microbiology research

MDPI

Review

Ice-Nucleating Gut Microbes in Insects: A Scoping Review

Matan Shelomi

Department of Entomology, National Taiwan University, Taipei 10617, Taiwan; mshelomi@ntu.edu.tw; Tel.: +886-0233665588

Abstract: (1) Background. At subzero temperatures, water crystallizes on ice nucleation agents. Researchers have identified ice-nucleating microbes (INMs) in insect digestive tracts that can raise the insect's supercooling point, causing freezing at higher temperatures but slower rates. For freeze-tolerant insects, such gut microbes should allow for slower freezing away from tissues and higher survival rates. For freeze-susceptible insects, however, such microbes could cause a fatal freeze at higher temperatures, and could possibly be used as biocontrol. (2) Methods. A first-ever scoping review was carried out of research on insect-associated INMs, from observational studies attempting to isolate these microbes, to experimental studies applying them and checking for increased mortality. (3) Results. Relatively few research groups have studied insect-associated INMs in any capacity. (4) Conclusions. Several authors hypothesized that such microbes are probably abundant, and their contribution to ice nucleation activity in insects is under-reported. Biocontrol assays using ice-nucleating microbes showed promise, but a risk to non-target organisms has been experimentally confirmed. Future surveys of insect–microbe interactions using molecular tools are likely to reveal new examples, if not new microbe species capable of ice nucleation.

Keywords: ice nucleation; ice-nucleating microbes; *Pseudomonas syringae*; insects; gut microbes; cold tolerance; freeze tolerance

Citation: Shelomi, M. Ice-Nucleating Gut Microbes in Insects: A Scoping Review. *Microbiol. Res.* **2024**, *15*, 708–720. https://doi.org/10.3390/microbiolres15020046

Academic Editor: Wataru Mitsuhashi

Received: 9 March 2024
Revised: 24 April 2024
Accepted: 29 April 2024
Published: 30 April 2024

1. Introduction

Freezing water can be lethal for organisms: ice crystals take more space than liquid water, physically damaging cells as they form while also disrupting the osmotic balance of tissues. Water in biological systems does not normally freeze at 0 °C due to the presence of solutes that lower the freezing point, causing supercooling (cooling below 0 °C without crystallization). Water can reach as low as −40 °C before it inevitably freezes spontaneously, so freezing above this point is typically initiated by an ice nucleation (IN) agent. An ice nucleation agent is a particle (dust, food, certain large molecules, and even ice itself) on which water molecules start to crystalize. Many ubiquitous species of microbes are capable of ice nucleation, and they are found worldwide even in climates that never approach freezing [1,2]. Ice-nucleating microbes (INMs) in the atmosphere even play a role in triggering precipitation [3]. Among ice-nucleating bacteria (INBs), strains of *Pseudomonas syringae* van Hall 1902 have particularly strong IN activity [4]: one of the first INBs to be discovered was *Ps. syringae* [5,6], and a commercially prepared, lyophilized, and UV-sterilized formulation of *Ps. syringae* called Snomax® (Genencor International, Rochester, NY, USA) is used for the artificial production of snow for ski slopes.

Insects that live in habitats where the temperature drops below freezing have evolved many different strategies to deal with the cold [7–9]. Some migrate or move to microhabitats where the local temperature is always above freezing. Others have evolved strategies to keep their bodies thawed even as the temperature around them reaches otherwise lethal levels. These "freeze-susceptible" insects use strategies such as producing antifreeze proteins to lower their supercooling point (SCP, the temperature at which the organism freezes) and ensure they stay unfrozen and alive at a greater range of subzero temperatures. Should they freeze, however, they will die. Other insects are "freeze-tolerant", able to

survive being frozen up to a point. They may use cryoprotectants like glycerol, sorbitol, or trehalose to stabilize the dehydrating cells [10], or even dehydrate as much as possible and enter a cryptobiotic state to minimize the damage to their cells from freezing water.

The relationship between ice nucleation and insect freeze tolerance is complicated. A small insect can supercool to −25 °C or lower before freezing. Some freeze-susceptible insects may survive winter by eliminating ice-nucleating agents from their body as much as possible, meaning that they are supercooled but hopefully not frozen [11]. Ice itself is an ice-nucleating agent, and some insects survive by having the ice outside their body passively serve as ice nucleation sites. Others, especially those that freeze at temperatures below −6 °C, will do the opposite and produce their own ice-nucleating agents in their extracellular space [like the hemolymph] or else depend on those in their gut. Once freezing starts, the damage to the insect tissues depends on how supercooled it is at that point: the lower the temperature, the faster the freezing once it starts, leading to the rapid production of ice crystals that pull water out of the cells, causing damage by osmotic shock, dehydration, and mechanical damage from ice itself [8]. Reduced supercooling, such as by ice-nucleating agents, causes freezing to begin at a higher temperature, which would slow crystal growth speed and limit damage to insect tissues by allowing time for the insect to adjust to the resulting osmotic and mechanical stresses as water from cells migrates to the extracellular spaces to freeze [10–12], reducing intracellular freezing and increasing cold tolerance [8] (Figure 1). Freeze-tolerant insects will overwinter frozen, conserving energy and reducing water loss [12], and would benefit from having ice nucleation agents in their body raising the supercooling point as much as possible, unlike freeze-susceptible insects that prefer to stay unfrozen at lower temperatures, and which would void their gut of food and microbes to remove the ice-nucleating agents [11,12] (Figure 2).

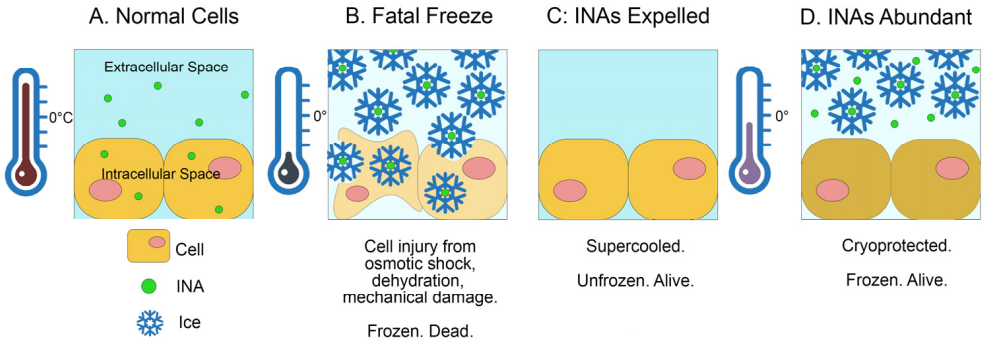

Figure 1. Diagram of how freezing damages cells, and two strategies to withstand freezing temperatures. (**A**) Normal cells above freezing. (**B**) Past an organism's supercooling point, ice rapidly crystalizes around ice-nucleating agents (INAs), damaging the cells. (**C**) Elimination of INAs, for example by clearing the gut, keeps the organism supercooled at an otherwise lethal temperature. (**D**) Concentrating INAs in the extracellular space causes water to start freezing at a higher temperature, giving the organism time to adapt to the change in osmotic pressure, build up cryoprotectants in the cells, and survive despite being frozen.

