

# Impact of black yeasts on the durability of polyurethane foam in tropical conditions in Vietnam

Hoai Nguyen Thu<sup>1\*</sup>, Hong Do Thi Thu<sup>1</sup>, Cuong Ngo Cao<sup>1</sup>, Thinh Do Tat<sup>2</sup>, and V.N. Sereda<sup>3</sup>

<sup>1</sup>Institute of biotechnology, Vietnam Russia Tropical Center, 63 Nguyen Van Huyen Str., Vietnam

<sup>2</sup>Institute of ecology, Vietnam Russia Tropical Center, 63 Nguyen Van Huyen Str., Vietnam

<sup>3</sup>The Russian Academy of Sciences, Moscow, Russia

**Abstract.** Polyurethane (PU) is a material that is widely used in industries. However, tropical climatic conditions in Vietnam often make the durability of PU lower than temperate climatic conditions. One of the causes of this problem is the biodegradation of microorganisms. In this study, biological characteristics of black yeast on PU is evaluated, thereby, initially assessing the impact of black yeast on the durability of PU in tropical conditions. Ten strains of black yeast were isolated from used PU foam samples in Vietnam. These black yeast strains were tested for enzyme activity including: urease activity, protease activity, polyurethanase activity. There were two strains selected to identify based on sequence analysis of the ITS1/ITS2 region, they belong to genus *Aureobasidium*. VN1Y3 was strain selected for biodegradability testing of PU foam samples. After 28 days of testing, the PU foam samples that have been infected with black yeasts have a change compared to the control in terms of color, surface, tensile strength, elongation. Research results have showed the impact of black yeast on the durability of PU.

## 1 Introduction

Polyurethane (PU) is a synthetic polymer that is formed by condensation polymerization reaction between polyisocyanate and polyol having intramolecular urethane bonds. Urethanes are derivatives of carbamic acids which exist only in the form of their esters [1]. In tropical climatic conditions in Vietnam, PU often has a short durability due to many reasons such as temperature, humidity, radiation and biodegradation of microorganisms,... Biodegradation is a chemical degradation of polymers by the action of microorganisms, such as bacteria, yeasts and fungi. Microorganisms degrade the polymers like polyurethane by using it as a substrate for their growth. J. R. Crabbe *et al.* found four species of fungi including *Curvularia senegalensis*, *Fusarium solani*, *Aureobasidium pullulans*, and *Cladosporium sp.* They were isolated based on their ability to utilize a colloidal polyester PU (Impranil DLN<sup>TM</sup>) as the sole carbon and energy source [2]. Halim *et al.* tested the growth of several species of bacteria on PU military aircraft paint. The investigators

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\* Corresponding author: [hongdt1009@gmail.com](mailto:hongdt1009@gmail.com)

isolated four species of bacteria including: *Acinetobacter calcoaceticus*, *Pseudomonas sp.*, *Pseudomonas cepacia*, and *Arthrobacter globiformis*. All species were capable of utilizing the polyurethane paint as a sole carbon and energy source with the exception of *P. cepacia* [3]. In addition, many authors argue that the biodegradation of PU is due to the activity of several extracellular enzymes, including polyurethanase [1-3]. In this paper, we described the biological characteristics of black yeast isolated from used PU foam samples and initially demonstrated their impact on the durability of PU.

## 2 Materials and methods

### 2.1 Materials

The materials that were used in this study included:

Samples: Used PU foam samples were collected in aircraft fuel compartment in Vietnam.

Media: Hansen medium [4], mineral medium with Impranil DLN™ (6 g/l) [1].

### 2.2 Methods

The study used the following methods:

Enrichment and isolation method: used PU foam samples are cut into small pieces of size 1.5 x 1.5 (cm) and enriched in mineral medium with Impranil DLN™ (6 g/l) at temperature 28°C and shaking 250 rpm for 7 days. Enrichment culture at the third inoculation was continued incubating on Hansen medium at temperature 28°C for 5 days to isolate black yeast colonies.

Determination of yeast morphology: Black yeasts were cultivated and differentiated by their macro and micro-morphologies according to Kurtzman *et al.*[5]

Determination of extracellular enzyme activity: the experiment was carried out by agar diffusion method according to Bauer *et al.*[4]

DNA extraction: the experiment was carried out using Zymo Research Kit (USA).

PCR: PCR is established using the ITS1/ITS4 primers and the corresponding heating programs.

Sequence analysis: The sequencing results were compared to related data in Genbank by the BLAST search on NCBI.

Biodegradation of PU test: according to ASTM G21-96 [6] and GOST 9049-91 [7].

Scanning electron microscopy: SEM was used to characterize surface modifications of polyurethane samples.

