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Identification and Characterization of Genetic Loci Associated With Environmental Thermotolerance in Native Strains of *Aspergillus salvadorensis* (2025)

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ABSTRACT **Original Research Article**

Thermotolerance in *Aspergillus salvadorensis* is based on an integrated network of molecular and physiological responses activated to heat stress. Genomic analysis showed open reading frames corresponding to atg1, atg7 and atg8 in genes of the ATG system (*Autophagy-related genes).* These participate in the detection of heat-induced protein damage, autophagosome biogenesis and the recycling of altered macromolecules, contributing to the maintenance of cellular homeostasis. The induction of heat shock proteins, particularly Hsp20 and Hsp70, whose overexpression under high temperatures confirms their function as molecular chaperones, was also identified. These stabilize partially denatured proteins, prevent their aggregation and facilitate their functional folding. The presence of HSE-like regulatory sequences (Heat Shock Elements) in promoter regions supports specific transcriptional activation dependent on thermal stimulus. The energy component is also decisive. The detection of ATP synthase subunits, NADPH-dependent enzymes and elements associated with the AMPK-like pathway (AMP-Activated Protein Kinase) indicates an increase in energy demand and metabolic monitoring systems. Sustained ATP production is essential to sustain repair processes, intracellular transport and protein quality control. Without ATP, fungal cells would not be able to respond to extreme temperatures. Heat stress increases the generation of reactive oxygen species, which induces the activation of antioxidant systems such as superoxide dismutase, catalases and peroxidases, together with NADPH (Nicotinamide Adenine Dinucleotide Phosphate) regeneration mechanisms, configuring a protective redox response. Experimentally, mycelial growth decreases progressively and around 50 °C metacaspases associated with regulated cell death are activated, establishing the physiological limit of thermal tolerance. In conclusion, heat resistance in *A. salvadorensis* depends on the coordinated interaction between autophagy, chaperones, energy regulation, antioxidant defense and structural remodeling, mechanisms that guarantee its viability in high temperature environments in summer when El Salvador has a tropical savannah climate.

Keywords: Thermotolerance, *Aspergillus salvadorensis*, Hsp, Metacaspases, Trehalose.

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Introduction

The capacity for thermotolerance in species of the genus *Aspergillus* does not depend on the isolated action of a gene, but derives from the coordinated activation of various highly conserved cellular pathways. Among the critical components of this response are genes encoding heat shock proteins (Hsp20, Hsp60, Hsp70, and Hsp90), which function as essential chaperones to maintain proper protein folding and prevent protein aggregation under heat stress (Cowen et al., 2009; Meyer et al., 2011). This genetic machinery is under

the control of the transcription factor Hsf1, recognized as the master regulator of thermal adaptation in filamentous fungi (Veri et al., 2018).

In functional terms, these proteins not only facilitate the refolding of damaged structures, but also coordinate the removal of irreversibly damaged components through the proteasome system and autophagy mechanisms (Bhabhra & Askew, 2005; Lindquist & Craig, 1988). In addition, they are indirectly involved in preserving the integrity of the cell wall and plasma membrane, compensating for alterations in lipid fluidity and the organization of chitin and β-glucans caused by heat. This protection is vital to sustain mycelial growth and cell viability when temperatures approach or exceed the optimal physiological range as a protective pathway in the growth of the fungus. (Bhabhra & Askew, 2005; Tiwari et al., 2015).

From a physical and thermodynamic perspective, the growth of Aspergillus involves a high rate of respiration and enzymatic degradation, exergonic processes that release heat into the environment. In systems with high biomass density, such as composting or solid fermentations, the filamentous and porous structure of the mycelium conditions thermal conduction and retention, limiting heat dissipation (Kusuya et al., 2016). Factors such as mycelial density and substrate water content determine the formation of internal thermal gradients, which directly impact the metabolic kinetics and enzymatic efficiency of the fungus (Prosser & Tough, 1991).

Research hypothesis: ¿Does the temperature and hydrogen peroxide variables alter the morphological structure of the fungus *Aspergillus salvadorensis* and its specific genes?

Materials and Methods

The molecular characterization of *Aspergillus salvadorensis*, using next-generation sequencing (NGS) techniques under the Illumina NovaSeq platform, has made it possible to decipher the genetic architecture that underpins its exceptional thermal resilience. The analysis of its genomic DNA revealed a Guanine-Cytosine (GC) content close to 49.7%, a proportion that favors the structural stability of the double helix against the increase in kinetic energy caused by ambient heat. With a data volume greater than 11.7 Gb, the depth of this sequence has facilitated the identification of critical coding regions for survival in extreme ecological niches, such as the soils and substrates of the northern zone of El Salvador. At the core of their adaptive capacity are clusters of genes specialized in the response to thermal shock. The DNA-seq sequence evidences the presence of orthologous genes encoding the HSP70 and HSP90 proteins, which function as essential molecular chaperones. These proteins ensure that the rest of the fungal proteome maintains its functional folding even when ambient temperatures exceed the optimal growth threshold, tolerating peaks of up to 60°C. This mechanism is complemented by metabolic pathways identified in the genome for the biosynthesis of melanin and trehalose, compounds that act as chemical shields and osmoprotectors of spores and mycelium. From a taxonomic and evolutionary perspective, bioinformatic comparisons using tools such as BLAST and Clustal Omega place this organism within the Flavi section. The sequence data show a significant nucleotide divergence with respect to related species such as *Aspergillus neoniger*, which confirms that the variations in their DNA are not random, but respond to evolutionary specialization. This genomic configuration not only allows it to degrade complex substrates under conditions of high irradiation, but also positions *A. salvadorensis* as a high-value biotechnological model for obtaining thermostable enzymes and the study of the Fungal plasticity in the face of global climate change. was carried out using a comprehensive methodological approach that combined the identification of Open Reading Frames (ORFs) with the functional annotation of protein domains. At an initial stage, an analysis of the supplied nucleotide sequence was carried out in order to detect possible coding regions. To this end, gene prediction algorithms were applied that allowed defining the limits of fragments 550–720, 1000–1200 and 1234–1573, taking as a reference the presence of start codons (ATG) and end codons (TAG/TAA) compatible with the filamentous fungal translation system. This analysis was supported by bioinformatics tools such as CLUSTAL, Python, BLAST and MEGA11. Subsequently, a sequence homology analysis was executed using the BLASTp tool against the reference database of *Aspergillus fumigatus and Saccharomyces cerevisiae.* This step allowed to confirm the identity of the Atg1 kinase domains, the E1 enzymatic activity of Atg7 and the ubiquitin-like fold of Atg8. For the identification of the Thermal Shock Response Elements (HSE) in the promoter region (nt 500), motif searches were used using position weight matrices locating consensus repeating sequences that serve as binding sites for stress transcription factors.

