

# Induction of Alternative Oxidase Activity in *Candida albicans* by Oxidising Conditions

Simon Brown and Raewyn Tuffery

**Abstract**—*Candida albicans* ATCC 10231 had low endogenous activity of the alternative oxidase compared with that of *C. albicans* ATCC 10261. In *C. albicans* ATCC 10231 the endogenous activity declined as the cultures aged. Alternative oxidase activity could be induced in *C. albicans* ATCC 10231 by treatment with cyanide, but the induction of this activity required the presence of oxygen which could be replaced, at least in part, with high concentrations of potassium ferricyanide. We infer from this that the expression of the gene encoding the alternative oxidase is under the control of a redox-sensitive transcription factor.

**Keywords**—alternative oxidase, *Candida albicans*, enzyme induction; oxygen; redox potential.

## I. INTRODUCTION

CYANIDE-RESISTANT respiration has been reported in many organisms, especially in higher plants and fungi. The alternative oxidase (AOX) responsible for this activity is a quinol:oxygen oxidoreductase which is insensitive to cyanide, unlike most other terminal oxidases. Like the other enzymes of the respiratory electron transfer chain, the AOX is associated with the inner mitochondrial membrane [1], but, unlike several of those enzymes, it is not coupled to proton translocation.

Alternative oxidase activity is inhibited by compounds such as SHAM [2], disulfiram and *n*-alkyl gallates [3, 4], none of which inhibit cytochrome oxidase. Conversely, cyanide inhibits cytochrome oxidase, but does not inhibit AOX. These two inhibitors can be used to distinguish between O<sub>2</sub> uptake catalysed by AOX and cytochrome oxidase. The disruption of electron transfer by cyanide and SHAM would affect ATP synthesis and the activity of metabolic pathways, either of which would inhibit growth. It has been suggested that AOX is a viable target in the treatment of trypanosomes [5] and that it plays an important role in the stress response in various fungi [6] and in plants [7]. Moreover, hydroxamic acids (such as SHAM) inhibit the growth of some bacteria, some of which also have an AOX homologue [1], and other fungi [8, 9]. While does SHAM inhibit the growth of various *Candida*

spp., including *C. albicans*, this is not due to the inhibition of AOX [10, 11].

While the AOX of higher plants appears to be present constitutively in many species, this is not necessarily the case in fungi. For example, *Hansenula anomala* and *Magnaporthe grisea* appear to lack detectable levels of the enzyme in normal circumstances [12, 13], whereas others (such as *Aspergillus niger* and *Neurospora crassa*) generally have only low levels of AOX [14, 15]. In each of these species AOX activity can be induced or enhanced by a variety of treatments. For example, treatment with respiratory inhibitors such as antimycin A or cyanide, which inhibit the cytochrome *bc*<sub>1</sub> complex and cytochrome oxidase, respectively, results in the appearance of AOX activity after a lag of as much as several hours, depending on the experimental conditions and the species. *Candida albicans* ATCC 10231, like some other *Candida* spp., has a constitutive AOX and an inducible AOX encoded by different genes [16, 17].

The AOX of *C. albicans* has been reported to be sensitive to azide as well as SHAM [18], unlike that from other fungi, including *H. anomala* [19], and the induction of AOX activity is relatively rapid [18]. Here we show that the induction of the AOX activity in *C. albicans* is dependent on the strain, the growth phase, on oxygenation and on the redox state of the medium.

## II. MATERIALS AND METHODS

*Candida albicans* ATCC 10231 and *C. albicans* ATCC 10261 were grown in an orbital incubator at 30°C in liquid YPD (1% (w/v) yeast extract, 2% (w/v) bacteriological peptone, 2% (w/v) glucose). Routinely, cultures (50 mL YPD in a 250 mL conical flask) were inoculated with sufficient cells from an overnight culture to get an initial A<sub>600</sub> of 0.4 and grown for 15 h, unless otherwise specified, at 30°C in a shaking incubator (at 200 min<sup>-1</sup>).

