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REVIEW

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Metabolic manipulation by *Pseudomonas fluorescens*: a powerful stratagem against oxidative and metal stress

Alex MacLean, Anondo Michel Bley, Varun P. Appanna and Vasu D. Appanna*

Abstract

Metabolism is the foundation of all living organisms and is at the core of numerous if not all biological processes. The ability of an organism to modulate its metabolism is a central characteristic needed to proliferate, to be dormant and to survive any assault. *Pseudomonas fluorescens* is bestowed with a uniquely versatile metabolic framework that enables the microbe to adapt to a wide range of conditions including disparate nutrients and toxins. In this mini-review we elaborate on the various metabolic reconfigurations evoked by this microbial system to combat reactive oxygen/nitrogen species and metal stress. The fine-tuning of the NADH/NADPH homeostasis coupled with the production of α-keto-acids and ATP allows for the maintenance of a reductive intracellular milieu. The metabolic networks propelling the synthesis of metabolites like oxalate and aspartate are critical to keep toxic metals at bay. The biochemical processes resulting from these defensive mechanisms provide molecular clues to thwart infectious microbes and reveal elegant pathways to generate value-added products.

INTRODUCTION

Environmental and intracellular stress is an ongoing challenge that all organisms have to confront. These abnormal situations have to be neutralized or at least mitigated if a living system is to survive. The very fact of consuming O_2 is rife with hazards as this process is inherently associated with oxidative stress [1–3]. The reducing factors such as NADH and $FADH_2$ resulting from the metabolism of a carbon source is oxidized in order to create a proton gradient and a membrane potential, the underlying power behind the synthesis of ATP, the universal energy currency in all cells. The flow of e^- from these reductants to O_2 is mediated by a series of e^- transporters located in the cytoplasmic membrane in bacteria and in the mitochondria of eukaryotes. This process is notorious for its inability to deliver all the e^- to O_2 where they are trapped as H_2O. Some of these e^- leak into the intracellular environment where they act as powerful oxidants. The partial reduction of oxygen can also contribute to the reactive oxygen species (ROS), H_2O_2 and that are the cause of numerous abnormalities.

To mitigate this situation, all aerobic organisms have evolved enzymes like catalase, superoxide dismutase and glutathione peroxidase aimed at the elimination of ROS. Reducing metabolites like glutathione and ascorbic acid are also utilized to act as ROS scavengers (Fig. 1) [4–6].

Environmental fluxes due to temperature changes and metal pollutants have to be dealt with if organisms are to survive these physical and chemical stresses. The remodelling of bacterial membrane allows microbes to adjust to temperature variation. While an increase in temperature is characterized by the saturation and lengthening of the acyl chain associated with the phospholipids, a decrease of temperature results in the shortening and unsaturation of these chains. These modifications of the lipids help maintain the fluidity of the cellular membrane and hence the activity of the proteins within [7, 8]. The synthesis of anti-freeze metabolites like polyols is aimed at preventing the cytoplasm from freezing when the temperature dips below freezing point [9]. The presence of elevated concentrations of metals elicits a variety of detoxification mechanisms including intracellular sequestration of these toxicants and their biotransformation into innocuous moieties. The presence of excess Cu, Cd and Zn orchestrates the enhanced synthesis of intracellular metal chelators like metallothionein known to sequester these metals. The immobilization of Mn and Al with the aid of biopolymers affords another pathway to the detoxification of metal pollutants. Ca and Cd are precipitated while Ga is sequestered by chelators (Fig. 2)

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Author affiliations: [1]Department of Chemistry and Biochemistry, Laurentian University, Sudbury, Ontario P3E 2C6, Canada.
***Correspondence:** Vasu D. Appanna, vappanna@laurentian.ca
Keywords: *Pseudomonas fluorescens*; metabolic networks; NADH; NADPH; oxidative stress; metal toxicity.
Abbreviations: ACN, aconitase; GOD, glyoxylate dehydrogenase; ICDH, isocitrate dehydrogenase; ICL, isocitrate lyase; MDH, malate dehydrogenase; MS, malate synthase; O.P., oxidative phosphorylation; PEPC, phosphoenolpyruvate carboxylase; RNS, reactive nitrogen species; ROS, reactive oxygen species; SLP, substrate level phosphorylation; SOD, superoxide dismutase.

