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Direcred Micro-Ecology Application System: On-site One-step Fermentation Facilitating Microbial Application Through Theoretical and Equipment Innovation

Yang Yang[1], Xian-feng[1] ZENG[1], Yun-feng LUO[1], Wei-min ZHAO[1], Jian-feng GUO[1], Rui-qiang MA[1,*], Wen-jing CUI[1,*]

(1. *Beijing Green Nitrogen Biotech Co., Ltd, Beijing 100094*)

*Correspondence: cuiwenjing@agreenbio.com；maruiqiang@agreenbio.com

Abstract

Functional microbial agents played a crucial role in various fields such as cultivation, feed fermentation, aquaculture and environmental protection. However, traditional microbial agents confronted with critical challenges such as limited shelf-life, reduced activity, and inconsistent efficacy. In this case, we innovatively proposed the concept of Directed Microbial Ecology (DME) and developed its application system, including a core module named DME intelligent fermentor (DME25). Over 40 functional strains, including Gram-negative strains, Gram-positive strains and fungus strains, were successfully cultured to 10-50 $\times 10^8$ CFU/mL within 20-48 h and maintained a relative low contamination rate (<2.5%). Finally, the stability and effectiveness of these DME fermented strains were validated in three different application area, all of which exhibited perfect functional characteristics. Firstly, the bacillus strains inhibited the progression of wilt disease and significantly improved the growth of tomatoes. Secondly, all tested lactobacillus strains improved the nutrition and quality of fermented feed, complying feed industry standards. Lastly, the ammonia concentration, nitrate concentration of aquaculture water and the phosphate concentration, COD of aquaculture tail water were significantly reduced within 1-4 d. The successful application of DME and its intelligent fermentor in different fields marks a pivotal breakthrough in technological innovation of the microbial on-site one-step fermentation. This technological advancement opens new avenues for enhancing the stability and effectiveness of microbial agents, infusing powerful impetus to the development of the microbial application.

Keywords: microbial agents; Directed Microbial Ecology; DME intelligent fermentor; on-site one-step fermentation; microbial application

Introduction

Microbial agents played a dispensable role in multiple fields. Increasing numbers of farmers and agricultural enterprises are adopting microbial agents to replace chemical pesticides, achieving biological control and increasing crop yields (Gouda et al., 2018; Huang et al., 2020; Jiao et al., 2021). Simultaneously, microbial agents are widely used in projects such as sewage treatment and soil remediation, accelerating the degradation of organic waste and reducing environmental pollution (Hasr Moradi Kargar and Hadizadeh Shirazi, 2020; Ilyin et al., 2004; Kim and Cho, 2022; Zhang et al., 2023). In industrial production, particularly in the preparation of bioenergy, microbial agents are expected to replace traditional fossil energy production methods, reducing adverse environmental impacts (Matsushika et al., 2009; Muras et al., 2021). In the livestock industry and aquaculture, their role in improving feed utilization, enhancing animal immunity, improving environmental hygiene, and promoting sustainable livestock development will provide important support for industry healthy development (Shao et al., 2021). Therefore, microbial agents play a vital role in achieving sustainable

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development, promoting ecological balance, protecting the environment, and improving human quality of life (Pourfadakari et al., 2021). As the pursuit of sustainable development and environmental protection awareness increases, the prospects for their application will become even broader (Adedayo et al., 2022; Meng et al., 2022).

Currently used microbial strains mainly include Gram-negative strains (*Sinorrhizobium*, *Pseudomonas*, *Nitrobacter*), Gram-positive strains (*Bacillus*, *Lactobacillus*, *Paenibacillus*) and fungus strains (*Trichoderma*, *Saccharomyces*) (Dobrzyński et al., 2023; Sadare and Daramola, 2023; Song et al., 2022; Tyśkiewicz et al., 2022; Woo et al., 2023). In general, bacillus strains were not sensitive to various adverse stresses and were widely applied, due to its ability to produce spores (Jeżewska-Frąckowiak et al., 2018; Paul et al., 2019; Todorov et al., 2022). However, other non-spore-forming microbial strains were hyper-sensitive stress tolerance, resulting in shorter shelf-life of their products, unstable application effects, and poor adaptability in the environment, leading to ineffective survival and colonization (Du et al., 2021; Schumpp and Deakin, 2010; Tittabutr et al., 2007). Therefore, improving the activity and quality stability of microbial agent products was crucial for maintaining their stable and efficient application (Chen et al., 2021; Poole et al., 2018). In recent years, increasing numbers of researchers have been attempting to overcome the shelf-life limitations of microbial agents. Nowadays, there are three common methods, including adding protective agents to liquid formulations, spray drying or freeze-drying to produce microbial powder and on-site fermentation through industrial small-scale fermenters, were extensively adopted (Biradar, 2018; Elsakhawy et al., 2021; Perry, 1995). Firstly, adding protective agents was the most simple way with low cost and short period (Bellali et al., 2020), but the extension of the shelf-life achieved through this method was limited, and the high transportation costs of liquid formulations also limited it’s development. Secondly, spray drying technical process was mainly suitable for spore-producing bacteria (Adjallé et al., 2011; Broeckx et al., 2016; Dianawati et al., 2016) and the finished spore powder faces the risk of low spore germination rates during use. Although freeze-drying could effectively extend the shelf-life of non-spore-forming strains (Yang et al., 2023), its high production cost resulted in high usage costs . Finally, using industrial fermentors for on-site fermentation exhibited great enormous advantage of standardized pure culture cultivation (Nobre et al., 2018; Wang et al., 2019). In spite of that, the high equipment procurement cost, complex operation, and requirement for specialized fermentation personnel were the three stubborn barriers for customers. To date, there was still no efficient and straightforward solution to solve the short shelf-life and maintain activity of microbial agents. Interestingly, probiotics (*Lactobacillus*) was widely used for homemade milk fermentation, because of their role in gut microbiome modulation and gastrointestinal health benefits (Vasudha et al., 2023). Immediate fermentation and use may shedding light on smart solutions to solve the problem of shelf-life of microbial and its products.

