

Review

Major roles of isocitrate lyase and malate synthase in bacterial and fungal pathogenesis

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The glyoxylate cycle is an anaplerotic pathway of the tricarboxylic acid (TCA) cycle that allows growth on C₂ compounds by bypassing the CO₂-generating steps of the TCA cycle. The unique enzymes of this route are isocitrate lyase (ICL) and malate synthase (MS). ICL cleaves isocitrate to glyoxylate and succinate, and MS converts glyoxylate and acetyl-CoA to malate. The end products of the bypass can be used for gluconeogenesis and other biosynthetic processes. The glyoxylate cycle occurs in Eukarya, Bacteria and Archaea. Recent studies of ICL- and MS-deficient strains as well as proteomic and transcriptional analyses show that these enzymes are often important in human, animal and plant pathogenesis. These studies have extended our understanding of the metabolic pathways essential for the survival of pathogens inside the host and provide a more complete picture of the physiology of pathogenic micro-organisms. Hopefully, the recent knowledge generated about the role of the glyoxylate cycle in virulence can be used for the development of new vaccines, or specific inhibitors to combat bacterial and fungal diseases.

Introduction

More than half a century ago, Smith & Gunsalus (1954) reported the existence of the enzyme isocitrate lyase (ICL), which cleaves isocitrate to glyoxylate and succinate, in extracts prepared from *Pseudomonas aeruginosa*. Shortly thereafter Ajl (1956) showed that malate synthase (MS) is able to convert acetyl-CoA and glyoxylate to malate in *Escherichia coli*. In 1957 Kornberg and co-workers demonstrated that the synthesis of C₄ dicarboxylic acids from acetate occurs by a modified tricarboxylic acid (TCA) cycle that was termed the glyoxylate cycle or glyoxylate bypass (Kornberg & Krebs, 1957; Kornberg & Madsen, 1957; Fig. 1). The pathway consists of the two initial steps of the TCA cycle (catalysed by citrate synthase and aconitase) followed by ICL, MS and malate dehydrogenase (Fig. 1). The glyoxylate cycle serves to bypass the CO₂-generating steps of the TCA cycle and allow the net assimilation of carbon from C₂ compounds, allowing micro-organisms to replenish the pool of TCA cycle intermediates necessary for gluconeogenesis and other biosynthetic processes. The net result of the glyoxylate cycle is the production of malate and succinate from two molecules of acetyl-CoA derived from acetate or from the degradation of ethanol, fatty acids or poly- β -hydroxybutyrate (Fig. 1). During growth on these compounds, ICL competes with isocitrate dehydrogenase (IDH) for their common substrate, isocitrate. IDH has a much higher affinity for isocitrate and, in bacteria, it

is inactivated by phosphorylation by a bifunctional IDH kinase-phosphatase, thus directing isocitrate towards the biosynthetic reactions of the glyoxylate cycle. IDH dephosphorylation (activation) occurs when glycolytic and TCA cycle intermediates are present in the medium, causing isocitrate to be directed towards the energy-yielding TCA cycle (Cozzone, 1998).

The glyoxylate cycle is widespread and well documented in archaea, bacteria, protists, plants, fungi and nematodes [the latter contain an ICL–MS gene fusion (Kondrashov *et al.*, 2006)]. The presence of this metabolic pathway in animals is controversial. ICL and MS activities have been reported in birds and amphibians (Davis *et al.*, 1986, 1990) and a recent comparative genomic study showed the presence of an ICL gene in nematodes and cnidaria and an MS gene in nematodes, cnidaria, echinoderms, amphibians, fish, and insects. Interestingly, in placental mammals the MS gene is a pseudogene and the ICL gene is absent (Kondrashov *et al.*, 2006). In addition to allowing the growth of bacteria on C₂ compounds, the glyoxylate cycle is important for the growth of higher plant seedlings under most environmental conditions, since it participates in the conversion of stored lipids to carbohydrates that serve as a primary nutrient source prior to the commencement of photosynthesis (Eastmond *et al.*, 2000; Kornberg & Beevers, 1957a, b). In plants, the glyoxylate cycle enzymes are usually localized in peroxisomes, but recent studies with protein-targeting mutants have shown that they can also function effectively

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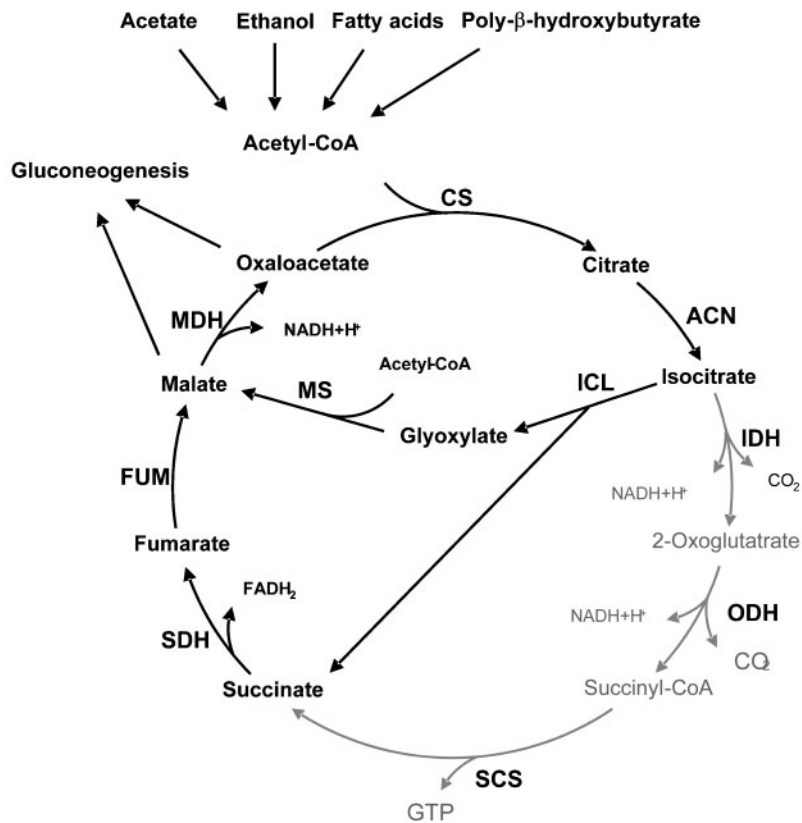


Fig. 1. Enzymic reactions of the glyoxylate and TCA cycles. A variety of metabolic processes can generate acetyl-CoA, the carbon from which can be preserved by metabolism via the glyoxylate cycle, which bypasses the CO₂-generating steps of the TCA cycle. Abbreviations: CS, citrate synthase; ACN, aconitase; IDH, isocitrate dehydrogenase, ODH, 2-oxoglutarate dehydrogenase; SCS, succinyl-CoA synthetase; SDH, succinate dehydrogenase; FUM, fumarase; MDH, malate dehydrogenase; MS, malate synthase; ICL, isocitrate lyase.

in the cytosol (Kunze *et al.*, 2002; McCammon *et al.*, 1990; Piekarska *et al.*, 2008). There is evidence supporting the expression of the glyoxylate cycle during embryogenesis of the nematode *Caenorhabditis elegans* (Kahn & McFadden, 1980; Liu *et al.*, 1997) and it was suggested that the glyoxylate cycle may allow the growth of halophytic archaea in hypersaline lakes (Oren & Gurevich, 1994; Serrano & Bonete, 2001).