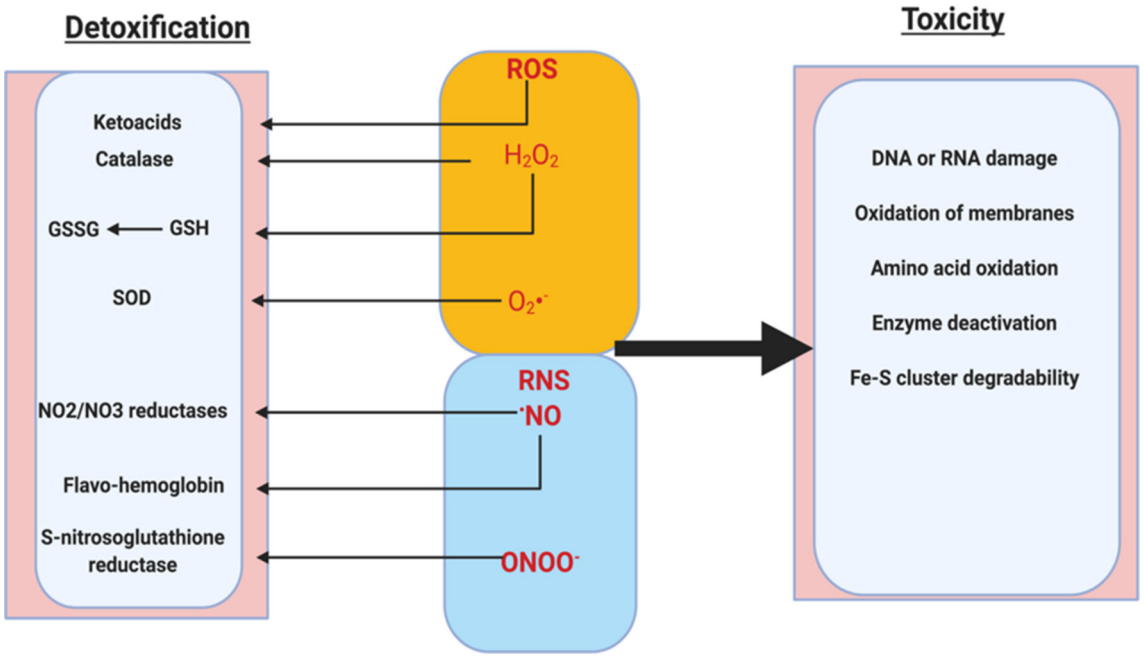

Fig. 1. RONS toxicity and detoxification. The ROS and RNS interact to generate powerful oxidants like $ONOO^-$. GSH: glutathione; SOD: superoxide dismutase. RNS: reactive nitrogen species; ROS: reactive oxygen species; GSSG: glutathione disulfide.

[10–13]. The stress imposed by a lack of essential metal like Fe evokes the synthesis of siderophores. For instance, *Aerobactin aerogenes* is known to secrete aerobactin, a hydroxamate derived from lysine to help this microbe acquire this vital nutrient [14].

Metabolic malleability *of P. fluorescens*

Although these adaptation strategies have been relatively well-characterized, the metabolic reprogramming that contributes to these stress-induced altered lifestyles is still awaiting further elucidation. As part of our research program to understand how the metabolic force fuels most cellular adaptations, we have adopted *P. fluorescens* as a model system to assist us in this endeavour. This organism is uniquely endowed with a variety of biochemical attributes that enables it to overcome numerous physical and chemical challenges. The metabolic malleability that this microbe exhibits lends itself for further molecular exploration specially