Generally, the misconception of using pure microorganisms for applications was commonly existed and unsuspicious. However, from a microbial functional standpoint, as long as the core population comprises the desired functional microorganisms, equivalent functionality can be attained. This concept bypassing the complex aseptic processes and will accelerate the conversion of functional microorganisms. Based on this concept, Green Nitrogen proposed directed micro-ecology (DME) and developed a DME intelligent fermentor through equipment and fermentation process innovation. In detail, the target functional microorganisms would dominant the final fermentation population under directed selection pressures, including values of fermentaion Tm, pH, EC, DO and selective nutrition, even under simple conditions of pasteurization. This DME intelligent fermentor with rapid proliferation and fermentation of target microorganisms in application scenarios, ensuring the activity of microorganisms, greatly

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improving product quality, and ensuring the application stability and efficiency of microbial agents. The DME application system was suitable for many fields and may helping customers to achieve desirable application performance of most agricultural microbial agents.

Materials and Methods

Strains and Materials

The strains used in this study are shown in Table S1. All DME directional medium 1~5 was shown in Table S2. The fermentation yield of these strains can reach 1.5 to 5.9×10^9 CFU/mL, with a contamination rate controlled below 2.5% (Fig. S1).

The tomato seeds were purchased from Chinese Academy of Agricultural Sciences. The feed materials were provided by Zhongnong Chuangda (Beijing) Environmental Protection Technology Co., Ltd. The aquaculture water and the aquaculture tail water samples were collected from small-scale greenhouse aquaculture ponds for South American white shrimp in Dongying, Shandong Province.

DME Fermentation of Different Strains

Validate the effects of fermenting different strains using the DME fermentation. Firstly, turn on the DME and click on the "feed" button to allow the machine to automatically add water. Next, add the culture medium into the fermentation vessel and put the inoculant into the inoculation chamber. The DME fermentation machine will then start intelligent fermentation. Once the water level reaches the set standard, begin heating for sterilization. Sterilization is conducted at 90°C for 15 mins. After sterilization, the equipment will start cooling down until the temperature drops to 37°C (for bacillus strains). At this point, the inoculant in the inoculation chamber will be automatically injected into the fermentation vessel. Follow the preset program for aeration and agitation during fermentation. After the program is completed, click the "output" button to obtain the usable inoculant.

Microbial Population and Contamination Rate Calculation

The viable microrial count was determined using the dilution plate method. Microbial suspension was serially diluted from 10^{-1} to 10^{-7}, and appropriate dilutions were selected to pipette 100 μL onto corresponding solid plates. The plates were then inverted and incubated at 28°C for 2 days in a constant temperature incubator. Microbial colonies were counted, and the final microbial population and contamination rate were calculated.

The Biocontrol and Growth-Promoting Test of Tomato

Initially, the seeds were subjected to a disinfection process, which included agitation and immersion in 95% ethanol for 1 min, followed by agitation and immersion in 2.5% NaClO for 3 min. The seeds were then rinsed 8-10 times with sterile water. Subsequently, the pre-treated seeds were placed on damp gauze, covered with an additional layer of gauze, and positioned within a petri dish. The dish was then covered with a breathable membrane to maintain moisture. The seeds were incubated at 25°C for 3 days until they began to germinate. Seeds with uniform sprout lengths were selected for sowing. Following planting, the nutrient soil was thoroughly watered and covered with a film. The seeds were then cultivated in a controlled environment chamber to ensure optimal growth conditions. Each treatment consisted of 40 tomato seedlings.

