



## **Turval B0399 Probiotic Additive**

**Probiotic additive for or all feeds with active typified Lactic Yeast  
(*Kluyveromyces B0399*)**

### **INFORMATIVE SHEET FOR TECHNICIANS**

STRAIN DEPOSITED IN ACCORDANCE TO THE TREATY OF BUDAPEST and DIRECTIVE 70/524 CEE.-

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### **INTRODUCTION**

**Turval B0399** is a new ingredient composed of an exclusive balanced mix of typified and selected lactic yeasts (*Kluyveromyces B0399*), supported by a wide range of cellular metabolites, fermenting products and hydrolyzed vegetable proteins.

The lactic fermenting yeasts (or lactic yeasts) belong to the *Kluyveromyces* genus and have been used in the food and pharmaceutical industries for quite some time now, giving excellent results. (Chart 1)

**Chart 1. List of yeast sources of industrial enzymes (Larpent, 1991)**

<b>Microorganisms</b>	<b>Enzymes</b>
<i>Saccharomyces cerevisiae</i>	Invertasi
<i>Kluyveromyces lactis</i>	Beta-glicosilasi
<i>Saccharomyces alluvius</i>	Amilasi
<i>Saccharomyces diastaticus</i>	Glicoamilasi
<b><i>Kluyveromyces Fragilis (B0399)</i></b>	<b>Beta-galattosidasi</b>
<i>Candida cylindraceae</i>	Lipasi
<i>Pichia guilliermondii</i>	Alfa-galattosidasi
<i>Cryptococcus albidus</i>	Beta-1,4 xilanoidrolasi

Up until now, the practical use of these yeasts in the animal diet (*K. Fragilis* or *marxianus*, in particular) was limited due to the difficulty in stabilizing these microorganisms (and the relative final products) using the standard industrial processes.

A lengthy process of research and experimentation resulted in the refinement of a particular biotechnological method to select and typify the lactic yeast and moreover to produce the yeast on the industrial scale which stabilizes the product and exalts the modulation activity of the intestinal flora. The result of this innovative and laborious procedure is the probiotic additive **Turval B0399** with active typified lactic yeast ***Kluyveromyces marxianus/fragilis B0399***.

**FUNCTIONAL AND STRUCTURAL CHARACTERISTICS OF  
TYPIFIED LACTIC YEASTS *Kluyveromyces B0399***

The principal characteristics of *Kluyveromyces fragilis B0399* (which sets it apart from brewer's yeast, for example) are:

- The capacity to grow and develop with extreme ease on a whey medium or other dairy by-products;
- The ability to enzymatically degrade lactose (due to the presence of the enzyme beta-galactosidase or lactase in the citosol) in its constituents galactose and glucose see (fig.1 and chart 2);

In regards to the structural components of these active yeasts, it should be noted that the cellular wall contains a considerable quantity of polymers of mannose ( glucomannani, galactomannani e peptidomannani) and of N- acetil-glucosammina organized in complex structures (chitin), as well as the endocellular presence of the natural vitamins C, E and B complex (especially B1 and B2

Fig. 1: Mechanism of action of the enzyme beta-galactosidase



**Chart 2. Glicosidase identified in the yeasts (Bartnett 1981, Suomalainen and Oura 1971)**

Enzyme	Yeast	Substratum	Localization
Alfa -amilasi	<i>Candida tropicalis</i>	Amido, destrine	Extracellulare
Treleasi	<i>Saccharomyces cerevisiae</i>	Trealosio	Citosol, m.citoplasmatica
Glicoamilasi	<i>Saccharomyces cerevisiae</i>	Amido, destrine	Extracellulare
Inulinasi	<i>Kluyveromyces fragilis (B0399)</i>	Inulina	Extracellulare
Maltasi	<i>Saccharomyces cerevisiae</i>	Maltosio, saccarosio	Citosol
Isomaltasi	<i>Saccharomyces cerevisiae</i>	Isomaltosio	Citosol
Invertasi	<i>Saccharomyces cerevisiae</i>	Saccarosio, raffiniosio	M. citoplasmatica
<b>Beta-galattosidasi Lattasi</b>	<b><i>Kluyveromyces fragili B0399</i></b>	<b>Cellobiosio Lattosio</b>	<b>Citosol</b>
Alfa-galattosidasi	<i>Saccharomyces cerevisiae</i>	Raffiniosio	M. citoplasmatica

## AN INNOVATIVE PRODUCT

Amongst the vast category of probiotic products, Turval B0399 stands out for its unique characteristics, which sets it apart from common brewer's yeasts and the major part of lactic yeasts.