Cells were harvested by centrifugation at 1300 × *g* for 15 min at 4°C, washed with 50 mM KH<sub>2</sub>PO<sub>4</sub>-KOH pH 5.6 and then resuspended to 5 mL in the same buffer. The O<sub>2</sub> uptake by cell suspensions (2 mg mL<sup>-1</sup> DW in 50 mM KH<sub>2</sub>PO<sub>4</sub>-KOH pH 5.6) was measured polarographically with a YSI 5300 O<sub>2</sub> electrode (Yellow Springs Instrument Co., Inc., Yellow Springs, USA) at (30 ± 1)°C. The O<sub>2</sub> concentration in the cuvette was digitised each second (each datum represented the average of 1000 determinations) using hardware and software developed in-house [20]. Where necessary, cell suspensions

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were oxygenated using a standard fish tank pump and the O<sub>2</sub> concentration was monitored using the oxygen electrode.

### III. RESULTS

#### A. Comparison of *C. albicans* strains

As isolated from various exponential phase cultures, *C. albicans* strains ATCC 10231 and ATCC 10261 consumed O<sub>2</sub> at similar rates, but the addition of 3 mM KCN to a suspension of *C. albicans* ATCC 10231 rapidly reduced the activity by about 85%, but had no significant effect on the rate of O<sub>2</sub> uptake by *C. albicans* ATCC 10261 (Fig 1). In either case, the residual rate of O<sub>2</sub> uptake in the presence of cyanide was insensitive to any further cyanide addition, but was partly inhibited by 3 mM SHAM, whereas SHAM in the absence of KCN had no significant effect on the rate of O<sub>2</sub> uptake by either strain (Fig 1).

While 3 mM KCN rapidly inhibited the rate of O<sub>2</sub> uptake by *C. albicans* ATCC 10231, after a period of sustained inhibition (Fig 1A), lasting as much as 20 minutes, the rate of O<sub>2</sub> uptake gradually increased, approaching the uninhibited rate (3 nmol O<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup> DW, Fig 2A), a phenomenon we have not observed with *C. albicans* ATCC 10261 in which the rate of O<sub>2</sub> uptake was not significantly affected by KCN (Figs 1 and 2B). The residual rate observed immediately after KCN addition to *C. albicans* ATCC 10231 could not be inhibited by SHAM, but the subsequent stimulated rate was SHAM-sensitive (Fig 2A). In the case of *C. albicans* ATCC 10261, the O<sub>2</sub> uptake activity observed in the presence of KCN (Fig 2B) was also SHAM-sensitive (Figs 1 and 2B). The rate of O<sub>2</sub> uptake by *C. albicans* ATCC 10261 was not significantly affected by SHAM in the absence of KCN (Fig 1).

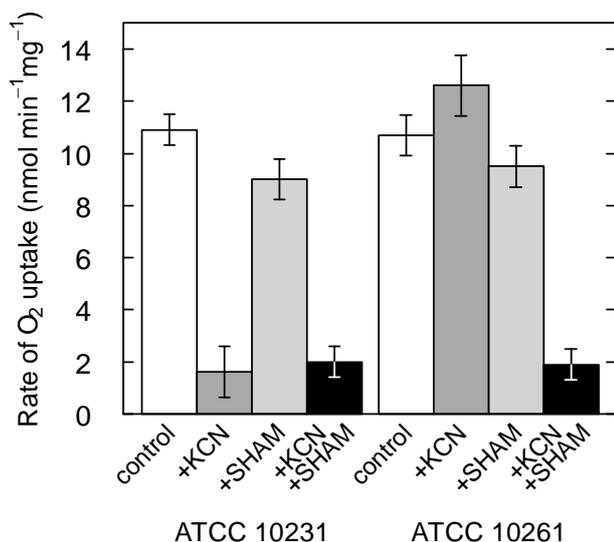


Fig. 1. Endogenous rates of O<sub>2</sub> uptake by *C. albicans* ATCC 10231 (left) and ATCC 10261 (right) and the effect of 3 mM KCN, 3 mM SHAM or both KCN and SHAM. The rate of O<sub>2</sub> uptake was determined as soon as practicable after KCN or SHAM addition. Oxygen uptake was followed using 2 mg mL<sup>-1</sup> DW in the cuvette in 50 mM KH<sub>2</sub>PO<sub>4</sub>-KOH pH 5.6. The errors represent ± SEM for at least 6 exponential phase cultures.