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Ralstonia solanacearum was inoculated into NA broth, 28°C , 150 rpm, 24 h. During the 20-day seedling stage, the tomato seedlings were inoculated with *R. solanacearum* at a concentration of 10^8 CFU/mL, with 0.5 mL per seedling applied to the tomato roots. The following day, each seedling was inoculated with the biocontrol agent 0.5 mL GN125 at a concentration of 5×10^8 CFU/mL to determine its disease resistance. Additionally, culture medium and the biocontrol agent GN125 was inoculated separately to assess its growth-promoting effect. Plant height was measured by taken of the distance from the soil surface to the top growth point of tomato plants. Additionally, stem diameter at 2 cm above the soil surface was measured. Starting from the bottom, the first fully developed trifoliate leaf was selected for chlorophyll content measurement (SPAD-502PLUS).

Silage and Corn Feed Experiment Process

The harvested corn at the early wax ripeness stage was cut into approximately 2 cm sections, and each section was thoroughly mixed with the respective microbial agent. The experiment included three treatment groups, control group without the addition of microbial agents, undergoing natural fermentation. Each treatment had three replicates, with each replicate contained in a bag, and each silage bag weighing approximately 40 kg. The bags were vacuum-sealed and stored in a cool, dark place for approximately two months, during which the highest daily temperature averaged 20°C, and the lowest averaged 11.3°C.

The quality of fermented silage was measured by Bayannur Hemao Muye Co. Ltd. The concentration of aflatoxin, vomitoxin of the corn feed was measured by kit (BA0141, Shanghai Youlong Biotech Co,.Ltd) according to it's manufacture.

Aquaculture Water and Aquaculture Tail Water Management and Parameter Analysis

GN2306 and GN104 were respectively added to containers with 300 mL of water samples, ensuring a bacterial concentration of 1×10^6 CFU/mL in the water. The containers were then placed in a water bath and incubated at 28°C with aeration. The concentrations of ammonia nitrogen and nitrite in the aquaculture water, and the concentrations of phosphate and COD in the aquaculture tail water were measured every day.

Multi-parameter water quality analyzer was purchased from Xiamen PanTian Biotech Co., Ltd. The determination of ammonia nitrogen was conducted using the Nessler reagent colorimetric method, while the determination of nitrite was carried out using the α-naphthylamine colorimetric method. The concentration of phosphate was measured by using the molybdenum blue method. COD was measured by COD on-line Monitor (Shanghai BoQu Instrument Co., Ltd, CODG-3000) according to it's manufacture.

Data Analysis

Graphs for experimental data were generated using GraphPad software. Analysis of variance (ANOVA) single-factor variance analysis and t-tests were conducted using SPSS software.

Results

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1. Basic Principle and Application Development System of Directed Micro-Ecology (DME)

In order to achieve standardized production and convenient operation of microbial products on-site, Green Nitrogen Biotech Co., Ltd. (referred to as "NBio") proposed the concept of Directed Micro-Ecology (DME) (Fig.1a). The principle was to supply directional selective pressure (Culture medium, fermentation time, temperature, EC value, rotation speed, dissolved oxygen level, etc.) conducive to the target functional microorganisms in a microbial ecosystem containing both the target microorganisms and various environmental microorganisms. This pressure enables the target microorganisms to gain an advantage in inter-species competition, occupy a dominant position in the microbial community within a specific cultivation period, manifesting as dominant microorganisms. Moreover, the low contamination rate from environmental microorganisms ensures that the target microorganisms perform effectively in practical applications. Based on the DME principle, NBio developed an application development system consisting of four major modules to establish the 'FNPP' cycle, achieving the iterative upgrading of a DME application system (Fig.1b). This system identifies target functional microorganisms (Function, F) based on application requirements, determines corresponding directed culture medium (Nutrition, N) and fermentation process models (Process, P), and accomplishing on-site standardized fermentation and application of target functional microorganisms through a DME intelligent fermentor (Production, P). By introducing the corresponding culture medium and inoculant into the DME intelligent fermentor , an integrated and intelligent fermentation process can be initiated (Fig.1c).