## 3 Results and discussions

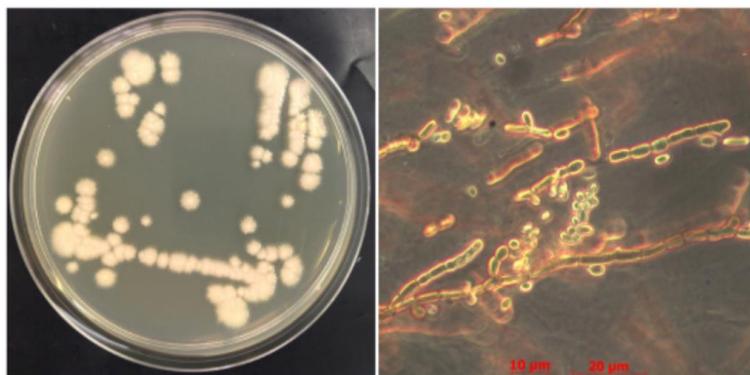
### 3.1 Isolation results

Ten black yeast strains were isolated from used PU foam sample in Vietnam (Table 1). They had a variety of colonial and cell morphologies with typical characteristics of black yeast group, such as porous colonies, diverse colors, turning black when old (after about 7 days of culture), rod or oval cells, cells in chain or multiple faults.

**Table 1.** Colony and cell of black yeast strains

№	Strains	Morphology	№	Strains	Morphology
1	VN1Y1	Rounded, yellow colony. Rod-shaped, sequined cells	6	VN2Y1	Milky white colony. Rod-shaped cells, many faults.
2	VN1Y2	Colony round, slightly glossy, turning black when old. Cells spherical, budding.	7	VN2Y2	Creamy white colony, turning black when old. Rod-shaped cells, many faults.
3	VN1Y3	Milky white colony, wrinkled, turning black when old. Oval cell and rod, budding.	8	VN2Y3	Yellow colony, the center has the mind. Rod cells, small, many faults
4	VN1Y4	Colony round, wrinkled, turning black when old. Cells spherical, budding.	9	VN2Y4	White colony, hard surface. Rod cells, small, many faults.
5	VN1Y5	Creamy white colony, wrinkled, turning black when old. Oval cells, sequined.	10	VN3H3	White colony, hard surface, turning black when old. Rod cells, small, many faults.

The morphology of colonies and cells of 10 strains of black yeast have complex differentiation. Many researchers believe that the complexity of the black yeast's life cycle (changes in colony color and cell shape that can occur during 3 weeks of culture) have led to grouping and identifying become less accurate. Therefore, to confirm the results, more biochemical tests and sequencing are needed [8]. In this study, an evaluation of enzyme activity and molecular identification was performed.



**Fig.1.** Colony (left) and cell (right) of strain VN1Y4

### 3.2 Determination of enzyme activity

The results showed that all strains had extracellular enzyme activity. In which, VN1Y3, VN1Y4 and VN2Y1 were the three strains with the highest polyurethanase activity, strain VN2Y1 and strain VN2Y4 had the highest protease activity, the urease activity of black yeast strains in this study was relatively low (except strain VN1Y3 ). VN1Y3 and VN1Y4 were the best enzyme activity strains, thus they continued to be identified by ITS1/ITS2 region sequences (Table 2).

**Table 2.** Enzyme activity of black yeast strains

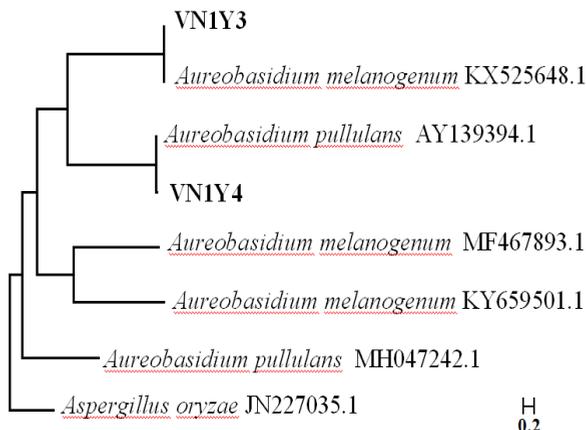
№	Strains	PU	Pr	Ure	№	Strains	PU	Pr	Ure
1	VN1Y1	-	++	+	6	VN2Y1	++	++++	-
2	VN1Y2	+	+	+	7	VN2Y2	+	+	-
3	VN1Y3	++	++	++	8	VN2Y3	-	-	+
4	VN1Y4	++	+++	+	9	VN2Y4	+	++++	-
5	VN1Y5	+	+++	+	10	VN3Y3	+	++	-

([+] positive, [-] negative; PU - polyurethanase, Pr - protease, Ure - urease)

Extracellular enzyme activity of microorganisms isolated from PU foam has been mentioned in many previous studies [9,10]. However, only a few studies have evaluated the polyurethanase activity of black yeast. Studies have also shown the role of polyurethanase in the use of PU materials by yeasts as carbon source for their growth [10]. Therefore, the research results can be considered as the first step to assess the impact of black yeast on the quality of PU during the use of this product.