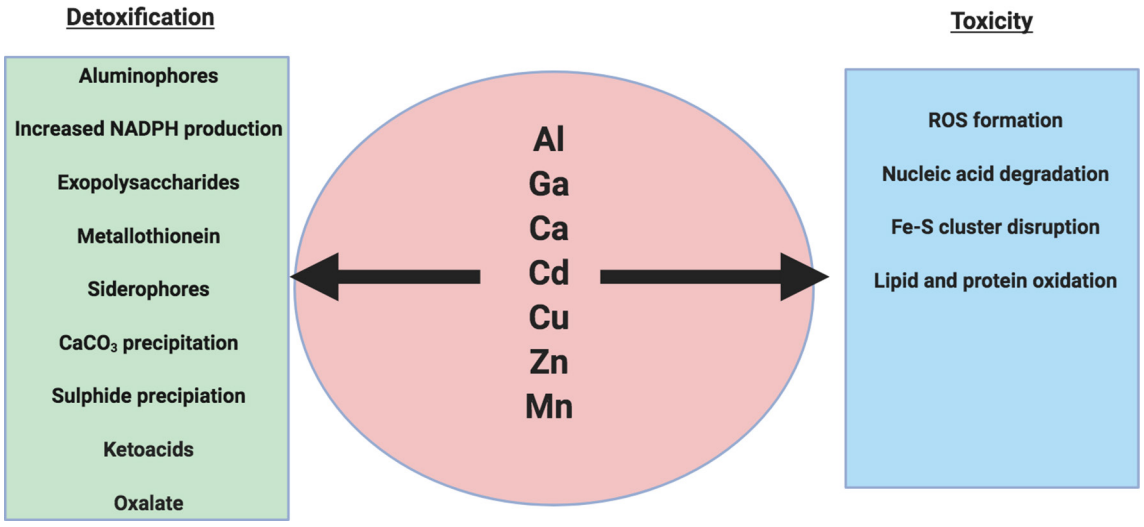

Fig. 2. Metal stress: toxicity and detoxification. NADPH helps promote a reductive environment, the immobilization of the metal toxicants and prevention of cellular damage.

in challenging conditions where other microbial system may succumb [15–17]. Although the microbe has been isolated from humans, *P. fluorescens* is essentially found in soil, water and plant. It produces a soluble green pigment, pyoverdine responsible for its fluorescence. The microbe has the ability to grow in diverse environments, utilizes a wide range of carbon sources, tolerates disparate temperatures and proliferates in pH ranging from 4 to 8 [18–20]. These characteristics have been tailored naturally in order to render *P. fluorescens* suitable for bioremediation, in pathogen control and as biofertilizers. This propensity to survive a plethora of adverse conditions makes this bacterium an ideal candidate to study metabolic reconfigurations responsive to abiotic stress [21–23]. In this mini-review, the metabolic networks that enable *P. fluorescens* to fend oxidative/nitrosative stress and to detoxify metal pollutants are discussed.

Oxidative and nitrosative stress

Controlled release of RONS is a natural process as these moieties are involved in numerous physiological functions aimed at signalling and transcription processes. However, their unregulated presence imposes an onerous burden on the cells. They are known to oxidize essential biomolecules resulting in their inactivation. RONS perturb membrane lipids, react with nucleic acids and release metal cofactors from their respective proteins. If the intracellular levels of RONS are not properly policed, living organisms are unable to survive. Although numerous organisms undergo this fate as they are ill-equipped to cope with the toxicity associated with RONS, microbes like *P. fluorescens* are known to possess intricate biochemical stratagems designed to neutralize these noxious toxicants. The malleable metabolic networks are repositioned to limit the production of ROS, increase the synthesis of NADPH, the intracellular reductive fuel, augment the formation of α-keto-acids, the scavengers of RONS and favour the generation of ATP via substrate level phosphorylation (SLP). SLP enables the synthesis of energy without the need of O_2, hence devoid of the liberation of ROS (Fig. 3) [24–26].