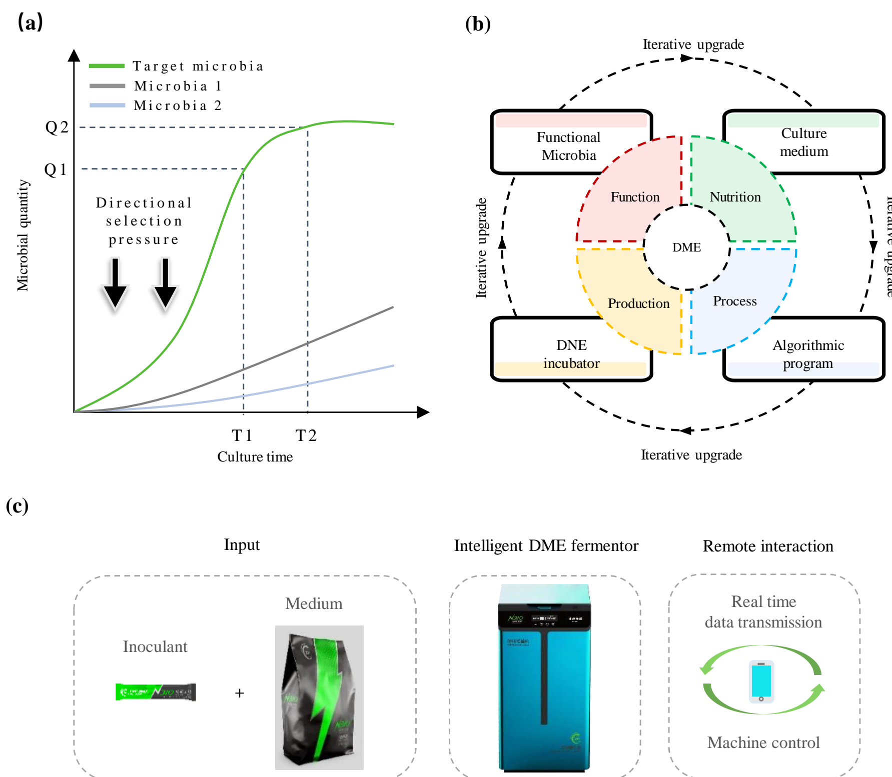

Fig. 1 Directed Micro-Ecology (DME) and it's application development system

a, Basic principle of Directed Micro-Ecology (DME). The directional selection pressure was applied to favor the dominance, within a specified cultivation time (T1-T2), of the desired population (Q1-Q2) of functional microorganism; b, The 'FNPP' development cycle, FNPP standing for Function-Nutrition-Process-Production; c, Intelligent DME incubator and fermentation consumables, including inoculant and culture medium.

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DME intelligent fermentor (DME-25), which includes temperature control components, heating and cooling modules, a stirring motor, an air pump, a filtration membrane, and automatic inoculation chamber. It operates within a temperature control range of RT to 95°C, with adjustable parameters for speed and aeration volume. The main parameters are summarized in Table 2. It can be operated by non-specialists, significantly reducing labor costs. Additionally, DME-25 was equipped with sensors for real-time data monitoring and recording to meet scientific research requirements. This advanced feature ensures precise control and documentation of the fermentation process, making the DME-25 an ideal tool for both practical applications and academic research.

Table 1 Parameter of the DME-25 fermentor

Category	Unit	Values
Size	mm	500×500×1000
Weight	kg	70
Power	kw	2.5
Voltage	V	220
Volume	L	25
Temperature range	°C	25-95
Rotation rate	r/min	150-300
Ventilation capacity	L/min	50-150

2. Fermentation Tests of Commonly Used Microbial Agents on DME Fermentor

We conducted DME fermentation tests on common microbial strains used in planting, aquaculture, forage, and environmental protection (Table 2). The results indicated that *Bacillus* adapted to medium 1 achieves a bacterial count of 20~100×10^8 CFU/mL after 16~18 h of fermentation in DME fermentor. *Rhizobium* adapted to medium 2 reaches a count of 30~40×10^8 CFU/mL after 20~22 h of DME cultivation. *Pseudomonas* adapted to medium 3 achieves a count of 30~50 ×10^8 CFU/mL after 20-22 h of cultivation. *Lactobacillus*, *Staphylococcus*, and *Lactobacillus* adapt to medium 5 and reach a count of 10-50×10^8 CFU/mL after 20~22 h of cultivation. As for several fungus strains adapted to medium 4, the required fermentation time was 36~48 h, slightly longer than bacterial fermentation, with a count of 10~20×10^8 CFU/mL. Excitingly, all tested strains' contamination rate was controlled below 3.5% (Table 2, Fig.S1). These results demonstrate the DME system's effectiveness in cultivating a wide range of microorganisms under optimal conditions, ensuring high yield and viability across different species.

Table 2 DME incubator partial matched strains

Species	Time (h)	Medium	Cell concentration (10^8 CFU/mL)	Contamination rate (%)	Application area			
					A	B	C	D
Bacillus subtilis	16-18	1	60-100	0.1-3.5	√	√		√
Bacillus licheniformis			20-40		√	√		√
Bacillus amyloliquefaciens					√			√
Bacillus mucilaginosus					√			√
Bacillus megateriumde					√			√
Bacillus velezensis					√			
Bacillus pumilus					√			

