Nutrition* 2017; 57(6): 1133-1152.

18 Jeoh-Zicari, T, Scher, HB, Santa-Maria, MC *et al.* Spray dry method for encapsulation of biological moieties and chemicals in polymers cross-linked by multivalent ions for controlled release applications. US Patent 10,610,492 (2020).

19 Jeoh-Zicari, T, Scher, HB, Strobel, SA *et al.* Coated granules produced by in-situ crosslinking process. US patent application 63085669 (2020).

20 Strobel, SA, Allen, K, Roberts, C *et al.* Industrially-scalable microencapsulation of plant beneficial bacteria in dry cross-linked alginate matrix. *Industrial Biotechnology* 2018; 14(3): 138-147.

21 Kawakita, R, Leveau, JHJ & Jeoh, T. Optimizing viability and yield and improving stability of Gram-negative, non-spore forming plant-beneficial bacteria encapsulated by spray-drying. *Bioprocess and Biosystems Engineering* 2021.

22 Santa-Maria, M, Scher, H & Jeoh, T. Microencapsulation of bioactives in cross-linked alginate matrices by spray drying. *Journal of Microencapsulation* 2012.

23 Wong, DE, Cunniffe, JC, Scher, HB *et al.* Chelator regulation of in situ calcium availability to enable spray-dry microencapsulation in cross-linked alginates. *ACS Omega* 2020; 5(38): 24453-24460.

24 Strobel, SA, Hudnall, K, Arbaugh, B *et al.* Stability of fish oil in calcium alginate microcapsules cross-linked by in situ internal gelation during spray drying. *Food and Bioprocess Technology* 2020; 13(2): 275-287.

25 Strobel, SA, Scher, HB, Nitin, N *et al.* In situ cross-linking of alginate during spray-drying to microencapsulate lipids in powder. *Food Hydrocolloids* 2016; 58141-149.

26 Kawakita, R, Strobel, S, Soares, B *et al.* Fluidized bed spray-coating of enzyme in a cross-linked alginate matrix shell (CLAMshell). *Powder Technology* 2021; 386372-381.

27 Strobel, SA, Knowles, L, Nitin, N *et al.* Comparative technoeconomic process analysis of industrial-scale microencapsulation of bioactives in cross-linked alginate. *Journal of Food Engineering* 2020; 266109695.

28 Doan, HK, Maharaj, NN, Kelly, KN *et al.* Antimycotal activity of *Collimonas* isolates and synergy-based biological control of Fusarium Wilt of tomato. *Phytobiomes Journal* 2020; 4(1): 64-74.

29 Mosquera, S, Stergiopoulos, I & Leveau, JHJ. Interruption of *Aspergillus niger* spore germination by the bacterially produced secondary metabolite collimomycin. *Environmental Microbiology Reports* 2020; 12(3): 306-313.

30 Akum, FN, Kumar, R, Lai, G *et al.* Identification of *Collimonas* gene loci involved in the biosynthesis of a diffusible secondary metabolite with broad-spectrum antifungal activity and plant-protective properties. *Microbial Biotechnology* 2020.

31 Fritsche, K, van den Berg, M, de Boer, W *et al.* Biosynthetic genes and activity spectrum of antifungal polyynes from *Collimonas fungivorans* Ter331. *Environmental Microbiology* 2014; 16(5): 1334-1345.

32 Mela, F, Fritsche, K, de Boer, W *et al.* Dual transcriptional profiling of a bacterial/fungal confrontation: *Collimonas fungivorans* versus *Aspergillus niger*. *The ISME Journal* 2011; 5(9): 1494-1504.

33 Uroz, S, Tech, JJ, Sawaya, NA *et al.* Structure and function of bacterial communities in ageing soils: Insights from the Mendocino ecological staircase. *Soil Biology and Biochemistry* 2014; 69265-274.

34 Fu, N & Chen, XD. Towards a maximal cell survival in convective thermal drying processes. *Food Research International* 2011; 44(5): 1127-1149.

35 Palmfeldt, J, Radstrom, P & Hahn-Hagerdal, B. Optimisation of initial cell concentration enhances freeze-drying tolerance of *Pseudomonas chlororaphis*. *Cryobiology* 2003; 47(1): 21-29.

36 Greffe, VRG & Michiels, J. Desiccation-induced cell damage in bacteria and the relevance for inoculant production. *Applied Microbiology and Biotechnology* 2020; 104(9): 3757-3770.

37 Moats, WA. Kinetics of thermal death of bacteria. *Journal of bacteriology* 1971; 105(1): 165-171.

38 Rocha, I, Ma, Y, Souza-Alonso, P *et al.* Seed coating: A tool for delivering beneficial microbes to agricultural crops. *Frontiers in Plant Science* 2019; 10(1357).

39 Deaker, R, Roughley, RJ & Kennedy, IR. Desiccation tolerance of rhizobia when protected by synthetic polymers. *Soil Biology & Biochemistry* 2007; 39(2): 573-580.

40 Rieck, C, Bück, A & Tsotsas, E. Estimation of the dominant size enlargement mechanism in spray fluidized bed processes. *AIChE Journal* 2020; 66(5): e16920.

41 Hede, PD, Bach, P & Jensen, AD. Fluidized-bed coating with sodium sulfate and PVA− TiO2, 1. Review and agglomeration regime maps. *Industrial & Engineering Chemistry Research* 2009; 48(4): 1893-1904.

42 Huang, S, Vignolles, M-L, Chen, XD *et al.* Spray drying of probiotics and other food-grade bacteria: A review. *Trends in Food Science & Technology* 2017; 631-17.

43 O'Callaghan, M. Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied Microbiology and Biotechnology* 2016; 100(13): 5729-5746.

Figure 1 Schematic of a Buchi B-290 benchtop spray-dryer. Outlet temperature is measured in the bridge between the drying chamber and the cyclone, marked by the ‘x’.

Figure 2 Schematic of UniGlatt fluidized bed spray-coater in Wurster configuration. Outlet temperature is measured between the filter and exhaust, marked by the ‘x’.

Figure 3 Feed and product characterization for each encapsulation method. (A) Viable cell count of Cal35 in the feed as log [CFU/g solids]. (B) Viable cell count of Cal35 in the encapsulated product as log [CFU/g solids] for spray-drying, and log [CFU/g coated solids] for spray-coating. (C) Yield as a percentage of recoverable solids. (D) Wet basis moisture content of spray-dried powder or spray-coated particles containing Cal35. (E) Water activity of spray-dried powder or spray-coated particles containing Cal35. Asterisks denote statistically significant differences between encapsulation methods.

Figure 4 Viability of Cal35 during the fluidized bed spray-coating process. Viability of the coating suspension before spraying is represented at 0% Batch Progress. Once the coating suspension was fully sprayed (20 min), the coated particles continued to fluidize for two extra minutes to complete drying, represented at 100% Batch Progress.

Figure 5 Viability of Cal35 heated between 35 to 60°C for three hours. The limit of detection is indicated by the horizontal red line. Data points on the limit of detection marked with an ‘x’ indicate no viable bacteria were recovered at this timepoint. Lines are drawn to guide the eye.

Figure 6 SEM images of, (A) CLAMs with 30 µm scale bar, and (B) CLAMshell particles with 500 µm scale bar.