NADH and NADPH homeostasis: the metabolic way

The tricarboxylic acid (TCA) cycle is a major generator of NADH, a pro-oxidant. In fact, this metabolic module oxidizes acetyl CoA into CO_2 and the reducing factors NADH and $FADH_2$. The latter is then consumed during oxidative phosphorylation to create a membrane potential responsible for the synthesis of ATP [27, 28]. However, this process is prone to unregulated ROS leakage. *P. fluorescens* down-regulates key enzymes driving these metabolic networks. Aconitase, α- ketoglutarate dehydrogenase (KGDH), isocitrate dehydrogenase (ICDH)-NAD dependent, complex I and complex IV are severely impeded. The latter two enzymes that are part of the electron transport chain (ETC), ICDH-NAD and KGDH limit NADH formation, while the e^- transporters restrict the uncontrolled release of ROS. The decreased expression of these enzymes supports the view that transcription control is involved in this process. The reprogramming of the TCA cycle is further impacted by the unavailability of NAD. This nicotinamide nucleotide is a key that switches on the consumption of acetyl CoA that fuels the TCA cycle. This is attained by the phosphorylation of NAD to NADP, a reaction mediated by NAD kinase (NADK). This enzyme is up-regulated when the microbe is subjected to RONS stress. The metabolic shift effected by this event not only starves the TCA cycle of a critical cofactor but also makes NADP readily available to enzymes mediating the synthesis of NADPH, the reductive intracellular force [29]. Enzymes like ICDH-NADP dependent, glutamate dehydrogenase (GDH)-NADP dependent, malic enzyme (ME) and glucose 6 phosphate dehydrogenase (G6PDH) are known to be activated. In fact, isoenzymes of ICDH and G6PDH are a common occurrence in organisms subjected to oxidative challenge (Fig. 4) [30, 31].

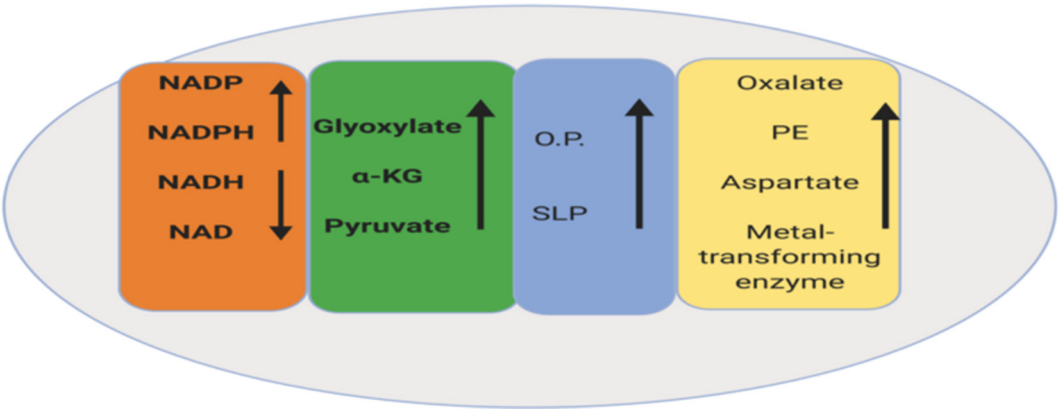

Fig. 3. Metabolic strategies deployed in *P. fluorescens* challenged by RONS and metal stress. Enhanced production of NADPH, keto-acids and metal chelators are essential if the organism is to survive. NAD: nicotinamide adenine dinucleotide; O.P: oxidative phosphorylation; SLP: substrate level phosphorylation; PE: phosphatidyl ethanolamine.

