

RESEARCH & DEVELOPMENT OF *Lactobacillus Hilgardii* CNCM I-4785 At Lallemand Animal Nutrition



DRIVEN BY INNOVATION, SUPPORTED BY R&D

Lallemand Animal Nutrition is committed to innovation by providing specific, research-based microbial solutions to optimize animal performance and well-being.

- No Lallemand product is brought to market without a substantial base of research and proof of performance.
- > We have experts across multiple disciplines who are skilled in molecular microbiology, omics and other techniques.
- Lallemand has a specific R&D process to ensure we produce quality products with practical applications.
- > Our products and services are supported by more than 100 years in microbial production and more than 25 years of expertise in the animal probiotic and forage inoculant fields.





THE LALLEMAND R&D PROCESS IN ACTION L. HILGARDII CNCM I-4785

PRODUCT DEPLOYMENT

SCALE-UP AND REGISTRATION

DISCOVERY AND PROOF OF CONCEPT

WORKING TOGETHER



The Lallemand Animal Nutrition R&D team develops partnerships with key opinion leaders, renowned universities and research centers around the world. Privileged collaborative partnerships have been established with leading research organizations, which allowed us to create Centers of Excellence to foster innovation in all areas of microbial solutions.

It was through such partnerships that the R&D team began the search for the next generation of forage inoculants to succeed the highly successful range utilizing *L. buchneri* NCIMB 40788.

In the following pages, we detail this exhaustive search to address unmet needs in the global silage market. It's a process that spanned more than a decade and included high-caliber research partners and next-generation technologies.

Bringing customers around the world *L. hilgardii* CNCM I-4785 is an example of how Lallemand Research & Development is truly SPECIFIC FOR YOUR SUCCESS.



DISCOVERY TO PATENT

2001: IDENTIFYING AREAS FOR IMPROVEMENT

- L. buchneri NCIMB 40788 was introduced globally and proven to improve aerobic stability across a variety of forages
- It was the first bacterium proven to improve aerobic stability registered by the European Union (EU) and U.S. Food and Drug Administration (FDA)
- The strain is highly efficient but requires a 60-day fermentation period

2009: An idea is born

Federal University of Lavras (Brazil) presented results from microbial screenings of naturally stable sugar cane silage at the International Silage Conference in Madison, Wisconsin

2011: A COLLABORATIVE PARTNERSHIP BEGINS

- Lallemand Animal Nutrition and University of Lavras entered into a joint R&D project
- The project searched for new bacterial strains that could improve aerobic stability quickly and efficiently

• 2012 TO 2013: BACTERIAL IDENTIFICATION AND SCREENING

- Researchers screened naturally well-fermented, aerobically stable sugar cane silage to identify the *Lactobacillus* species present
- Various techniques were used and resulted in 81 strains isolated
- Results were published in peer-reviewed journals to further validate the conclusions
- These strains were narrowed to 14 based on their ability to grow; fermentation characteristics; and ability to preserve and improve aerobic stability in sugar cane silage
- Strains were identified by DNA sequencing and classified as belonging to three different species: *L. plantarum, L. brevis* and *L. hilgardii*









2012 TO 2013: NARROWING THE FIELD

- Each strain was evaluated for its ability to ferment, improve aerobic stability, reduce yeast and mold levels and reduce dry matter (DM) losses in mini silos
- Strains were evaluated in both 61-day and 126-day experiments
- After this analysis, only two strains met these criteria: *L. hilgardii* CNCM I-4784 and *L. hilgardii* CNCM I-4785





Dynamics of temperature during aerobic of surgarcane silages without inoculants and inoculated with strains of lactic acid bacteria (LAB)

*The mean values with different capital letters are significant at P<0.05 according to the Scott-Knott test.

2014-2015: Proving the concept in different forages And inoculant combinations

- To ensure both strains could produce the results seen in sugar cane forage, each were tested with various forages, individually and in combination with *L. buchneri* NCIMB 40788
- Trials were conducted in North America and Europe and included forages such as maize, sorghum and whole-crop cereals
- The team focused on the strains' ability to improve aerobic stability earlier in the fermentation cycle





DISCOVERY TO PATENT



2015: SELECTING THE NEXT GENERATION OF FORAGE INOCULANT

- Results of a meta-analysis of nine trials demonstrated that *L. hilgardii* CNCM I-4785 significantly improved aerobic stability after just 15 days fermentation and also significantly improved stability at 30 and 100 days in combination with *L. buchneri* NCIMB 40788
- L. hilgardii CNCM I-4785 was chosen as the new aerobic strain to be used in combination with L. buchneri NCIMB 40788 and a patent application was granted to Lallemand Animal Nutrition



