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PHYSIOLOGICAL AND BIOCHEMICAL ALTERATIONS DURING THE GERMINATION OF ASPERGILLUS NIGER CONIDIA

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ABSTRACT: *The provided analytical data pertains to the germination of Aspergillus niger van Tieghem spores, focusing on various components measured by dry weight. These components include sugars, organic acids, amino acids, proteins, and lipids. Additionally, indicators of spore activity, such as respiration and permeability changes, were assessed. It is noteworthy that A. niger spores do not initiate germination utilizing their internal reserves. The introduction of glucose triggers heightened oxygen consumption and an increase in the overall concentration of organic acids. The initial 3-hour germination period is characterized by an augmentation in protein synthesis, along with a reduction in free amino acid concentration, which suggests the synthesis of proteins to facilitate germination. Furthermore, changes in permeability are observed to influence the release of various substances from the germinating spores. Significant alterations occur in the concentration of free sterols and the sterol-to-lipid ratio in germinating A. niger spores. During the early stages of germination, there is extensive degradation of phospholipids, particularly phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, and their lyso-derivatives. However, these phospholipids are subsequently resynthesized in the later stages of germination. Regarding nonpolar lipids in A. niger spores, they consist primarily of hydrocarbons, triglycerides, fatty acids, sterol esters, and free sterols. Notably, the content of free sterols increases while the content of hydrocarbons decreases during the germination process in A. niger spores.*

KEYWORDS: Physiology, Biochemistry, Metabolic Changes, Germinating Spores, *Aspergillus niger*.

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INTRODUCTION

The exploration of fungal spore germination, especially in *Aspergillus niger*, is a crucial area of study with profound implications across various disciplines like medical microbiology, physiology, and biochemistry. This thorough investigation delves into the complex dynamics and biochemistry governing the germination process, offering valuable insights into spore metabolism and activity. One of the main challenges lies in comparing data across different studies due to variations in experimental conditions, spore age, and collection methods. The process of spore washing, in particular, highlights the need for standardized protocols to ensure consistency and reliability across research endeavors. Through meticulous examination, researchers have uncovered changes in permeability and various physiological and biochemical markers, shedding light on the intricate processes underlying spore metabolism during germination. Key studies by Strobel (1965), Singh and Salim (1980), Salim and Singh (1984), Singh and Salim (1985), Parween *et al*. (2014, 2015a & b) and Salim *et al*. (2016) have significantly enriched our understanding in the field [1-8]. This investigation focused on physiological and biochemical alterations during *Aspergillus niger* spore germination, utilizing fresh spores but calibrating the data to standardized spore dry weights based on previous works by Strobel (1965) [8] and Parween *et al*. (2014) [4]. Unlike earlier studies that predominantly used washed spores, this research provided quantitative biochemical assessments based on the dry weight of unwashed spores, referencing works by Salim and Singh (1984) [2] and Parween *et al.* (2015b) [6]. A wide range of constituents including sugars, organic acids, amino acids, proteins, and lipids were scrutinized, alongside monitoring indicators of spore activity such as respiration and permeability changes. This study stands out as unique, as it comprehensively examined numerous physiological and biochemical parameters simultaneously within the context of a single investigation, setting new benchmarks for the analysis of *A. niger* spores. In essence, this research signifies the relentless pursuit of knowledge and the continuous effort to unravel the mysteries surrounding microbial life, particularly in understanding the intricate biology of fungal spores.

MATERIALS AND METHODS

Experimental Design

In this experimental study, *Aspergillus niger* van Tiegh., a specific type of fungus, served as the primary organism under investigation. To cultivate this fungus, we employed a carefully prepared agar medium, which was subjected to sterilization at 121°C for a duration of 15 minutes. The composition of this medium consisted of glucose (5g), KNO_3 (3.5g), KH_2PO_4 (1.75g), $MgSO_4$ (0.75g), $MnCl_2.4H_2O$ (1mg), $ZnSO_4.7H_2O$ (1mg), $FeSO_4.7H_2O$ (0.5mg), and agar (20g) dissolved in one Liter of double-distilled water. The pH of this mixture was adjusted to 5 using HCl. The resulting medium was then poured into glass Petri dishes, each with a diameter of 7.5 cm. To initiate the growth process, each Petri dish received an inoculation of 1 ml from an aqueous spore suspension, which contained a concentration of 10^6 spores per milliliter. These dishes were subsequently placed in an incubator, maintaining a constant temperature of 25±2°C in complete darkness. Under these controlled conditions, sporulation of the fungus began within 2 to 3 days following inoculation and reached completion within 50 hours thereafter. It is important to note that the spore age mentioned in this study corresponds to the age of the culture from which the spores were originally collected.

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Therefore, the actual age of the spores used in our experimentation is approximately three days less than the stated duration. Finally, on the 15th day from the initial inoculation, we collected the spores using an improved vacuum collector, as described in previous works by Singh and Salim in 1980 [1] and Parween *et al.* in 2015a [5]. This process allowed us to obtain a representative sample of the spores for further analysis and research purposes.

Germination was initiated by dispersing the spores on Petri dishes containing a medium devoid of agar but supplemented with 1 mg/ml concentration, which had previously been filtered through Whatman No. 1 filter paper. The use of filter paper in preparing the germination medium was essential to achieve consistent and synchronized spore germination on a large scale, as described by Wiese and Daly in 1967 [9]. Notably, germ tube protrusion became evident within a relatively short time frame of 7 to 11 hours, and the germination process exhibited a high degree of synchronicity. Throughout the examined time intervals of 3 to 12 hours, germ tube elongation occurred continuously, maintaining a normal appearance. Remarkably, germination consistently reached an approximate rate of 90%. After subjecting spores to dark incubation periods of 3, 6, 9, and 12 hours at a controlled temperature of 25±2 degrees Celsius, the spores were meticulously separated from the germination medium through a filtration process. Great care was taken to ensure that no spores remained in the filtered medium. Subsequently, both the spores and the medium were promptly utilized for a variety of chemical assays. The germination period was defined as the duration from the moment the spores were dispersed onto the medium until they were collected for analysis.

Each experimental condition involved no fewer than six replicate samples, with each sample consisting of 100 milligrams of fresh spores. It is important to note that throughout this study, all analyses were conducted using fresh spores. However, the final data were adjusted to align with the dry weight of spores, which was determined through calibration curves established between the fresh and dry weight of parallel spore samples from the same experiment (Parween *et al*., 2014) [4]. The respiration rate of the germinating spores was ascertained utilizing a Differential Capillary Microrespirometer, following the methodology detailed by Grunbaum *et al*. in the year 1955 [10], at a controlled temperature of 25±2 degrees Celsius. Simultaneously, the protein content was quantified using the Lowry method, as established by Lowry *et al.* in 1951 [11], employing crystalline bovine serum albumin as the standard reference.

A comprehensive analysis was conducted on a shared 80% (v/v) ethanol extract, encompassing the examination of sugars, organic acids, and free amino acids. The determination of bound amino acids was achieved through the utilization of a 6N HCl hydrolysate of the ethanol-extracted spore residues. Qualitative assessment of sugars was conducted according to the methodology outlined by Buchan and Savage in 1952 [12], while quantitative measurements were ascertained using the anthrone method, as established by Yemm and Willis in 1954[13]. The estimation of organic acids followed the procedure described by Ranjan and Laloraya in 1960 [14]. All amino acids and amides, with the exception of proline, were separated via paper chromatography in accordance with the method outlined by Consden and colleagues in 1944 [15], utilizing Partridge's solvent as per Partridge's 1948 work [16]. Detection of these amino acids and amides was accomplished using 0.1% ninhydrin in n-butanol, followed by heating the chromatograms at 80°C for 10 minutes. Subsequently, quantitative measurements of these amino acids and amides were obtained from corresponding spots on the untreated duplicate chromatograms, following the approach established by Rosen in 1957[17]. The estimation of proline was carried out in accordance with the methodology outlined by Wren and Wiggall in 1965 [18].

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The spore lipids were meticulously extracted and refined using the methodology outlined by Folch *et al.* in 1957 [9]. Subsequently, a qualitative thin-layer chromatography (TLC) analysis was conducted to discern neutral lipids and phospholipids, adhering to the protocols elucidated by Malins and Mangold in 1960 and Gentner *et al.* in 1981, respectively [20,21]. For a more in-depth analysis, a quantitative assessment of glycerides, free fatty acids, sterols, hydrocarbons, and phospholipids was meticulously performed. These assessments followed the established methods delineated by Vanhandel and Zilversmit in 1957, Chakravorty *et al.* in 1969, Stadtman in 1957, Amenta in 1964, and Ame's in 1966, each tailored to their respective lipid components [22-26].

To assess the permeability of spore samples, specimens weighing 0.1±0.01 grams were collected at intervals of 3, 6, 9, and 12 hours after germination. These spores were then immersed in a 0.05 mM $CaSO_4$ solution for 30 minutes, subjected to centrifugation, and subsequently transferred to a 0.5 mM KCl solution for a duration of 1 hour. After another round of centrifugation, the spores were thoroughly rinsed twice with distilled water, again employing centrifugation. Throughout the immersion in the $CaSO_4$ and KCl solutions, aeration was consistently maintained. The resulting spore samples were then transferred to glass chambers with a diameter of 2 centimetres, where they were stirred magnetically using a small stirring device. The test medium employed for this investigation consisted of 14 milliliters of distilled water with a conductivity of less than 5 micro siemens. To this, 1 milliliter of ethanol was added. Additionally, each experiment included a control sample lacking ethanol. The assessment of permeability was based on the measurement of electrolyte leakage, monitored using a conductivity meter equipped with a micro dipping-type conductivity cell featuring a cell constant of 1.0. The specific conductance was expressed in units of reciprocal ohms, also known as mhos.