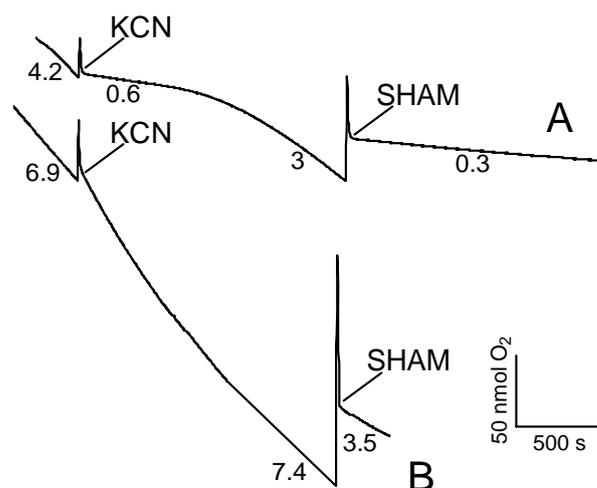


Fig. 2. Oxygen electrode traces of suspensions of *C. albicans* ATCC 10231 (A) and ATCC 10261 (B) illustrating the induction of cyanide-insensitive O<sub>2</sub> uptake in the former (A) but not the latter (B). At the times indicated 3 mM KCN or 3 mM SHAM (A) or 0.5 mM SHAM (B) were added (the sharp spikes are addition artefacts). As SHAM was dissolved in 2-methoxyethanol, in which O<sub>2</sub> is more soluble than it is in water, the addition of SHAM was necessarily associated with an addition of O<sub>2</sub> resulting in an increase in O<sub>2</sub> concentration. The numbers on each O<sub>2</sub> electrode trace represent the rate of O<sub>2</sub> uptake (nmol O<sub>2</sub> min<sup>-1</sup> mg<sup>-1</sup> DW).

#### B. Induction of AOX activity in relation to growth phase

The endogenous rate of O<sub>2</sub> uptake of *C. albicans* ATCC 10231 declined approximately exponentially with culture age (Fig 3). The rate of O<sub>2</sub> uptake observed following the addition of KCN is a measure of the activity of the AOX, which also declined as the culture aged (Fig 3). Cells taken from exponential phase cultures (10-45 mg mL<sup>-1</sup> DW) were more sensitive to KCN (exhibiting only 20% of the endogenous rate of O<sub>2</sub> uptake after KCN addition) than those taken from stationary phase cultures (>45 mg mL<sup>-1</sup> DW), which were relatively insensitive to the addition of KCN (exhibiting >50% of the endogenous rate of O<sub>2</sub> uptake after KCN addition).

The induction of SHAM-sensitive AOX activity also varied with the growth phase of the culture (Fig 4). Cells from exponential phase cultures developed relatively high rates of SHAM-sensitive O<sub>2</sub> uptake within about 1000 s (Fig 2A) and so the stimulation of the KCN-inhibited rate 1000 s after KCN addition was arbitrarily used to monitor the induction of AOX activity after the addition of KCN (Fig 4). Cells from stationary phase cultures failed to exhibit any further induction of AOX activity within 1000 s (Fig 4) and *C. albicans* ATCC 10261 showed no evidence of any induction of AOX activity within 1000 s (Fig 2A).

The kinetics of the appearance of SHAM-sensitive O<sub>2</sub> uptake after the addition of KCN also depended on the growth phase of the culture, but this will be described elsewhere.