While insects can produce their own ice-nucleating agents, which are often proteinaceous [11,13], others may rely on INMs in the gut, as the gut lumen is an extracellular space and may be a safer location for ice to form as the insect freezes. While INMs could thus be advantageous for freeze-tolerant insects, they would for the same reason be lethal for freeze-susceptible species, raising the temperatures at which they freeze and increasing mortality at relatively higher subzero temperatures. Researchers have already considered INMs as a form of biocontrol, testing topical and oral applications of these microbes as a means of freezing and killing insects that would otherwise survive winter unfrozen [14,15].

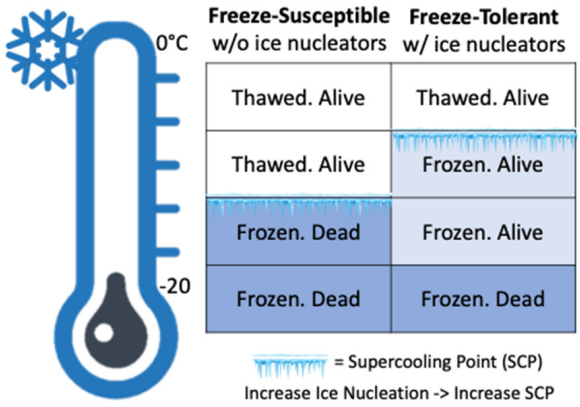

Figure 2. Diagram of the effects of temperature on the supercooling point (SCP, the temperature at which an organism freezes) and survival of freeze-susceptible and freeze-tolerant insects. Freeze-susceptible insects may eliminate active ice nucleation bacteria in their gut to lower their SCP and increase the range of temperatures where they are unfrozen and alive, while freeze-tolerant species may instead benefit from ice-nucleating bacteria that increase their SCP and lead to more survivable freezing at higher temperatures.

INMs are considered to be environmentally common [8,12], as the proteins responsible for IN activity may not have evolved specifically for IN functions and could be incidentally ice-nucleating. An example is human low-density lipoprotein, which induces ice nucleation at temperatures far below lethal for humans [16]. Despite this, the number of known INMs is low, and the number of insects with known INMs in the gut even lower. Nor are INMs widespread tools in pest management yet. This scoping review seeks to map the depth and breadth of research on insect-associated INMs. The research questions are as follows: (1) Which insects are known to have what species of ice-nucleating microbes in their gut, and how does this affect their freeze tolerance? (2) What kind of research has been carried out on INMs as biocontrol, and what obstacles remain ahead of bringing INMs to field trials? (3) Where has the research on ic- nucleating microbes in insects been carried out, how has this impacted the state of knowledge in the field, and where should future insect-associated INM research focus?

2. Materials and Methods

A scoping review was chosen, as the goal is to comprehensively search the literature with uniformly applied search criteria, extensively describe and interpret prior studies, identify parameters and gaps in the research, and set the stage for future work. This scoping review was carried out according to the PIECES framework [17,18] and following published guidelines for scoping reviews [19,20]. The publication of a scoping review's protocol is considered optional [21,22], but this was done anyway and it is available on https://osf.io/zhqnj (accessed on 28 April 2024).

The databases searched for references were Google Scholar, PubMed, Science Direct, Scopus, and Web of Science. To search for papers about ice-nucleating microbes in insects, the following search string was used [modified for ScienceDirect where wildcards are not allowed]: "ice nucleat*" AND insect* AND (bacteria OR microb* OR yeast OR fung*). No limitations were placed on the date range. The search was performed on 28 February 2024. The Covidence online platform (www.covidence.org, Accessed on 28 April 2024) was used to manage the initial screening and Microsoft Excel for the full-text analysis [23]. For the initial screening, duplicates and papers clearly off-topic based on title and abstract were excluded.

For full-text analysis, the following inclusion and exclusion criteria were applied. Only peer-reviewed journal articles and theses were included. Review papers were excluded, but still mined for relevant articles in the references that the database search may have missed. Works with only an abstract were included, even though less useful information could be

gleaned from these works. Language restrictions were not needed, as any non-English text found by the English search string could be translated if it did not already have an accompanying translation. Encyclopedia entries, book reviews, patents, and editorials were excluded (though none were identified from the search). Content criteria were any study involving hexapods (insects and Endognatha like Collembola) that used culturing to identify gut or topical microbes and then tested them for ice-nucleating ability, used molecular techniques to identify the microbes and checked the strains for known/hypothesized ice-nucleating ability, checked the ice-nucleating ability of the gut contents of insects without identifying the microbes responsible, or applied INMs to insects orally and/or topically and checked if this affected the insect SCPs, though this latter group was analyzed separately. Studies referring exclusively to non-microbial ice-nucleating agents, including those made by insects endogenously, were excluded. Data to be extracted included the type of study, the location of the study, the species of insect involved, and the species and strain, if known, of the INMs identified or used.

3. Results

The results of the review are summarized in the PRISMA flowchart (Figure 3) [20,24]. A search of five databases found a total of 246 hits, of which 90 were duplicates. The 156 unique hits were screened for relevance based on title and abstract, and 91 removed for being off-topic. Of the 65 remaining, full text was only obtainable from 56, but all were sent for full-text screening. An additional nine relevant hits were identified from other sources, of which six were only abstracts. Of the 74 hits sent for full-text screening, 15 were off-topic, 17 were review, 2 were conference proceedings possibly redundant with included journal papers, and 1 was a title with no abstract, and these were all excluded. In total, 39 publications (31 from the database search) were included for full analysis, the results of which are summarized in Tables 1 and 2. [The author notes that, while this paper was undergoing peer review in April 2024, an additional article on the topic was published online as in press [25]].

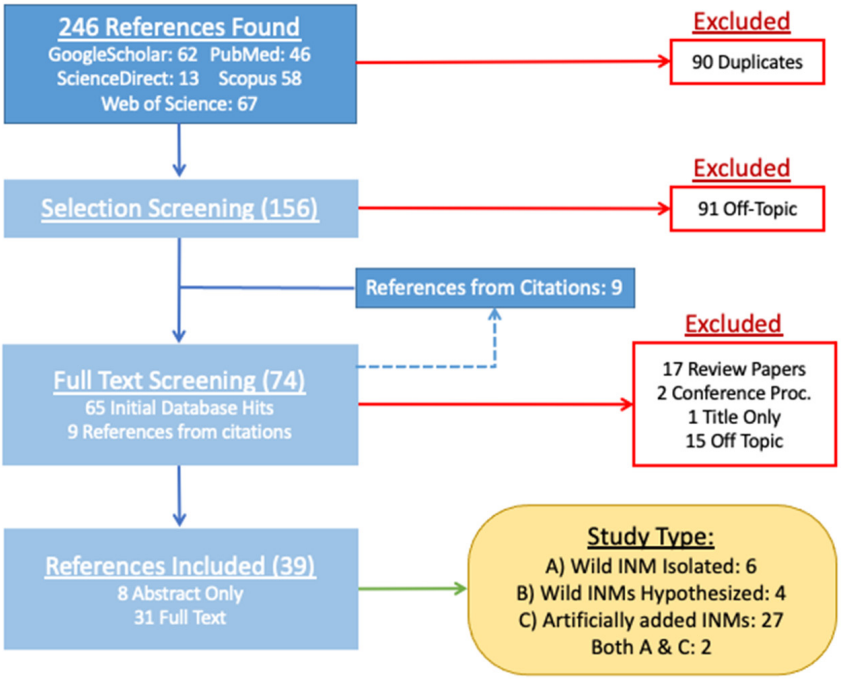

Figure 3. PRISMA flowchart for the scoping review process. Search performed on 28 February 2024 using the protocol registered with Open Science Framework (https://osf.io/zhqnj/ accessed on 28 April 2024).