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Bacillus thuringiensis				√			
Brevibacillus laterosporus				√			
Paenibacillus polymyxa				√			
Bradyrhizobium japonicum	20-22	2	30-40	√			
Sinorhizobium fredii				√			
Pseudomonas stutzeri	20-22	3	30-50	√			
Pseudomonas aeruginosa							√
Pseudomonas putida							√
Beauveria bassiana	36-48	4	10-20	√			
Trichoderma harzianum				√			
Purpureocillium lilacinum				√			
Saccharomyces cerevisiae				√	√	√	
Candida utilis						√	
Lactobacillus plantarum	20-22	5	30-50	√	√	√	
Lactobacillus acidophilus					√	√	
Lactobacillus casei			10-20		√	√	
Lactobacillus buchneri						√	
Lactobacillus delbrueckii						√	
Lactobacillus reuteri						√	
Lactobacillus paracasei						√	
Enterococcus faecalis			30-50			√	
Pediococcus acidilactici					√	√	
Bifidobaterium longum			10-15			√	
Bifidobaterium breve						√	
Bifidobaterium animalis						√	

Note: Medium id was indicated by 1-5; Application area A, B, C, D were represent for planting, forage, aquaculture and environmental protection, respectively.

3. The Application Effect of Functional Microorganisms by Using DME intelligent Fermentor

3.1 Validation of GN125 Efficacy in Tomato Growth-Promoting and Disease Resistance Using DME Fermentor

To validate the efficacy of functional microorganisms expanded using the DME fermentor, we designed a tomato pot experiment. Data were collected at the seedling stage and harvest stage for verification, with the results presented in Figure 2. Following inoculation with *Ralstonia solanacearum* and subsequent treatment with GN125, significant increases were observed in plant height, stem diameter, fresh weight, and leaf chlorophyll content compared to plants inoculated solely with Ralstonia solanacearum. These findings suggest that GN125 fermented by DME exhibits disease resistance properties. Furthermore, growth-promotion assays indicated that plants irrigated with GN125 demonstrated significant enhancements in plant height, stem diameter, fresh weight, and leaf chlorophyll content compared to untreated controls. These results confirm that GN125 fermented by DME possesses growth-promoting properties.

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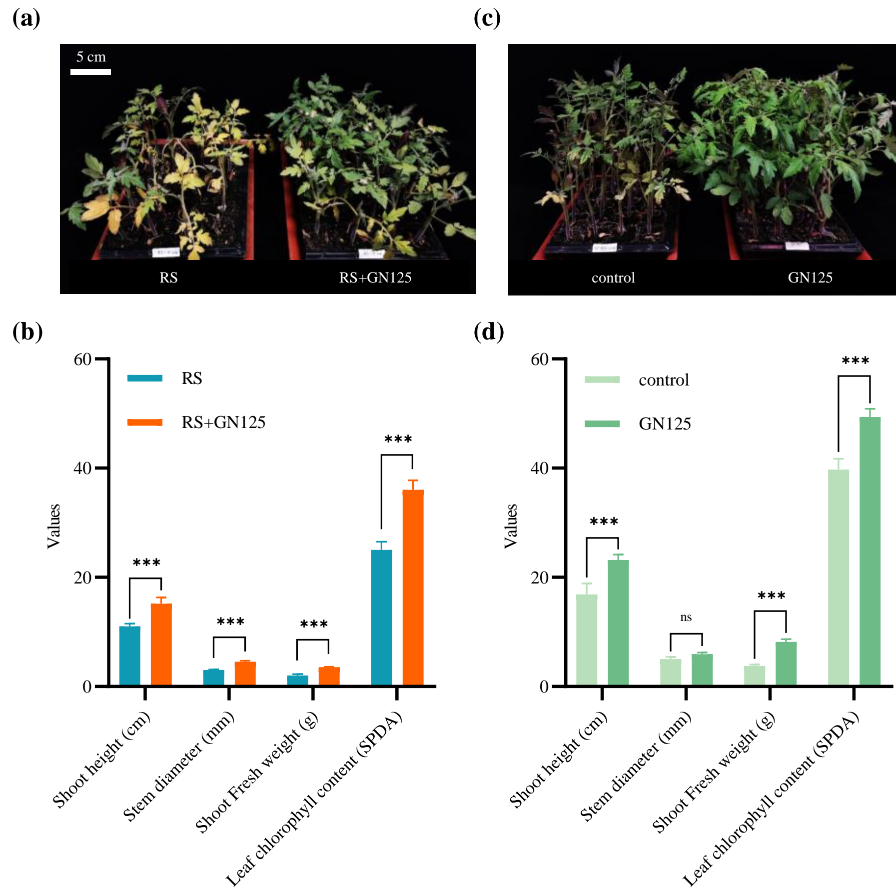

Fig. 2 Tomato growth promotion and tomato bacterial diseases biocontrol

The phenotype (a) and the physiological indexes (b) of tomato treated with bacterial wilt pathogen *Ralstonia solanacearum* (RS) and bacillus strain GN125. The phenotype (c) and the physiological indexes (d) of tomato treated with growth promoting bacillus strain GN125. Physiological indexes including the shoot height, stem diameter, shoot fresh weight and leaf chlorophyll content (measurement indicated in bracket). Error bars represents standard deviation (SD). Significance test was accomplished by *t*-test. *, significant *p* value at 0.05 level; ***, significant *p* value at 0.001 level; ns, no significant difference.