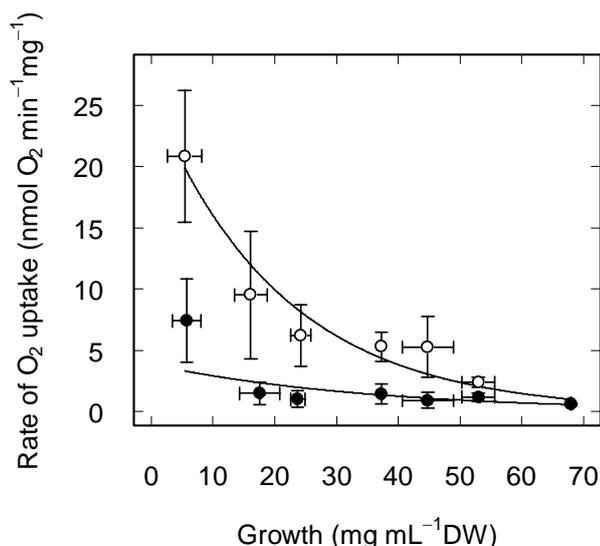


Fig. 3. The endogenous rate of  $O_2$  uptake ( $\circ$ ) and the residual activity immediately after the addition of 3 mM KCN ( $\bullet$ ) during the growth of batch cultures of *C. albicans* ATCC 10231. The data represent the mean ( $\pm$  95% confidence intervals) and the exponential curves are intended only as a guide. Data were obtained from at least 4 independent cultures.

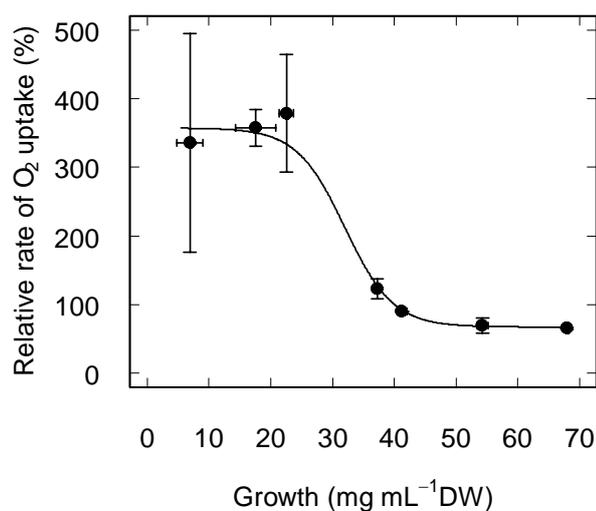


Fig. 4. The relative rate of  $O_2$  uptake 1000 s after the addition of 3 mM KCN during the growth of batch cultures of *C. albicans* ATCC 10231. The data represent the mean of at least 4 independent cultures ( $\pm$  95% confidence intervals) and the smooth curve is intended only as a guide. The values are expressed relative to the rate observed immediately after the addition of KCN (Fig 3).

#### C. Effect of redox state on the induction of AOX activity

The induction of the AOX activity in *C. albicans* ATCC 10231 generally required oxygen (Fig 5). Cells incubated anaerobically in the presence of cyanide for 1 hour exhibited a slow rate of oxygen consumption ( $2.9 \text{ nmol } O_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ DW}$ ) compared with cells which had been incubated aerobically in the presence of cyanide ( $9.8 \text{ nmol } O_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ DW}$ ). Furthermore, aerating cells which had been incubated anaerobically for 1 hour in the presence of cyanide caused a

gradual SHAM-sensitive increase in the rate of  $O_2$  uptake, consistent with the induction of AOX activity (Fig 5). In these experiments, the lag before the appearance of AOX activity following the transfer of the anaerobically-incubated cells to aerobic conditions in the  $O_2$  electrode (Fig 5) was consistently shorter than that observed in the standard induction assay (an example of which is shown in Fig 2A).

The  $O_2$  required in the induction of AOX activity could be at least partially replaced with potassium ferricyanide (FeCN). The presence of 50 mM FeCN in the incubation medium had no significant effect on the rate of  $O_2$  uptake by cells incubated aerobically with KCN (Fig 6). However, cells incubated anaerobically with KCN, but without FeCN (or with 50mM potassium ferrocyanide) exhibited a rate of oxygen consumption (about  $3 \text{ nmol } O_2 \text{ min}^{-1} \text{ mg}^{-1} \text{ DW}$ ) which was only 22% of that of cells incubated anaerobically with 50 mM FeCN. Since the FeCN was slowly reduced during the incubation, the conditions gradually became less oxidising. In contrast, FeCN had little effect on the rate of  $O_2$  uptake of oxygenated suspensions incubated with KCN (Fig 6).