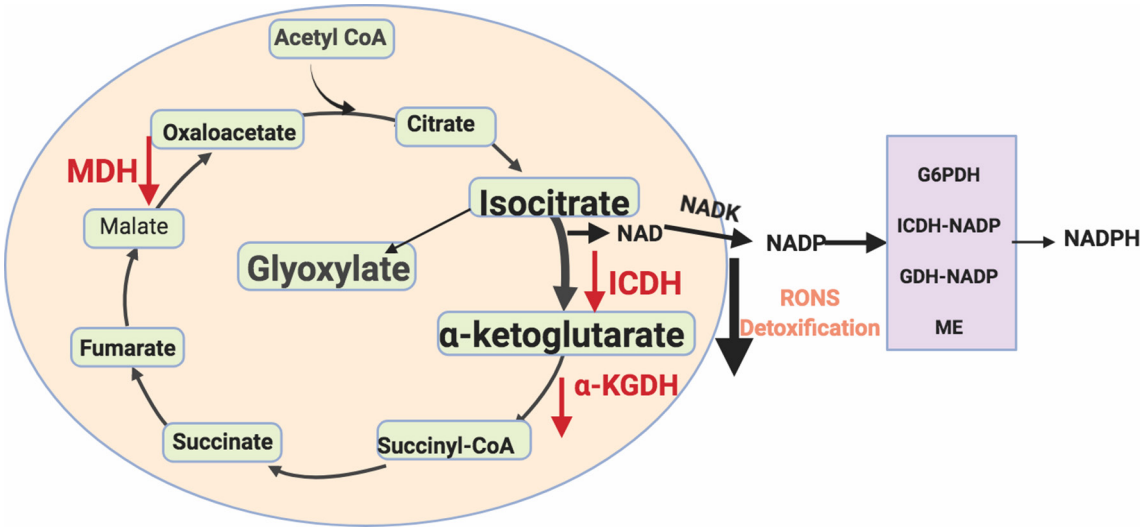

Fig. 4. NADK, a potent metabolic modulator of NADH and NADPH homeostasis. The ability of NADK to synthesize NADP favours the activity of NADPH-generating enzymes at the expense of those producing NADH. MDH: malate dehydrogenase; ICDH: isocitrate dehydrogenase; α-KGDH: alpha-ketoglutarate dehydrogenase; GDH: glutamate dehydrogenase; ME: malic enzyme; G6PDH: glucose 6-phosphate dehydrogenase decrease.

Enhanced production of α-keto-acids and ATP synthesis devoid of ROS formation

Metabolic reconfiguration aimed at NADPH production and impeding NADH formation is coupled with the ability of the microbe to produce α-keto-acids. These moieties can readily neutralize RONS via decarboxylation. The corresponding carboxylic acids and CO_2 are the main products with the concomitant destruction of the oxidative or nitrosative toxins. *P. fluorescens* is known to produce copious amounts of KG, pyruvate and glyoxylate when stressed with RONS [32, 33]. The nature of the carbon source in the environment dictates which specific α-keto-acid is elaborated. For instance, citrate promotes KG formation while glycerol favours the synthesis of pyruvate. Furthermore, enzymes like α-KGDH, pyruvate