Table 1. Ice-nucleating microbes isolated from or hypothesized to exist in wild insects.

Order/Family	Insect Species	FT/FS	Microbe Species	Location	Citation
Collembola; Tullbergiidae	*Tullbergia antarctica*	FS	Unidentified	Kerguelen archipelago	[26]
Blattodea; Rhinotermitidae	*Reticulitermes flavipes*	FS	Unidentified	NE, USA	[27]
Coleoptera; Chrysomelidae	*Cerotoma trifurcata*	FS [28]	*Pseudomonas fluorescens*	-	[29] ^
			Pseudomonas putida	-	
			Pseudomonas syringae	-	
			Enterobacter taylorae	OH, USA	[30]
			Enterobacter agglomerans		
Coleoptera; Perimylopidae	*Hydromedion sparsutum*	FT	*Pseudomonas* sp.	South Georgia	[31,32] *
	Perimylops antarcticus	FT	*Unidentified*		
Coleoptera; Pyrochroidae	*Dendroides canadensis*	FT [33]	*Pseudomonas fluorescens*	IN, USA	[34]
Lepidoptera; Crambidae	*Glyphodes pyloalis*	-	*Pseudomonas syringae* pv. *Mori*	Ibarakai, Japan	[35]
			Erwinia herbicola		
			Erwinia ananas		
	Chilo suppressalis	FT [36]	*Fusarium moniliforme* var. *subglutinans*	Okayama, Japan	[37,38] *
Lepidoptera; Plutellidae	*Plutella xylostella*	-	*Erwinia herbicola*	Japan	[39–41] *
Orthoptera; Gryllidae	*Dianemobius mikado*	-	Unidentified	Hokkaido, Japan	[42]
† Malacostraca; Isopoda; Porcellionidae	*Porcellio scaber*	FS [43]	Unidentified		

If known, the insects are described as being freeze-susceptible (FS) or freeze-tolerant (FT). A dash in the third column means that the information is unknown, and a citation means that this information came from a different source than the INA experiment paper cited in the last column. ^ = The source of these data is cited in this reference as "M.R.L., unpublished data". * = Includes a follow-up paper that further identified the microbes isolated in the older paper. † = Not an insect, but included in the table, as it was part of a study that did include an insect.

Table 2. Ice-nucleating microbes used in biocontrol experiments.

Microbe Species	Strain	Source	Effect	Citation
Bacteria:				
Enterobacter agglomerans	BBI	*Cerotoma trifurcata* (Coleoptera: Chrysomelidae)	↑ SCP	[44,45]
	[same study]		↑ SCP	[30]
Enterobacter cloacae †	Enc181-I	Transgenic	↑ SCP	[46]
	Enc2022-I		↑ SCP	
	WBMH-3-CMr (pICE6S13)		↑ SCP	[47]
Enterobacter taylorae	[same study]	*Cerotoma trifurcata*	↑ SCP	[30]
Erwinia ananas	Mei7-CM	*Glyphodes pyloalis* (Lepidoptera: Pyralidae)	↑ SCP	[48]
	TM2		↑ SCP	[47,48]
Erwinia ananas ††	110	*Zea mays* leaves	↑ SCP	[46]
	IN10-CM	*Thea sinensis* buds	↑ SCP	[47,48]
Erwinia herbicola	265G-2	-	↑ SCP	[30,49]
Erwinia pyrifoliae	1.3333	-	↑ SCP	[50]

Table 2. *Cont.*

Microbe Species	Strain	Source	Effect	Citation
Erwinia herbicola	265G-2	-	↑ SCP	[30,49]
Erwinia pyrifoliae	1.3333	-	↑ SCP	[50]
Pseudomonas fluorescens	F-12	*Rana sylvatica* (wood frog)	↑ SCP	[44,45]
	F26-4C		↑ SCP	[29,51,52]
	88–335	*Cerotoma trifurcata*	↑ SCP	[29]
Pseudomonas putida	F-31	*Rana sylvatica*	↑ SCP	[44,45]
	Hr6-1	*Cerotoma trifurcata*	↑ SCP	[29,52]
Pseudomonas syringae	1.32	-	↑ SCP	[50]
	1.7277	-	↑ SCP	
	C-9 [ATCC 39254]	-	↑ SCP	[53]
	cit 7	Plant rot/Epiphyte	↑ SCP	[29,30,44,45,49,54,55]
	Hr6-3B	*Cerotoma trifurcata*	↑ SCP	[29]
	Mei40-CM	*Glyphodes pyloalis*	↑ SCP	[47,48]
	Ni23	-	↑ SCP	[56]
	Snomax®	Genencor International, Rochester, NY, USA	↑ SCP	[45,57–60]
	unknown	-	↑ SCP	[61–63]
Xanthomonas campestris	unknown	-	None	[63]
Fungi: *Fusarium acuminatum*	303	-	↑ SCP	[64]
Fusarium avenaceum	411	-	↑ SCP	[45,64]
	-	-	↑ SCP	[54]

Microbe species names are as given by the original paper. See Section 3 of text for new taxonomic designations. "Same study" marks cases where the microbe was isolated from the source and then applied in biocontrol experiments in the same study, without being given a strain designation. † = transgenic strain, where the wildtype is not ice-nucleating. †† = source of the ice nucleation transgene. - = Data not available.

Of the 39 studies, 8 attempted to isolate and identify INMs associated with wild insects. Notably, this included the first report of INBs from any animal: the isolation of IN *Enterobacter* spp. (Gammaproteobacteria; Enterobacterales; Enterobacteriaceae) from the bean leaf beetle *Cerotoma trifurcata* (Forster 1771) (Coleoptera: Chrysomelidae) [30]. Solid nutrient agar supplemented with 2.5% glycerol was commonly used, as it is known to enhance IN activity [52,65]. From these eight studies, as well as unpublished data cited by one paper [29], bacteria in the genera *Pseudomonas*, *Enterobacter* (Gammaproteobacteria; Enterobacterales; Enterobacteriaceae), and *Erwinia* (Gammaproteobacteria; Enterobacterales; Erwiniaceae) plus one strain of *Fusarium* fungus (Ascomycota: Sordariomycetes; Hypocreomycetidae) were cultured from six species of insect. Another four studies checked wild insects for IN activity, and hypothesized, speculated, or at least could not rule out that these were microbial in origin, or determined they were microbial but did not identify them. For example, a study used antibiotics and oxygen treatment to defaunate the guts of the termite *Reticulitermes flavipes* (Kollar 1837) (Blattodea; Rhinotermitidae), and found that this caused a decrease in SCP similar to that of starving the termites [27]. The authors concluded that the protozoa and bacteria in the termite gut included IN strains, though other authors note that dead INMs often retain their IN activity, with Snomax® being just as effective as living *Ps. syringae* [54]. It should be noted that the one study made available online after the original database search [25] also examined *Reticulitermes flavipes*, using metagenomics to identify gut microbes at different times of year. That study found a correlation between the gut microbiome and SCP, but could not prove causality.