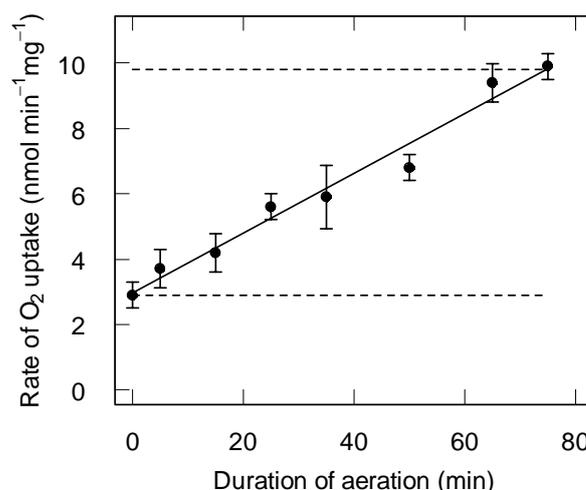


Fig. 5. The effect of oxygenation on the induction of KCN-insensitive  $O_2$  uptake by *C. albicans* ATCC 10231. Five millilitres of cell suspension ( $15 \text{ mg mL}^{-1} \text{ DW}$ ) were placed in each of two vials for 10 minutes to ensure that the suspension was anaerobic before 2.4 mM KCN was added to each, after which one was aerated (using a fish tank pump) and the other was kept tightly sealed. After one hour, samples were taken from each vial and the rate of  $O_2$  uptake was determined (indicated by the upper and lower dashed lines). At this time aeration of the anaerobic vial was started and samples were taken intermittently after the start of bubbling and the rate of  $O_2$  uptake was measured using  $1.5 \text{ mg mL}^{-1} \text{ DW}$  in the cuvette in 50 mM  $KH_2PO_4$ -KOH pH 5.6. The data shown are representative at least 4 experiments ( $\pm$  95% confidence intervals). The straight line is the least squares fit to the data ( $r = 0.988$ ,  $p < 0.001$ ) and is intended only to guide the eye.

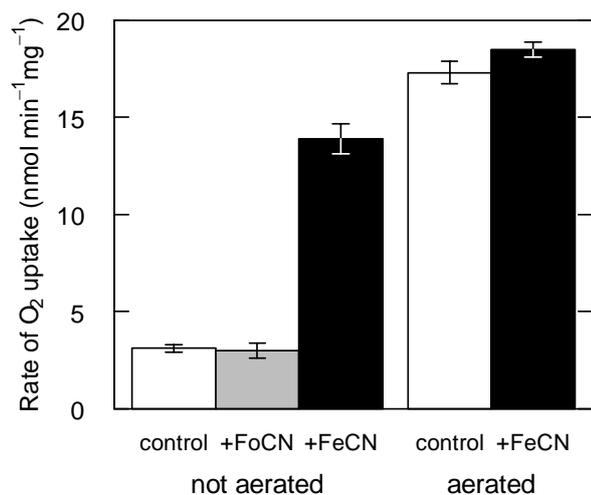


Fig. 6. The effect of ferri- and ferrocyanide on the induction of AOX activity. Five millilitres of culture (15 mg mL<sup>-1</sup> DW) were placed in each of five vials for 10 minutes to ensure that the suspension was anaerobic, before 2.4 mM KCN was added to each. At this time, 50 mM potassium ferricyanide (FeCN) was added to two vials, 50 mM potassium ferrocyanide (FoCN) was added to one vial and an equal volume of water was added to each of the other two vials (control). Two vials (a control and one containing FeCN) were aerated (using a fish tank pump) and the others were kept tightly sealed. After one hour, samples were taken from each vial and the rate of O<sub>2</sub> uptake was measured using 1.5 mg mL<sup>-1</sup> DW in the cuvette in 50 mM KH<sub>2</sub>PO<sub>4</sub>-KOH pH 5.6. The data shown are representative of at least four experiments and the errors represent ± 95% confidence intervals.