The genetic regulation of the glyoxylate cycle during bacterial growth on acetate has been reviewed (Cozzone, 1998) and in the last several years it has become evident that the pathway is important in fungal and bacterial pathogenesis (Lorenz & Fink, 2002; Vereecke *et al.*, 2002a). This review focuses on the latter aspect and summarizes the functional role of the glyoxylate cycle in human, animals and plant pathogens as well as in symbionts.

Biochemical characteristics of ICL and MS

As described above, the glyoxylate cycle is a specialized pathway that has been extensively studied in connection with bacterial growth on C₂ compounds, and ICL and MS are the signature enzymes of the pathway (Fig. 1). ICL is a homotetramer requiring Mg²⁺ or Mn²⁺ and a thiol for activity. During catalysis, isocitrate is deprotonated, forming succinate and glyoxylate. In the ICL of *E. coli*, Lys-193, Cys-195, His-197 and His-356 are catalytic, active-site residues, while His-184 is involved in the assembly of

the tetrameric enzyme (Diehl & McFadden, 1993, 1994; Rehman & McFadden, 1996, 1997). The recent structural determination of the ICLs from *E. coli* (Britton *et al.*, 2001), *Mycobacterium tuberculosis* (Sharma *et al.*, 2000) and *Aspergillus nidulans* (Britton *et al.*, 2000) has revealed key aspects of their functionality. For instance, a remarkable difference between prokaryotic and eukaryotic ICLs is the presence of an additional approximately 100 amino acids located near the centre of the eukaryotic enzyme that have been proposed to function in the localization of ICL in peroxisomes. This also explains the subunit molecular mass difference between prokaryotic (48 kDa) and eukaryotic (67 kDa) enzymes.

The second enzyme of the glyoxylate cycle is MS, which condenses glyoxylate with an acetyl group from acetyl-CoA to produce malate. MS requires Mg²⁺ for activity and is competitively inhibited by oxalate, a glyoxylate analogue (Dixon *et al.*, 1960). Two MSs are present in *E. coli*: MSG and MSA. MSG, with a molecular mass of around 80 kDa, is a monomeric enzyme (encoded by *glcB*) that functions during growth on glycolate as sole carbon source, and has been found only in bacteria. The active site is composed of the catalytic Asp-631, the residues that bind Mg²⁺ (Glu-427, Asp-455), Arg-338, which hydrogen bonds with glyoxylate, and the residues interacting with acetyl-CoA (Tyr-126, Pro-538, Val-18 and Pro-536) (Howard *et al.*, 2000; Tugarinov *et al.*, 2005). Genetic data support these functional assignments, since a D631N mutant had no

detectable activity and an R338K mutant showed only 6 % of wild-type activity (Anstrom *et al.*, 2003).

MSA is a multimeric enzyme with a molecular mass of about 65 kDa (per subunit) that is indispensable for growth on acetate and is found in plants, fungi and bacteria. Lohman *et al.* (2008) showed recently that the structures of MSA and MSG are very similar and that the difference in size is due to the presence of an additional α/β domain in the G isoform. The active site is formed by Glu-250 and Asp-278, which bind Mg^{2+} , while the adenine ring or ribose of acetyl-CoA binds to residues Pro-369, Met-102, Thr-95 Ala-367, Asn-105, Lys-101, Tyr-154 and His-368. MSA contains a highly conserved Cys-438 in the active site, which corresponds to Cys-617 in MSG.

Importance of ICL and MS in fungal pathogens

Plant-pathogenic fungi

Several reports indicate an important role for ICL in fungal virulence on plants. Studies with *Leptosphaeria maculans*, causal agent of blackleg of crucifers (e.g. broccoli, canola and cauliflower) have shown that the *icl1* gene is expressed during its infection of *Brassica napus* cotyledons and inactivation of this locus causes low germination rates of pycnidiospores, reducing the pathogenicity of the fungus on cotyledons as well as limiting its hyphal growth on canola. It was suggested that the reduced pathogenicity of the mutant is due to its inability to utilize carbon sources provided by the plant (Idnurm & Howlett, 2002). Another role for ICL in fungal phytopathogenesis has been reported in *Magnaporthe grisea*, the rice blast pathogen, which can also infect a number of other agriculturally important cereals including wheat, rye and barley. *M. grisea* reproduces both sexually and asexually to produce specialized infectious structures known as appressoria. The appressoria attach to the cuticle of the host and from them hyphae emerge and penetrate the plant, the inside of which is rapidly colonized by the fungus, with disease symptoms being observable in a few days. During infection by *M. grisea*, significant ICL gene expression was found in conidia, appressoria, mycelia and hyphae. Deletion of the *M. grisea* *ICL1* gene caused a reduction in appressorium formation, conidiogenesis and cuticle penetration, and an overall decrease in damage to leaves of rice and barley. Thus ICL is essential for full virulence in this organism (Rauyaree *et al.*, 2001; Wang *et al.*, 2003).

Stagonospora nodorum is a necrotropic fungal pathogen producing leaf and glume blotch disease on wheat and other cereals of economic importance. Infection begins by germination of pycnidiospores on the leaf followed by hyphal penetration of the host and sporulation at the end of the infection cycle. In the initial steps of infection the pathogen depends on internal, stored carbon sources and it is thought that lipids are metabolized via the glyoxylate pathway to produce glucose and support fungal development. Expression analysis of the MS gene (*mls1*) in this pathogen during its interaction with wheat showed an

increased expression in ungerminated spores followed by a dramatic decrease in transcription after germination. Paradoxically, the opposite pattern was seen using MS activity measurements, where activity was undetectable in ungerminated spores but increased to a significant level after germination. Spores of an *mls1* null mutant inoculated onto wheat seedlings and leaves were unable to induce necrotic lesions on either tissue, indicating that this gene is essential for virulence on wheat. The biological reason for this phenotype is that the *mls1*-deficient strain has dramatically decreased spore germination and a reduction in the length of hyphae (Solomon *et al.*, 2004).