As the *in vitro* tests conducted at the University of Udine (Italy) have demonstrated, Turval B0399 produces (from the fermentation of the organic substrata) lactic acid and acetic acid much more efficiently than many of the *Saccharomyces cerevisiae* (Brewer's yeast) currently used in zootechnical diets. (Chart 3)

**Chart 3. Comparization of fermentation profil of Turval B0399 vs Brewer's yeast ( pH6,8, 39° C, 24 ore). (Univ. Udine 1999).**

	<b>TURVAL B0399</b>	Brewer's yeast
Acid latic (mg/l)	2152	214
Ac. Latic + ac. acetic (mg/l)	3483	330

Compared to other products with a lactobacilli basis (see chart 4 ), Turval B0399 stands out for its elevated stability at room temperature (at least 24 months) and for the possibility, thanks to its particular resistance, of combining it with antibiotics such as the Ampicillin, Erythromycin, Tetracyclines and Amoxicillin (to which the major part of lactic bacteria is sensitive) and others (see Chart 5).

Further more it produces 2 molecules of lactic acid for every molecule of glucose and in the intestine it increases the local lactobacteria.

Chart : 4 Summary chart of comparisons between lactic ferments and lactic yeasts



	LACTIC FERMENTS (lactobacteria)	LACTIC YEASTS (KLUYVEROMYCES B0399)
CLASSIFICATION	PROCARYOTES	<i>EUCARYOTES</i>
WALL	WITHOUT CHITIN	WITH CHITIN
MOTILITY	IMMOBILE	IMMOBILE
SPOROGENESIS	NON SPORIGENIC	SPORIGENIC (ascosporigenic)
RESPIRATION	ANAEROBIC AEROTOLLERANTI	ANAEROBIC AEREOTOLLERANTI
METABOLISM	THEY FERMENT LACTOSE. Mostly are heterofermenting and produce 1 ATP for every molecule of glucose Only few are homofermenting and produce 2 ATP from one molecule of glucose	THEY FERMENT LACTOSE Only homofermenting They produce 2 ATP for every molecule of glucose
ENZYMATIC ACTIVITY (BETA GALACTOSIDASE)	BETA GALACTOSIDASE + ONLY SOME SPECIES	BETA GALACTOSIDASE+++ ALL SPECIES
ACID PRODUCTION	THEY PRODUCE LACTIC ACID  Mostly produce 1 molecule of lactic acid for every molecule of glucose Some, which are homofermenting, produce 2 molecules of lactic acid for every molecule of glucose. Only a few species produce L-LACTIC acid, some produce D-LACTIC acid, numerous produce DL-LACTIC acid- (racemic or inactive form)	THEY PRODUCE LACTIC ACID  They produce 2 molecules of lactic acid for every molecule of glucose.  Further more, in the intestine they increase the local lactobacteria.
STORAGE AT ROOM TEMPERATURE	3-4 months	More than 24 months
TEMPERATURE FOR STORAGE	3 – 8 ° C	3 – 30 ° C
RESISTANCE TO THERMAL SHOCK	NONE	RESISTANCE UP TO 65°C
RESISTANCE TO GASTRIC SHOCK I (pH 3)	NONE	EXCELLENT
PATHOGENIC TO MAN	POSSIBLY	NO
OPTIMUM pH OF GROWTH.	5.5-7.0	4.8-7.5
RESISTANCE TO INFECTING AGENTS	NO RESISTANCE TO BACTERIOPHAGI	RESISTANCE TO BACTERIOPHAGI
RESISTANCE TO ANTIBIOTICS	NON RESISTANT	RESISTANT



**Chart 5 - Antibigram of the strain *Kluyveromyces fragilis* (B0399)**

(cfr. Department of Vegetation Biology of the University of Perugia, Italy.

**ANTIBIOTIC RESISTANCE/SENSITIVITY**
**Resistance Sensitivity (mg/l)**

<b>Bacitracin</b>	R
<b>Colistin</b>	R
<b>Penicillin</b>	R
<b>AMP</b> (Ampicillin)	R
<b>Oxytetracycline</b>	R
<b>AMX</b> (Amoxicillin)	R
<b>GM</b> (Gentamicin)	R
<b>CMP</b> (Chloramphenicol)	R
<b>Erythromycin</b>	R
<b>Tetracycline</b>	R
<b>Clorotetracycline.HCL</b>	R
<b>Tartared Tilosin</b>	R
<b>Nalidixic Acid</b> (Chinolons)	R
<b>Lincomycin</b> (Lincomycins)	R
<b>Rifampicin</b> (Rifamycins)	R
<b>Quinupristin/Dalfopristin</b> (Streptogramins)	R
<b>Linezolid</b> (Oxazolidinones)	R
<b>Teicoplanin</b> (Glycopeptides)	R
<b>5FCT</b> (Flocitosin)	S<2
<b>AMB</b> (Amphotericin)	S<1
<b>NYS</b> (Nystatin)	S<4
<b>MIC</b> (Myconazol)	S<1
<b>KET</b> (Ketonazol)	S<1