3.2 Enhancement of Silage Quality and Nutrition through DME-Fermented Microbial Agents

We evaluated the application effects of DME-fermented microbial solutions on fermented silage from sensory characteristics, quality, and nutrient aspects, with results shown in Table 3. The *Lactobacillus plantarum* GN1125, GN251 and GN1367 fermentation treatments had less uniform color but a stronger acid aroma compared to the control, with no noticeable texture difference. The pH values of the treated silages were slightly higher yet remained within a beneficial range to effectively inhibit harmful microorganisms. The Ammonia N/Total N ratio remained unchanged, indicating that protein degradation did not increase, thus maintaining feed quality. Dry matter content improved to varying degrees, with GN251 showing the best results, followed by GN1367 and GN1125 with the least improvement at 3%. Crude protein content increased with GN1125 and GN1367 treatments, while GN251 had no significant

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effect. Nutritionally, starch content increased and both Neutral Detergent Fiber and Acid Detergent Fiber contents decreased, improving feed digestibility and palatability. The fermented silage by all strains meet the requirements of level 1 feed standard while the unfermented silage dropped to level 2 feed standard. In conclusion, the microbial treatments enhanced feed quality, with GN251 demonstrating the most significant improvements.

Table 3 The evaluation of fermented silage

Evaluation category		Control	*Lactobacillus plantarum*		
			GN1125	GN251	GN1367
		Scores			
Sensory (30)	Color	10 (green)	8 (yellow)	8 (yellow)	8 (yellow)
	Smell	8 (good)	10 (fresh)	10 (fresh)	10 (frsh)
	Texture	10 (soft)	10 (soft)	10 (soft)	10 (soft)
Quality (30)	pH	13 (3.63)	13 (3.68)	13 (3.78)	13 (3.78)
	Ammonia N/Total N (%)	15 (4%)	15 (4%)	15 (4%)	15 (4%)
	Dry matter/DM (%)	6 (29%)	8 (32%)	8 (38%)	8 (36%)
Nutrient (40)	Crude protein (%)	8 (8%)	8 (11%)	8 (8%)	8(11%)
	Starch (% DM)	4 (16%)	4 (17%)	8 (33%)	6 (20%)
	Neutral detergent fiber (% DM)	0 (60%)	6 (50%)	8 (44%)	6 (48%)
	Acid detergent fiber (% DM)	4 (28%)	6 (24%)	8 (21%)	8 (22%)
Total score		78	88	96	92
Grade		2	1	1	1

Note: The fermented silage was evaluated by three indicators, including sensory, quality and nutritional composition. The maximum score for evaluation was 100 points. 85-100 points, grade 1; 70-84 points, grade 2; 60-69 poimts, grade 3. Control group represent for unfermented silage. GN1125, GN251 and GN1367 belonging to *Lactobacillus plantarum*.

3.3 Application testing of the DME fermentor in fermented corn feed

Table 3 The evaluation of fermented corn feed

Treatment	Testing index				
	pH	aflatoxin (ppb)	vomitoxin (ppm)	acides	contamination
L. buchneri GN1061	4.7	0.9	0.10	+++	-
L. plantarum GN232	4.2	0.6	0.06	+++	-
L. casei GN1710	4.3	0.3	0.16	+++	-
L. Faecalis GN456	4.3	0.9	0.13	+++	-
control	4.7	1.7	0.39	+	-

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Note: ppb, parts per billion, 1 ppb equals 1ug/kg; ppm, parts per million, 1 ppm equals 1mg/kg. The acides degree of the fermented feed was represented by different number of '+'. -, no contamination was found.

The efficacy of fermented corn feed with commonly used strains on DME was evaluated (Table 4). The results indicated that, compared to the control, all treatments, except L. buchneri GN1061, effectively reduced the pH of the fermented corn feed and better inhibited the growth of harmful microorganisms. Furthermore, the levels of aflatoxin and vomitoxin in all four treatments were lower than those in the control, thereby ensuring the feed safety. It is noteworthy that the addition of microbial agents did not result in contamination, which not only ensured both the quality and efficiency of the fermentation process.

3.4 Enhancing Aquaculture Water and Aquaculture Tail Water Management with DME-Fermented Microbial Agent

Similarly, we conducted the efficacy verification of functional microbia in aquaculture water and aquaculture tail water management. The results showed that after the addition of *B. subtilis* GN2036, the levels of ammonia nitrogen, nitrite, phosphate, and COD in both the aquaculture water and the aquaculture tail water were significantly reduced compared to control (Fig.3 a, b). In contrast, when *L. plantarum* GN104 was added, there was a noticeable reduction in ammonia nitrogen and nitrite levels of aquaculture water, while the reductions in phosphate and COD levels of aquaculture tail water were slight and not as significant (Fig.3 c, d). These findings indicated that the addition of these microbial agents can improve the water quality in aquaculture environments, particularly in reducing the level of ammonia nitrogen, nitrate, phosphate and COD which was harmful to aquaculture and environment.