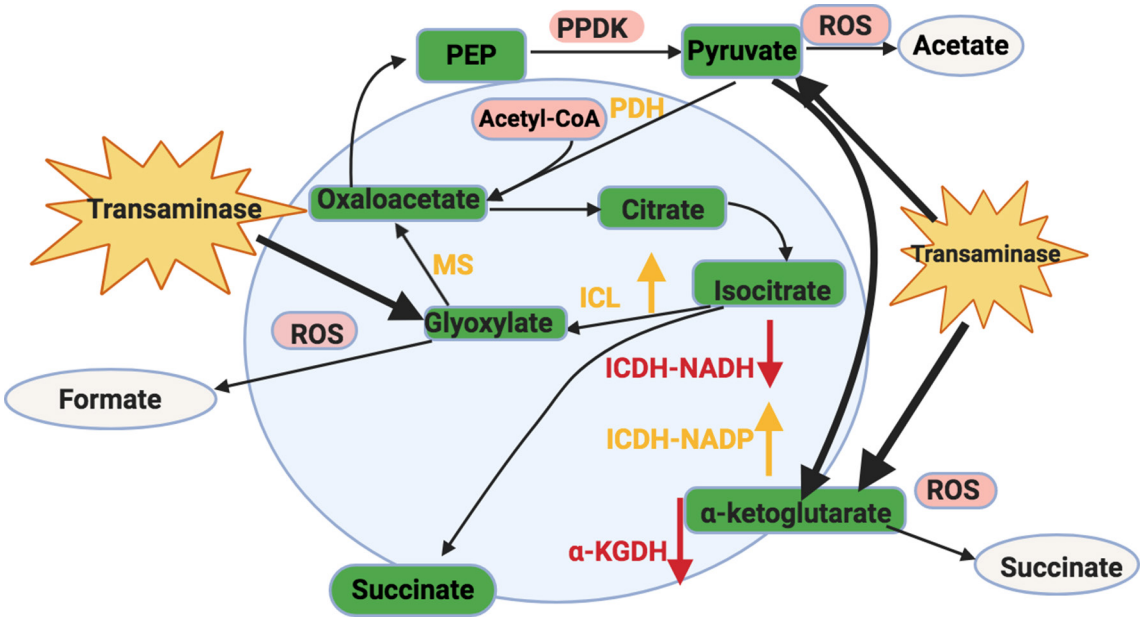

Fig. 5. Keto-acid production and ROS detoxification. The increased synthesis of glyoxylate, pyruvate and KG enables the scavenging of RONS with the formation of metabolites that can be readily utilized by the microbe. PDH: pyruvate dehydrogenase; MS: malate synthase: increase: decrease.

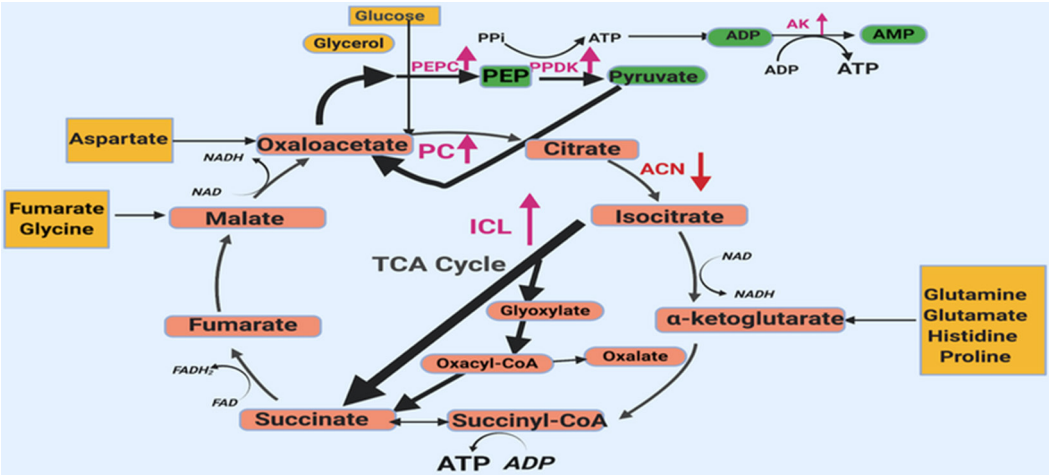

Fig. 6. Phospho-transfer networks in *P. fluorescens* to generate ATP via SLP under RONS stress. PEPC: phosphoenolpyruvate carboxylase; PPDK: pyruvate, phosphate dikinase; AK: acetate kinase; ACN: aconitase: increase: decrease.

dehydrogenase (PDH) and malate synthase (MS) that limit the utilization of these keto-acids coupled with the concomitant increase in such enzymes as GDH, pyruvate, phosphate dikinase (PPDK) and numerous transaminases (aspartate amino transaminase, glycine amino transaminase) result in an enhanced formation of these moieties (Fig. 5) [34, 35].