	AEROBIC STABILITY (HC		IRS)	
INOCULATION TREATMENT	GRP	P FERMENTATION TIME		
		15 DAYS	30 DAYS	100 DAYS
Negative control	Control	60.3 ^{b1}	65.8 ^b	102.5 °
Positive control (L. buchneri 40788)	LB	66.1 ^{ab}	87.7 ª	168.1 ^{ab}
L. hilgardii 4784	S1	64.1 ^{ab}	80.0 ^{ab}	128.5 ^{abc}
L. hilgardii 4785	S2	65.6 ^{ab}	82.4 ^{ab}	116.0 ^{bc}
L. buchneri 40788 + L. hilgardii 4784	LB+S1	65.7 ^{ab}	72.5 ^{ab}	173.6 ^{ab}
L. buchneri 40788 + L. hilgardii 4785	LB+S2	77.7 ª	95.5 °	177.3 ª
	Р	0.052	0.003	< 0.001
	SEM	5.43	7.43	17.89

Results from global trials

¹Values with different letters in one column are significantly different ($P \le 0.05$) according to a Turkey HSD test.



SCALE-UP AND REGISTRATION **2**

2016: Farm Trials

- Lallemand Animal Nutrition and its research partners conducted farm-scale trials in North America and in Europe to ensure the performance of *L. hilgardii* CNCM I-4785 in combination with *L. buchneri* 40788 could be replicated outside of experimental silos
- Farm-scale trials confirmed *L. hilgardii* CNCM I-4785 in combination with *L. buchneri* 40788 worked at the commercial scale



Trials continue with various DMs and forages

2017 TO 2018: PRODUCTION SCALE-UP

- All bacteria utilized in Lallemand Animal Nutrition forage inoculants are produced in its fermentation plants in North America and Europe
- The first production-scale fermentations of *L. hilgardii* CNCM I-4785 were produced in 2018

2019: COMMERCIAL AUTHORIZATION

- Before commercially launching or selling any product containing *L. hilgardii* CNCM I-4785, the European Food Safety Authority's (EFSA) opinion is required to ensure claims are proven by independent scientific studies
- Authorization was granted by the EFSA in June 2019
- In July 2019, Lallemand Animal Nutrition launched a global forage inoculant brand called MAGNIVA
- The MAGNIVA Platinum range contains a combination of *L. hilgardii* CNCM I-4785 and *L. buchneri* NCIMB 40788







PRODUCT DEPLOYMENT





The MAGNIVA Platinum Forage Inoculant range exclusively utilizes *L. hilgardii* CNCM I-4785 in combination with the industry gold standard *L. buchneri* NCIMB 40788. Together, these strains deliver powerful and unrivaled improvements to forage preservation and aerobic stability across a range of forage types and conditions.

The combination of *L*. *hilgardii* CNCM I-4785 and *L*. *buchneri* NCIMB 40788 in MAGNIVA Platinum Forage Inoculants deliver assured silage quality and proven on-farm benefits including:

- Significant improvements to feedout stability after just 15 days of fermentation
- Enhanced long-term feedout stability
- Decreased DM losses through fermentation and reduced waste
- Better preservation and nutrient retention across a range of forages



WORLDWIDE RESOURCES AND COLLABORATIVE NETWORK

Developing, producing and launching the MAGNIVA Platinum range containing L. hilgardii CNCM I-4785 and L. buchneri NCIMB 40788 took a worldwide network of researchers and professionals. These global connections helped prove the benefits of MAGNIVA Platinum across a range of forages and climates.



Lallemand Animal Nutrition is committed to optimizing animal performance and well-being with specific natural microbial product and service solutions. Using sound science, proven results and knowledge, Lallemand Animal Nutrition develops, produces and markets high value yeast and bacteria products — including probiotics, forage inoculants and yeast derivatives. These innovative solutions positively benefit animal nutrition and well-being, forage management and animal environment. Lallemand offers a higher level of expertise, leadership and industry commitment with long-term and profitable solutions to move our partners *Forward*. Lallemand Animal Nutrition is *Specific for your Success*.

KEY FIGURES

10+	YEARS FROM CONCEPT TO CUSTOMER DELIVERY	10 YEARS
81	STRAINS SCREENED	6:30
57	ISOLATES EVALUATED FOR FERMENTATION CHARACTERISTICS	
30+	RESEARCHERS	
14	STRAINS STUDIED IN EXPERIMENTAL SILOS	
11+	PUBLISHED ARTICLES	
12+	GLOBAL RESEARCH LOCATIONS	
3	MAGNIVA PLATINUM FORAGE INOCULANTS	Марина
1	NEW FORAGE INOCULANT STRAIN: LHILGARDI CNCM I-4785	

Lallemand Animal Nutrition is part of the Lallemand group, a privately owned Canadian company founded at the end of the 19th Century. Lallemand develops, produces and markets specific yeast, bacteria and derivatives of micro-organisms for applications in:



Not all products are available in all markets nor all claims allowed in all regions.

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