IN microbes were not always easy to find: studies examining the guts of diamondback moth *Plutella xylostella* (L. 1758) (Lepidoptera; Plutellidae) found three INBs out of 719 isolates, and only by using techniques to increase the likelihood of finding INMs such as precooling [39–41]; and another study culturing microbes from the gut of surface-sterilized striped rice borer *Chilo suppressalis* (Walker 1863) (Lepidoptera: Crambidae) only found a single IN microbe, a strain of *Fusarium* sp., out of over 600 isolates [37]. The insects surveyed were mostly either pests or cold-weather species. One study examined the interactions of the fire-colored beetle *Dendroides canadensis* Latreille 1810 (Coleoptera; Pyrochroidae), which notably alternates between freeze-tolerant and freeze-susceptible [66], with its gut INM, identified as *Pseudomonas fluorescens* Migula 1895 (Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae) [34]. The study found that cryoprotectant and antifreeze proteins produced by the insect's gut actively inhibited the IN activity of the *Ps. fluorescens* isolates (an effect replicated by a later study on exogenous *Ps. syringae* [67]), and that the insect cleared its gut of bacteria before winter, which would lower its SCP and enable it to stay unfrozen at lower temperatures. Studies on cold-weather insects from the sub-Antarctic suggested that INMs may contribute to higher SCPs in the insects [32], but not all INBs in the gut were equally effective: a survey of the beetles *Hydromedion sparsutum* (Müller 1884) and *Perimylops antarcticus* (Müller 1884) (Coleoptera; Perimylopidae) found the former had a strongly IN *Pseudomonas* sp., but the latter's INBs were not as active [31]. A survey of bacteria in the subantarctic springtail *Tullbergia antarctica* Lubbock 1876 found bacteria in all individuals, many of which were INBs, but the presence or absence of INBs did not correlate with increased SCPs in the springtails [26].

Of the 39 studies, 29 were about applying known INMs to insects topically and/or orally and testing the effect on the insect SCP, including the aforementioned 2 where these microbes were isolated from wild insects in the same study [30,37]. [In cases where insects live completely surrounded by their food, the application of INMs through inoculated food could infect them both topically and orally, and not all studies made efforts to distinguish between these.] Non-IN microbes used in these studies were most frequently strains of *Escherichia coli* or, occasionally, non-IN strains of the same genus as one of the used INMs. Three studies used IN fungi: *Fusarium avenaceum* (Fr.) Sacc. 1886 in all three and *F. acuminatum* Ellis & Everhart 1895 in one. All other artificially applied INMs were bacteria from the limited pool of known IMB genera. One study used Xanthomonas campestris (Pammel 1895) Dowson 1939 (Approved Lists 1980) emend. Vauterin et al., 1995, nom. approb. (Gammaproteobacteria; Xanthomonadales; Xanthomonadaceae). The genus *Erwinia* used in six studies included *Erwinia ananas*, *Er. herbicola*, and *Er. pyrifoliae* Kim et al., 1999, although the first two names are no longer valid. Synonyms for *Erwinia ananas* include *Erwinia herbicola* var. *ananas* and *Erwinia ananatis*, and the currently accepted name is *Pantoea ananatis* corrig. (Serrano 1928) Mergaert et al., 1993 [NCBI:txid553]. *Erwinia herbicola* is now *Pantoea agglomerans* (Beijerinck 1888) Gavini et al., 1989 [NCBI:txid549]. INBs of the genus *Enterobacter* were used in six studies: these included *Enterobacter agglomerans*, which has also been synonymized with *Erwinia herbicola* into *Pantoea agglomerans*; *En. taylorae*, which is now *Enterobacter cancerogenus* (Urosevic 1966) Dickey and Zumoff 1988 in [Skerman VBD et al. (1980)] [NCBI:txid69218]; and transgenic strains of normally non-IN *Enterobacter cloacae* (Jordan 1890) Hormaeche and Edwards 1960 with IN protein genes from *Erwinia ananas*. Nineteen studies used at least one of three species of *Pseudomonas*: four used *Ps. fluorescens* Migula 1895, four used *Ps. putida* (Trevisan 1889) Migula 1895, and all nineteen used *Ps. syringae* van Hall 1902. The latter was by far the most popular, with *Ps. syringae* strain cit 7 the most common, and five studies [though four of these were the same research group] using *Ps. syringae*-derived Snomax®.

The results from these studies found that INM application almost invariably led to significantly higher SCPs compared to the application of non-IN microbes or the microbe-less control, with differences sometimes exceeding 10 °C [30,45]. The one ineffective exception reported was *Xanthomonas campestris* taken orally by the clover leaf weevil, *Hypera zoilus* Scopoli 1763 [syn. *H. punctata* Fabricius 1775] (Coleoptera; Curculionidae) [63]. The SCP-

raising effect of INMs sometimes persisted for at least seven months after application [61]. Mortality increased with SCP accordingly. One study noted that their topical INM treatment also led to increased trehalase activity in the insect, which would use their stored energy and further raise mortality [50]. Studies that applied INMs to specific parts of the body, especially with insects whose mouths had been blocked to prevent the accidental ingestion of INM, found that the location of inoculation mattered, with application by the spiracles more effective than on parts of the abdomen with thick cuticles [54,58]. One study on freeze-susceptible *Hippodamia convergens* Guérim-Méneville 1842 (Coleoptera; Coccinellidae) found that topical INM treatment was more effective than oral, raising SCP from approximately −16 °C to −3 °C (topical) compared to −10 °C (oral) [55]. Transgenic INMs were as effective as the source of the IN gene [46,47]. Some INMs performed better than others: a study on silkworm *Bombyx mori* L. 1758 (Lepidoptera; Bombycidae) exposed orally to INMs found that *Ps. syringae* could not colonize the gut, while *Erwinia ananas* [*Pantoea ananatis*] could, causing twice as great a change in SCP and being more suitable for use as biocontrol [48]. Several authors concluded favorably on INM application as a form of biocontrol for freeze-susceptible species [51,57,60,62,68,69]. One research team, however, noted a risk to using INMs as biocontrol: Spiders fed prey exposed to INMs, either from the wild or through laboratory inoculation, experienced equally significant increases in SCP, meaning that natural predators of pest insects could become nontarget casualties of INM-based pest management [42,56].

4. Discussion

Since the discovery of INMs in 1974, and the isolation of the first insect-associated INM in 1991, few research groups have identified insect-associated INMs. Identifying INMs from cultured insect gut microbes is labor-intensive: two studies reported ratios of one strain out of hundreds being IN. Even in a species of microbe known to be IN-active, not all strains of the species will have this activity, and even then not all individual microbe cells will show this IN activity at the same temperatures, with IN activity often measured as a proportion of droplets of a given concentration of bacterial cells that freeze at a given temperature [3]. Nonetheless, a research group that isolated several INMs from insects as well as from a freeze-tolerant frog [70] concluded that, considering the "relative ease" with which the INMs can be found from insects and the "apparent fact" that few research groups have searched for them, it is possible that they are environmentally common [12]. As IN activity can be incidental, INMs can be found in habitats and climates that never approach freezing; and as the known INMs are in highly cosmopolitan genera like *Pseudomonas*, *Erwinia*, *Pantoea*, and *Enterobacter* already widely reported as insect gut microbes [71], this author concurs that insect-associated INMs are probably widespread.