RESULTS AND DISCUSSION

The current investigation incorporates several notable advancements in spore collection and quantification techniques, including the use of an enhanced vacuum collector, as pioneered by Singh and Salim in 1980 [1]. The criterion for quantifying spores involves measuring their dry weight, a method established by Parween *et al*. in 2014 and 2015a [4,5]. Furthermore, the study extensively examines various physiological and biochemical parameters. One significant improvement over conventional methods is the prevention of pre germination events triggered by hydration during spore collection, as observed in the conventional washing procedures introduced by Yanagita in 1957 and Ohmori and Gottlieb in 1965 [27,28]. Therefore, it is imperative to contextualize our results and ensuing discussions within the aforementioned framework before drawing comparisons with any similar studies on *A. niger* spores conducted previously by Parween *et al*. in 2014, 2015a & b and 2016, and Salim *et al*. in 2016 as well as by Novodvorska *et al.*2016 [4-7,29].

The spores of *A. niger*, unable to initiate germination solely through internal reserves, necessitate external nutrients for the process to commence. This dependency on external nutrients for germination has been underscored by various studies [2, 30-34]. Prior to germination, ungerminated spores exhibit minimal rates of respiration [35]. Upon suspension in an aqueous medium, these spores promptly initiate glucose uptake and exude traces of fructose and sucrose (Fig. 1). The zenith of glucose absorption per unit of spore dry weight

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occurs at the 9-hour mark of germination, followed by a gradual decline. This early surge in glucose uptake during germination triggers heightened oxygen consumption [36] and an overall augmentation in the concentration of organic acids, primarily citric acid. Notably, a substantial portion of these organic acids, in accordance with findings by Allen 1965 [37] and Karaffa and Kubicek 2003 [38], is relinquished into the suspension medium (Figs. 2 & 3). While the endogenous levels of individual organic acids exhibit variability, the cumulative organic acid concentration escalates as spore germination progresses. Specifically, the concentrations of endogenous oxalic acid and oxalosuccinic acid show marked increases during germination, contrasting with the notable reduction in endogenous citric acid levels, particularly within the initial 3 hours of germination (Fig. 2) [34].

Upon suspension in the medium, *A. niger* spores exhibit a notable surge in respiration rates during the initial 3 hours of germination, followed by a subsequent decrease to a lower optimal level thereafter (Fig. 3). Concurrently, the total lipid content decreases to approximately 50% of its initial concentration during the early 3-hour germination phase. Notably, neutral lipids display a similar temporal pattern of concentration change. However, in contrast to total and neutral lipids, phospholipid concentration diminishes with the progression of spore germination (Fig. 3 to 6). Among the neutral lipids of *A. niger* spores, the concentration of free sterols escalates during germination (Fig. 4). A remarkable decline in hydrocarbon concentration during the initial 3 hours of germination is succeeded by a subsequent increase, albeit at a lower optimal level compared to the initial level. Free fatty acids, sterol esters, and triglycerides vanish from germinating *A. niger* spores (Fig. 4 & 5). The decreasing trend in phosphatidylcholine concentration stabilizes after the early germination period. There is a steady decline in phosphatidylethanolamine and phosphatidylserine concentrations until 9 hours of germination, followed by an increase thereafter. However, the lyso-derivatives of these phospholipids exhibit a different pattern of concentration change. Lysophosphatidylethanolamine concentration decreases until 3 hours of germination and returns to the control level (0 hours or ungerminated spores) at 9 hours, followed by a decrease until 12 hours. Conversely, lysophosphatidylserine concentration rises until 3 hours, decreases by 9 hours, but increases thereafter (Fig. 4 & 6). Permeability, measured as electrical conductance, increases within 1 hour after the spores are suspended in the medium (Fig. 5). A gradual increase in spore permeability over the 12-hour germination period culminates in a peak increase between 3 and 9 hours.

Numerous studies have delved into the alterations in lipid composition during the germination of fungal spores, as extensively reviewed by Weber and Hess in 1976, Morozova *et al.* in 2002, Feofilova *et al*. in 2012 and Iwama *et al.* in 2023 [39-42]. Lipids, being a significant component of fungal spores, undergo a reduction in concentration as *A. niger* spores germinate, indicating their pivotal role as substrates in the germination process. The primary constituents of lipids in *A. niger* spores encompass triglycerides, free fatty acids, free sterols, sterol esters, phospholipids, and hydrocarbons. The elevated total lipid content in ungerminated *A. niger* spores (4.28%) compared to previously reported lower values 4.1%, [43] is attributed to the utilization of unhydrated spores in our investigation. The disappearance of fatty acids during the germination of *A. niger* spores may be attributed to their conversion from lipids to carbohydrates, as proposed by Reisener and Jager in 1966 [44] and Gunasekaran *et al.* in 1972 [45], facilitated by the activity of glyoxylate path enzymes, as indicated by the studies of Frear and Johnson in 1961 [46] and Caltrider and Gottlieb in 1963 [47]. However, the resultant carbohydrates may not accumulate to supraoptimal levels, as they are likely utilized as

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respiratory substrates. Similarly, the decline in triglycerides implies their extensive degradation during the germination of *A. niger* spores, a notion supported by the decreasing lipid concentration and increasing respiratory rate observed (Figs. 3 to 6).

Lipidology, as highlighted by Tevini and Lichtenthaler in 1977 [48], intertwines significantly with membranology [42]. Hence, phospholipids and sterols hold paramount importance as membrane constituents (Fig. 5). Observing fluctuations in total phospholipid levels (Fig. 3) during the initial stages of *A. niger* spore germination, followed by subsequent resynthesis, implies their dual functional and structural roles. Similar variations in phospholipid concentration have been documented in *A. niger* spores previously [49]. Furthermore, our investigation delved into alterations in the concentration of other metabolites and physiological processes within dry harvested spores. Of particular relevance in this context is the respiratory rate, serving as a gauge of spore activity. As established for spores of other fungi by Maheswari and Sussman in 1970 [50], and Langenbach and Knoche in 1971[43], the uptake of O_2 by germinating *A. niger* spores seems intricately linked with phospholipid catabolism. Given that phospholipids predominantly reside in cellular membranes, shifts in phospholipid levels reflect the degradation and synthesis of membranes. Consequently, the temporal ultrastructural changes observed in germinating *A. niger* spores [51] appear tightly correlated with phospholipid catabolism during the early 3-hour germination period. Assuming a fundamental similarity of events in germ tube growth between *A. niger* spores and other fungi, it is logical to surmise that the energy and precursors required for germ tube wall formation would be most demanded at the apical portion of the germ tube. The increase in phospholipid concentration between the 9- and 12-hour germination period, aligning with optimal germ tube emergence and growth, suggests that, akin to rust fungi [52], germinating *A. niger* spores predominantly utilize phospholipids during rapid germ tube elongation. However, the subsequent elevation in phospholipid levels implies a transition towards their structural role in the membrane.

Sterols exert a pivotal role in both the structural integrity [53] and permeability modulation of membranes [54-56]. Variations in the proportions of sterols to lipids prompt distinct molecular arrangements within the lipophilic constituents of the phospholipid layer, consequently altering membrane permeability [57]. The configuration of the membrane's micelle structure is contingent upon the interaction between phospholipids and proteins [58], with sterols capable of penetrating phospholipids having the capacity to alter micelle arrangement [59] and thereby membrane permeability. Consequently, alterations in free sterol concentration, the sterol-to-phospholipid ratio, and the permeability of germinating *A. niger* spores (Fig. 5) emerge as significant phenomena. Permeability fluctuations of germinating *A. niger* spores were quantified over the 12-hour period required for optimal germination. The pronounced efflux of substances from the germinating spores into the medium at the 3-hour mark (Fig. 5) underscores a regulatory aspect of permeability rather than mere diffusion or a passive process, as might be erroneously assumed in the absence of permeability measurements [52]. However, the diminished level of total metabolites lost to the medium post-3-hour germination, despite a substantial increase in spore permeability, further corroborates the regulatory nature of permeability. This assertion appears plausible, considering that permeability reflects the equilibrium between efflux and influx contingent upon the direction of the concentration gradient. Regrettably, the study of permeability changes in germinating fungal spores has not garnered extensive attention in previous research endeavors [40-42].

Protein synthesis during fungal spore germination, as observed in studies by Allen (1965) [37] and Lovett (1976) [60], emerges as a delayed response to spore hydration and nutrient

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absorption in *A. niger* spores. This phenomenon is underscored by the initial decrease in protein levels within the first 2 hours of germination, followed by a subsequent increase at the 3-hour mark, corroborating findings from recent investigations [61]. Beyond the 3-hour threshold, there is a notable two to threefold augmentation in protein levels within germinating *A. niger* spores, as depicted in Figures 6 and 7. The early phase of *A. niger* spore germination, specifically within the initial 3 hours, is marked by vigorous proteolysis, leading to the liberation of numerous amino acids into the surrounding medium. Both qualitative and quantitative disparities are evident in the array of released amino acids throughout the 12-hour germination period. With the exception of proline and cysteine, all other amino acids are detected in the germination medium at various time intervals. The collective concentration of free amino acids within germinating *A. niger* spores notably declines during the initial 3-hour germination period, remaining relatively stable until the 9-hour mark, followed by a gradual increase thereafter (Figures 7 and 8). However, the temporal pattern of amino acid release indicates a significant decrease in the concentration of released amino acids into the medium from the 3rd to the 9th hour of germination. In contrast, the concentration of total protein-bound amino acids, as well as proteins themselves, experiences an increment until the 9-hour mark, followed by a slight decline thereafter (Figures 6 to 8).