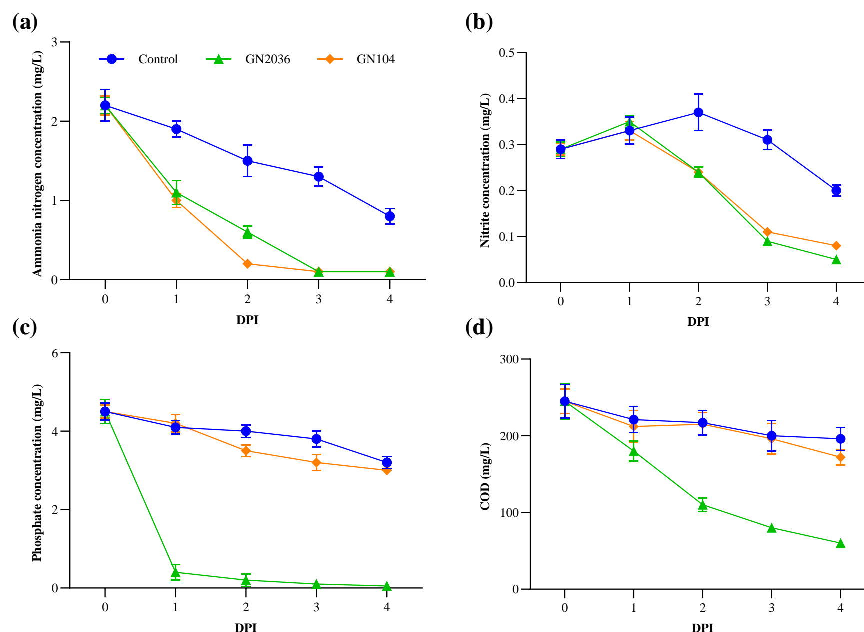

Fig. 3 The aquaculture water and aquaculture tail water management

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B. subtilis GN2036 and *L. plantarum* GN104 were used to treat with the aquaculture water and aquaculture tail water. Ammonia nitrogen concentration of aquaculture water (a), nitrate concentration of aquaculture water (b), phosphate concentration of aquaculture tail water (c) and COD (d) indexes of aquaculture tail water were selected to evaluate the performance of each strains. DPI, days post inoculation; COD, chemical oxygen demand.

Conclusions and Discussion

This study introduced the innovative concept of DME and developed the DME application system, which was composed of a core hardware named DME intelligent fermentor. We conducted fermentation tests on nearly 40 commonly used microbial agents, reaching a fermentation yield of $1{\sim}10\times10^8$ CFU/mL within 16~48 hours, in the fields of planting, aquaculture and Silage. Selected strains were then applied in fermentation trials, demonstrating excellent results in disease prevention and growth promotion for tomatoes, the improvement of fermented silage quality and the improvement of water quality in aquaculture and aquaculture tail water.

The "on-site" + "one-step" fermentation of DME intelligent fermentor ingeniously avoided the issues of short shelf-life and unstable application effects of non-spore forming functional microorganisms, while also bypassing the complicated sterile operation processes required in traditional large-scale or small-scale factory fermentation production. This model not only enhances production efficiency but also ensures the activity and functionality of the microorganisms, providing reliable support for practical applications. Previously, the American company Pivot Bio developed a portable fermentation system for facultative anaerobic nitrogen-fixing bacteria, capable of achieving an expansion of 200 million bacteria per milliliter per day in room temperature (https://www.pivotbio.com/product-proven40-corn). However, this system was limited to anaerobic or facultative anaerobic bacteria, resulting in relatively limited application coverage. What's more, the medium sterilization and aseptic filling was indispensable. Therefore, they through on-site fermentation solved the effectiveness and activity of functional strains while the complex aseptic filling progress and high transport cost still remained. In contrast, NBio accomplished the high pure fermentation for functional microorganisms bypassing complex sterilization process through adopting directional selection pressure under simple pasteurization process (90℃, 30 min). In addition, the DME system fully considered the fermentation needs of different types of strains by setting different aeration conditions to meet diverse fermentation requirements. This design makes the DME system more flexible and efficient in handling various types of microorganisms, expanding its application scope and potential market.

In total, the DME intelligent fermentor not only exhibits superior application outcomes across multiple fields but also overcomes numerous challenges inherent in traditional fermentation methods through its innovative design and operational models. This positions the DME intelligent fermentor as a promising technology with substantial application prospects and market potential. The DME system was crucial for bridging the gap between basic research and industrial development of agricultural microorganisms, lowering the industrialization threshold for functional microorganisms, accelerating the application of functional strains, and promoting the healthy development of the agricultural microbial industry.