**INGREDIENTS**

The additive TURVAL B0399 is composed of a balanced mix of natural products, such as: tipified active lactic yeast *Kluyveromyces m/f B0399*, wheat bran pre-treated and sterilized, whey powder, lactose, hydrolysed casein

**ANALYSIS of additive**(approximate value)

<b>Kluyveromyces marxianus fragilis B0399</b>	<b>&gt; 5 x 10<sup>6</sup> CFU/g</b>
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Parameter	Percentage value % on additive as is
Humidity	10.0%
Total fats	2,0%
Proteins	22,0
Raw fiber	9,5%
Ashes	8,0%

**Analysis of heavy metals** (approximate value)

Parameter	Value on additive as is (mg/kg)
Cd	0,08
Hg	0,05
Cr tot	1,00
Cu	0,50
Pb	0,25

**PHYSICAL STATE, DIMENSION OF PARTICLES**

The additive is under the form of powder which is easily miscible.  
Regarding the powder quality, an absence of particles under 36 micron is observed.

**Granulometric analysis** (approximate value)

Granulometry (µm)	Percentage fraction
> 170	58 %
> 100	26 %
> 36	16 %
< 36	0 %

**CHARACTERISTICS OF THE SELECTED STRAIN.**

The active ingredient is a yeast,, *Kluyveromyces marxianus* (B0399) (Hansen) van der Walt, synonymous of *Kluyveromyces fragilis*; Genus *Kluyveromyces*, ascosporigenous, of which morphological characteristics and reproduction system are described.

Isolation, purification, preservation, identification and classification were executed according to the dictates of "Bergey's Manual of Systematic Bacteriology" and according to Lodder e Kreger van Rij, at the Universitt di Perugia, Italy, Department of Botanical Biology , Applied Microbiology Sector.

The mother strain (B0399) is deposited at BCCM – Belgian Coordinated Collection of Microorganism – Colture Collection Mycotheque de l'Université Catholique de Louvain.

**Biological activity:**

- CFU of *Kluyveromyces marxianus/fragilis* (B0399) :  $> 5 \times 10^6$  CFU/gr
- production of  $\beta$ -galactosidase ( lactase )
- demolition of lactose in glucose and galactose

**Morphological characteristics:**

The microorganism *Kluyveromyces Marxianus/Fragilis* B0399 is not MGO.

The morphology of the cell and the mode of reproduction varies depending on whether the strain was cultivated in liquid culture or on "morphology agar".

- *Kluyveromyces marxianus* ( Hansen ) van der Walt var. *Marxianus* ( synonymous of *Kluyveromyces fragilis* ) registration number B0399 at BCCM.
- Genus: *Kluyveromyces* ( Ascosporigenous ).
- Vegetative cells: spheroidal, ovoidal, ellipsoidal or cylindrical ( 2,0 - 6,0 )  $\times$  ( 3,0 - 10,0 )  $\mu\text{m}$
- Reproduction : through single budding ( monopolar ) or in pairs ( bipolar ) and occasionally ( in conditions of anaerobiosis ) in short chains.
- Asci : jugated or not jugated, with one or more ascospores ( 4 max. ) , evanescent.
- Ascospores: crescent-shaped, kidney-shaped, ellipsoidal or spheroidal with a tendency to stick together after liberation.
- Culture in agar : colonies of a creamy type, cream colored, with wavy or lobe-shaped border .
- Base nitrogenous percentages (mol % G+C):  $43.91 \pm 0,10$  which include values characteristic to the species .

**Physiological characteristics :**

- ☐ vegetative phase : cells predominantly spheroid but also ellipsoidal
  - ☐ reproduction by monopolar budding.
  - ☐ aschi : jugated, of the faded kind, with one or two ascospores up to a maximum of four.
- ascospores : kidney-shaped and ellipsoidal with chitinose membrane.

**RESISTANCE TO PELLETIZATION**

It has been demonstrated that **TURVAL B0399** is fir for pelletization as the cell wall is rich in chitin and provides a useful protective barrier thus making the cell similar to a spore.

- In cold conditions ( $t^{\circ}\text{C} < 45^{\circ}\text{C}$ ): mortality  $< \log 1$
- In hot conditions ( $t^{\circ}\text{C} < 70^{\circ}\text{C}$ ): mortality  $< \log 3$



#### **DURATION AND CONSERVATION**

The product is **thermo stabilized** - Expiry time: **9month**

Conservation: store in a dry and **room-temperature place (3-25°C)**.

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*More information and detailed reports of trials are available.*