The concentration of individual amino acids within the free amino acid pool within *A. niger* spores fluctuates at different periods during the 12-hour germination period, either increasing, decreasing, or remaining static (Figure 8). With the exception of proline and threonine, whose peak concentrations in the protein-bound fraction decline after the 3-hour germination period, the levels of other amino acids rise until the 9-hour mark, forming intricate dynamics within the germination process. The revelation of fungal and bacterial spore amino acids in the germination medium was previously acknowledged [52], yet quantitative data regarding the dry weight of unhydrated spores have not been systematically presented before. The quantities observed in our current study are notably significant. Notably, the pinnacle, constituting 25% of the total amino acids found in the ungerminated spore, could be discerned in the medium within a mere 3 hours of germination. The underlying mechanisms responsible for this occurrence could fundamentally alter our comprehension of spore metabolism during the germination process. However, elucidating this phenomenon is impeded by our inability to ascertain whether the exogenous amino acids originate from all spores within the population or only from a specific percentage thereof. In our investigation, only 90% of the spores underwent germination. Consequently, it is highly probable that a certain proportion of amino acids also trace their origins back to the ungerminated spores. This complexity underscores the intricate nature of spore physiology and metabolism during the critical phase of germination [56].

While the process of protein synthesis supporting germination is extensively documented, the precise timeline for amino acid synthesis, the fundamental constituents of proteins, remains inadequately explored in germinating fungal spores [33, 60-62]. The increased presence of proteins and amino acids observed in our current investigation, exceeding those present in dormant *A. niger* spores, not only confirms the concomitant nature of these interconnected processes but also underscores the absorption of nitrogen from the surrounding medium as a prerequisite for protein synthesis. Evidently, the reduction in amino acid concentration within the spores' internal free pool during germination likely arises from their incorporation into proteins and their release into the medium, rather than from a deficit in their synthesis [33]. The observed trend of a notable increase in free amino acids during the initial 3-hour

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germination phase, followed by a slight decline thereafter (Figure 7), mirrors findings from the studies conducted by Yanagita (1957) [27] and Weber (1966) [63]. These studies, respectively focusing on the germination of spores of *A. niger* and *Rhizopus arrhizus*, reported analogous patterns. Drawing from the elevated concentration of bound amino acids, our inference suggests that the reduction in free amino acids over the 12-hour period of *A. niger* spore germination arises from their accelerated assimilation into proteins and cellular constituents [41, 64].

Glutamic acid stands as the primary product of inorganic nitrogen assimilation, assuming a pivotal role in intermediary nitrogen metabolism. The net synthesis of amino acids, rooted in nitrogen uptake from the germination medium, typically comprised of NO^{-}_{3} sourced from KNO_3, hints at the dormant *A. niger* spores either harboring the complete set of glutamic acid synthesizing enzymes or inducing their synthesis during germination. This likelihood gains credence from the documented presence of enzymes involved in glutamate metabolism in fungi, such as glutamate dehydrogenase, glutamine synthetase, and glutamate synthase [65]. Moreover, the germination of *A. niger* spores hinges on the synthesis of enzymes absent in the dormant state [66]. Fungi exhibit notably high glutamate dehydrogenase activity [67]. Although alanine-glutamic transaminase is initially lacking in dormant *A. niger* spores, its presence emerges during the swelling phase of germination [68].

However, the limited accumulation of glutamic acid within the first 3 hours of *A. niger* spore germination (Fig. 8) implies its swift conversion into other amino acids. Notably, during the initial 3-hour germination period, glutamic acid seems not to integrate into proteins. Yet, its net synthesis becomes evident from its overall concentration in the free endogenous pool and the fraction released into the germination medium [39,65]. Among the amino acids found in *A. niger* spores, α-alanine displays the most consistent and substantial synthesis throughout the 12-hour period, as depicted in Figure 8. This occurrence is unsurprising, given the concurrent increase in spore respiration, as illustrated in Figure 3, which supplies pyruvate, the precursor for alanine synthesis through a specific enzymatic reaction involving an aminotransferase:

$$\underset{\text{Pyruvate}}{CH_3\text{-}C(O) - COOH} + \text{amino acid} \longleftrightarrow \underset{\text{Alanine}}{CH_3\text{-}CH(NH_2) - COOH} + \text{Oxo acid}$$

The loss of alanine from the free amino acid pool within *A. niger* spores into the germination medium, along with its integration into proteins, follows a consistent trend without any irregularities during the 12-hour germination period [60]. Leucine, isoleucine, threonine, and valine are absent from the free amino acid pool in dormant *A. niger* spores but emerge during germination. The behaviour of these amino acids during the 12-hour germination period is erratic:

- At 3 hours into germination:

i. Proteolysis of proteins containing leucine and isoleucine does not result in a proportional increase in these amino acids within the endogenous pool of free amino acids, nor are they released into the medium.

ii. Despite the absence of proteolysis of proteins containing valine, its concentration in the endogenous free pool remains remarkably constant without being released into the medium.

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iii. Threonine concentration increases both in protein and the released fraction without being detectable in the free amino acid pool inside the spore initially.

These findings suggest that the synthesis and degradation of these amino acids are activated to varying degrees during *A. niger* spore germination [37, 41].

Proline assumes heightened importance due to its unique characteristics within *A. niger* spores. Despite existing at only a tenth of the total amino acid concentration, proline is remarkably incorporated into proteins to an extent of approximately 98% of its total concentration within the free pool after 3 hours, with no detectable traces released into the surrounding medium. Proline serves multifaceted roles, acting as a source of carbon, nitrogen, and energy, as highlighted in studies by Caltrider *et al*. (1963) [69], Weber (1966) [63], and Lang and Lang (1958) [70]. The synthesis of proline and its predominant integration into proteins underscore a probable necessity for the synthesis of proline-specific proteins, indicating an early stage in *A. niger* spore germination, as elucidated by Lang and Lang (1958), Weber (1966), Salim and Singh (1984), and Singh and Salim (1985) [2, 3, 63, 70]. In the realm of microorganism biology, a significant regulatory mechanism governing amino acid biosynthesis involves the suppression of enzyme synthesis when one or more end products of a pathway are present, as noted by Stewart et al. in 1980 [65]. The synthesis of amino acids necessitates a considerable reduction in the pool size of these foundational compounds before the enzymes can effectively catalyze the process. In the context of germinating *A. niger* spores, amino acid release plays a crucial role in alleviating the metabolic block imposed by substrate accumulation, thereby facilitating both net amino acid and protein biosynthesis. This phenomenon has been studied by Daly *et al.* in 1967, Mirkes in 1974, Lovett in 1976, Abdel-Rahim in 1985, Feofilova *et al*. in 2012, Kimran Hayer *et al.* in 2014, Baltussen *et al.* in 2020 and Jata Shankar in 2022 [30, 33, 41, 52, 56, 60-62].

CONCLUSION

In summary, the investigation presented a comprehensive analysis of *A. niger* spore germination, emphasizing novel methodologies and insights into physiological and biochemical dynamics. Through improved vacuum collection and dry weight quantification, the study circumvented pre germination events encountered in conventional washing procedures, enhancing the accuracy of results. The study delineated the metabolic intricacies of *A. niger* spore germination, highlighting the reliance on external nutrients for germination initiation and the subsequent metabolic shifts, notably in lipid and amino acid metabolism. Notably, the rapid glucose uptake and lipid degradation underscored the energetic demands during early germination phases, while the dynamic changes in phospholipid and sterol concentrations reflected membrane remodeling and permeability alterations. Furthermore, the investigation provided nuanced observations on protein synthesis dynamics, revealing a complex interplay between amino acid utilization, incorporation into proteins, and release into the medium. The patterns of amino acid synthesis and release underscored the intricate regulation of metabolic pathways during spore germination, suggesting mechanisms for substrate utilization and metabolic flux regulation. Overall, the study elucidated the multifaceted metabolic adaptations underlying *A. niger* spore germination, offering insights into the interplay of nutrient uptake, metabolic transformation, and cellular remodeling. The findings not only contribute to the understanding of fungal spore physiology but also

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underscore the complexity and sophistication of microbial metabolic regulation during germination processes.

ABBREVIATIONS

g - Gram
mg - Milligram
^{o}C - Degree Centigrade
α - Alpha
µg - Microgram
pH - Hydrogen ion concentration
cm - Centimetre
ml - Millilitre
mM - Millimole
NO_3 - Nitrate
HCl - Hydrochloric acid
KCl - Potassium chloride
TLC - Thin layer chromatography
KNO_3 - Potassium nitrate
$MgSO_4$ - Magnesium sulphate
$CaSO_4$ - Calcium sulphate
KH_2PO_4 - Potassium hydrogen phosphate
A. niger - *Aspergillus niger*
$MnCl_2.4H_2O$ - Manganese sulphate
$ZnSO_4.\ 7H_2O$ - Zinc sulphate
$FeSO_4.\ 7H_2O$ - Ferrous sulphate
CH_3-C (O)-COOH - Pyruvic acid
CH_3-CH (NH_2)-COOH - Alanine