### 3.3 Molecular identification

The sequence of ITS1/ITS2 of two black yeast strains (VN1Y3, VN1Y4) was sequenced based on primers ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3'). Phylogenetic tree was established by comparing the sequencing results on Genbank using the BLAST search on NCBI (Fig. 2).



**Fig.2** Phylogenetic tree of yeast, based on the ITS1/ITS2 region sequences

VN1Y3 and VN1Y4 were closely related to species in the following order: *A. melanogenum*, *A. pullulans*. Combined with the results of analyzing the morphology of colonies, the morphology of cells and checking the extracellular enzyme activity of the black yeast strains in the above section (section 3.1 and 3.2), the results of identification by sequencing was completely reliable. These results were similar to the results of J.R. Crabbe *et al.* about the existence of yeast belonging to genus *Aureobasidium* on PU foam [2].

### 3.4 Biodegradation of PU test results

VN1Y3 was the black yeast strain selected for biodegradation testing because it had high extracellular enzyme activity for all enzymes studied and determined to be closely related

to species *A. melanogenum* (black yeast species was assessed as being capable of producing polymerases [11]). After 28 days of testing with strain VN1Y3, PU foam samples were changed in color, surface, tensile strength and elongation compared to the control PU foam.

### 3.4.1 Colour of PU

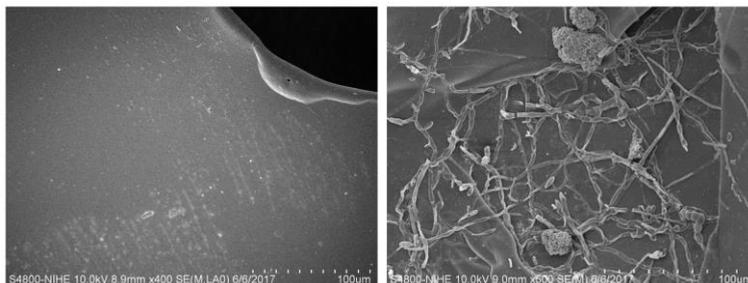
After drying, the PU foam sample tested with strain VN1Y3 has changed its color from light yellow to dark yellow (Fig. 3). The cause of this problem may be due to the yeast adhesion on the surface of PU and the PU material has been modified due to the growth of yeast. To clarify this problem, PU foam surfaces continue to be observed by scanning electron microscopes.



**Fig.3** Colour of PU foam with VN1Y3 (below) and control (above)

### 3.4.2 Surface of PU

The surface of the PU foam after testing was observed by scanning electron microscopy at magnification 500x (Fig.4). Fig 4 showed the adhesion of yeast biomass on PU foam surfaces. Compared with the control under the same test conditions, it can be seen that the PU foam samples tested with VN1Y3 appear to have plaque on the PU surface, their fiber systems form dense networks on the foam surface. In addition, at higher magnifications, pits and dimples can be observed while the control PU foam had a smooth and flat surface after 28 days of testing.



**Fig. 4** Surface of control (left) and PU foam with VN1Y3 (right)

The results of this study are completely similar to those of T. H. Gary and A. Nakkabi *et al.* on the growth of microorganisms on PU. A. Nakkabi *et al.* observed a much net growth of the bacterium *Bacillus safensis* on the polyurethane surface after 7 days of testing [1]. T. H. Gary also suggested that microorganisms have used PU as a source of carbon and energy for their growth [10].

### 3.4.3 Tensile strength and elongation of PU

Many studies suggest that the extracellular enzymes of microorganisms can cut the chemical bonds of PU, thereby reducing their durability [3,10,12]. In this study, tensile strength and elongation of foam samples were checked by specialized equipment. The results showed that the presence of yeast VN1Y3 caused the tensile strength of PU foam sample was 4,9 N/cm<sup>2</sup> corresponding to 38,9% compared to the control (12,6 N/cm<sup>2</sup>). The elongation was 175% while the control reaches 335%. The results demonstrated the obvious impact of black yeast VN1Y3 on the durability of PU foam.

## 4 Conclusions

Ten strains of black yeast were isolated from used PU foam samples with biological characteristics that showed their significant impact on the durability of PU foam, especially the ability to produce polyurethanase. After 28 days of testing with black yeast VN1Y3, PU foam was changed in color, tensile strength and elongation compared to the control PU foam. In which, tensile strength decreases to 38.9%. The strain VN1Y3 is closely related to species *Aureobasidium melanogenum*.

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