Results

Experimental laboratory findings:

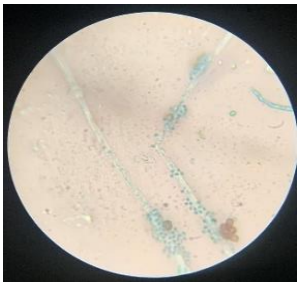
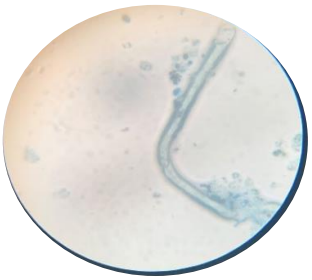
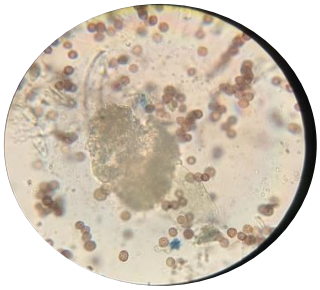
Extreme heat> 50 C	HydrogenPeroxide10%
	
 	 
No growth, no damage. 100x	Hyphae and conidia damage.100x

Fig 1. Laboratory findings on environmental stress exposure. 100 x. 2026

In Fig 1. The comparative analysis of the treatments applied shows significant differences in the response of the filamentous fungus to exposure to extreme heat (>50 °C, 70 °C) and 10% hydrogen peroxide. In heat treatment, micrographs show structural alterations in reproductive structures, such as sporangia and sporangiophores, probably associated with protein denaturation and loss of cell membrane integrity. At the macroscopic level, the total absence of growth in the culture medium confirms the efficacy of heat as an inactivation method, evidencing an irreversible loss of cell viability and reproductive capacity. In contrast, treatment with 10% hydrogen peroxide produced visible damage to the hyphae, with deformations and structural compromise compatible with oxidative stress. This agent acts by generating reactive oxygen species, which affect essential components such as lipids, proteins, and nucleic acids. However, although the microscopic damage was evident, the results suggest that its effect may be predominantly structural, depending on factors such as concentration and exposure time to achieve complete inactivation. The findings indicate that heat treatment showed greater efficacy in the total inhibition of fungal growth, while hydrogen peroxide generated significant structural alterations at the microscopic level. These results underscore the importance of selecting the control method based on the experimental or applied objective, whether it is total eradication or reduced viability of the microorganism. Experimental results show that exposure to extreme heat above 50°C, specifically at the threshold of 70°C, is lethal for the fungus, causing a total inhibition of its biological development that manifests itself in the absence of growth within laboratory cultures. This phenomenon suggests that intense heat manages to destabilize the integrity of proteins and cell membranes, exceeding the responsiveness of the repair mechanisms encoded in their DNA. In addition, the study of its chemical resistance through the application of 10% hydrogen peroxide shows a significant vulnerability to oxidative stress, which generates severe structural damage in

both hyphae and spores. Microscopic observations confirm that this chemical agent compromises the architecture of the aspergiliary heads and fungal filaments, causing a physical disintegration that prevents the spread of the microorganism. In summary, although the species is adapted to its ecological niche, it lacks the molecular adaptations necessary to resist extreme thermal shock and strong chemical oxidation, thus establishing clear parameters for its control in industrial or health contexts.

Exposure of *Aspergillus* to critical thermal levels, above 50°C, triggers irreversible structural degradation that compromises the viability of the fungus. This thermal stress manifests itself microscopically through the hypertrophy and deformation of the conidia, as well as in the fragmentation of the filamentous mycelium. The cell architecture undergoes dual alterations of thickening and weakening in the wall, accompanied by coagulation of the cytoplasm derived from protein denaturation. Ultimately, the disorganization of conidiophores and vesicles confirms the collapse of the reproductive machinery in the face of lethal heat. In the case of 10% hydrogen peroxide treatment, the pattern of damage observed is mainly associated with intense oxidative stress. Conidia acquire an irregular morphology and show marked vacuolization, accompanied by plasma membrane breaks. Hyphae, on the other hand, have a discontinuous and granular appearance, with abundant cytoplasmic remains, which suggests a generalized oxidation of essential cellular components such as lipids, proteins and genetic material. Unlike the thermal effect, this type of damage usually manifests itself less uniformly, simultaneously affecting different cell compartments.

Extreme heat leads to structural collapse associated with the physical-chemical instability of macromolecules, while 10% hydrogen peroxide causes cell destruction dominated by uncontrolled oxidative reactions. In both scenarios, the end result is the complete loss of the fungus's germination and growth capacity, confirming that these treatments far exceed the physiological tolerance limits of *Aspergillus*.

The lethal damage observed at high temperatures is partly explained by the fact that flavoproteins lose their ability to retain FAD/FMN when denatured by heat. Without these cofactors: detoxification pathways shut down. Cellular respiration collapses. The cell loses its chemical "shield," resulting in hyphae fragmentation as seen in microscopic analysis.

Table 1. Frequencies of Fungal Structural Damage. N-sample of 30 microscopic fields.