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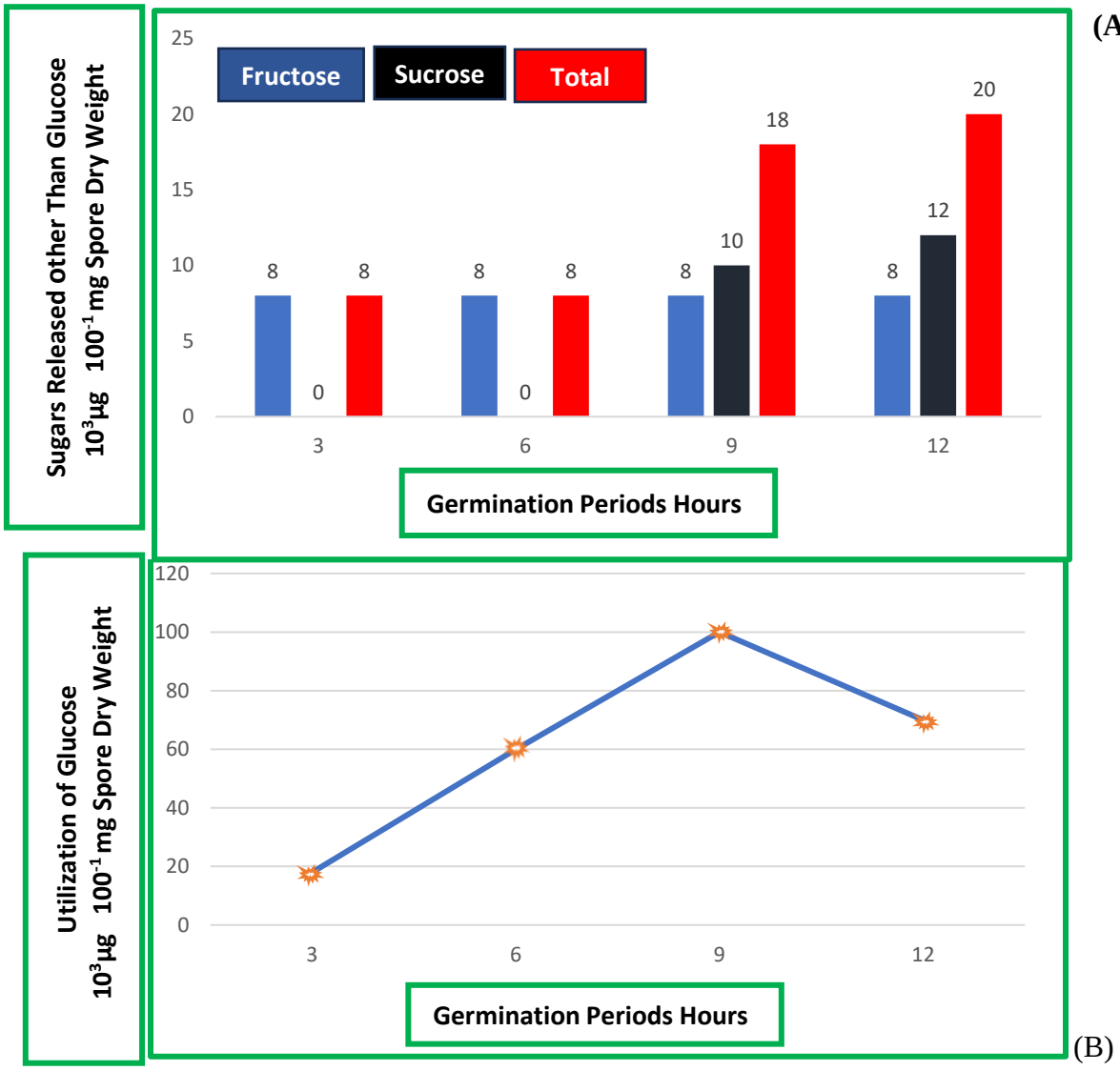

Fig. 1: Sugars other than glucose released in the medium (A) and utilization of glucose (B) by germinating *A. niger* conidia

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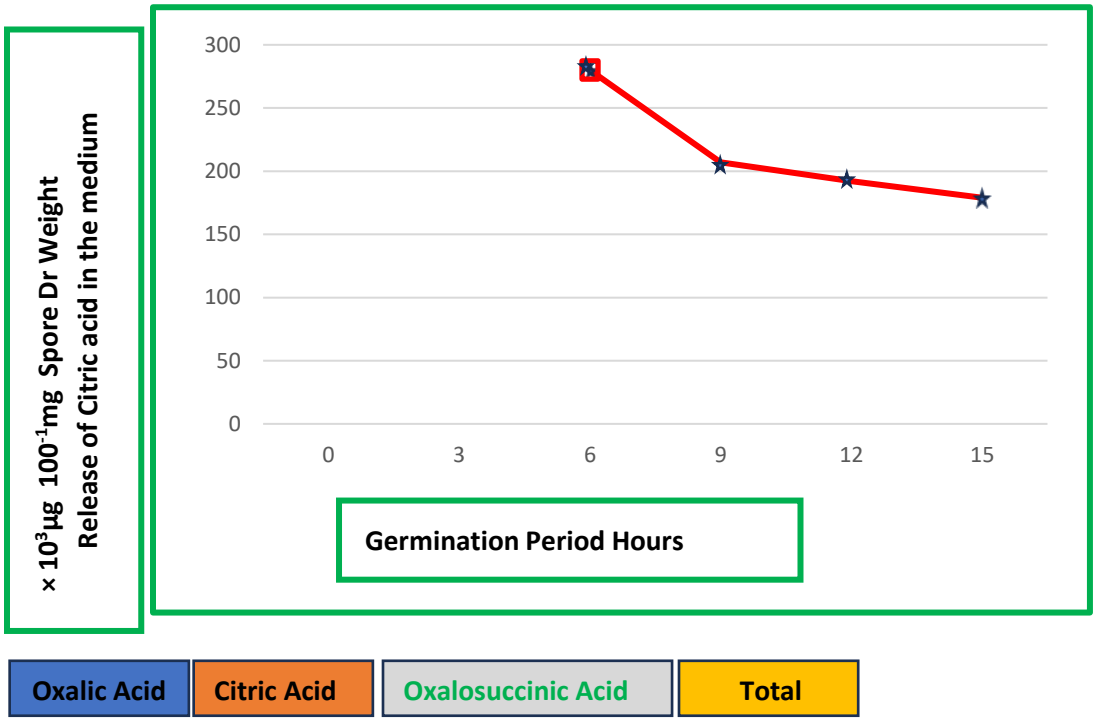

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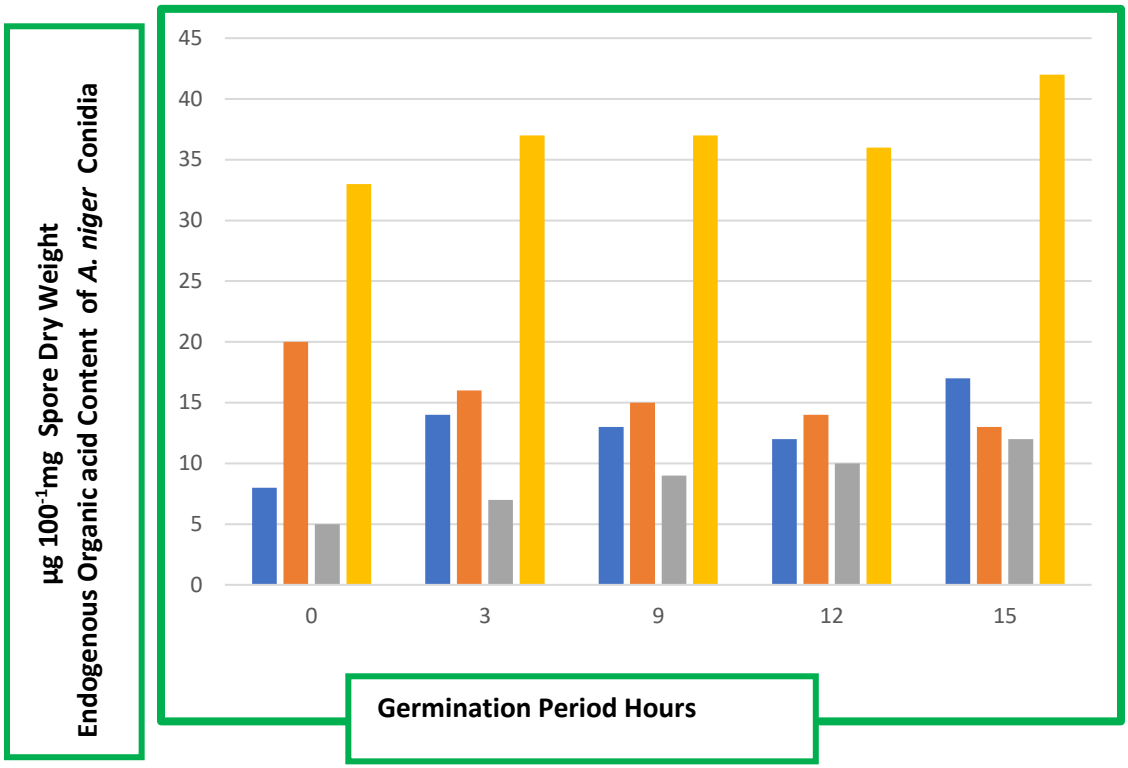

(B)

Fig. 2: Released (A) and endogenous (B) organic acid content of germinating *A. niger* conidia