The metabolic reprogramming aimed at maintaining a reductive environment under oxidative assault severely perturbs ATP synthesis via aerobic respiration as this process is inherently associated with the further release of ROS. Oxidative phosphorylation (O.P) exacerbates the intracellular oxidative environment [36–38]. The need for the two ingredients namely NADPH and α-ketoacids that are the basis of the oxidative defense restrict the ability of the organism to generate NADH, a critical fuel that propels ATP formation during O.P. Hence, *P. fluorescens* has to resort to alternative ATP-forming mechanisms if it is to survive this stress. In this instance, the microbe adopts the strategy of SLP whereby a high-energy phosphate is produced to be eventually converted to ATP via the phosphorylation of ADP or AMP [39–42]. The energy-rich substrates such as phospho-enol pyruvate (PEP), acetyl phosphate (Ace~P) and acetyl CoA are commonly utilized in SLP and their synthesis is independent of the reduction of O_2; hence contributes to the diminution of ROS in the cell. When subjected to the energy dilemma imposed by RONS, *P. fluorescens* is known to orchestrate a variety of metabolic pathways dedicated to the formation of high-energy metabolites with the aim of eventually trapping them as ATP, the universal energy currency in the cells (Fig. 6) SLP is known to be utilized by a variety of living organisms exposed to stress [43–46].

Metabolism and adaptation to metal stress

Metal pollution is a major challenge that organisms have to face on an ongoing basis. While numerous organisms tend to succumb, *P. fluorescens* utilizes its metabolic arsenal to neutralize or mitigate the toxic impact of metal pollutants [47, 48]. All the major metabolic networks are fine-tuned in an effort to deal with metal stress. A specific strategy is devised to counter the presence of a given metal. However, if different metals are present at the same time, the microbe invokes a common detoxification stratagem aimed at all the metallic pollutants. Aluminum (Al) is a common pollutant in acidic soil and interferes with iron (Fe) metabolism and is known as a pro-oxidant. Enzymes and proteins that require Fe to function are severely impeded. As Al replaces Fe in these biomolecules, processes like oxidative phosphorylation are affected and the rise of free Fe gives rise to an oxidative environment. To circumvent Al toxicity, *P.fluorescens* adopts a metabolic reconfiguration aimed at producing oxalate, a dicarboxylic acid that can immobilize Al [12, 13, 49–53]. As aconitase, a Fe-containing enzyme that is sharply affected, the microbe upregulates ICDH-NADP and isocitrate lyase (ICL). This arrangement not only allows the metabolism of citrate resulting in the production of glyoxylate, a critical precursor of oxalate but also limits the formation of NADH, a pro-oxidant. The enhanced activity of these enzymes helps augment the formation of α-KG, a moiety responsible for the decomposition of ROS (Fig. 7) [54–56]. The oxidation of glyoxylate by glyoxylate dehydrogenase to oxalate is dependent on NADP and Co-enzyme A, a process that produces NADPH and the high energy oxalyl-CoA . The latter is an important intermediate in the formation of ATP via SLP. This metabolic adaptation produces oxalate to sequester Al, NADPH to generate a reductive environment, α-KG to neutralize ROS and ATP independent of O_2 [57].

Gallium, an Fe mimetic also imposes a severe toxic burden on a cell. To survive, the microbe utilizes an up-regulated ICDH and ICL counter the ineffectiveness of aconitase. However, in this instance as the organism utilizes an aspartate-rich