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Table S1 Strains used in this study

Strains ID	Function	Species	Sources
GN125	Plant growth, biocontrol	*Bacillus velezensis*	Green Nitrogen biotech Co., Ltd.
GN2036	Water treatment	*Bacillus subtilis*	Green Nitrogen biotech Co., Ltd.
GN104	Water treatment	*Lactobacillus plantarum*	Green Nitrogen biotech Co., Ltd.
GN251	Feed fermentation	*Lactobacillus plantarum*	Green Nitrogen biotech Co., Ltd.
GN1367	Feed fermentation	*Lactobacillus plantarum*	Green Nitrogen biotech Co., Ltd.
GN232	Feed fermentation	*Lactobacillus plantarum*	Green Nitrogen biotech Co., Ltd.
GN1125	Feed fermentation	*Lactobacillus plantarum*	Green Nitrogen biotech Co., Ltd.
GN1061	Feed fermentation	*Lactobacillus buchneri*	Green Nitrogen biotech Co., Ltd.
GN1710	Feed fermentation	*Lactobacillus casei*	Green Nitrogen biotech Co., Ltd.
GN456	Feed fermentation	*Lactobacillus Faecalis*	Green Nitrogen biotech Co., Ltd.
RS	Pathogenesis	*Ralstonia solanacearum*	Green Nitrogen biotech Co., Ltd.

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Table S2 DME medium and its formula

Medium ID	Medium formula (g/L)
1	yeast extract 10 g, tryptone15 g, glucose 20 g, amylase 1 g, protease 1 g, K_2HPO_4 1 g, KH_2PO_4 1 g, $CaCl_2$ 0.6 g, organosilicon 3 g, DME ADDITIVES1
2	yeast extract 3 g, tryptone 5 g, $CaCl_2$ 0.6 g, K_2HPO_4 1 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 0.5 g, organosilicon 3 g, DME ADDITIVES2
3	yeast extract 10 g, tryptone 10 g, sucrose 15 g, NaCl 10 g, organosilicon 3 g, DME ADDITIVES3
4	sucrose 40 g, yeast extract 10 g, (NH4)2SO4 3 g, KH_2PO_4 1 g, $MgSO_4 \cdot 7H_2O$ 1 g, $MnSO_4 \cdot H_2O$ 0.05 g, $CaCl_2$ 0.4 g, $FeSO_4$ ·7H2O 0.05 g, ZnSO4 ·7H2O 0.014 g, chloramphenicol 0.1 g, organosilicon 3 g, DME ADDITIVES4
5	yeast extract 10 g, tryptone 15 g, sucrose 20 g, glucose 10 g, NaCl 2 g, CH_3COONa 3 g, L-$HSCH_2CH(NH_2)COOH$-HCl 0.5 g, starch1.5 g, organosilicon 3 g, DME ADDITIVES5

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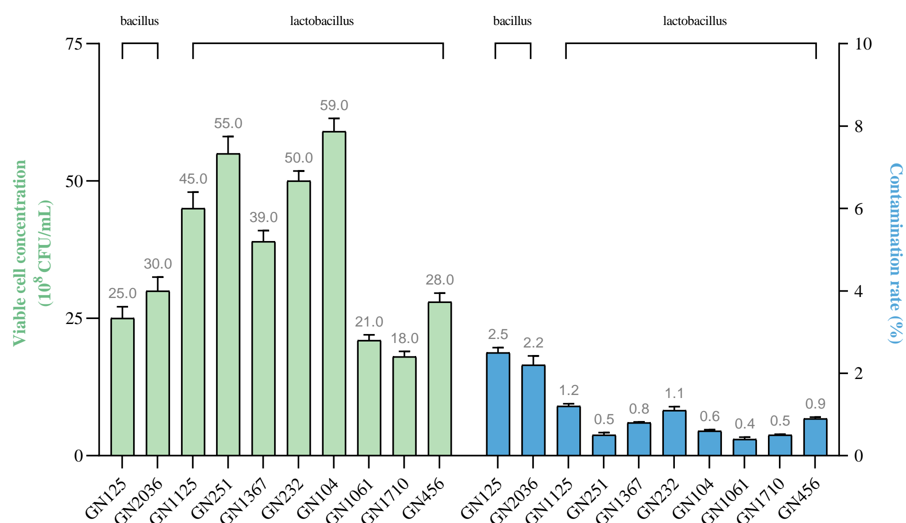

Fig. S1 The used strains cultured by DME intelligent fermentor

The final cell concentration and contamination rate of used strains, including 2 bacillus strains and 8 lactobacillus strains. Error bars represents standard deviation (SD).