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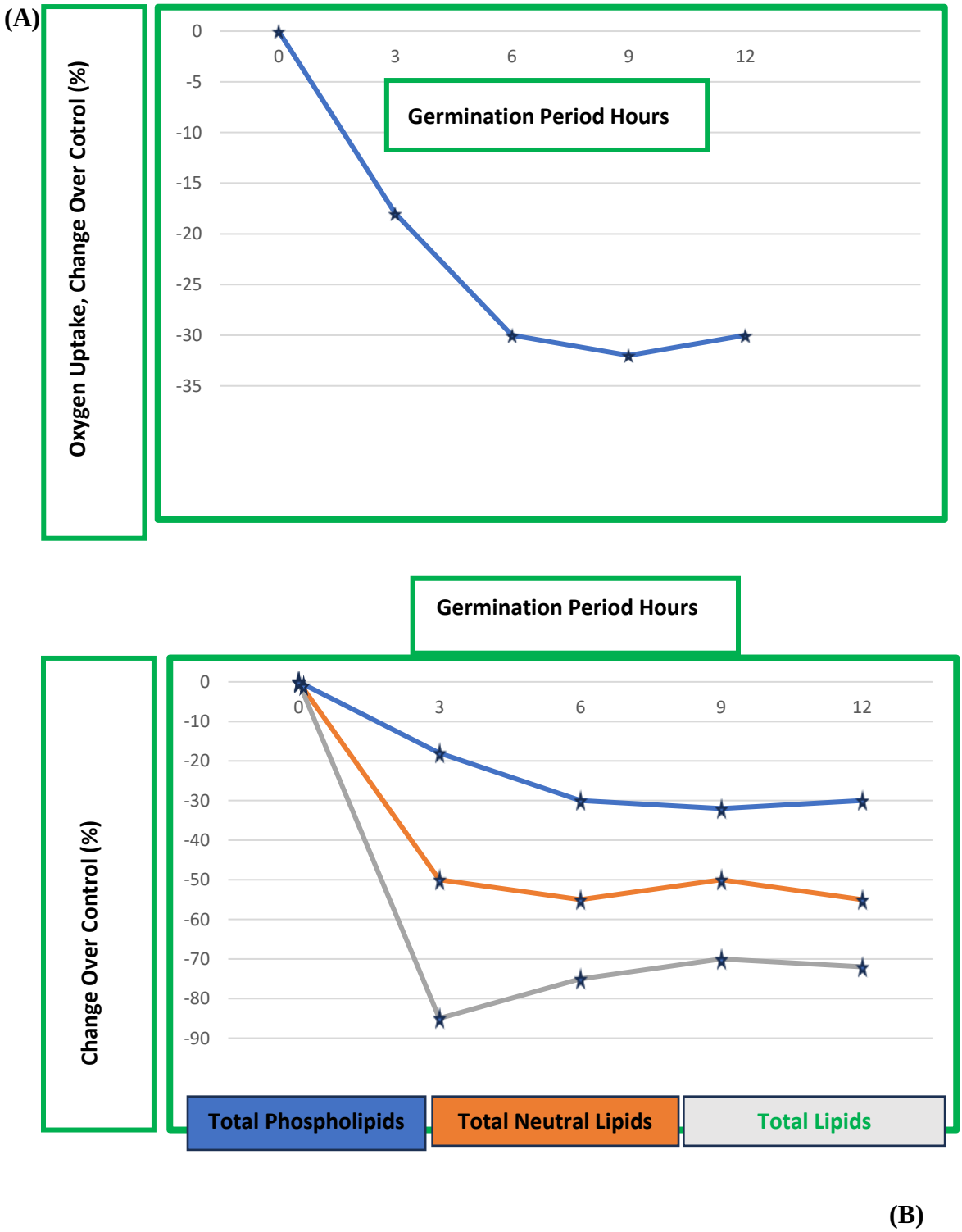

Fig. 3: Oxygen uptake (A) and total lipid content (B) of germinating *A. niger* conidia

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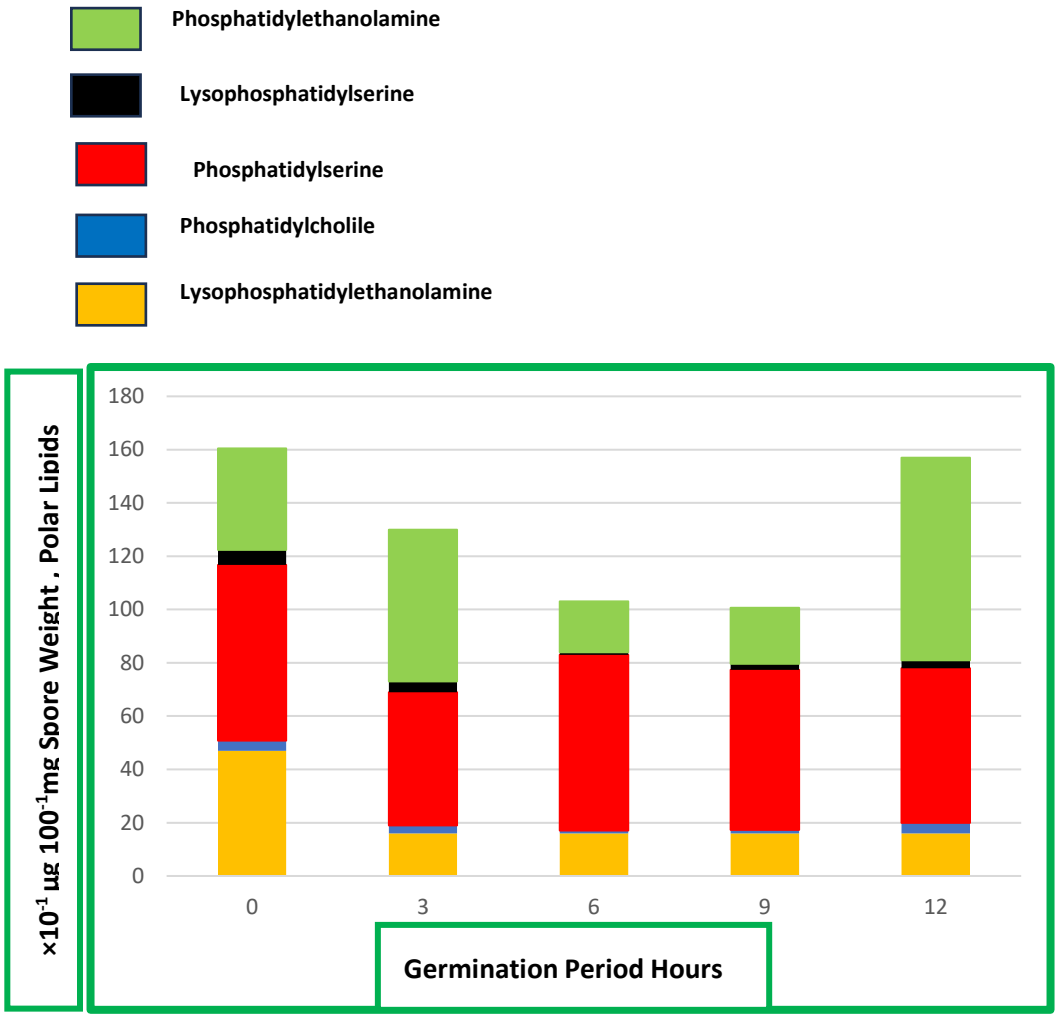

Fig. 4 (A): Changes in phospholipid composition of germinating *A. niger* conidia

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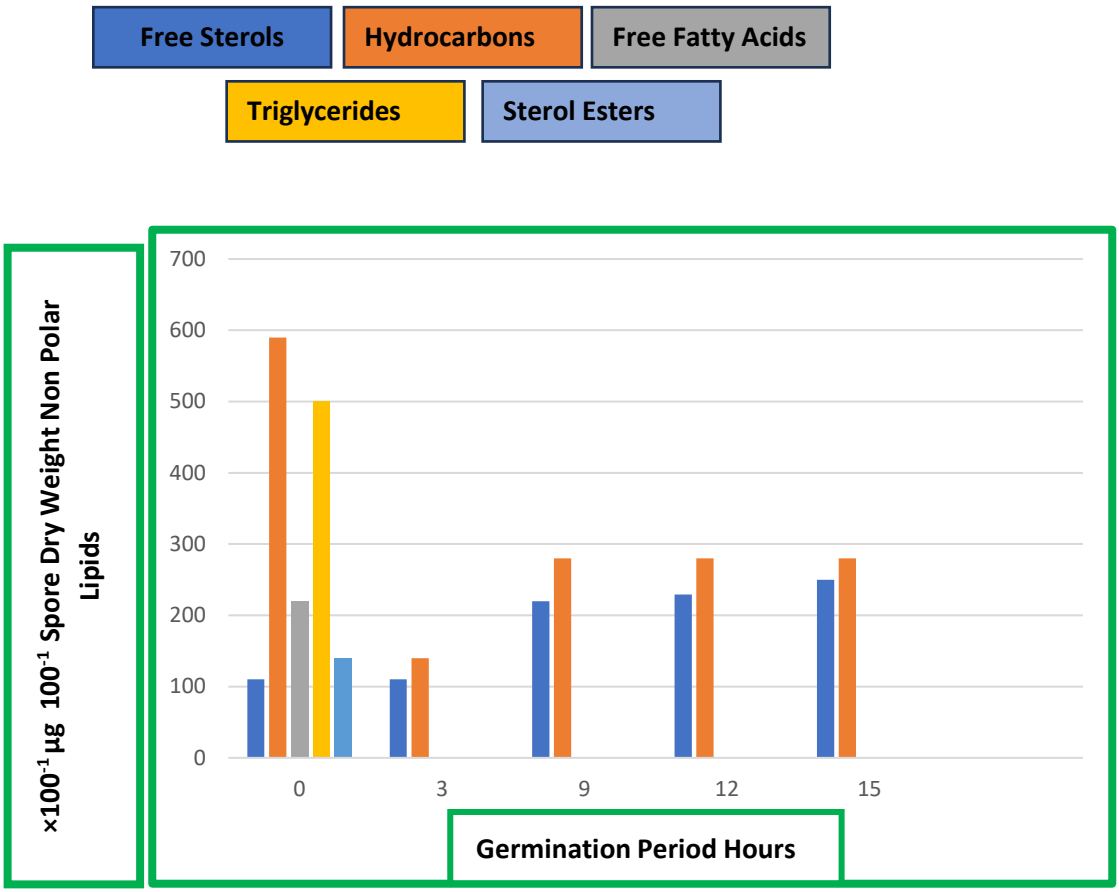