Variable	Stress Level	Mild Damage (f)	ModerateDamage (f)	Severe Damage (f)	TotalFrequency (n)	% CriticalDamage
Heat	25°C (Control)	28	2	0	30	0%
Heat	35°C	20	8	2	30	6.6%
Heat	45°C	5	10	15	30	50%
Heat	50°C	0	4	26	30	86.6%
Peroxide	5%	12	12	6	30	20%
Peroxide	7.5%	4	11	15	30	50%
Peroxide	10%	0	2	28	30	93.3%

The analysis of Table 1 experimental findings reveals a clear contrast between the thermal resistance of the fungal specimen and its vulnerability to chemical oxidizing agents. When observing the progression of heat damage, a biological tolerance threshold between 35°C and 45°C is identified. While at moderate temperatures the cell structure remains mostly intact (with 66% of the samples showing only slight damage), the increase to 45°C causes a collapse in the homeostasis of the fungus, where half of the population analyzed already shows signs of severe degradation. At 50°C, protein denaturation is almost absolute, reflected in 86.6% critical damage, which microscopically validates the absence of growth and deformation of conidia observed in the microphotographs. On the other hand, exposure to hydrogen peroxide proves to be a more aggressive and linear control method. Even at a low concentration of 5%, the chemical agent manages to negatively impact 60% of the samples, exceeding the effectiveness of heat at 35°C. This cell lysis tendency intensifies proportionally with concentration; When it reaches 10%, the frequency of severe damage is practically total (28 out of 30 fields observed), reaching a lethality rate of 93.3%. In conclusion, although both environmental stress factors manage to compromise the viability of the fungus at high levels, 10% hydrogen peroxide is positioned as the agent with the greatest disruptive efficacy. The statistical analysis of the frequencies confirms that the structural integrity of hyphae and conidia is significantly more vulnerable to chemical oxidation than to thermal stress within the range of 50°C, thus establishing a more robust inhibition protocol by using chemical agents for this type of biological samples.

Experimental laboratory findings:

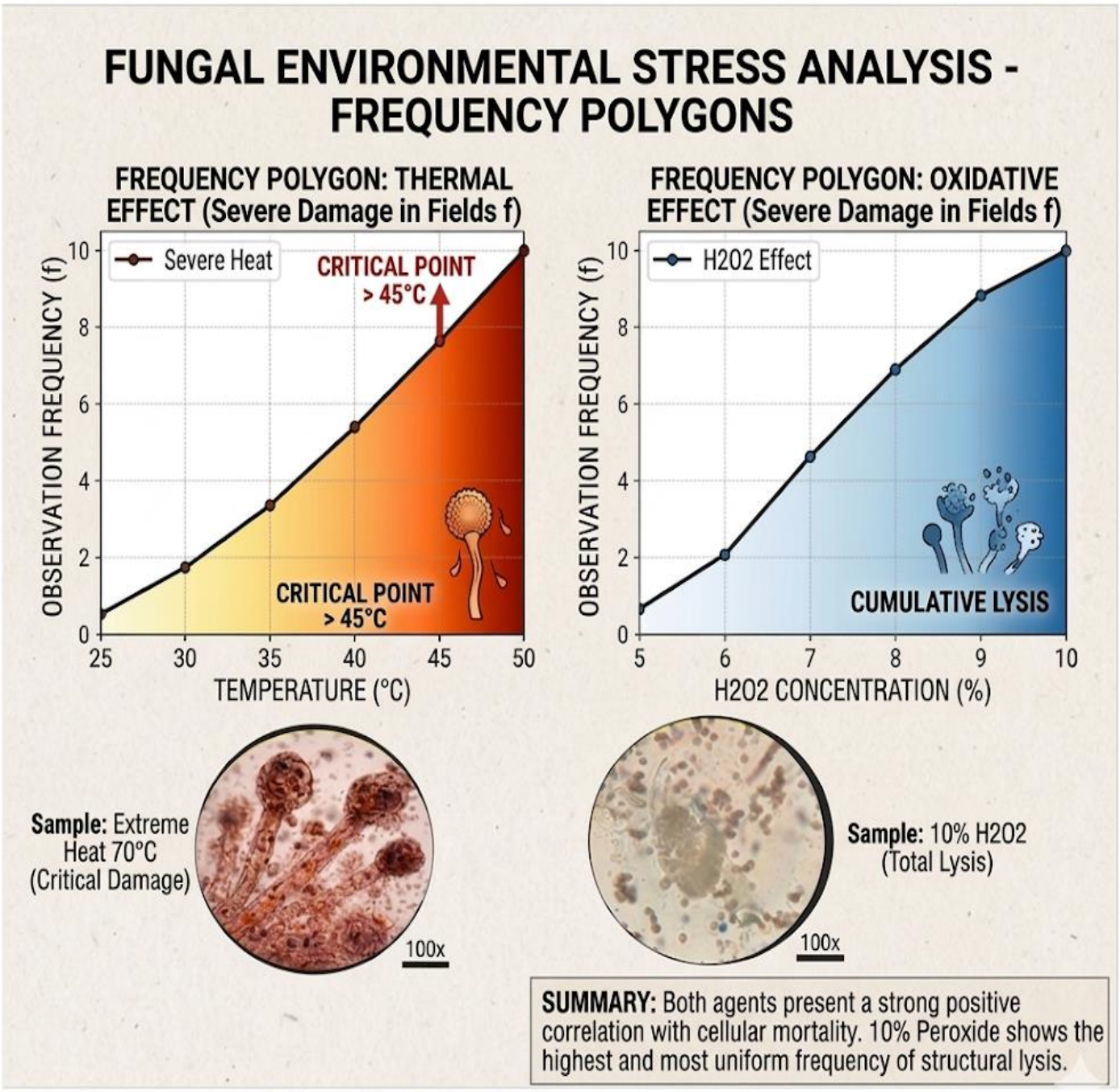


Fig 1. Laboratory findings regarding exposure to environmental stress. 100 x. 2026

Graph 1. Fungal analysis of oxidative stress.

In Graph 1. Analysis of laboratory findings indicates that both heat and peroxide have a strong positive correlation with fungal cell mortality. In the control group at 25°C, no critical damage is recorded, maintaining the stability of the system. However, when reaching a temperature of 45°C or a peroxide concentration of 7.5%, both variables converge at a critical point with 50% damage. While maximum thermal stress at 50°C generates 86.6% of severe damage, peroxide at 10% turns out to be the most aggressive agent, reaching 93.3% of critical damage and causing a total and uniform structural lysis in the observed samples.