The shift in microbiology research from culturing-based to metagenomics may partly explain why most of the studies on INMs are from before the year 2000. Applying 21st century techniques could renew interest in the field while speeding up the rate of INM discovery. Newer tools to identify INMs from samples include metagenomics and quantitative PCR targeting the *ina* gene found in all known INB strains [3,29,72,73], and these have the advantage of working on possible INBs that cannot be cultured [29], though these primers would not work on IN fungi and may not work for other, yet to be discovered, INB clades. Checking previously published insect gut metagenomic data could even reveal evidence of INMs and identify priority targets for future culturing effects. It should, however, be noted that the presence of INMs in an insect gut does not necessarily mean that the microbes are affecting the physiology or ecology of the insect [25], even in climates that routinely reach subzero temperatures [32].

INMs are only known in a few genera, all of which were observed in insect guts. As several of the few known INBs were first isolated from insects, the possibility exists that novel INM genera, species, or strains will be found in as yet unstudied insects. Somewhat complicating the task is that, besides *Pseudomonas* and the fungus *Fusarium*, the other genera are taxonomically complicated. Past work stated the difficulty in differentiating

among common Enterobacteriaceae found in insect gut microbiomes [among other sites] such as *Enterobacter*, *Pantoea*, *Serratia*, *Klebsiella*, and *Erwinia* with just 16S barcoding. That some of these have been merged into the same species may explain why (e.g., *Enterobacter agglomerans* and *Erwinia herbicola* are now merged into *Pantoea agglomerans* [74–76]). Another complication is that not all species in these large genera are nucleating [29], nor are all strains of species with known ice-nucleating members necessarily also ice-nucleating.

A low number of insect species were analyzed for INMs. These were predominantly agricultural pests, both for applied reasons and as INMs are strongly associated with plant surfaces [77], and cold-climate insects for which supercooling can be significantly [mal]adaptive. Any insect that overwinters or otherwise regularly survives subzero temperatures in its natural habitat could have unique relationships with INMs. Future research should more closely examine these potential insect–INM interactions, as many questions remain. It should be considered how INMs that increase SCP in insects are nonetheless present in freeze-susceptible arthropods in cold-winter climates like *Reticulitermes flavipes* [25,27], that would in theory be better served by ridding their guts of INMs to prevent freezing. In the case of termites, eliminating IN gut microbes with essential digestive roles may not be possible, so they should have evolved different mechanisms to balance their freeze susceptibility with their symbiosis. In freeze-tolerant insects, researchers should check for strategies that could minimize the impact of any gut INMs or non-microbial IN particles, from gut clearing (starvation and/or defecation) [78] to the production of antifreeze proteins that actively suppress IN activity, as seen in *Dendroides canadensis* [34,67]. It may be worth re-examining freeze-susceptible insects for which past publications confirmed IN activity in the gut but did not check whether the IN particles were microbial or endogenous in origin [79,80]. In a review of freeze-tolerant insects from Aotearoa/New Zealand, the authors suspected that "the role of exogenous ice-nucleating agents has been neglected" in many studies of IN compounds in insects [8]. Any such studies of IN in insects that did not include a mention of microbes, bacteria, yeasts, or fungi in the text would also have been missed by the search string in this review, so one cannot speculate on how many such studies exist. The authors hypothesized that insects that freeze above −6 °C likely rely on environmental sources of INAs, like microbes, rather than endogenous IN compounds, especially if the gut and frass have higher SCPs or a higher concentration of IN particles than the hemolymph or the rest of the body. These suggestions, combined with published lists of insect SCPs [7], can help guide future research directions.

Many papers tested the application of INMs as biocontrol of freeze-susceptible insects, with the results broadly positive and with effects lasting days or even months. The bulk of this research was carried out in the late 1990s and early 2000s, which raises the question of whether the state of research in this field is still on track to bringing practical applications to market in the future. INMs have a number of possible applications [81], with Snomax®'s use in artificial snow generation a case of successful commercialization of an INB's potential, but field trials of IN-based biocontrol have not yet been performed. Importantly, non-target effects have been demonstrated, as IN-active particles can spread up the food chain [42,56]; and this must be taken into consideration before INMs are deployed as biocontrol, if not also for snow production and weather modification. Insect-associated INMs still have possible applications, for example, to aid the cold-temperature, long-term storage of live, farmed insects like parasitoid wasps for biocontrol [82] and edible insects as food or feed.

In conclusion, this review found reason to believe that the number of known insect-INM associations is an underestimate. As the known INMs are in cosmopolitan microbe genera, among other reasons, new INMs should be relatively easy to find, especially using molecular methods. The search need not be limited to agricultural pests or cold-weather insects, as IN activity does evolve incidentally, though the role of INMs in the survival of freeze-susceptible or freeze-tolerant insects has not been fully determined and merits deeper investigation. Interest in the use of INMs as biocontrol has waned since the 20th century, and a study found non-target effects in arthropods higher up the food chain, though non-target effects also exist in other insecticides. Given the high rate of success

of INM experiments [accounting, of course, for positive results bias], the use of INMs in biocontrol deserves further attention. The presence of these non-target effects also suggests another field of research: looking into the possible negative effects on native arthropods of using INMs like Snomax® in weather modification.

Funding: The APC was funded by the Taiwan Ministry of Education, Grant 113L7801.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created in this study.

Conflicts of Interest: The author declares no conflicts of interest.