Fig. 4 (B): Changes in neutral lipids of germinating *A. niger* conidia

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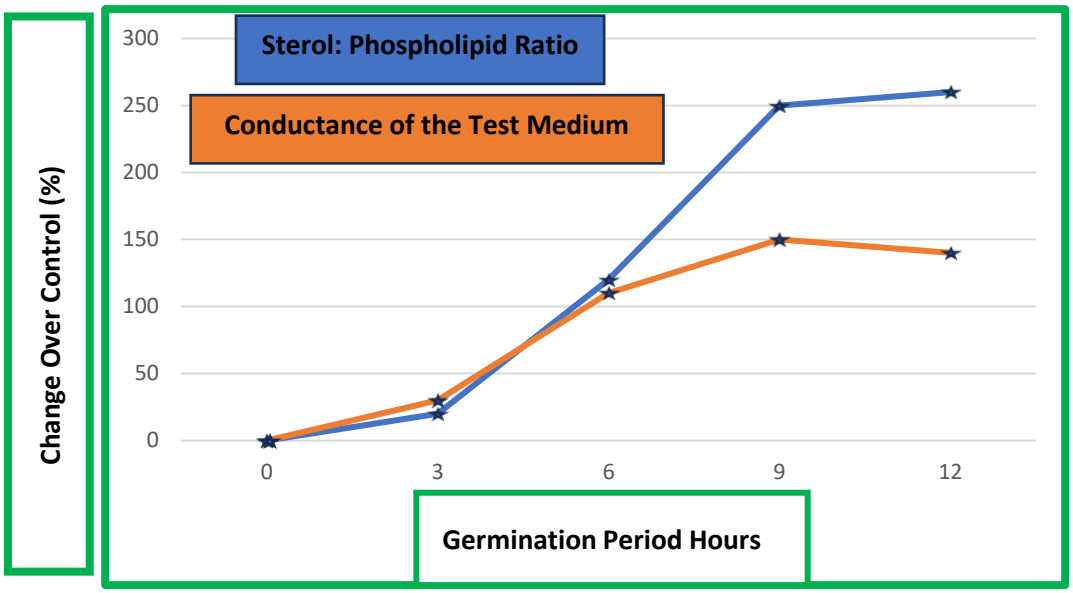
(A)



(B)

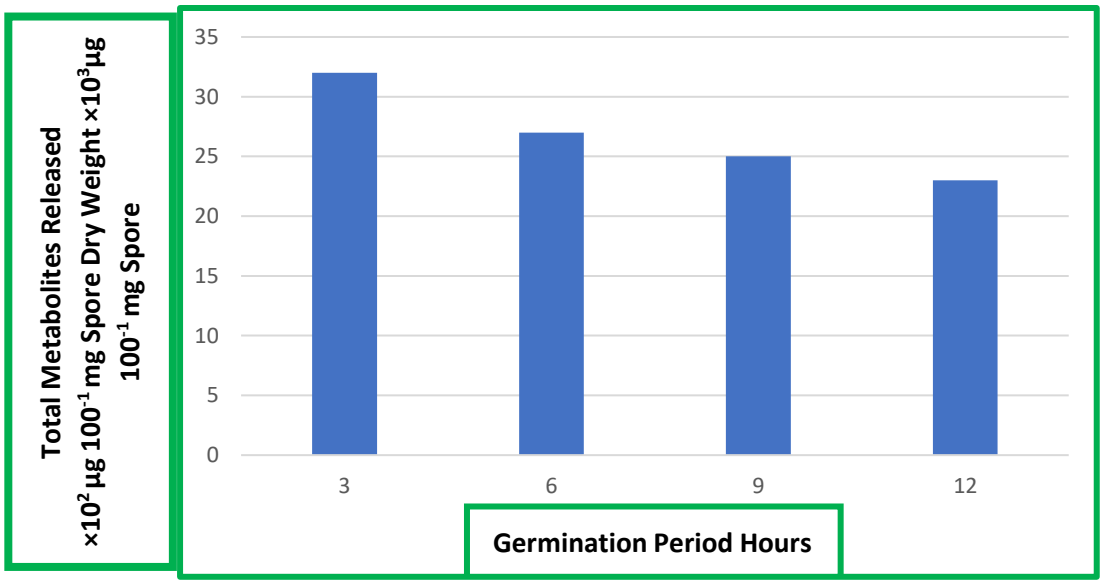


Fig. 5: Timecourse of conductance of the test medium, sterol: phospholipid ratio (A) with the total metabolites released (B) by the germinating *A. niger* conidia

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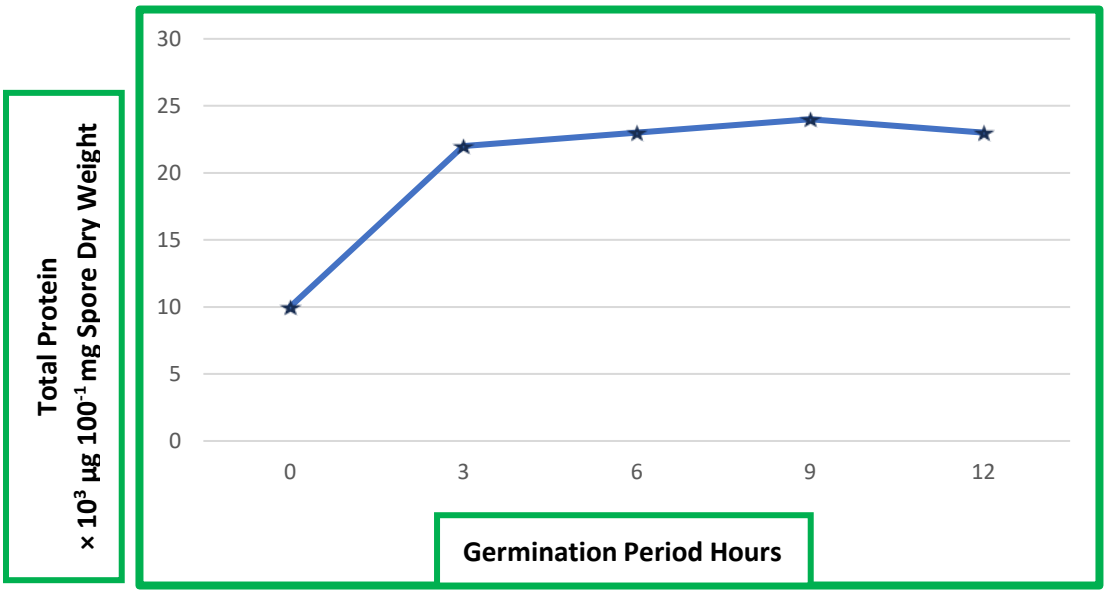

Fig. 6: Changes in the total protein content of geminating *A. niger* conidia

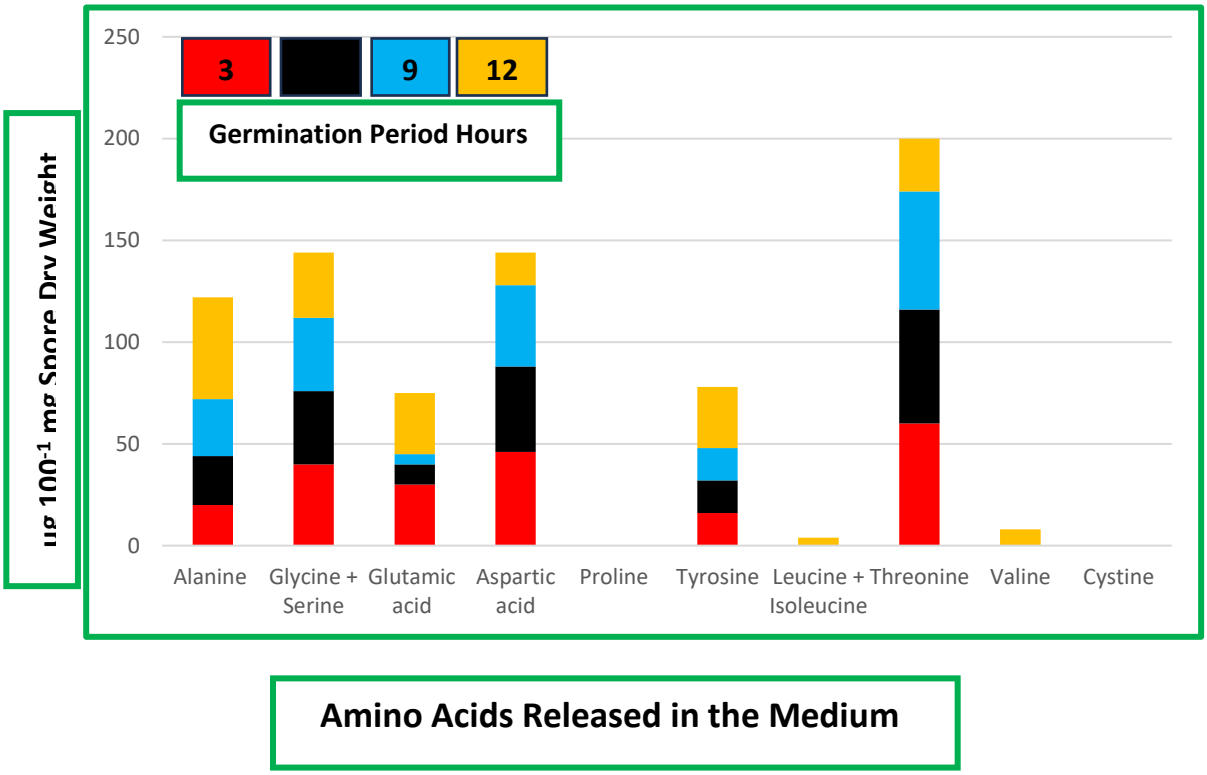

Fig. 8 (A): Changes in the amino acid composition of amino acids released in the medium of germinating *A. niger* conidia