Asakura *et al.* (2006) demonstrated the functional role of ICL in *Colletotrichum lagenarium*, which causes anthracnose on a considerable number of plants of agricultural interest such as cucumber, watermelon, muskmelon, cantaloupe, winter squash and bittermelon. An *icl1* mutant of *C. lagenarium* failed to grow on acetate or fatty acids, similar to other ICL mutants of fungi and prokaryotes. For the cellular localization of ICL, the encoding gene was fused to that of the green fluorescent protein (GFP) and *icl1-gfp* expression was detected in peroxisomes, conidia, appressoria and hyphae of the fungus. The *icl1* mutant was able to germinate and develop appressoria and was capable of degrading lipid bodies as well as the wild-type strain. However, conidia from the *icl1*-deficient mutant inoculated onto cucumber leaves and cotyledons formed a reduced number of lesions on leaves, and especially on cotyledons, but nevertheless remained pathogenic. In invasive experiments such as the inoculation of conidia into wound sites, no defect was observed in the *icl1* mutant, while in penetration assays on cucumber cotyledons the mutant was unable to develop penetrating hyphae, indicating a requirement for ICL at this early stage of *C. lagenarium* infection.

Candida albicans

Support for the direct involvement of ICL in virulence has also come from studies of *Candida albicans*, a commensal of the mammalian microbiota inhabiting the skin, mouth, gastrointestinal tract, gut and vagina. In immunocompromised patients, this diploid fungus is responsible for mucosal surface infections as well as life-threatening systemic infections. *C. albicans* is able to survive and grow inside macrophages. Transcriptional profiles of phagocytosed populations of *C. albicans* showed that all the steps of the glyoxylate cycle are induced (Lorenz *et al.*, 2004). Northern blot and differential display experiments with *C. albicans* in the presence of macrophages revealed that both ICL and MS are induced (Lorenz & Fink, 2001; Prigneau *et al.*, 2003). In addition, both enzymes are induced in *C. albicans* exposed to human neutrophils (Fradin *et al.*, 2005). High enzymic activities of ICL and MS were detected in *C. albicans* strains isolated from diabetic patients suffering from vulvovaginal candidiasis (Lattif *et al.*, 2006). Furthermore, evaluation of ICL mutants in a

mouse model demonstrated that activity of this enzyme is essential for fungal virulence (Lorenz & Fink, 2001). In a model that mimics *C. albicans* bloodstream infection, ICL and MS were downregulated in the initial stages of infection (10 min), upregulated beginning about 20 min after infection and reached a 20-fold increase after 60 min, suggesting blood-specific expression and an important biological role for the glyoxylate cycle genes in bloodstream infections (Fradin *et al.*, 2003). In an interesting study Barelle *et al.* (2006) showed a specific activation of the *C. albicans ICL1* when the pathogen was exposed to neutrophils or macrophages, but because *icl* was not expressed in infected kidney cells it was concluded that *ICL1* contributes to virulence but is not essential for systemic infection.

A clear role for ICL in the pathogenesis of *C. albicans* emerges from the data summarized above. However, the function of this enzyme in *C. albicans* is peculiar because it is probably involved in processes other than lipid utilization or gluconeogenesis, since an ICL-deficient mutant is unable to utilize acetate, ethanol, citrate, glycerol, oleate, lactate, pyruvate, peptone, glutamate or alanine for growth, unlike the parental strain (Ramírez & Lorenz, 2007; Piekarska *et al.*, 2006, 2008; Brock, 2009). ICL expression is also detected during growth on Casamino acids, glutamate or peptone, and under starvation conditions (Barelle *et al.*, 2006; Brock, 2009). Interestingly a *fox2* mutant lacking the second enzyme of the β -oxidation pathway is also unable to utilize acetate, ethanol, lactate and oleic acid and is significantly attenuated in virulence. Therefore both the *fox2* mutant and the ICL-deficient mutant are unable to utilize nonfermentable carbon sources and have reduced virulence in mice, indicating a role for the β -oxidation pathway in virulence. This contention is not supported by the finding that a mutant in peroxisome biogenesis (*pex5*), which is strongly reduced in β -oxidation activity and is unable to utilize oleic acid, is able to use acetate, ethanol or lactate and is not affected in virulence (Piekarska *et al.*, 2006). This shows that acetyl-CoA derived from β -oxidation is not the carbon source inducing the glyoxylate cycle in the *pex5* mutant. Thus, *ICL1* expression in virulence may result not from lipid metabolism (Piekarska *et al.*, 2006; Ramírez & Lorenz, 2007; Brock, 2009) but from the conversion of carbon sources such as lactate into C₂ units for metabolism by the glyoxylate cycle (Piekarska *et al.*, 2006). ICL in *C. albicans*, unlike other organisms, is necessary for the utilization of a large variety of carbon sources. The fact that ICL is probably interconnected with multiple metabolic networks important in virulence encourages the development of specific inhibitors against this enzyme.

Other fungal pathogens

Upregulation of the glyoxylate cycle genes has also been detected in other intracellular fungal pathogens such as *Paracoccidioides brasiliensis*, which causes paracoccidioido-

mycosis in humans. RT-PCR analysis showed that transcript levels of the ICL and MS genes in this fungus increased following phagocytosis by murine macrophages (Derengowski *et al.*, 2008). *Penicillium marneffeii* is a dimorphic fungus that can cause systemic mycosis in humans. The incidence of this fungal infection has increased substantially during the past few years, occurring most often in patients infected with HIV. To evaluate if the glyoxylate cycle was involved in the virulence of *P. marneffeii*, Northern blot experiments were performed; these showed that after macrophage internalization of conidia the ICL-encoding gene (*acuD*) was highly expressed, suggesting a potential role for the cycle in the pathogen's adaptation inside macrophages (Thirach *et al.*, 2008). Together, these data directly or indirectly support the relevance of the glyoxylate pathway in fungal virulence in plants, animals and humans.

While a functional role for the glyoxylate cycle in fungal virulence is widespread, it is not universal. In the animal pathogen *Aspergillus fumigatus*, ICL expression was detected in hyphae and in conidia (Ebel *et al.*, 2006) but tissues of patients infected with the fungus were negative for the enzyme after immunostaining, and a mutant deleted of the ICL gene (*acuD*) was fully virulent in a murine model (Schöbel *et al.*, 2007). Similar results were obtained with the human-pathogenic fungus *Cryptococcus neoformans*, where ICL in a rabbit meningitis model was upregulated after 7 days in the subarachnoid space but an *icl1* mutant showed the same number of subarachnoidal yeast cells as the wild-type after 10 days in immunosuppressed rabbits. In addition, in an inhalation model of murine cryptococcosis, no differences in survival were observed between an *icl1* mutant and the wild-type, and similar growth was observed for both *C. neoformans* strains inside macrophages (Rude *et al.*, 2002). These findings show a lack of correlation between ICL gene expression and biological function in these systems.