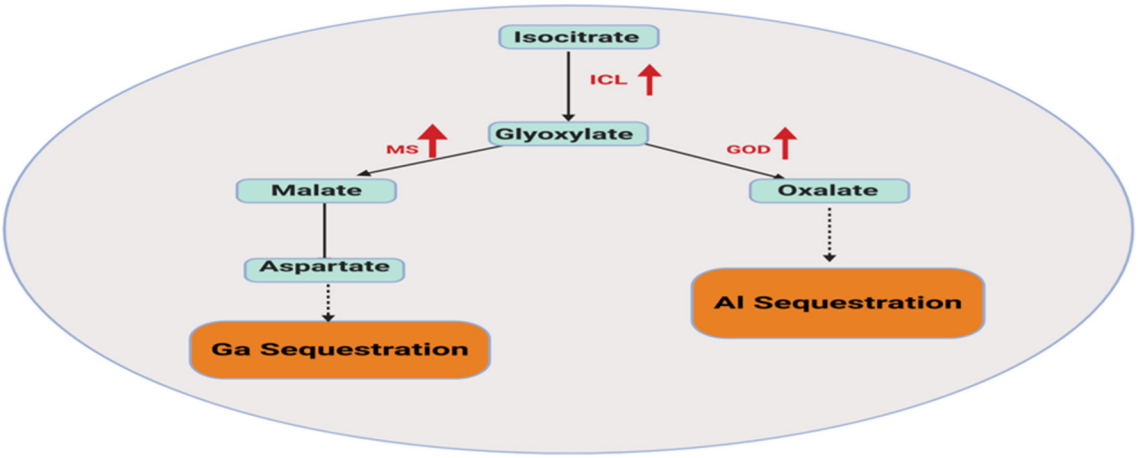

Fig. 7. Gallium and aluminum sequestration; ICL: isocitrate lyase; MS: malate synthase; GOD: glyoxylate dehydrogenase-CoA NADP-dependent: increase.

derivative to immobilize Ga, the glyoxylate generated from the ICL reaction is rapidly converted to malate, a biotransformation facilitated by MS. The malate is processed into oxaloacetate that becomes the source of aspartat[[58–61]]. Calcium (Ca) is a known intracellular signalling molecule when present in micromolar amounts. However, when present in elevated concentrations Ca is toxic. To combat this situation, *P. fluorescens* elaborates a detoxification strategy reliant on the conversion of the divalent metal into calcite, a crystalline insoluble calcium carbonate. To achieve this metabolic feat, the microbe initially immobilizes the Ca in an epicellular organic matrix where the presence of the up-regulated carbonic anhydrase enables the controlled formation of calcite where the toxic divalent metal is locked [62–65].

Metabolic reprogramming, a pathogenic attribute of *Pseudomonas aeruginosa*

P. aeruginosa is another widely occurring organism, which is known to utilize its metabolic plasticity to survive a variety of stress conditions including antibiotic toxicity and the defense mechanisms of the host. This opportunistic pathogen takes advantage of individuals whose immune system is compromised to cause severe infections [66]. In order to unleash its pathogenicity, the microbe deploys a plethora of biochemical strategies aimed at thwarting the oxidative stress imposed by the host. The up-regulation of NADPH-producing enzymes with the concomitant reduction of NADH-generating enzymes is central to maintaining a reductive environment. The decrease in the TCA cycle, the uncoupling of electron transport chain and oxidative phosphorylation help promote a diminution of ROS. This metabolic rearrangement is assisted by the carbon catabolite repression (CRC) protein that orchestrates the consumption of a variety of carbon sources that are dedicated to the synthesis of NADPH [67–69]. The glyoxylate shunt, a metabolic pathway essentially driven by the enzymes isocitrate lyase and malate synthase is actively utilized by *P. aeruginosa* not to only diminish the formation of NADH but to also produce glyoxylate, a scavenger of ROS [70]. These metabolic networks are aided by enzymes like catalases, alkyl hydroperoxide reductases and methionine sulfoxide reductases all committed to neutralizing ROS. Hence, it is quite evident that these two pseudomonads share common stratagems to survive an oxidative environment that can be exploited to arrest the proliferation of the opportunistic pathogen.

Conclusions

In conclusion, the ability of *P. fluorescens* to manipulate its vast metabolic reservoir is marshalled to thwart the dangers associated with RONS and metal stress. The malleable metabolic networks are reorganized to fend against the toxic influence of these stressors. The limiting of NADH synthesis coupled with the enhanced synthesis of NADPH not only creates a reductive environment but ensures that the further production of RONS is quelled. The convergence of metabolic pathways to produce α-keto-acids provides for an ideal RONS scavenger and also presents an effective route to ATP formation without necessitating O_2. Similarly, adaptation to metal stress orchestrated by the reshuffling of the metabolic routes creates metabolites with multi-pronged functions. The enhanced formation of pyruvate, α-KG and oxalate from disparate carbon sources provide routes to generate value-added products. Thus, the metabolic networks can be tailored for biotechnological applications as bioreactors with free-living or immobilized *P. fluorescens* can be a source of these value-added products. Furthermore, the metabolic modules also reveal therapeutic targets against infectious microbes including *P. aeruginosa*, resistant to host-imposed oxidative stress.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

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