References

1. Forbes, J.; Bissoyi, A.; Eickhoff, L.; Reicher, N.; Hansen, T.; Bon, C.G.; Walker, V.K.; Koop, T.; Rudich, Y.; Braslavsky, I.; et al. Water-organizing motif continuity is critical for potent ice nucleation protein activity. *Nat. Commun.* **2022**, *13*, 5019. [CrossRef]
2. Khosasih, V.; Prasetyo, N.; Sudianto, E.; Waturangi, D.E. Prevalence and characterization of Ice Nucleation Active (INA) bacteria from rainwater in Indonesia. *BMC Microbiol.* **2022**, *22*, 116. [CrossRef]
3. Hill, T.C.; Moffett, B.F.; Demott, P.J.; Georgakopoulos, D.G.; Stump, W.L.; Franc, G.D. Measurement of ice nucleation-active bacteria on plants and in precipitation by quantitative PCR. *Appl. Environ. Microbiol.* **2014**, *80*, 1256–1267. [CrossRef] [PubMed]
4. Cochet, N.; Widehem, P. Ice crystallization by *Pseudomonas syringae*. *Appl. Microbiol. Biotechnol.* **2000**, *54*, 153–161. [CrossRef]
5. Maki Leroy, R.; Galyan Elizabeth, L.; Chang-Chien, M.-M.; Caldwell Daniel, R. Ice Nucleation Induced by *Pseudomonas syringae*. *Appl. Microbiol.* **1974**, *28*, 456–459. [CrossRef]
6. Lindow, S. History of Discovery and Environmental Role of Ice Nucleating Bacteria. *Phytopathology* **2023**, *113*, 605–615. [CrossRef] [PubMed]
7. Danks, H.V.; Kukal, O.; Ring, R.A. Insect Cold-Hardiness: Insights from the Arctic. *Arctic* **1994**, *47*, 391–404. [CrossRef]
8. Morgan-Richards, M.; Marshall, C.J.; Biggs, P.J.; Trewick, S.A. Insect Freeze-Tolerance Downunder: The Microbial Connection. *Insects* **2023**, *14*, 89. [CrossRef] [PubMed]
9. Zhao, Z. Progress In The Research On Mechanism Of Insect Cold-hardiness. *Insect Sci.* **1997**, *4*, 265–276. [CrossRef]
10. Klowden, M.J. (Ed.) Chapter 7—Circulatory Systems. In *Physiological Systems in Insects*, 2nd ed.; Academic Press: San Diego, CA, USA, 2008; pp. 357–401.
11. Duman, J.G. Insect antifreezes and ice-nucleating agents. *Cryobiology* **1982**, *19*, 613–627. [CrossRef]
12. Lee, R.E., Jr.; Costanzo, J.P.; Mugano, J.A. Regulation of supercooling and ice nucleation in insects. *Eur. J. Entomol.* **1996**, *93*, 405–418.
13. Duman, J.G.; Patterson, J.L. The role of ice nucleators in the frost tolerance of overwintering queens of the bald faced hornet. *Comp. Biochem. Physiol. Part A Physiol.* **1978**, *59*, 69–72. [CrossRef]
14. Lee, R.E., Jr.; Lee, M.R.; Strong-Gunderson, J.M. Insect cold-hardiness and ice nucleating active microorganisms including their potential use for biological control. *J. Insect Physiol.* **1993**, *39*, 1–12. [CrossRef]
15. Andreadis, S.S.; Athanassiou, C.G. A review of insect cold hardiness and its potential in stored product insect control. *Crop Prot.* **2017**, *91*, 93–99. [CrossRef]
16. Lundheim, R. Physiological and ecological significance of biological ice nucleators. *Philos. Trans. R. Soc. Lond. B* **2002**, *357*, 937–943. [CrossRef] [PubMed]
17. Foster, M.J.; Jewell, S.T. (Eds.) *Assembling the Pieces of a Systematic Review: A Guide for Librarians*; Rowman & Littlefield: Lanham, MD, USA, 2017; p. 223.
18. Parker, R.; Sikora, L. Literature Reviews: Key Considerations and Tips from Knowledge Synthesis Librarians. *J. Grad. Med. Educ.* **2022**, *14*, 32–35. [CrossRef] [PubMed]
19. Mak, S.; Thomas, A. Steps for Conducting a Scoping Review. *J. Grad. Med. Educ.* **2022**, *14*, 565–567. [CrossRef]
20. Tricco, A.C.; Lillie, E.; Zarin, W.; O'Brien, K.K.; Colquhoun, H.; Levac, D.; Moher, D.; Peters, M.D.; Horsley, T.; Weeks, L. PRISMA extension for scoping reviews (PRISMA-ScR): Checklist and explanation. *Ann. Intern. Med.* **2018**, *169*, 467–473. [CrossRef] [PubMed]
21. Peters, M.D.J.; Godfrey, C.M.; Khalil, H.; McInerney, P.; Parker, D.; Soares, C.B. Guidance for conducting systematic scoping reviews. *JBI Evid. Implement.* **2015**, *13*, 141–146. [CrossRef] [PubMed]
22. Peters, M.D.J.; Marnie, C.; Tricco, A.C.; Pollock, D.; Munn, Z.; Alexander, L.; McInerney, P.; Godfrey, C.M.; Khalil, H. Updated methodological guidance for the conduct of scoping reviews. *JBI Evid. Synth.* **2020**, *18*, 2119–2126. [CrossRef]
23. Macdonald, M.; Martin Misener, R.; Weeks, L.; Helwig, M. Covidence vs Excel for the title and abstract review stage of a systematic review. *JBI Evid. Implement.* **2016**, *14*, 200–201. [CrossRef]
24. Moher, D.; Liberati, A.; Tetzlaff, J.; Altman, D.G. Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *Int. J. Surg.* **2010**, *8*, 336–341. [CrossRef] [PubMed]