Functional role of ICL and MS in phagocytosed bacteria

Mycobacterium tuberculosis

The actinobacterium *Mycobacterium tuberculosis* kills 3 000 000 people worldwide every year and is assumed to utilize fatty acid degradation products when growing in the host (Segal & Bloch, 1956; Srivastava *et al.*, 2008). The genome sequence of this bacterium (Cole *et al.*, 1998) contains more than 250 genes encoding proteins annotated as being involved in fatty acid metabolism, providing indirect support for the role of fatty acid degradation and C₂ metabolism in the pathogenesis of *M. tuberculosis*. ICL activity increases in pellicles in synthetic media as a consequence of fatty acid degradation (Murthy *et al.*, 1973) as well as under microaerophilic growth conditions (Wayne & Lin, 1982). Increased *aceA* (*icl*) mRNA expression in response to human macrophages is also documented (Dubnau *et al.*, 2002; Graham & Clark-

Curtiss, 1999). *M. tuberculosis* expresses a 50 kDa protein during intracellular infection (Sturgill-Koszycki *et al.*, 1997) and this was shown to be encoded by a second copy of an ICL gene that is present in several *Mycobacterium* species. Expression analysis and biochemical characterization of ICL activity clearly show that *M. tuberculosis* and *Mycobacterium avium* have two functional ICLs, ICL and AceA (Höner Zu Bentrup *et al.*, 1999). ICL seems to be the principal enzyme in the processing of isocitrate and McKinney *et al.* (2000) reported that single ICL mutations had no dramatic effect on the growth of *M. tuberculosis* in mouse lung during the first 2 weeks of infection. However, lungs infected with the mutant showed few changes between 2 and 16 weeks, suggesting that the ICL mutant had a reduced ability to sustain the infection. In contrast, at 16 weeks the lungs of mice infected with the virulent Erdman strain showed inflammatory lesions, enlargement and multiple expanding and coalescing tubercles. *icl* mRNA levels markedly increase in lungs of mice (Timm *et al.*, 2003) and in human lung granulomas, as well as in the lymphocyte region of necrotic granulomas. In contrast, *icl* expression was not detected in the transition zone and in the central region of necrotic granulomas, supporting the notion that *icl* has a pivotal role in bacterial persistence in the host (Fenhalls *et al.*, 2002). Muñoz-Elías & McKinney (2005) reported that single mutations in *icl* or *aceA* had no dramatic effect on bacterial growth on fatty acids, while an *icl aceA* double mutant was unable to grow on this carbon source. The double mutant inoculated into mice was eliminated from lungs and spleen and was unable to induce splenomegaly or alterations in lungs. ICL activity is thus essential for *M. tuberculosis* survival in the host. Additionally, ICL and to a lesser extent AceA were required for the growth of *M. tuberculosis* on propionate and on odd-chain fatty acids as a carbon source (Muñoz-Elías & McKinney, 2005; Muñoz-Elías *et al.*, 2006). The propionate or propionyl-CoA derived from β -oxidation of odd-chain fatty acids can be catabolized by the methylcitrate cycle, consisting of the enzymes 2-methylisocitrate lyase (MICL), methylcitrate synthase and methylcitrate dehydratase, encoded by the *prpB*, *prpC* and *prpD* genes, respectively. The *M. tuberculosis* genome contains homologues of *prpC* and *prpD*, but not *prpB*. However, structural and biochemical studies have demonstrated that unlike other ICLs and MICLs, the *M. tuberculosis* ICL possesses dual ICL/MICL activity and can support growth on acetate and propionate (Gould *et al.*, 2006). The *prpC* and *prpD* genes are upregulated during infection of macrophages (Schnappinger *et al.*, 2003), suggesting that the methylcitrate cycle could be important in *M. tuberculosis* pathogenesis. However, studies with a *prpC prpD* double mutant show that the methylcitrate cycle is required for *M. tuberculosis* replication in non-activated murine bone-marrow-derived macrophages, but that in IFN- γ -activated macrophages or in the lungs and spleen of inoculated mice the double mutant shows no alteration of *in vivo* growth, persistence or virulence. Thus, the functional role of ICL in *M. tuberculosis* virulence is in the glyoxylate cycle rather

than the methylcitrate cycle (Muñoz-Elías *et al.*, 2006). Recent reports show that *M. tuberculosis phoP* mutants are attenuated but persist in macrophages and mouse organs apparently because the mutant expresses higher levels of *icl*. The PhoP-deficient strain would be a good candidate for vaccine production, since prolonged exposure of the immune system to the persistent, attenuated strain could result in long-term immunogenicity (Gonzalo-Asensio *et al.*, 2008).

The single MS present in *M. tuberculosis* may also contribute to pathogenicity. Studies *in vitro* show that MS is secreted and enhances the adherence of the pathogen to lung epithelial cells, supporting the notion that it may function as an adhesin as well as an enzyme (Kinshikar *et al.*, 2006). MS can also be used as a biomarker because it is recognized in the humoral response of tuberculosis patients, making possible its use in serodiagnostic assays for identification of this pulmonary disease (Achkar *et al.*, 2006; Melo Cardoso Almeida *et al.*, 2008; Samanich *et al.*, 2001; Wanchu *et al.*, 2008).

Other bacterial pathogens

The importance of a functional glyoxylate cycle in some intracellular human pathogens such as *Salmonella* has been evaluated, and the results obtained with an ICL-deficient strain of *Salmonella enterica* serovar Typhimurium show that ICL is required for persistence during chronic infection, but not for acute lethal infection in mice (Fang *et al.*, 2005; Kim *et al.*, 2006; Tchawa Yimga *et al.*, 2006). In *Brucella suis*, the glyoxylate cycle has been shown to be unnecessary for virulence (Kohler *et al.*, 2002). The pathogens *Yersinia enterocolitica* and *Yersinia pseudotuberculosis* induce ICL and MS during growth on acetate but not on xylose, while *Yersinia pestis* synthesizes both enzymes on both carbon sources (Hillier & Charnetzky, 1981b). A natural mutation in *iclR*, encoding a repressor protein, explains why *Y. pestis* constitutively produces ICL (Sebbane *et al.*, 2004), and this constitutive enzymic activity has been used to identify *Y. pestis* in humans, animals, water, soil and food (Hillier & Charnetzky, 1981a; Quan *et al.*, 1982). Sebbane *et al.* (2004) showed that mutations in the sole ICL gene (*aceA*) of *Y. pestis* prevented growth on acetate but did not affect pathogenesis in a mouse model.

Rhodococcus equi is a Gram-positive intracellular bacillus causing pneumonia and enteritis in foals and is also able to infect cats, dogs and pigs, producing submandibular adenitis. *R. equi* causes infection in immunocompromised humans and is easily isolated from soil contaminated with faeces. The fact that *R. equi* uses acetate (Kelly *et al.*, 2002) and probably lipids as carbon sources in soil and within macrophages suggests that it may utilize the glyoxylate cycle in its interaction with foals. To validate this hypothesis an ICL-deficient (*aceA* mutant) strain of *R. equi* was evaluated in macrophages (Wall *et al.*, 2005). The population of the ICL-deficient strain increased in macro-

phages after 12 h but then declined significantly, indicating that ICL is essential for long-term survival and proliferation in macrophages, consistent with the finding that the *aceA* mutant was partially attenuated in a mouse model. In foals, the wild-type *R. equi* strain induced severe lesions of suppurative to pyogranulomatous bronchopneumonia in lung, while the *aceA* mutant was unable to produce any alteration, probably because the mutant population was six orders of magnitude lower than that of the wild-type strain (Wall *et al.*, 2005).