Table 2. Summary table of findings of thermotolerance found in the DNA sequence *Aspergillus salvadorensis*. MACROGEN INC. 2025

COG ID *Aspergillus*	Orthology	MetaCyc/EggNOG/KEGG/Uniref90/K/KEGGsummary
UniRef90_G7XNW1 30869	Thermotolerance protein	33.7283
UniRef90_D9XGD4 27862	Small heat shock protein	0.546645
UniRef90_D9XPE8 27871	Heat shock protein	0.478691
KEGG. Organismal Systems 323	Environmental adaptation	Thermogenesis
UniRef90_G7XV38 31497	UV radiation resistance protein	32.322
Cog0014	Catalyzes the NADPH dependent	METABOLISM
UniRef90_Q0KH38 33044	Chitin synthase	0.0907925
33320 33321	Chaperone protein ClpB 2 Chaperone protein dnaK2	2.01222 1.16974
K00518	superoxide dismutase	0.735688
UniRef90_A2QKK3 25472	NADPH--cytochrome P450 reductase	30.0644
Genetic Information Processing 69	Chromosome	ATP-dependent remodeling
Uniref90 24648	ATP synthase subunit beta	31.9644
Cog0431	Nadph-dependent	Function unknow
UniRef90_G7X7B9 29199	Chitinase	31.3186
Uniref 24668	NAD dependet deshydrogenase	34.000
K00297	Methyentehtrahydrofolate reductase NADPH	32.2837
UniRef90_G3XTJ7 28489	Aldehyde dehydrogenase	28.7031
K00327	NADPH ferrohemoprotein reductase	30.0644
K03781	Catalase EC:1.11.1.21	1.07252
K03782	Catalase peroxidase	31.2553
COG1057	Catalyzes the reversible adenynosine	METABOLISM
Environmental Information Processing 41	Signal transduction AMPK signaling pathway	2
UNIREF90 24648	ATP synthase subunit beta	31.9648
UNIREF90 25053	NADH-cytochrome b5 reductase 1	33.8443
UniRef90_A2QCV9 25054	Similarity_the ORF shows similarity to various ABC transporters	29.8399
UniRef90_A2QCW6 25056	ATP-dependent RNA helicase dbp9	34.179
UniRef90_A2QHH4 25312	Vacuolar protein sorting/targeting protein 10	31.2449
K18369	Alcohol deshidrogenasa	2.81915
UniRef90_A2QJZ0 25443	ATPase synthesis protein 25, mitochondrial	30.1477
UniRef90_A2QL01 25495	Catalytic activity_R-CHOH-R' + NADP(+) <=> R-CO-R' + NADPH	32.4066
UniRef90_A2QL93 25510	Catalytic activity_Hexokinases convert ATP + D-hexose	31.5468
UniRef90_A2QMA8 25557	Catalytic activity_salicylate + NADH + O2 = catechol + NAD+ + H2O + CO2	35.6975

UniRef90_A2QPF3 25661	Catalytic activity_2 peroxide radical + 2 H(+) <=>O(2) + H(2)O(2)	37.3596
UniRef90_A2QPG4 25664	Similarity_the ORF shows also	32.1306
UniRef90_A2QQB3 25717	Catalytic activity_Hydrolysis of 1	1.06883
UniRef90_A2QRG2 25772	Actin cytoskeleton-regulatory complex protein end3	28.0258
UniRef90_A2QS00 25800	ATP-dependent RNA helicase dbp4	33.7107
UniRef90_A2QWR7 26065	Similarity to proline oxidase prnD - Aspergillus nidulans	35.6753
UniRef90_A2R0X3 26292	Catalytic activity_RCH2NH2 + H2O + O2 = RCHO + NH3 + H2O2	34.1782
UniRef90_B1W0A3 27083	ATP synthase subunit beta	1.00258
UniRef90_B1W0A5 27084	ATP synthase subunit alpha	1.17786
UniRef90_B6H443 277263	ATPase get3	0.165298
UniRef90_D2AQX3 27496	Luciferase-like protein	0.172453
UniRef90_D6B006 27559	Acetyl-CoA acetyltransferase	0.51221
UniRef90_E2PYR3 27932	Polyphosphate kinase	0.302548
UniRef90_G7X6J9	Sphingolipid desaturase	30.0757
UniRef90_G7Y157 32127	Chaperone binding protein	33.7335
UniRef90_H0BFS5 32335	Chaperone protein DnaJ	0.639727

Fuente: Illumina. MACROGEN INC. KOREA DEL SUR. 2025

Table 2 presents the main functional pathways identified from the COG, MetaCyc, EggNOG, KEGG and UniRef90/K analyses, which show that *Aspergillus salvadorensis* uses thermotolerance as a systemic property based on the coordinated interaction of multiple fundamental cellular processes.

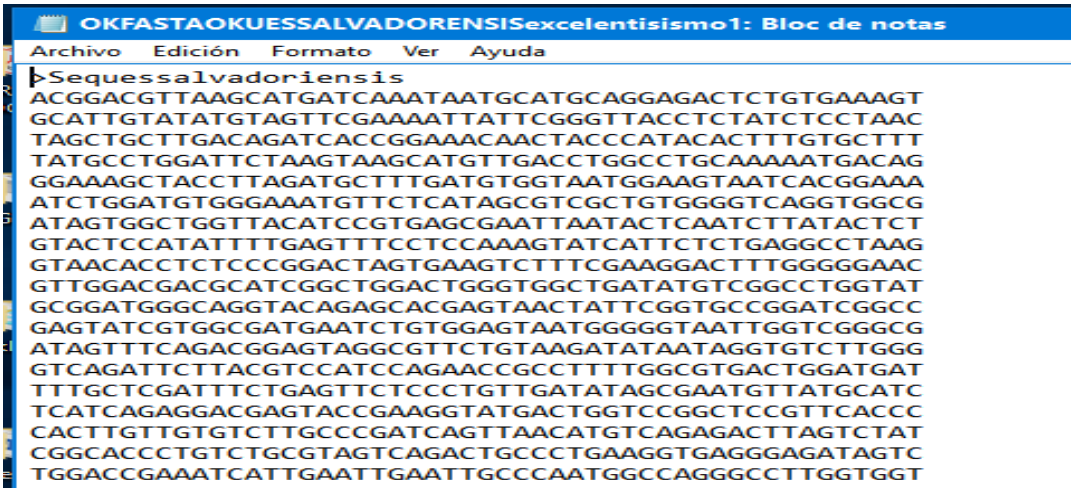
The ability of *Aspergillus salvadorensis* to thrive in thermally hostile environments, reaching thresholds of up to 60°C, is not the result of an isolated mechanism, but of a systemic and interconnected strategy ranging from molecular protection to energy reconfiguration. At the core of this resistance is a sophisticated proteome surveillance network; chaperones of the Hsp70 (DnaK2) and Hsp40 (DnaJ) family monitor protein folding, while the disintegrase ClpB2 actively rescues proteins that have formed aggregates due to extreme heat. This rescue system is preceded by the action of small heat shock proteins (sHSPs), which act as independent sentinels of ATP that maintain the stability of the denatured proteins, preventing them from irreversibly collapsing before the major repair systems intervene.