#### IV. DISCUSSION

We have shown that not all strains of *C. albicans* have comparable constitutive AOX activities (Fig 1), but SHAM-sensitive O<sub>2</sub> uptake can be induced by the addition of cyanide in at least *C. albicans* ATCC 10231 (Fig 2A). Both the activity (Fig 3) and the induction of the activity (Fig 4) of the AOX in *C. albicans* vary with growth phase, and the induction of the activity following cyanide addition requires aerobic (Fig 5) or oxidising conditions (Fig 6).

Cells in the exponential growth phase, which are actively dividing, have the lowest proportion of cyanide-insensitive activity (about 20% of the uninhibited rate) and cells in the stationary phase have the highest relative activity of the AOX (Fig 3). This is consistent with the observations of Minagawa and Yoshimoto [12] with *H. anomala* and Kirimura *et al.* [21] with *A. niger*. Since the AOX is not coupled to proton translocation across the inner mitochondrial membrane, it does not contribute to ATP synthesis. Therefore, it is reasonable to suppose that rapidly dividing cells, with a high demand for ATP, should have the least AOX activity. Similarly, stationary phase cells, which are not dividing rapidly, have a lower requirement for ATP and are able to sustain the metabolic cost of AOX activity.

The data indicate that AOX activity in *C. albicans* ATCC 10231 is induced by oxidising conditions. While we have not

shown that there is *de novo* protein synthesis, we suspect that this is the most likely explanation for at least three reasons. First, *C. albicans* lacks the cysteine in the N-terminal domain of the AOX that is associated with pyruvate activation and dimerisation of the enzyme in plant mitochondria [1, 22]. Second, if the enzyme were activated by pyruvate, it should occur essentially instantaneously, as is the case in plant mitochondria [23], but AOX activity of the enzyme remained low for up to 20 min after KCN addition (Fig 2A). Third, where the activity of existing enzyme is increased, this is mediated by reducing conditions such as a reduced quinone pool [24], elevated pyruvate concentrations [23, 25] and reduction of the enzyme [25, 26].

In various yeast, AOX activity is induced by factors which reduce the activity of the mitochondrial electron transfer chain. These include inhibitors of cytochrome oxidase or the cytochrome *bc*<sub>1</sub> complex [12, 18, 19]; copper-limited growth [27]; low glucose concentration [28]; chloramphenicol, which inhibits the synthesis of the mitochondrially-encoded subunits of the electron transfer chain enzymes [15, 29]; and various sulphur compounds [30]. Conversely, induction of the AOX activity is blocked by factors which prevent the expression of the nuclear gene(s) encoding the polypeptide. Such factors include: conditions which reduce the ATP supply, such as anaerobiosis [12] and the presence of uncouplers [14, 31]; inhibitors of extra-mitochondrial protein synthesis, such as cycloheximide [15] and antibiotics [14]; and Fe(II) chelators such as *o*-phenanthroline [32], that prevent the incorporation of iron atoms that could be involved in the oxygen chemistry at the catalytic centre of the enzyme [33]. It has been argued [34] that the signal for the induction of the AOX is the accumulation of superoxide anion, however, an alternative explanation, which is consistent with our own data, is that oxidising conditions (low glucose concentrations resulting in a low intracellular [NADH]/[NAD<sup>+</sup>] ratio; high O<sub>2</sub> concentration; high ferricyanide concentration; high concentration of oxidised glutathione [30]) promote the induction of AOX activity, whereas reducing conditions (high substrate supply, anaerobiosis) tend to block the induction.

Bunn and Poyton [35] presented a model of the oxygen regulation of transcription in yeast which could be mediated either by oxygen binding to, or through the redox state of, specific haem proteins. The data we have presented here imply that it is possible that the induction of AOX activity could be mediated by an analogous redox-sensitive process. However, such a model does not take into account the effects of inhibitors of electron transfer and protein synthesis.