Another pathogen infecting lungs is *Pseudomonas aeruginosa*, which produces lung dysfunction and mortality in humans with cystic fibrosis. *P. aeruginosa* upregulates genes *in vivo* that are needed for replication in the lung environment, including those encoding enzymes for fatty acid and lipid metabolism (alcohol dehydrogenase), choline metabolism (betaine aldehyde dehydrogenase), amino acid degradation (arginine deiminase), nitrogen metabolism (respiratory nitrate reductase) and the glyoxylate cycle (ICL). These metabolic genes almost certainly contribute to carbon and nitrogen nutrition, allowing the replication and persistence of *P. aeruginosa* inside the host (Son *et al.*, 2007). Support for this hypothesis was obtained by a study in which several metabolic mutants of *P. aeruginosa* were isolated and evaluated on alfalfa seedlings and in a mouse model (Lindsey *et al.*, 2008). An ICL (*aceA*) mutant derived from strain PAO1 displayed reduced virulence on alfalfa seedlings and a reduction in histopathology in rat lungs. Thus the glyoxylate cycle has a pivotal role in the interaction of *P. aeruginosa* with both plant and mammalian hosts.

Inhibitors of ICL and MS

The development of specific inhibitors against ICL and MS is an attractive prospect, since in a variety of human-pathogenic bacteria and fungi the expression of the glyoxylate cycle genes is detected in specific stages of the interaction (Table 1). Several inhibitors of ICL have been identified, including itaconate, itaconic anhydride, bromopyruvate, nitropropionate, oxalate and malate (Höner Zu Bentrup *et al.*, 1999; McFadden & Purohit, 1977). However, these are not pharmacologically suitable for use *in vivo* since they are toxic and non-specific. For instance, nitropropionate inhibits ICL but also inhibits succinate dehydrogenase, a pivotal enzyme of the TCA cycle (Alston *et al.*, 1977; Fig. 1). Efforts to isolate natural ICL inhibitors from plants revealed that extracts of *Illicium verum* and *Zingiber officinale* inhibit the ICL of *Mycobacterium tuberculosis* (Bai *et al.*, 2007).

Because the glyoxylate cycle is important in many types of fungal pathogenesis, natural inhibitors of fungal ICLs have been sought. Those isolated from the tropical sponge *Hippospongia* sp. are halisulfates that are able to inhibit ICL activity, appressorium formation and C₂ utilization in the rice blast fungus *Magnaporthe grisea* (Lee *et al.*, 2007). Natural glyoxylate cycle inhibitors such as 5-hydroxyin-

dole-type alkaloids are potent inhibitors of the *Candida albicans* ICL (Lee *et al.*, 2009).

It was recently proposed that instead of ICL or MS, other enzymes could make better targets for bringing about inhibition of the glyoxylate cycle. For instance, inactivation of the kinase-phosphatase that phosphorylates and inactivates isocitrate dehydrogenase would be a good candidate, since its absence would promote carbon flow through the full TCA cycle and avoid its assimilation by ICL (Singh & Ghosh, 2006). Other proposed targets for indirect inhibition of the glyoxylate cycle are the enzymes of the PHB cycle, since this metabolic route can provide acetyl-CoA in a manner that circumvents the link between glycolysis and the TCA cycle (Purohit *et al.*, 2007).

Roles of ICL and MS in other plant–bacteria interactions

The functionality of the glyoxylate cycle was evaluated in the plant pathogen *Rhodococcus fascians*, which causes leafy gall disease on a variety of monocots and dicots, including *Nicotiana tabacum* (tobacco). A malate synthase mutant of *R. fascians* gave a diminished number of bacteria inside symptomatic tobacco tissues in comparison to tissues infected by the wild-type (Vereecke *et al.*, 2002a, b). In the plant pathogen *Xanthomonas campestris*, MS was induced during infection of tomato plants and a MS-deficient strain induced fewer and smaller lesions on 75 % of inoculated leaves as compared to the wild-type strain (Tamir-Ariel *et al.*, 2007). Thus, in these two pathogens the glyoxylate cycle has an important role in the plant–microbe interaction.

Carbon metabolism has long been studied in *Rhizobium* spp., bacteria that form a nitrogen-fixing symbiosis with leguminous plants. Large quantities of acetate and fatty acids were reported in soybean nodules formed by *Bradyrhizobium japonicum* (Johnson *et al.*, 1966), and radiorespirometric studies of *B. japonicum* bacteroids indicated that up to 50 % of the acetyl-CoA entering the TCA cycle was metabolized via MS (Stovall & Cole, 1978). It was also shown that acetate can be used by isolated *B. japonicum* bacteroids to support *ex planta* nitrogen fixation (Peterson & LaRue, 1981, 1982). The existence of a complete glyoxylate cycle in nitrogen-fixing bacteroids is in doubt because ICL activity is not detected in the microsymbionts isolated from soybean, pea, alfalfa and clover nodules (Green *et al.*, 1998; Johnson *et al.*, 1966). However, ICL activity has been detected in bacteroids from senesced nodules formed by *B. japonicum* (Wong & Evans, 1971). In contrast, MS activity was found in bacteroids isolated from pea, alfalfa and clover nodules, and substantially higher activities were detected in bacteroids isolated from bean, cowpea and soybean nodules (Green *et al.*, 1998; Johnson *et al.*, 1966). Based on these data we decided to genetically evaluate the role of the glyoxylate cycle in the *Rhizobium*–Leguminosae symbiosis. We showed that neither MS nor ICL is required for symbiosis, since MS (*glcB*) mutants of *Rhizobium legumi-*

Table 1. ICL and MS gene expression and mutant virulence phenotypes during interactions with host organisms