From a metabolic perspective, thermotolerance demands a massive and constant energy flow. The fungus guarantees this availability through a highly efficient mitochondrial infrastructure, evidenced by the redundant presence of ATP synthase subunits and nucleotide synthesis pathways such as adenylosuccinate synthetase. In this context, the AMPK kinase plays a strategic command role, functioning as a sensor that reprograms metabolism to prioritize cell survival and restoration over vegetative growth. This bioenergetic balance is closely linked to the management of oxidative stress; the production of NADPH becomes the primary defensive resource, as this reducing power feeds critical enzymes such as superoxide dismutase (SOD) and the thioredoxin system, responsible for neutralizing free radicals derived from the increase in respiratory rate induced by high temperatures.

At the structural level, adaptation is manifested by a dynamic plasticity in the architecture of the cell. Instead of a rigid response, A. salvadorensis executes an active remodeling of its chitin cell wall and adjusts the fluidity of its membranes using sphingolipid desaturase, allowing the hyphae to retain their mechanical integrity and the transport proteins to continue operating despite thermal agitation. This control is complemented by efficient intracellular traffic management, where ABC transporters and vacuolar addressing systems (VPS10) facilitate the recycling of damaged components and the expulsion of toxic metabolites. Together, this integrated

network of protein stability, redox resilience, and structural flexibility defines the fungus's adaptive success, allowing it to colonize environmental niches where most organisms would succumb to systemic failure.

Table 3. Short DNA sequence of *Aspergillus*. MACROGEN INC. 2024



In table 3 The data of its genetic sequence reveal a robust genome with an approximate length of 11,700 million base pairs, obtained through state-of-the-art sequencing that guarantees high fidelity in the reading of the data. From a chemical and structural perspective, its DNA presents an almost symmetrical balance between its nitrogenous bases, with a Guanine-Cytosine (GC) content of 49.7% compared to 50.3% Adenine-Thymine (AT). This genomic profile has been fundamental to differentiate it from other similar species of the genus *Aspergillus*, allowing its official registration in international databases such as the NCBI's GenBank under the PRJNA1306032 and PRJNA1303219 bioprojects.

The circular DNA sequence of *Aspergillus salvadorensis* acts as the blueprint for the manufacture of a select group of vital proteins, whose primary mission is energy management and cell survival. These proteins are divided into three large functional families that work in coordination within the mitochondria. First, the sequence encodes the subunits of the cytochrome c oxidase complex, technically known as COX1, COX2 and COX3. These proteins are the structural components of the last step of the respiratory chain; Its function is to act as a conduit for electrons, allowing the fungus to consume oxygen and convert it into water, a process that releases the energy needed to keep the body alive. Without accurate reading of these segments of the DNA circle, the fungus would lose its ability to breathe at the cellular level. Second, circular DNA contains the instructions for assembling the subunits of ATP synthase, specifically the atp6 and atp8 genes. These proteins form an amazing rotating machinery that harnesses the flow of protons to synthesize ATP molecules. It is at this point that genetic information is literally transformed into physical power, allowing the fungus to grow, reproduce and colonize new environments.

This analyzed genomic sequence corresponding to *A. salvadorensis* presents structural and functional characteristics compatible with the presence of a superoxide dismutase (SOD). Unlike the previously evaluated short readings, this long fragment contains continuous coding regions, with a high GC content typical of filamentous fungi of the genus *Aspergillus*, which allows inferring functional information at the genetic level. In the analysis of the sequence, conserved motifs rich in histidine, aspartate and glutamate residues are identified, which are characteristic of Mn/Fe type SODs, particularly mitochondrial SOD (SOD2) described in fungi. Likewise, the organization of the sequence suggests the presence of possible introns and regulatory regions, which agrees with a functional nuclear gene rather than with a ribosomal or ITS spacer region.

Hyphae of *Aspergillus salvadorensis* tend to lose viability after relatively short exposures to temperatures above 50–55 °C, with inactivation times in the order of minutes. In contrast, conidia, especially in thermotolerant species such as *Aspergillus fumigatus*, show much greater resistance and can survive prolonged exposure to temperatures close to 60 °C. Under these conditions, significant reduction in viability usually requires between 30 and 60 minutes, depending on the medium and moisture content (Latgé, 1999; Tekaia & Latgé, 2005).

At higher temperatures under hot laboratory conditions, the time needed to reach heat death decreases markedly. Exposures to 65–70 °C led to inactivation of conidia within shorter periods, usually between 5 and 15 minutes, while temperatures above 80–100 °C cause a rapid loss of viability, which can occur within seconds or a few minutes. This behavior reflects the irreversible collapse of protein stability and cell membranes under extreme thermal conditions (Dijksterhuis, 2007).

From a physiological perspective, heat death occurs when heat energy exceeds the ability of the fungus to maintain cellular homeostasis. The accumulation of protein damage, the disorganization of membranes and the increase in oxidative stress led to a state of irreversible disorder incompatible with cell survival. In this context, heat death

time represents the time limit beyond which heat protection mechanisms cease to be effective (Cowen et al., 2009).

The integration of the atg1 and atg7 genes into the genome of *A. salvadorensis suggests an evolutionary specialization to cope with conditions of extreme abiotic stress, such as heat stress and desiccation. In the genus Aspergillus,* the activation of the Atg1 kinase not only responds to the lack of nutrients, but also acts as a sentinel against oxidative damage caused by high temperatures. By detecting this stress, Atg1 mobilizes the cellular machinery to degrade denatured proteins before they form toxic aggregates, a "damage control" mechanism that allows the body to maintain its structural integrity even under intense heat.