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REFERENCES

- [1] S.-Y. M. Pang, S. Tristram, and S. Brown, "An *in silico* model of the alternative oxidase," *International Journal of Biosciences and Technology*, vol. 2, pp. 139-148, 2009.
- [2] G. R. Schonbaum, W. D. Bonner, Jr., B. T. Storey, and J. T. Bahr, "Specific inhibition of the cyanide-insensitive respiratory pathway in plant mitochondria by hydroxamic acids," *Plant Physiology*, vol. 47, pp. 124-128, 1971.
- [3] J. N. Siedow and M. E. Girvin, "Alternative respiratory pathway: its role in seed respiration and its inhibition by propyl gallate," *Plant Physiology*, vol. 65, pp. 669-674, 1980.
- [4] J. N. Siedow and D. M. Bickett, "Structural features required for inhibition of cyanide-insensitive electron transfer by propyl gallate," *Archives of Biochemistry and Biophysics*, vol. 207, pp. 32-39, 1981.
- [5] N. Sen and H. K. Majumder, "Mitochondrion of protozoan parasite emerges as potent therapeutic target: exciting drugs are on the horizon," *Current Pharmaceutical Design*, vol. 14, pp. 839-846, 2008.
- [6] A. Veiga, J. D. Arrabaca, and M. C. Loureiro-Dias, "Stress situations induce cyanide-resistant respiration in spoilage yeasts," *Journal of Applied Microbiology*, vol. 95, pp. 364-371, 2003.
- [7] V. N. Popov, R. A. Simonian, V. P. Skulachev, and A. A. Starkov, "Inhibition of the alternative oxidase stimulates H<sub>2</sub>O<sub>2</sub> production in plant mitochondria," *FEBS Letters*, vol. 415, pp. 87-90, 1997.
- [8] R. M. Nervig and S. Kadis, "Effect of hydroxamic acids on growth and urease activity in *Corynebacterium renale*," *Canadian Journal of Microbiology*, vol. 22, pp. 544-551, 1976.
- [9] C. Y. Wang and L. H. Lee, "Mutagenicity and antibacterial activity of hydroxamic acids," *Antimicrobial Agents and Chemotherapy*, vol. 11, pp. 753-755, 1977.
- [10] S.-Y. M. Pang, S. Tristram, and S. Brown, "Salicylhydroxamic acid inhibits the growth of *Candida albicans*," *International Journal of Biological and Life Sciences*, vol. 6, pp. 40-46, 2010.
- [11] S.-Y. M. Pang, S. Tristram, and S. Brown, "Inhibition of the growth of pathogenic *Candida* spp. by salicylhydroxamic acid," *International Journal of Biological and Life Sciences*, vol. 7, pp. 1-7, 2011.
- [12] N. Minagawa and A. Yoshimoto, "The induction of cyanide-resistant respiration in *Hansuela anomala*," *Journal of Biochemistry*, vol. 101, pp. 1141-1146, 1987.
- [13] H. Yukioka, S. Inagaki, R. Tanaka, K. Katoh, N. Miki, A. Mizutani, and M. Masuko, "Transcriptional activation of the alternative oxidase gene of the fungus *Magnaporthe grisea* by a respiratory-inhibiting fungicide and hydrogen peroxide," *Biochimica et Biophysica Acta*, vol. 1442, pp. 161-169, 1998.
- [14] K. Kirimura, T. Matsui, S. Sugano, and S. Usami, "Enhancement and repression of cyanide-insensitive respiration in *Aspergillus niger*," *FEMS Microbiology Letters*, vol. 141, pp. 251-254, 1996.
- [15] D. L. Edwards, E. Rosenberg, and P. A. Maroney, "Induction of cyanide-insensitive respiration in *Neurospora crassa*," *Journal of Biological Chemistry*, vol. 249, pp. 3551-3556, 1974.
- [16] W.-K. Huh and S.-O. Kang, "Molecular cloning and functional expression of alternative oxidase from *Candida albicans*," *Journal of Bacteriology*, vol. 181, pp. 4098-4102, 1999.
- [17] W.-K. Huh and S.-O. Kang, "Characterization of the gene family encoding alternative oxidase from *Candida albicans*," *Biochemical Journal*, vol. 356, pp. 595-604, 2001.
- [18] M. G. Shepherd, C. M. Chin, and P. A. Sullivan, "The alternate respiratory pathway of *Candida albicans*," *Archives of Microbiology*, vol. 116, pp. 61-67, 1978.