Organism	Host	Gene*	Expression†	Mutant phenotype virulence†	Reference
<i>Aspergillus fumigatus</i>	Human	<i>icl</i>	Hyphae and conidia	Virulent	Schöbel <i>et al.</i> (2007)
<i>Brucella suis</i>	Human	<i>icl</i>	ND	Virulent	Kohler <i>et al.</i> (2002)
<i>Candida albicans</i>	Human	<i>icl</i>	Macrophages	Less virulent	Lorenz & Fink (2001)
		<i>ms</i>	Macrophages	ND	
<i>Cryptococcus neoformans</i>	Human	<i>icl</i>	Rabbit subarachnoid space	Virulent	Rude <i>et al.</i> (2002)
<i>Mycobacterium tuberculosis</i>	Human	<i>icl1</i>	Lung of mice and human	Double mutant (<i>icl1 icl2</i>)	McKinney <i>et al.</i> (2000)
		<i>icl2</i>		Avirulent	Muñoz-Elías & McKinney (2005)
<i>Paracoccidioides brasiliensis</i>	Human	<i>ms</i>	Lung	ND	Kinhikar <i>et al.</i> (2006)
		<i>icl</i>	Macrophages	ND	Derengowski <i>et al.</i> (2008)
<i>Penicillium marneffei</i>	Human	<i>ms</i>	Macrophages	ND	Thirach <i>et al.</i> (2008)
		<i>icl</i>	Macrophages	ND	
<i>Pseudomonas aeruginosa</i>	Human	<i>icl</i>	ND	Less virulent	Lindsey <i>et al.</i> (2008)
<i>Salmonella enterica</i> serovar Typhimurium	Human	<i>icl</i>	ND	Virulent	Fang <i>et al.</i> (2005); Kim <i>et al.</i> (2006)
<i>Yersinia pestis</i>	Human	<i>icl</i>	Constitutive	Virulent	Sebbane <i>et al.</i> (2004)
		<i>ms</i>	Constitutive	ND	
<i>Rhodococcus equi</i>	Foals	<i>icl</i>	ND	Avirulent	Wall <i>et al.</i> (2005)
<i>Colletotrichum lagenarium</i>	Cucumber	<i>icl</i>	Appressorium, conidia, hyphae	Less virulent	Asakura <i>et al.</i> (2006)
<i>Leptosphaeria maculans</i>	Canola	<i>icl</i>	Cotyledons	Less virulent	Idnurm & Howlett (2002)
<i>Magnaporthe grisea</i>	Rice	<i>icl</i>	Appressorium, conidia, mycelia, hyphae	Less virulent	Wang <i>et al.</i> (2003)
<i>Rhodococcus fascians</i>	Tobacco	<i>ms</i>	ND	Less virulent	Vereecke <i>et al.</i> (2002)
<i>Stagonospora nodorum</i>	Wheat	<i>ms</i>	Ungerminated spores	Avirulent	Solomon <i>et al.</i> (2004)
<i>Xanthomonas campestris</i>	Tomato	<i>ms</i>	ND	Less virulent	Tamir-Ariel <i>et al.</i> (2007)
<i>Rhizobium leguminosarum</i>	Pea	<i>ms</i>	ND	No effect on symbiosis	García-de los Santos <i>et al.</i> (2002)
<i>Rhizobium tropici</i>	Bean	<i>icl</i>	ND	No effect on symbiosis	Ramírez-Trujillo <i>et al.</i> (2007)
<i>Sinorhizobium meliloti</i>	Alfalfa	<i>icl</i>	ND	No effect on symbiosis	Ramírez-Trujillo <i>et al.</i> (2007)
		<i>ms</i>	ND	No effect on symbiosis	Ramírez-Trujillo <i>et al.</i> (2007)

*Various names have been used in the literature for the genes encoding ICL and MS in different organisms; for clarity, the designations *icl* and *ms* are used here.

†ND, Not determined.

nosarum evaluated during their interaction with *Pisum sativum* have the same levels of nitrogen fixation and nodulation as the wild-type strain (García-de los Santos *et al.*, 2002). Similarly, a *glcB* mutant of *Sinorhizobium meliloti* is able to normally nodulate and fix nitrogen in symbiosis with *Medicago sativa*. To evaluate the role of ICL, null mutants were constructed in *S. meliloti* and *Rhizobium tropici* and their symbiotic performance was evaluated on *M. sativa* and *Phaseolus vulgaris*, respectively. The results demonstrated that in both symbiotic models ICL is not involved in nodulation or nitrogen fixation (Ramírez-Trujillo *et al.*, 2007).

Conclusions

The study of the metabolic pathways involved in the pathogenesis of bacterial and fungal infections is critical for public health, crop productivity and animal welfare. The

glyoxylate cycle is an important metabolic pathway in this regard, since substantial evidence supports its importance in many host–pathogen systems. The knowledge generated about the role of this pathway in pathogenesis is important, since it provides the opportunity to develop specific inhibitors of ICL and MS that could be used to combat fungal and bacterial diseases.