25. Arango, R.A.; Bishell, A.B.; Ohno, K.M.; Shelton, T.G.; Schoville, S.D.; Carlos-Shanley, C. Seasonal shifts in gut microbiota and cold tolerance metrics in a northern population of *Reticulitermes flavipes* (Blattodea: Rhinotermitidae). *Environ. Entomol.* **2024**, *In Press*, nvae027. [CrossRef]
26. Worland, M.R. Factors that influence freezing in the sub-Antarctic springtail *Tullbergia antarctica*. *J. Insect Physiol.* **2005**, *51*, 881–894. [CrossRef]
27. Cabrera, B.J.; Kamble, S.T. Supercooling Differences in the Eastern Subterranean Termite (Isoptera: Rhinotermitidae). *J. Entomol. Sci.* **2004**, *39*, 525–536. [CrossRef]
28. Carrillo, M.A.; Koch, R.L.; Burkness, E.C.; Bennett, K.; Ragsdale, D.W.; Hutchison, W.D. Supercooling Point of Bean Leaf Beetle (Coleoptera: Chrysomelidae) in Minnesota and a Revised Predictive Model for Survival at Low Temperatures. *Environ. Entomol.* **2005**, *34*, 1395–1401. [CrossRef]
29. Castrillo, L.A.; Lee, R.E., Jr.; Lee, M.R.; Rutherford, S.T. Identification of Ice-Nucleating Active *Pseudomonas fluorescens* Strains for Biological Control of Overwintering Colorado Potato Beetles (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **2000**, *93*, 226–233. [CrossRef] [PubMed]
30. Lee, R.E., Jr.; Strong-Gunderson, J.M.; Lee, M.R.; Grove, K.S.; Riga, T.J. Isolation of ice nucleating active bacteria from insects. *J. Exp. Zool.* **1991**, *257*, 124–127. [CrossRef]
31. Worland, R.; Block, W.; Rothery, P. Ice nucleation studies of two beetles from sub-antarctic South Georgia. *Polar Biol.* **1993**, *13*, 105–112. [CrossRef]
32. Worland, M.R.; Block, W. Ice-Nucleating Bacteria from the Guts of Two Sub-Antarctic Beetles, *Hydromedion sparsutum* and *Perimylops antarcticus* (Perimylopidae). *Cryobiology* **1999**, *38*, 60–67. [CrossRef]
33. Duman, J.G. Factors involved in overwintering survival of the freeze tolerant beetle, *Dendroides canadensis*. *J. Comp. Physiol.* **1980**, *136*, 52–59. [CrossRef]
34. Olsen, T.M.; Duman, J.G. Maintenance of the supercooled state in the gut fluid of overwintering pyrochroid beetle larvae, *Dendroides canadensis*: Role of ice nucleators and antifreeze proteins. *J. Comp. Physiol. B* **1997**, *167*, 114–122. [CrossRef]
35. Takahashi, K.; Watanabe, K.; Sato, M. Survival and Characteristics of Ice Nucleation-active Bacteria on Mulberry Trees (*Morus* spp.) and in Mulberry Pyralid (*Glyphodes pyloalis*). *Ann. Phytopathol. Soc. Jpn.* **1995**, *61*, 439–443. [CrossRef]
36. Izumi, Y.; Sonoda, S.; Yoshida, H.; Tsumuki, H. Identification of tissues showing the lowest tolerance to freezing in larvae of the rice stem borer, *Chilo suppressalis*. *Physiol. Entomol.* **2005**, *30*, 324–331. [CrossRef]
37. Tsumuki, H.; Konno, H.; Maeda, T.; Okamoto, Y. An ice-nucleating active fungus isolated from the gut of the rice stem borer, *Chilo suppressalis* Walker (Lepidoptera: Pyralidae). *J. Insect Physiol.* **1992**, *38*, 119–125. [CrossRef]
38. Tsumuki, H. Review of low temperature tolerance and ice nuclei in insects, with special emphasis on larvae of the rice stem borer, *Chilo suppressalis* Walker. *Jpn. J. Appl. Entomol. Zool.* **2000**, *44*, 149–154. [CrossRef]
39. Kaneko, J.I.; Kita, K.; Tanno, K. Bacteria in the gut determines the supercooling point of diamondback moth, *Plutella xylostella*, pupae reared on germinating radish seeds (*Raphanus sativus* L. var. *acanthiformis* Makino). *Jpn. J. Appl. Entomol. Zool.* **1989**, *33*, 82–91. [CrossRef]
40. Kaneko, J.I.; Kita, K.; Tanno, K. INA bacteria isolated from diamondback moth, *Plutella xylostella* L. pupae (Lepidoptera: Yponomeutidae). *Jpn. J. Appl. Entomol. Zool.* **1991**, *35*, 7–11. [CrossRef]
41. Kaneko, J.I.; Yoshida, T.; Owada, T.; Kita, K.; Tanno, K. *Erwinia herbicola*: Ice nucleation active bacteria isolated from diamondback moth, *Plutella xylostella* L. pupae. *Jpn. J. Appl. Entomol. Zool.* **1991**, *35*, 247–251. [CrossRef]
42. Tanaka, K. Supercooling ability in the house spider, *Achaearanea tepidariorum*: Effect of field-collected and laboratory-reared prey. *Naturwissenschaften* **2001**, *88*, 431–433. [CrossRef]
43. Tanaka, K.; Udagawa, T. Cold adaptation of the terrestrial isopod, *Porcellio scaber*, to subnivean environments. *J. Comp. Physiol. B* **1993**, *163*, 439–444. [CrossRef]
44. Costanzo, J.P.; Humphreys, T.L.; Lee, R.E., Jr.; Moore, J.B.; Lee, M.R.; Wyman, J.A. Long-term reduction of cold hardiness following ingestion of ice-nucleating bacteria in the Colorado potato beetle, *Leptinotarsa decemlineata*. *J. Insect Physiol.* **1998**, *44*, 1173–1180. [CrossRef] [PubMed]
45. Lee, R.E., Jr.; Litzgus, J.D.; Mugnano, J.A.; Lee, M.R.; Horton, D.R.; Dunley, J. Evaluation of Ice-nucleating Microorganisms For Reducing The Supercooling Capacity And Cold-hardiness Of *Cacopsylla pyricola* (Hemiptera: Psyllidae). *Can. Entomol.* **1999**, *131*, 715–723. [CrossRef]
46. Tang, C.; Sun, F.; Zhang, X.; Zhao, T.; Qi, J. Transgenic ice nucleation-active *Enterobacter cloacae* reduces cold hardiness of corn borer and cotton bollworm larvae. *FEMS Microbiol. Ecol.* **2004**, *51*, 79–86. [CrossRef] [PubMed]
47. Watanabe, K.; Abe, K.; Sato, M. Biological control of an insect pest by gut-colonizing *Enterobacter cloacae* transformed with ice nucleation gene. *J. Appl. Microbiol.* **2000**, *88*, 90–97. [CrossRef]
48. Watanabe, K.; Sato, M. Gut Colonization by an Ice Nucleation Active Bacterium, *Erwinia (Pantoea) ananas* Reduces the Cold Hardiness of Mulberry Pyralid Larvae. *Cryobiology* **1999**, *38*, 281–289. [CrossRef]
49. Strong-Gunderson, J.M.; Lee, R.E.; Lee, M.R.; Tammy, J.R. Ingestion of ice-nucleating active bacteria increases the supercooling point of the lady beetle *Hippodamia convergens*. *J. Insect Physiol.* **1990**, *36*, 153–157. [CrossRef]
50. Shao, H.; Liu, Y.; Liu, Y.; Lai, Y. The Effect of Ice-Nucleation-Active Bacteria on Metabolic Regulation in *Evergestis extimalis* (Scopoli) (Lepidoptera: Pyralidae) Overwintering Larvae on the Qinghai-Tibet Plateau. *Insects* **2022**, *13*, 909. [CrossRef] [PubMed]