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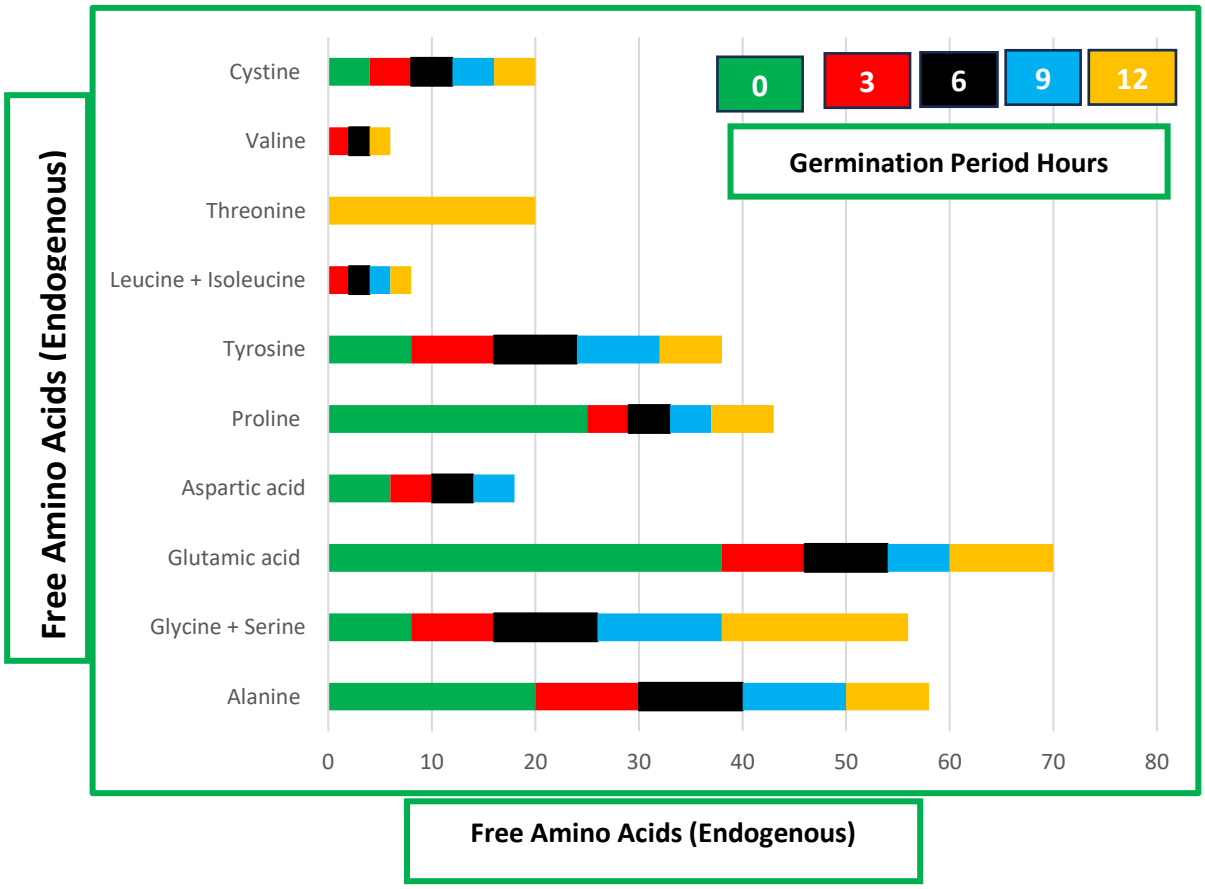

Fig. 8 (B): Changes in the free amino acids (endogenous) of germinating *A. niger* conidia

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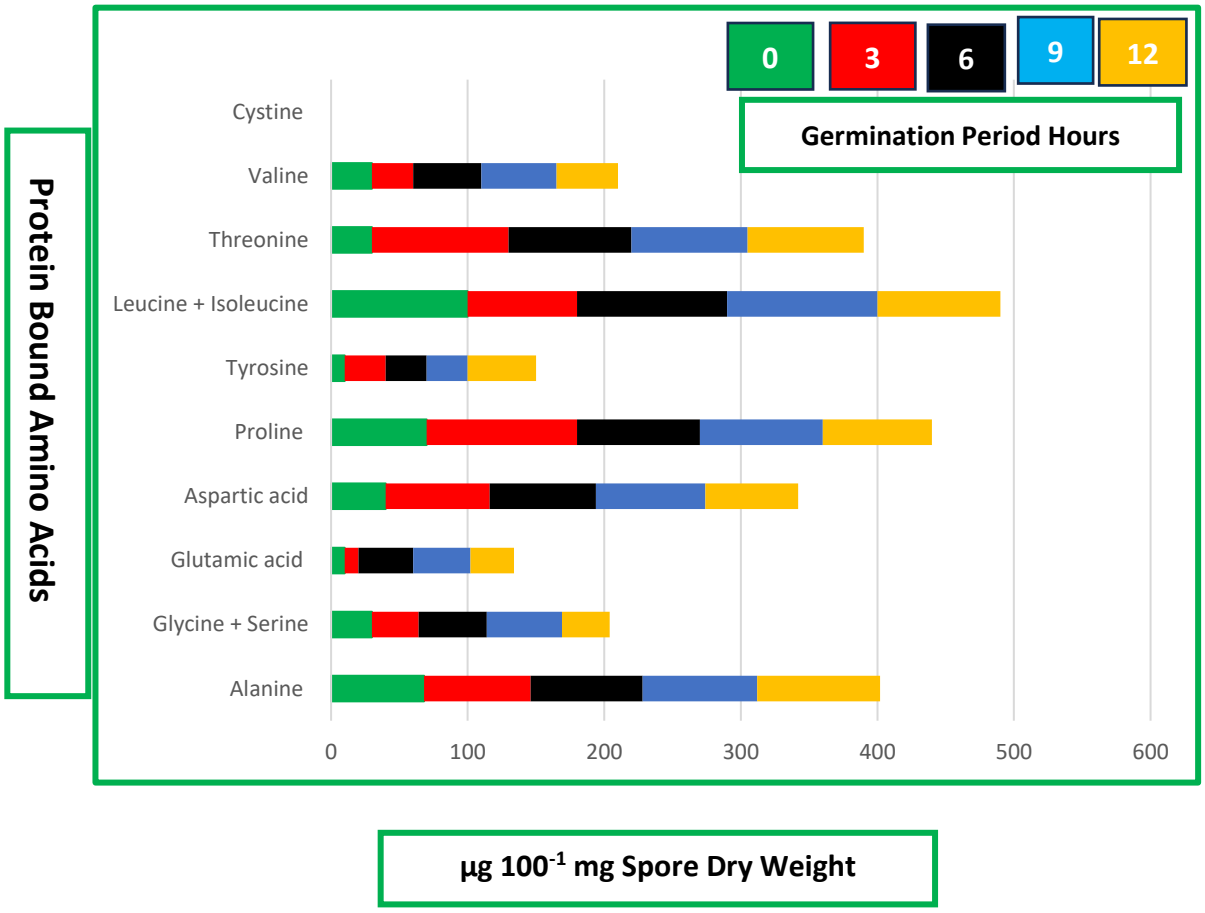

Fig. 8 (C): Changes in the protein bound amino acids of germinating *A. niger* conidia

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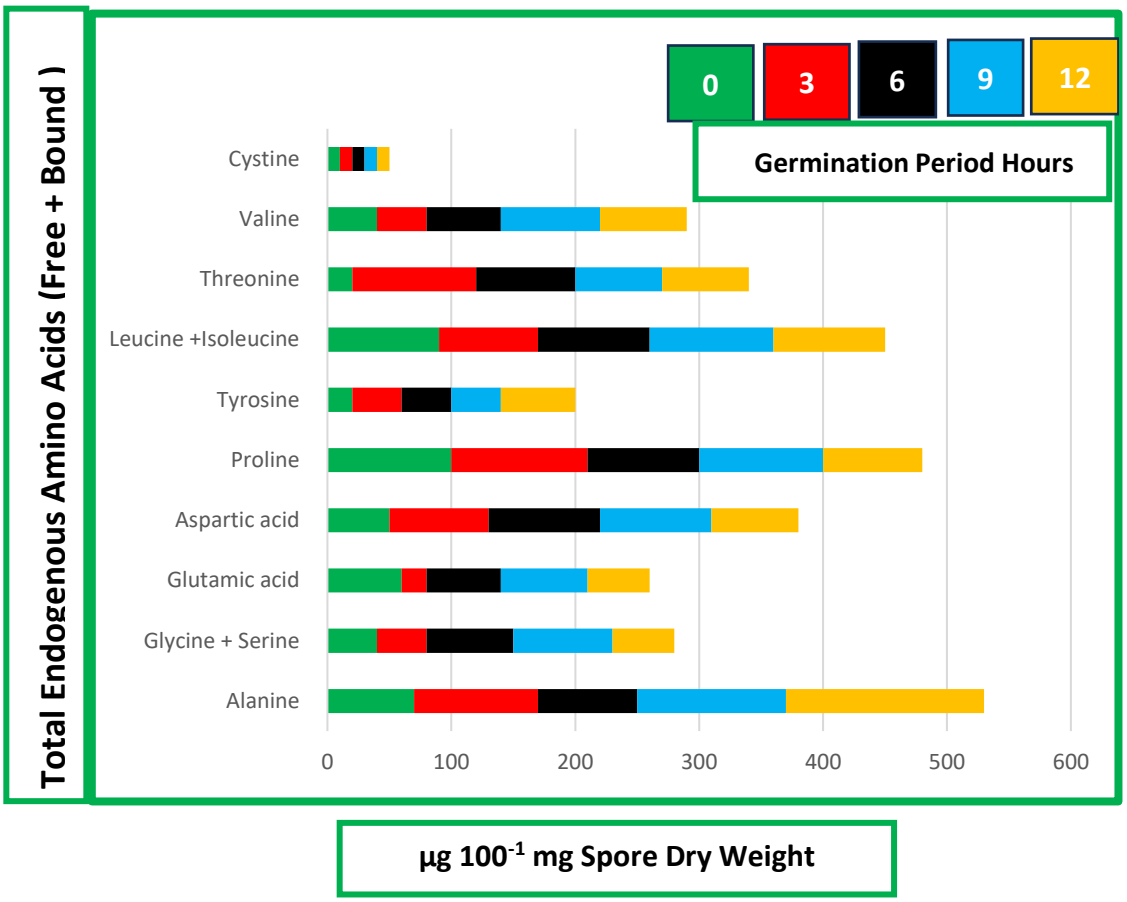