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References

- Achkar, J. M., Dong, Y., Holzman, R. S., Belisle, J., Kourbeti, I. S., Sherpa, T., Condos, R., Rom, W. N. & Laal, S. (2006). *Mycobacterium tuberculosis* malate synthase- and MPT51-based serodiagnostic assay as an adjunct to rapid identification of pulmonary tuberculosis. *Clin Vaccine Immunol* **13**, 1291–1293.
- Ajl, S. J. (1956). Conversion of acetate and glyoxylate to malate. *J Am Chem Soc* **78**, 3230–3231.
- Alston, T. A., Mela, L. & Bright, H. J. (1977). 3-Nitropropionate, the toxic substance of *Indigofera*, is a suicide inactivator of succinate dehydrogenase. *Proc Natl Acad Sci U S A* **74**, 3767–3771.
- Anstrom, D. M., Kallio, K. & Remington, S. J. (2003). Structure of the *Escherichia coli* malate synthase G: pyruvate: acetyl-coenzyme A abortive ternary complex at 1.95 Å resolution. *Protein Sci* **12**, 1822–1832.
- Asakura, M., Okuno, T. & Takano, Y. (2006). Multiple contributions of peroxisomal metabolic function to fungal pathogenicity in *Colletotrichum lagenarium*. *Appl Environ Microbiol* **72**, 6345–6354.
- Bai, B., Xie, J.-P., Yan, J.-F., Wang, H.-H. & Hu, C.-H. (2007). A high throughput screening approach to identify isocitrate lyase inhibitors from traditional Chinese medicine sources. *Drug Dev Res* **67**, 818–823.
- Barelle, C. J., Priest, C. L., Maccallum, D. M., Gow, N. A., Odds, F. C. & Brown, A. J. (2006). Niche-specific regulation of central metabolic pathways in a fungal pathogen. *Cell Microbiol* **8**, 961–971.
- Britton, K. L., Langridge, S., Baker, P. J., Weeradechapon, K., Sedelnikova, S. E., De Lucas, J. R., Rice, D. W. & Turner, G. (2000). The crystal structure and active site location of isocitrate lyase from the fungus *Aspergillus nidulans*. *Structure* **8**, 349–362.
- Britton, K. L., Abeyasinghe, I. S. B., Baker, P. J., Barynin, V., Diehl, P., Langridge, S. J., McFadden, B. A., Sedelnikova, S. E., Stillman, T. J. & other authors (2001). The structure and domain organization of *Escherichia coli* isocitrate lyase. *Acta Crystallogr D Biol Crystallogr* **57**, 1209–1218.
- Brock, M. (2009). Fungal metabolism in host niches. *Curr Opin Microbiol* **12**, 371–376.
- Cole, S. T., Brosch, R., Parkhill, J., Garnier, T., Churcher, C., Harris, D., Gordon, S. V., Eiglmeier, K., Gas, S. & other authors (1998). Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. *Nature* **393**, 537–544.
- Cozzone, A. J. (1998). Regulation of acetate metabolism by protein phosphorylation in enteric bacteria. *Annu Rev Microbiol* **52**, 127–164.
- Davis, W. L., Jones, R. G. & Goodman, D. B. (1986). Cytochemical localization of malate synthase in amphibian fat body adipocytes: possible glyoxylate cycle in a vertebrate. *J Histochem Cytochem* **34**, 689–692.
- Davis, W. L., Jones, R. G., Farmer, G. R., Dickerson, T., Cortinas, E., Cooper, O. J., Crawford, L. & Goodman, D. B. (1990). Identification of glyoxylate cycle enzymes in chick liver – the effect of vitamin D3: cytochemistry and biochemistry. *Anat Rec* **227**, 271–284.
- Derengowski, L. S., Tavares, A. H., Silva, S., Procópio, L. S., Felipe, M. S. & Silva-Pereira, I. (2008). Upregulation of glyoxylate cycle genes upon *Paracoccidioides brasiliensis* internalization by murine macrophages and in vitro nutritional stress condition. *Med Mycol* **46**, 125–134.
- Diehl, P. & McFadden, B. A. (1993). Site-directed mutagenesis of lysine 193 in *Escherichia coli* isocitrate lyase by use of unique restriction enzyme site elimination. *J Bacteriol* **175**, 2263–2270.
- Diehl, P. & McFadden, B. A. (1994). The importance of four histidine residues in isocitrate lyase from *Escherichia coli*. *J Bacteriol* **176**, 927–931.
- Dixon, G. H., Kornberg, H. L. & Lund, P. (1960). Purification and properties of malate synthetase. *Biochim Biophys Acta* **41**, 217–233.
- Dubnau, E., Fontán, P., Manganelli, R., Soares-Appel, S. & Smith, I. (2002). *Mycobacterium tuberculosis* genes induced during infection of human macrophages. *Infect Immun* **70**, 2787–2795.
- Eastmond, P. J., Germain, V., Lange, P. R., Bryce, J. H., Smith, S. M. & Graham, I. A. (2000). Postgerminative growth and lipid catabolism in oilseeds lacking the glyoxylate cycle. *Proc Natl Acad Sci U S A* **97**, 5669–5674.
- Ebel, F., Schwienbacher, M., Beyer, J., Heesemann, J., Brakhage, A. A. & Brock, M. (2006). Analysis of the regulation, expression, and localisation of the isocitrate lyase from *Aspergillus fumigatus*, a potential target for antifungal drug development. *Fungal Genet Biol* **43**, 476–489.
- Fang, F. C., Libby, S. J., Castor, M. E. & Fung, A. M. (2005). Isocitrate lyase (AceA) is required for *Salmonella* persistence but not for acute lethal infection in mice. *Infect Immun* **73**, 2547–2549.
- Fenhalls, G., Stevens, L., Moses, L., Bezuidenhout, J., Betts, J. C., van Helden, P., Lukey, P. T. & Duncan, K. (2002). In situ detection of *Mycobacterium tuberculosis* transcripts in human lung granulomas reveals differential gene expression in necrotic lesions. *Infect Immun* **70**, 6330–6338.
- Fradin, C., Kretschmar, M., Nichterlein, T., Gaillardin, C., d'Enfert, C. & Hube, B. (2003). Stage-specific gene expression of *Candida albicans* in human blood. *Mol Microbiol* **47**, 1523–1543.
- Fradin, C., De Groot, P., MacCallum, D., Schaller, M., Klis, F., Odds, F. C. & Hube, B. (2005). Granulocytes govern the transcriptional response, morphology and proliferation of *Candida albicans* in human blood. *Mol Microbiol* **56**, 397–415.
- García-de los Santos, A., Morales, A., Baldomá, L., Clark, S. R., Brom, S., Yost, C. K., Hernández-Lucas, I., Aguilar, J. & Hynes, M. F. (2002). The *glcB* locus of *Rhizobium leguminosarum* VF39 encodes an arabinose-inducible malate synthase. *Can J Microbiol* **48**, 922–932.
- Gonzalo-Asensio, J., Mostowy, S., Harders-Westerveen, J., Huygen, K., Hernández-Pando, R., Thole, J., Behr, M., Gicquel, B. & Martin, C. (2008). PhoP: a missing piece in the intricate puzzle of *Mycobacterium tuberculosis* virulence. *PLoS One* **3**, e3496.
- Gould, T. A., van de Langemheen, H., Muñoz-Elias, E. J., McKinney, J. D. & Sacchettini, J. C. (2006). Dual role of isocitrate lyase I in the glyoxylate and methylcitrate cycles in *Mycobacterium tuberculosis*. *Mol Microbiol* **61**, 940–947.
- Graham, J. E. & Clark-Curtiss, J. E. (1999). Identification of *Mycobacterium tuberculosis* RNAs synthesized in response to phagocytosis by human macrophages by selective capture of transcribed sequences (SCOTS). *Proc Natl Acad Sci U S A* **96**, 11554–11559.
- Green, L. S., Karr, D. B. & Emerich, D. W. (1998). Isocitrate dehydrogenase and glyoxylate cycle enzyme activities in *Bradyrhizobium japonicum* under various growth conditions. *Arch Microbiol* **169**, 445–451.
- Hillier, S. L. & Charnetzky, W. T. (1981). Rapid diagnostic test that uses isocitrate lyase activity for identification of *Yersinia pestis*. *J Clin Microbiol* **13**, 661–665.
- Hillier, S. & Charnetzky, W. T. (1981a). Glyoxylate bypass enzymes in *Yersinia* species and multiple forms of isocitrate lyase in *Yersinia pestis*. *J Bacteriol* **145**, 452–458.
- Höner Zu Bentrup, K., Miczak, A., Swenson, D. L. & Russell, D. G. (1999). Characterization of activity and expression of isocitrate lyase in *Mycobacterium avium* and *Mycobacterium tuberculosis*. *J Bacteriol* **181**, 7161–7167.
- Howard, B. R., Endrizzi, J. A. & Remington, S. J. (2000). Crystal structure of *Escherichia coli* malate synthase G complexed with