51. Castrillo, L.A.; Lee, R.E.; Wyman, J.A.; Lee, M.R.; Rutherford, S.T. Field Persistence of Ice-Nucleating Bacteria in Overwintering Colorado Potato Beetles. *Biol. Control* **2001**, *21*, 11–18. [CrossRef]
52. Castrillo, L.A.; Rutherford, S.T.; Lee, R.E.; Lee, M.R. Enhancement of Ice-Nucleating Activity in *Pseudomonas fluorescens* and Its Effect on Efficacy against Overwintering Colorado Potato Beetles. *Biol. Control* **2001**, *21*, 27–34. [CrossRef]
53. Landry, C.E.; Phillips, S.A., Jr. Potential of Ice-Nucleating Active Bacteria for Management of the Red Imported Fire Ant (Hymenoptera: Formicidae). *Environ. Entomol.* **1996**, *25*, 859–866. [CrossRef]
54. Steigerwald, K.A.; Lee, M.R.; Lee, R.E.; Marshall, J.C. Effect of biological ice nucleators on insect supercooling capacity varies with anatomic site of application. *J. Insect Physiol.* **1995**, *41*, 603–608. [CrossRef]
55. Strong-Gunderson, J.M.; Lee, R.E.; Lee, M.R. Topical Application of Ice-Nucleating-Active Bacteria Decreases Insect Cold Tolerance. *Appl. Environ. Microbiol.* **1992**, *58*, 2711–2716. [CrossRef] [PubMed]
56. Tanaka, K.; Watanabe, M. Transmission of ice-nucleating active bacteria from a prey reduces cold hardiness of a predator (Araneae: Theridiidae). *Naturwissenschaften* **2003**, *90*, 449–451. [CrossRef] [PubMed]
57. Lee, R.E., Jr.; Costanzo, J.P.; Kaufman, P.E.; Lee, M.R.; Wyman, J.A. Ice-Nucleating Active Bacteria Reduce the Cold-Hardiness of the Freeze-Intolerant Colorado Potato Beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* **1994**, *87*, 377–381. [CrossRef]
58. Lee, R.E., Jr.; Steigerwald, K.A.; Wyman, J.A.; Costanzo, J.P.; Lee, M.R. Anatomic Site of Application of Ice-Nucleating Active Bacteria Affects Supercooling in the Colorado Potato Beetle (Coleoptera: Chrysomelidae). *Environ. Entomol.* **1996**, *25*, 465–469. [CrossRef]
59. Lee, R.E., Jr.; Strong-Gunderson, J.M.; Lee, M.R.; Davidson, E.C. Ice-Nucleating Active Bacteria Decrease the Cold-Hardiness of Stored Grain Insects. *J. Econ. Entomol.* **1992**, *85*, 371–374. [CrossRef]
60. Mignon, J.; Haubruge, E.; Gaspar, C. Effect of ice-nucleating bacteria (*Pseudomonas syringae* Van Hall) on insect susceptibility to sub-zero temperatures. *J. Stored Prod. Res.* **1998**, *34*, 81–86. [CrossRef]
61. Castrillo, L.A.; Lee, R.E.; Lee, M.R.; Wyman, J.A. Long-term retention of ice-nucleating active *Pseudomonas fluorescens* by overwintering colorado potato beetles. *Cryo Lett.* **2000**, *21*, 5–12.
62. Strong-Gunderson, J.M.; Lee, R.E., Jr.; Lee, M.R. Ice nucleating active bacteria increase insect mortality at high subzero temperatures. *Cryo Lett.* **1994**, *15*, 385–392.
63. Watanabe, M. Effects of ingestion and excretion of ice-nucleating-active bacteria on the supercooling ability and cold hardiness in larvae of the clover leaf weevil, *Hypera punctata*. *Cryo Lett.* **1999**, *20*, 363–370.
64. Lee, M.R.; Lee, R.E., Jr.; Strong, J.M.; Minges, S.R.; Mugnano, J.A. Reduction of insect cold-hardiness using ice-nucleating active fungi and surfactants. *Entomol. Exp. Appl.* **1998**, *89*, 103–109. [CrossRef]
65. Chen, M.-L.; Chiou, T.-K.; Jiang, S.-T. Isolation of ice-nucleating active bacterium from mackerel and its properties. *Fish. Sci.* **2002**, *68*, 934–941. [CrossRef]
66. Horwath, K.L.; Duman, J.G. Yearly Variations in the Overwintering Mechanisms of the Cold-Hardy Beetle *Dendroides canadensis*. *Physiol. Zool.* **1984**, *57*, 40–45. [CrossRef]
67. Duman, J. The inhibition of ice nucleators by insect antifreeze proteins is enhanced by glycerol and citrate. *J. Comp. Physiol. B* **2002**, *172*, 163–168. [CrossRef]
68. Fields, P.; Pouleur, S.; Richard, C. The Effect Of High Temperature Storage On The Capacity Of An Ice-nucleating-active Bacterium And Fungus To Reduce Insect Cold-tolerance. *Can. Entomol.* **1995**, *127*, 33–40. [CrossRef]
69. Fields, P.G. Reduction of Cold Tolerance of Stored-Product Insects by Ice-Nucleating-Active Bacteria. *Environ. Entomol.* **1993**, *22*, 470–476. [CrossRef]
70. Lee, M.R.; Lee, R.E., Jr.; Strong-Gunderson, J.M.; Minges, S.R. Isolation of Ice-Nucleating Active Bacteria from the Freeze-Tolerant Frog, *Rana sylvatica*. *Cryobiology* **1995**, *32*, 358–365. [CrossRef]
71. Li, Y.; Chang, L.; Xu, K.; Zhang, S.; Gao, F.; Fan, Y. Research Progresses on the Function and Detection Methods of Insect Gut Microbes. *Microorganisms* **2023**, *11*, 1208. [CrossRef]
72. Stephanie, D.E.W. Distribution of Ice Nucleation-Active (INA) Bacteria from Rain-water and Air. *HAYATI J. Biosci.* **2011**, *18*, 108–112. [CrossRef]
73. Edwards, A.R.; Van den Bussche, R.A.; Wichman, H.A.; Orser, C.S. Unusual pattern of bacterial ice nucleation gene evolution. *Mol. Biol. Evol.* **1994**, *11*, 911–920. [CrossRef] [PubMed]
74. Gavini, F.; Mergaert, J.; Beji, A.; Mielcarek, C.; Izard, D.; Kersters, K.; De Ley, J. Transfer of *Enterobacter agglomerans* (Beijerinck 1888) Ewing and Fife 1972 to *Pantoea* gen. nov. as *Pantoea agglomerans* comb, nov. and Description of *Pantoea dispersa* sp. nov. *Int. J. Syst. Evol. Microbiol.* **1989**, *39*, 337–345. [CrossRef]
75. Harada, H.; Ishikawa, H. Phylogenetical relationship based on groE genes among phenotypically related *Enterobacter, Pantoea, Klebsiella, Serratia* and *Erwinia* species. *J. General. Appl. Microbiol.* **1997**, *43*, 355–361. [CrossRef] [PubMed]
76. Mergaert, J.; Verdonck, L.; Kersters, K. Transfer of *Erwinia ananas* (synonym, *Erwinia uredovora*) and *Erwinia stewartii* to the Genus *Pantoea* emend. as *Pantoea ananas* (Serrano 1928) comb. nov. and *Pantoea stewartii* (Smith 1898) comb. nov., Respectively, and Description of *Pantoea stewartii* subsp. *indologenes* subsp. nov. *Int. J. Syst. Evol. Microbiol.* **1993**, *43*, 162–173. [CrossRef]
77. Arnold, D.L.; Preston, G.M. *Pseudomonas syringae*: Enterprising epiphyte and stealthy parasite. *Microbiology* **2019**, *165*, 251–253. [CrossRef] [PubMed]
78. Gehrken, U. Correlative influence of gut appearance, water content and thermal hysteresis on whole body supercooling point of adult bark beetles, *Ips acuminatus* Gy11. *Comp. Biochem. Physiol. Part A Physiol.* **1995**, *112*, 207–214. [CrossRef]

79. Wharton, D.A.; Pow, B.; Kristensen, M.; Ramløv, H.; Marshall, C.J. Ice-active proteins and cryoprotectants from the New Zealand alpine cockroach, *Celatoblatta quinquemaculata*. *J. Insect Physiol.* **2009**, *55*, 27–31. [CrossRef] [PubMed]
80. Hodkova, M.; Hodek, I.V.O. Temperature Regulation of Supercooling and Gut Nucleation in Relation to Diapause of *Pyrrhocoris apterus* (L.) (Heteroptera). *Cryobiology* **1997**, *34*, 70–79. [CrossRef]
81. Gurian-Sherman, D.; Lindow, S.E. Ice nucleation and its application. *Curr. Opin. Biotechnol.* **1992**, *3*, 303–306. [CrossRef]
82. Colinet, H.; Boivin, G. Insect parasitoids cold storage: A comprehensive review of factors of variability and consequences. *Biol. Control* **2011**, *58*, 83–95. [CrossRef]