Fig. 8 (D): Changes in the total endogenous amino acids (Free + Bound) of germinating *A. niger* conidia

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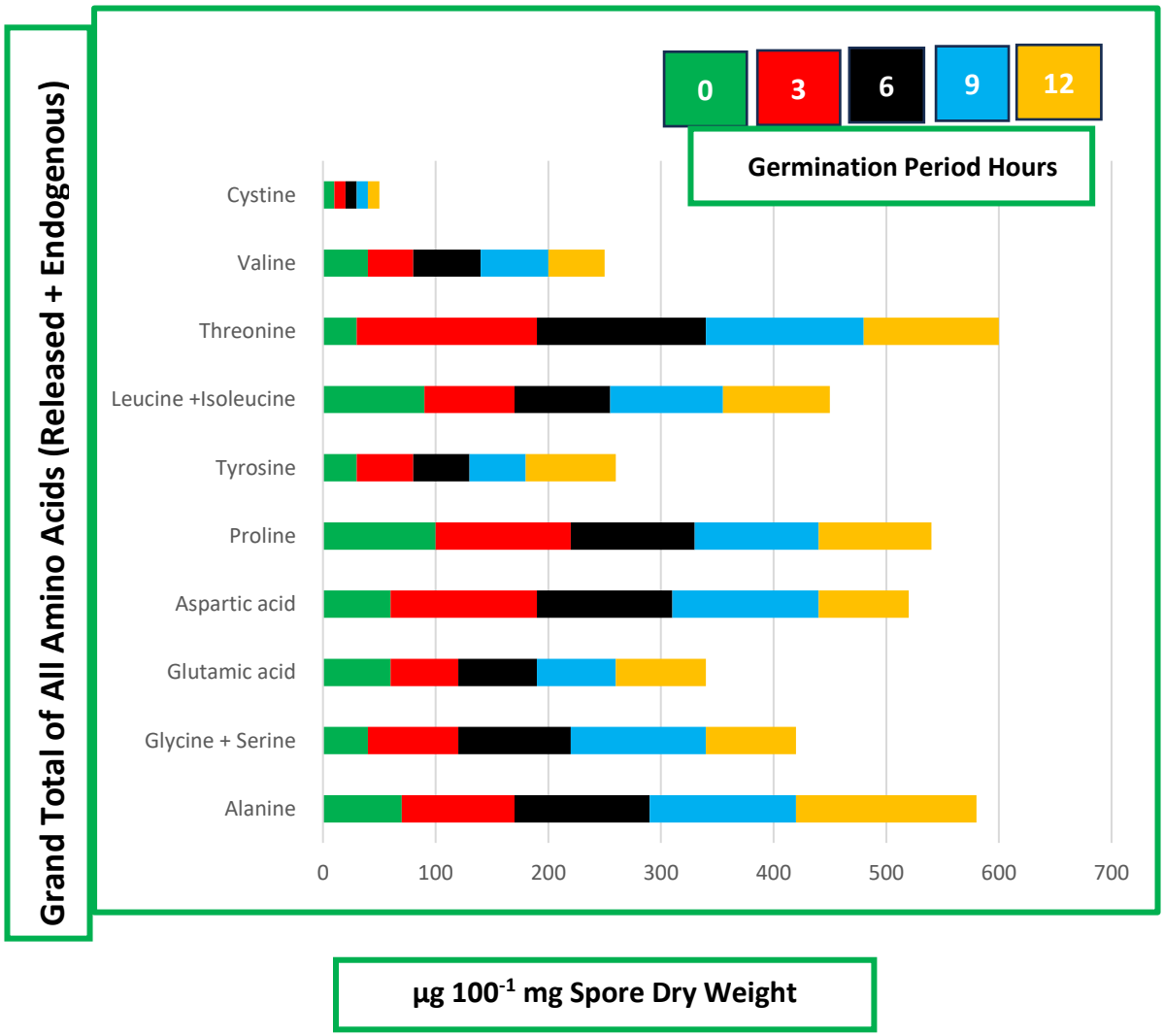

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Fig. 8 (E): Changes in the grand total of all amino acids (released + endogenous) of germinating *A. niger* conidia

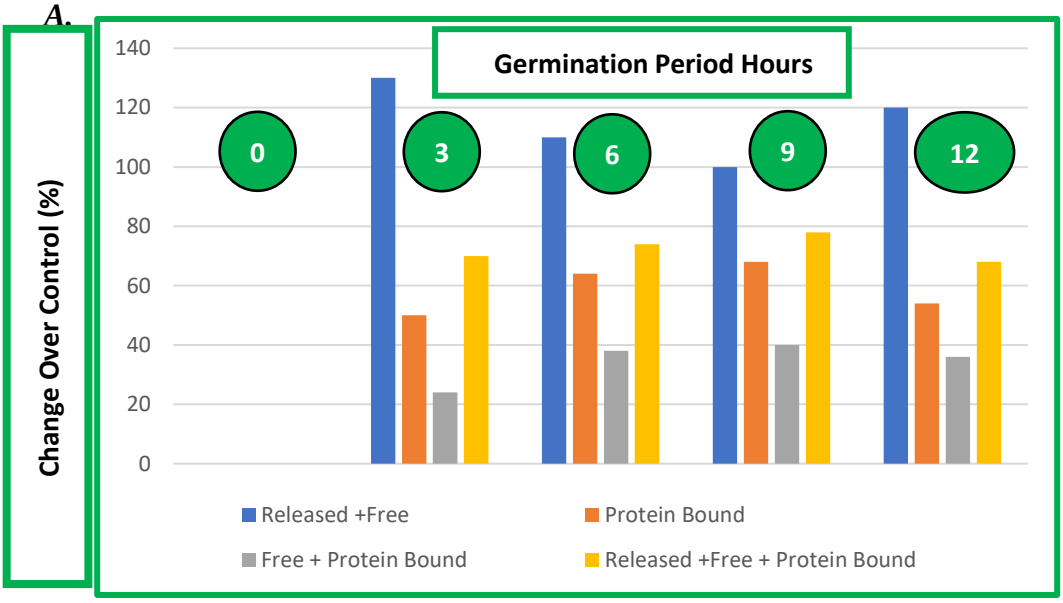

Fig. 7 (A): Total amino acids of germinating *A. niger* conidia

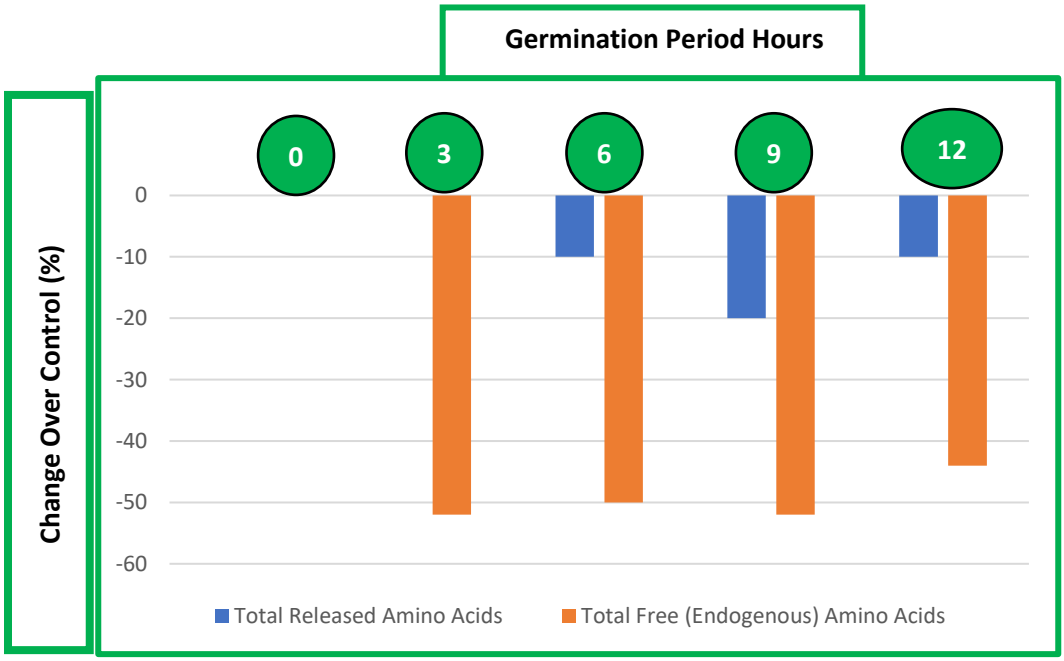

Fig. 7 (B): Total released and free (endogenous) amino acids of germinating *A. niger* conidia

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