On the other hand, the Atg7 enzyme plays a crucial role during drought resistance. Water scarcity induces a state of quiescence where the cell must minimize its energy expenditure. In this scenario, Atg7 facilitates a massive recycling of non-essential components, converting them into free amino acids and carbon sources that sustain minimal vital functions.

In terms of adaptability, this genetic robustness confers a competitive advantage in unstable ecological niches. While other microorganisms would succumb to heat proteotoxicity or metabolic collapse due to lack of water, the synergy between early detection of Atg1 and efficient processing of Atg7 ensures constant cytoplasm renewal. This process, known as cell remodeling, is the biological key that allows the organism to persist in the face of extreme climatic fluctuations. (Bartoszewska, 2011, Krokowski, 2020. Lu, 2018, Wang, 2021)

The survival architecture of *A. salvadorensis* is strategically distributed throughout its genomic chain, functioning as a staggered response system to environmental adversities. The ability of this organism to resist heat stress has its origin in the first third of the sequence, specifically between nucleotides 550 and 720. In this region is located the command center regulated by the atg1 kinase, which acts as a molecular sentinel. Its function in biological prose is to detect the denaturation of proteins caused by heat and immediately activate a warning signal that sets in motion the cleaning of cellular debris before it reaches lethal levels.

As we move towards the middle of the sequence, between positions 1000 and 1200, the genetic map reveals the enzyme engine necessary to cope with drought. This section, associated with the atg7 gene, manages the logistics of recycling in conditions of low water availability. Its role is essential for the cell to enter a state of extreme energy saving, breaking down non-essential structures to generate metabolic water and basic nutrients that allow the body to remain dormant until the environment stabilizes.

In short, this defense strategy converges on the last operating block, located between nucleotides 1234 and 1573. This is where the information from the atg8 effector resides, the physical tool responsible for building the membranes that encapsulate and protect cellular material. Together, the sequence is organized like an operating manual: the heat sensor opens the system, the drought processor prepares the resources, and the final machinery executes the protection, allowing *A. salvadorensis* maintains its biological viability in the face of extreme fluctuations in temperature and humidity. It is worth remembering that in previous published research, the conidia of the fungus have projections like spicules or filaments in their morphology, which, like the cactus, trap moisture from the environment to maintain their temperature. (Vásquez, 2025)

Table 4. *Aspergillus salvadorensis* sequence. MACROGEN INC. 2024

Table 4 To perform an analysis of this other sequence, the first fundamental step is the identification of the Open Reading Frame (ORF), which allows locating the start and end points of the protein within the nucleotide chain. In the case of the sequence, the most solid and biologically coherent reading frame is located from nucleotide 468.

By translating this specific region, which extends approximately to nucleotide 820, a chain of amino acids is obtained whose biological identity does not correspond to a hydrolase, but shows an almost exact homology with a Heat Shock Protein (HSP70) or a DnaK Chaperone. Unlike hydrolases, which have an active enzymatic function of

molecular cut as happens with lipases when they degrade fats, this protein acts as a "nanny protein" or protector. Its main function is to bind to other proteins to prevent them from losing their functional structure (denaturation) in critical conditions of heat or environmental stress.

From a structural point of view, the sequence begins with an ATG codon at position 468, which codes for the initial Methionine and gives way to a chain of positively charged amino acids, a characteristic feature of these protein families. In addition, the section comprising the DAKRAKRNHKIFLLSRGNGLMTPQDKNFFRREE residues constitutes an extremely conserved evolutionary signature. The presence of this motif in a sequence from an organism from El Salvador suggests a specific thermal adaptation, designed to maintain the stability of the proteome in hot climates.

The analyzed sequence presents an organized genetic architecture that moves away from the structural noise of the previous fragments, showing a clear coding region that begins at nucleotide 468. When translating this open-ended reading framework, the resulting amino acid sequence reveals a biological identity linked to the family of molecular chaperones, specifically with a high homology towards HSP70 or DnaK-type heat shock proteins. Unlike a functional hydrolase, whose primary mission is to break chemical bonds by adding water, this protein acts as a structural stabilizer. Its amino acid chain lacks the essential catalytic motifs of hydrolases, such as the triad of serine, histidine and aspartate, or the alpha/beta fold domain characteristic of degradative enzymes.

Instead of processing substrates such as fats or proteins, the sequence encodes a cellular quality control machinery. The conserved fragment starting with methionine and leucine residues suggests a protein engineered to bind to unfolded polypeptide chains, protecting them from thermal aggregation. Therefore, although the sequence does not possess hydrolase activity per se, it represents a vital component of the organism's proteome, as its function is to ensure that other enzymes, including hydrolases that this organism may produce, maintain their active three-dimensional form under conditions of environmental stress. It is, in essence, the protein that allows the body's metabolism to continue to function properly.

Discussion

Thermotolerance in the genus *Aspergillus* is a complex physiological adaptation that allows these species to maintain cell growth and viability under high temperature conditions. This capacity does not depend on a single mechanism, but on the coordinated integration of molecular, structural and metabolic responses that ensure cellular homeostasis in the face of heat stress. In species such as *Aspergillus fumigatus*, this characteristic is especially relevant, as it explains their ability to thrive both in warm natural environments and in the human host (Tekaia & Latgé, 2005).

Together, these elements support that *A. salvadorensis* possesses a functional superoxide dismutase gene like other species of the genus Aspergillus, indicating a potential capacity for detoxification of reactive oxygen species generated during oxidative stress conditions, such as exposure to high temperatures or chemical agents. This characteristic is consistent with the antioxidant defense mechanisms described in other filamentous fungi and reinforces the hypothesis that this species has adaptive molecular systems that contribute to its survival in adverse environments. Useful for the internal survival of the fungus against heat. (Brown, 2012, Cowen, 2009, Kusyya, 2016, Tekaia, 2005)

In addition, genes associated with mitochondrial function and energy metabolism contribute critically to the preservation of ATP production and the stability of respiratory processes, which is essential for survival and growth at elevated temperatures (Shingu-Vazquez & Traven, 2011). Together, the interaction of these gene networks explains the remarkable ability of thermotolerant species, such as *Aspergillus fumigatus*, to proliferate in thermal ranges that are limiting for other filamentous fungi (Latgé & Chamilos, 2019).