- [19] N. Minagawa and A. Yoshimoto, "The induction of cyanide-resistant respiration by carbon monoxide in *Hansenula anomala*," *Agricultural and Biological Chemistry*, vol. 53, pp. 2025-2026, 1989.
- [20] S. Brown and N. L. Taylor, "Inhibition of mitochondrial electron transfer by antipsychotic medication," *Human and Veterinary Toxicology*, vol. 42, pp. 209-211, 2000.
- [21] K. Kirimura, Y. Hirowatari, and S. Usami, "Alterations of respiratory systems in *Aspergillus niger* under the conditions of citric acid fermentation," *Agricultural and Biological Chemistry*, vol. 51, pp. 1299-1303, 1987.
- [22] T. Joseph-Horne, J. Babij, P. M. Wood, D. Hollomon, and R. B. Sessions, "New sequence data enable modelling of the fungal alternative oxidase and explain an absence of regulation by pyruvate," *FEBS Letters*, vol. 481, pp. 141-146, 2000.
- [23] A. H. Millar, J. T. Wiskich, J. Whelan, and D. A. Day, "Organic acid activation of the alternative oxidase of plant mitochondria," *FEBS Letters*, vol. 329, pp. 259-262, 1993.
- [24] M. H. N. Hoefnagel and J. T. Wiskich, "Activation of the plant alternative oxidase by high reduction levels of the Q-pool and pyruvate," *Archives of Biochemistry and Biophysics*, vol. 355, pp. 262-270, 1998.
- [25] G. C. Vanlerberghe, L. McIntosh, and J. Y. H. Yip, "Molecular localization of a redox-modulated process regulating plant mitochondrial electron transport," *The Plant Cell*, vol. 10, pp. 1551-1560, 1998.
- [26] A. L. Umbach and J. N. Siedow, "Covalent and non-covalent dimers of the cyanide-resistant alternative oxidase protein in higher plant mitochondria and their relationship to enzyme activity," *Plant Physiology*, vol. 103, pp. 845-854, 1993.
- [27] J. A. Downie and P. B. Garland, "An antimycin A- and cyanide-resistant variant of *Candida utilis* arising during copper-limited growth," *Biochemical Journal*, vol. 134, pp. 1051-1061, 1973.
- [28] N. Minagawa and A. Yoshimoto, "The induction of cyanide-resistant respiration in the absence of respiratory inhibitors in *Hansenula anomala*," *Agricultural and Biological Chemistry*, vol. 51, pp. 2263-2265, 1987.
- [29] J.-P. Schwitzguébel and J. M. Palmer, "Rotenone- and cyanide-insensitive respiration in mitochondria from *Neurospora crassa*," *Journal of General Microbiology*, vol. 129, pp. 2387-2397, 1983.
- [30] N. Minagawa, S. Sakajo, and A. Yoshimoto, "Induction of cyanide-resistant respiration by sulfur compounds in *Hansenula anomala*," *Agricultural and Biological Chemistry*, vol. 55, pp. 1573-1578, 1991.
- [31] N. Minagawa, S. Sakajo, T. Komiyama, and A. Yoshimoto, "A 36-kDa mitochondrial protein is responsible for cyanide-resistant respiration in *Hansuela anomala*," *FEBS Letters*, vol. 264, pp. 149-152, 1990.
- [32] N. Minagawa, S. Sakajo, T. Komiyama, and A. Yoshimoto, "Essential role of ferrous iron in cyanide-resistant respiration in *Hansuela anomala*," *FEBS Letters*, vol. 267, pp. 114-116, 1990.
- [33] J. N. Siedow, A. L. Umbach, and A. L. Moore, "The active site of the cyanide-resistant oxidase from plant mitochondria contains a binuclear iron center," *FEBS Letters*, vol. 362, pp. 10-14, 1995.
- [34] N. Minagawa, S. Koga, M. Nakano, S. Sakajo, and A. Yoshimoto, "Possible involvement of superoxide anion in the induction of cyanide-resistant respiration in *Hansuela anomala*," *FEBS Letters*, vol. 302, pp. 217-219, 1992.
- [35] H. F. Bunn and R. O. Poyton, "Oxygen sensing and molecular adaptation to hypoxia," *Physiological Reviews*, vol. 76, pp. 839-885, 1996.