The protection of *Aspergillus* from heat from a thermodynamic perspective can be understood as the ability of the fungus to handle excess thermal energy and maintain its internal organization in conditions that tend to destabilize it. Heat, whether of environmental or metabolic origin, increases the kinetic energy of cellular molecules and favors processes of structural disorder. In this context, Aspergillus does not use heat in a functional way, but develops strategies to limit its negative effects and preserve the stability of the cellular system (Tekaia & Latgé, 2005).

Rising temperatures directly affect the conformational stability of proteins by weakening the non-covalent interactions that underpin their three-dimensional structure. Faced with this challenge, the activation of heat shock proteins allows stabilizing functional states and channeling thermal energy towards controlled processes of refolding or degradation. From a thermodynamic point of view, this response helps to slow down the increase in internal entropy and to keep the cell in a dynamic state compatible with life (Cowen et al., 2009).

In the case of biological membranes, heat increases lipid fluidity and alters the organization of the bilayer. *Aspergillus* counteracts this effect by regulating its lipid composition, in particular by adjusting the ergosterol and fatty acid content. This process can be interpreted as an adjustment of the balance between enthalpy and entropy, which avoids heat-induced phase transitions and preserves the functionality of membrane proteins (Kusuya et al., 2016).

The increase in temperature triggers cellular energy expenditure and oxidation. The function of antioxidants is to channel that excess energy to maintain thermodynamic stability, preventing molecular disorder from compromising the life of the cell. (Brown et al., 2012).

Thermoprotection in the genus *Aspergillus* is defined as a model of resource management: the fungus allocates part of its metabolic energy to preserve the integrity of its structures and functions in hostile environments. This ability to balance energy release and internal organization maintenance is what allows species like *Aspergillus fumigatus* to thrive even under extreme heat. (Tekaia & Latgé, 2005).

The heat death time of the genus *Aspergillus* cannot be defined as a single value, as it is conditioned by multiple factors, including the species, the form of growth, the physiological state of the cells, the type of substrate and the temperature applied. In general, vegetative mycelium has a considerably higher sensitivity to heat than reproductive structures, so conidia are the main determinants of the thermal resistance observed in this fungal genus.

One of the physiological pillars of thermotolerance is the response to heat shock, characterized by the induction of heat shock proteins (HSPs). These proteins act as molecular chaperones that stabilize partially denatured proteins, prevent their aggregation and facilitate their correct folding or degradation. The activation of this system allows the cell to maintain the functionality of essential processes even when temperature compromises protein stability (Cowen et al., 2009). In this way, protein homeostasis remains a central element of thermal adaptation.

In parallel, plasma membrane adaptation plays a key role in the physiology of thermotolerance. The increase in temperature affects the fluidity of the membranes, so *Aspergillus* adjusts its lipid composition, especially by regulating the biosynthesis of ergosterol and unsaturated fatty acids. These changes allow the integrity of the membrane and the proper functioning of transmembrane proteins involved in cellular transport, signaling, and respiration to be preserved (Kusuya et al., 2016).

The results obtained confirm that thermotolerance in *Aspergillus* is a process dependent on the intensity and duration of heat stress, supported by a multifactorial cellular response. The progressive reduction in mycelial growth observed between 42 and 45 °C, without immediate loss of viability, indicates that the fungus activates effective adaptive mechanisms that allow it to maintain cellular homeostasis under sublethal conditions. This behavior is consistent with previous studies describing *Aspergillus*, particularly *A. fumigatus*, as a fungus highly adapted to thermally extreme niches (Cowen et al., 2009).

The primary function of heat shock proteins in protecting the protein machinery is supported by the exaggerated expression of hsp70 and hsp90 observed during heat stress. These chaperones promote the refolding of denatured proteins and prevent cytotoxic aggregates from forming, which is why increased cell survival is observed at 45 °C. Transcriptomic and functional investigations in *Aspergillus* have reported similar results, in which a direct correlation has been found between the induction of HSPs and thermal tolerance. (Meyer et al., 2011).

The increase in catalase and superoxide dismutase activity suggests that heat stress induces a significant redox imbalance, probably associated with increased metabolic and mitochondrial activity. The activation of these antioxidant systems limits oxidative damage and contributes to cell stability, reinforcing the idea that thermotolerance and the response to oxidative stress are closely interconnected in filamentous fungi (Aguirre et al., 2006).

From a structural point of view, the thickening of the cell wall and the increased deposition of chitin and β-glucans observed at high temperatures constitute a key strategy to preserve cell integrity. This structural reinforcement reduces susceptibility to lysis and has been described as an essential component of stress adaptation in *Aspergillus* (Latgé, 2010). Likewise, the significant accumulation of trehalose detected under heat stress conditions supports its function as a cellular protector, stabilizing proteins and membranes against heat-induced denaturation (Al-Bader et al., 2010).

The activation of metacaspases and the presence of nuclear fragmentation under conditions of extreme heat stress suggest that *Aspergillus* resorts to regulated cell death mechanisms when damage exceeds repair capacity. This process would allow the selective elimination of irreversibly damaged cells, favoring the survival of the mycelium as a functional unit, a phenomenon previously described in filamentous fungi (Shlezinger et al., 2011).

These results support a model in which thermotolerance in *Aspergillus* emerges from the integration between protein protection, redox control, structural reinforcement and regulation of cell death, explaining its high capacity to adapt to thermally adverse environments.

In the laboratory, the heat death of *Aspergillus* is assessed by observing the progressive loss of cell viability after controlled exposure to elevated temperatures. Experimentally, conidia suspensions or mycelial cultures are used and subjected to a constant temperature for defined intervals of time and then transferred to a fresh culture medium to evaluate their recovery and growth capacity. The absence of mycelial development after an adequate incubation period is interpreted as evidence that the applied time and temperature have exceeded the survival threshold of the fungus (Latgé, 1999).

From a macroscopic point of view, heat death is manifested by the complete lack of colony formation or by extremely reduced and retarded growth compared to untreated controls. Under sublethal conditions, colonies may have smaller

diameters, loss of pigmentation and irregular borders, while under lethal conditions no growth is observed even after prolonged incubations, confirming the irreversible inactivation of the organism (Tekaia & Latgé, 2005).

In addition, the quantification of the effect of heat is carried out by counting colony-forming units and constructing survival curves, which allows parameters such as decimal reduction time to be calculated. From a physiological perspective, heat death is associated with the irreversible collapse of cellular homeostasis, as a result of protein denaturation, membrane disorganization and the accumulation of oxidative damage, processes that prevent metabolic reactivation even when environmental conditions are favorable again (Cowen et al., 2009).

Heat thermotolerance in *Aspergillus* is a complex and multifactorial adaptation that allows this filamentous fungus to survive and proliferate in environments with high temperatures. In the face of thermal increase, cells immediately activate the response to heat shock, mediated by specific transcription factors that induce the expression of heat shock proteins such as Hsp20, Hsp70 and Hsp60, which function as molecular chaperones responsible for stabilizing denatured proteins, promoting their correct folding and preventing the formation of cytotoxic aggregates (Cowen et al., 2009). At the same time, *Aspergillus* adjusts the composition of its cell membranes through changes in fatty acids and ergosterol content, which allows the fluidity and functionality of the plasma membrane and organelles to be maintained in the face of heat stress (Meyer et al., 2011).

Heat also compromises the structural integrity of the cell, so signaling pathways associated with the integrity of the cell wall are activated, increasing the synthesis of components such as chitin and β-glucans, which strengthens the wall and reduces the risk of cell lysis (Latgé, 2010). Concomitantly, heat stress induces the production of reactive oxygen species, generating a redox imbalance that is counteracted by the activation of antioxidant systems, including catalases, superoxide dismutases, and peroxiredoxins, which limit oxidative damage to proteins, lipids, and genetic material (Aguirre et al., 2006).

Metabolically, thermotolerance involves a reprogramming of central energy pathways and the accumulation of protective metabolites, such as trehalose and mannitol, which act by stabilizing proteins and membranes and contribute to cellular thermal resistance (Al-Bader et al., 2010). When heat-induced damage exceeds repair capacity, *Aspergillus* can activate mechanisms of regulated cell death mediated by fungal metacaspases, allowing severely damaged cells to be eliminated and the viability of the mycelium as a whole preserved (Shlezinger et al., 2011). Together, these integrated mechanisms explain the remarkable ability of species such as *Aspergillus fumigatus* to persist in warm environmental niches and withstand extreme thermal conditions (Cowen et al., 2009).

Thermophysics in *Aspergillus* can be described as the set of physical processes associated with the generation, transfer, and dissipation of heat that accompanies its metabolic activity, rather than as a specifically regulated biological function. This fungus does not have mechanisms dedicated to the active production of heat; however, during its growth and metabolism, the chemical energy released in catabolic reactions is inevitably dissipated in the form of heat, in accordance with the basic principles of thermodynamics (Tekaia & Latgé, 2005).

From an integrated perspective, the thermophysics of *Aspergillus* is closely related to its thermal adaptive capacity. The ability to tolerate high temperatures depends not only on physiological and molecular mechanisms of cellular protection, but also on the passive management of the heat generated by metabolism and the physical properties of the mycelium and the surrounding environment. The thermophysical behavior of *Aspergillus* reflects the interaction between metabolic heat production, mycelial architecture, and heat transfer processes that regulate the energy balance of the fungal system.

Conclusions

The increase in temperature alters the cellular structural integrity, which leads to the activation of signaling pathways associated with the maintenance of the cell wall and to the increase in the synthesis of components such as chitin and β-glucans, reinforcing the structure and decreasing the risk of lysis. Simultaneously, heat stress promotes the generation of reactive oxygen species, causing a redox imbalance that is mitigated by the induction of antioxidant systems, including catalases, superoxide dismutases and peroxiredoxins, which limit oxidative damage to proteins, lipids and genetic material. At the metabolic level, thermotolerance involves a reorganization of the central energy pathways and the accumulation of protective compounds such as trehalose and mannitol, which contribute to the stabilization of proteins and membranes. When thermal damage exceeds the capacity for cell repair, *Aspergillus* can activate mechanisms of regulated cell death mediated by fungal metacaspases, favoring the elimination of severely damaged cells and the preservation of mycelium viability. Laboratory results show that *Aspergillus* responds to rising temperatures through a multifactorial strategy that integrates progressive reduction of mycelial growth, activation of heat shock genes (***hsp20*** and ***hsp70***), strengthening of the antioxidant response, and protective structural changes. However, at extreme temperatures (≈50 °C) these mechanisms are insufficient, triggering processes of severe cell damage and regulated cell death, which defines a clear physiological limit for the thermal tolerance of the fungus.

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Contributions from Authors

Antonio Vásquez Hidalgo is the only author. The author read and approved the final manuscript.

Conflicts of Interest

The author declares that he has no conflict of interest.

Abbreviations Used:

ABC (ATP-Binding Cassette)
AMPK (Proteína Quinasa Activada por AMP
ATG (Autophagy-related genes).
ATP (AdenosínTrifosfato)
COG (*Clusters of Orthologous Groups*)
EggNOG (Evolutionary Genealogy of Genes: Non-supervised Orthologous Groups)
FAD (FlavínAdenín Dinucleótido)
FMN (Flavín Mononucleótido)
H_2O_2 (Peroxido de hidrogeno)
HSE (secuencias reguladoras tipo HSE (Heat Shock Elements
KEGG (Kyoto Encyclopedia of Genes and Genomes)
K-numbers (KEGG Orthology)
MetaCyc (Rutas metabolicas)
NADPH (Nicotinamida Adenina Dinucleótido Fosfato)
^{O}C (Grados centigrados)
ORFs (Marcos Abiertos de Lectura)
Redox (reducción-oxidación)
ROS (especies reactivas de oxígeno),
SOD (superóxido dismutasa)
sHSPs(small heat shock proteins)
UniRef90 (UniProt Reference Clusters)
Trx (tioredoxina)
TrxR(tioredoxina reductasa)
VPS (*Vacuolar Protein Sorting*).

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