- magnesium and glyoxylate at 2.0 Å resolution: mechanistic implications. *Biochemistry* **39**, 3156–3168.
- Idnurm, A. & Howlett, B. J. (2002).** Isocitrate lyase is essential for pathogenicity of the fungus *Leptosphaeria maculans* to canola (*Brassica napus*). *Eukaryot Cell* **1**, 719–724.
- Johnson, G. V., Evans, H. J. & Ching, T. (1966).** Enzymes of glyoxylate cycle in rhizobia and nodules of legumes. *Plant Physiol* **41**, 1330–1336.
- Kahn, F. R. & McFadden, B. A. (1980).** Embryogenesis and the glyoxylate cycle. *FEBS Lett* **115**, 312–314.
- Kelly, B. G., Wall, D. M., Boland, C. A. & Meijer, W. G. (2002).** Isocitrate lyase of the facultative intracellular pathogen *Rhodococcus equi*. *Microbiology* **148**, 793–798.
- Kim, Y. R., Brinsmade, S. R., Yang, Z., Escalante-Semerena, J. & Fierer, J. (2006).** Mutation of phosphotransacetylase but not isocitrate lyase reduces the virulence of *Salmonella enterica* serovar Typhimurium in mice. *Infect Immun* **74**, 2498–2502.
- Kinhikar, A. G., Vargas, D., Li, H., Mahaffey, S. B., Hinds, L., Belisle, J. T. & Laal, S. (2006).** *Mycobacterium tuberculosis* malate synthase is a laminin-binding adhesin. *Mol Microbiol* **60**, 999–1013.
- Kohler, S., Foulongne, V., Ouahrani-Bettache, S., Bourg, G., Teyssier, J., Ramuz, M. & Liautard, J. P. (2002).** The analysis of the intramacrophagic virulome of *Brucella suis* deciphers the environment encountered by the pathogen inside the macrophage host cell. *Proc Natl Acad Sci U S A* **99**, 15711–15716.
- Kondrashov, F. A., Koonin, E. V., Morgunov, I. G., Finogenova, T. V. & Kondrashova, M. N. (2006).** Evolution of glyoxylate cycle enzymes in Metazoa: evidence of multiple horizontal transfer events and pseudogene formation. *Biol Direct* **1**, 31.
- Kornberg, H. L. & Beevers, H. (1957a).** The glyoxylate cycle as a stage in the conversion of fat to carbohydrate in castor beans. *Biochim Biophys Acta* **26**, 531–537.
- Kornberg, H. L. & Beevers, H. (1957b).** A mechanism of conversion of fat to carbohydrate in castor beans. *Nature* **180**, 35–36.
- Kornberg, H. L. & Krebs, H. A. (1957).** Synthesis of cell constituents from C₂-units by a modified tricarboxylic acid cycle. *Nature* **179**, 988–991.
- Kornberg, H. L. & Madsen, N. B. (1957).** Synthesis of C₄-dicarboxylic acids from acetate by a glyoxylate bypass of the tricarboxylic acid cycle. *Biochim Biophys Acta* **24**, 651–653.
- Kunze, M., Kragler, F., Binder, M., Hartig, A. & Gurvitz, A. (2002).** Targeting of malate synthase 1 to the peroxisomes of *Saccharomyces cerevisiae* cells depends on growth on oleic acid medium. *Eur J Biochem* **269**, 915–922.
- Lattif, A. A., Prasad, R., Banerjee, U., Gupta, N., Mohammad, S. & Baquer, N. Z. (2006).** The glyoxylate cycle enzyme activities in the pathogenic isolates of *Candida albicans* obtained from HIV/AIDS, diabetic and burn patients. *Mycoses* **49**, 85–90.
- Lee, H. S., Lee, T. H., Yang, S. H., Shin, H. J., Shin, J. & Oh, K. B. (2007).** Sesterterpene sulfates as isocitrate lyase inhibitors from tropical sponge *Hippospongia* sp. *Bioorg Med Chem Lett* **17**, 2483–2486.
- Lee, H. S., Yoon, K. M., Han, Y. R., Lee, K. J., Chung, S. C., Kim, T. I., Lee, S. H., Shin, J. & Oh, K. B. (2009).** 5-Hydroxyindole-type alkaloids, as *Candida albicans* isocitrate lyase inhibitors, from the tropical sponge *Hyrtios* sp. *Bioorg Med Chem Lett* **19**, 1051–1053.
- Lindsey, T. L., Hagins, J. M., Sokol, P. A. & Silo-Suh, L. A. (2008).** Virulence determinants from a cystic fibrosis isolate of *Pseudomonas aeruginosa* include isocitrate lyase. *Microbiology* **154**, 1616–1627.
- Liu, F., Thatcher, J. D. & Epstein, H. F. (1997).** Induction of glyoxylate cycle expression in *Caenorhabditis elegans*: a fasting response throughout larval development. *Biochemistry* **36**, 255–260.
- Lohman, J. R., Olson, A. C. & Remington, S. J. (2008).** Atomic resolution structures of *Escherichia coli* and *Bacillus anthracis* malate synthase A: comparison with isoform G and implications for structure-based drug discovery. *Protein Sci* **17**, 1935–1945.
- Lorenz, M. C. & Fink, G. R. (2001).** The glyoxylate cycle is required for fungal virulence. *Nature* **412**, 83–86.
- Lorenz, M. C. & Fink, G. R. (2002).** Life and death in a macrophage: role of the glyoxylate cycle in virulence. *Eukaryot Cell* **1**, 657–662.
- Lorenz, M. C., Bender, J. A. & Fink, G. R. (2004).** Transcriptional response of *Candida albicans* upon internalization by macrophages. *Eukaryot Cell* **3**, 1076–1087.
- McCammon, M. T., Veenhuis, M., Trapp, S. B. & Goodman, J. M. (1990).** Association of glyoxylate and beta-oxidation enzymes with peroxisomes of *Saccharomyces cerevisiae*. *J Bacteriol* **172**, 5816–5827.
- McFadden, B. A. & Purohit, S. (1977).** Itaconate, an isocitrate lyase-directed inhibitor in *Pseudomonas indigofera*. *J Bacteriol* **131**, 136–144.
- McKinney, J. D., Höner zu Bentrup, K., Muñoz-Eliás, E. J., Miczak, A., Chen, B., Chan, W. T., Swenson, D., Sacchetti, J. C., Jacobs, W. R., Jr & Russell, D. G. (2000).** Persistence of *Mycobacterium tuberculosis* in macrophages and mice requires the glyoxylate shunt enzyme isocitrate lyase. *Nature* **406**, 735–738.
- Melo Cardoso Almeida, C., Vasconcelos, A. C., Jr, Kipnis, A., Andrade, A. L. & Junqueira-Kipnis, A. P. (2008).** Humoral immune responses of tuberculosis patients in Brazil indicate recognition of *Mycobacterium tuberculosis* MPT-51 and GlcB. *Clin Vaccine Immunol* **15**, 579–581.
- Muñoz-Eliás, E. J. & McKinney, J. D. (2005).** *Mycobacterium tuberculosis* isocitrate lyases 1 and 2 are jointly required for in vivo growth and virulence. *Nat Med* **11**, 638–644.
- Muñoz-Eliás, E. J., Upton, A. M., Cherian, J. & McKinney, J. D. (2006).** Role of the methylcitrate cycle in *Mycobacterium tuberculosis* metabolism, intracellular growth, and virulence. *Mol Microbiol* **60**, 1109–1122.
- Murthy, P. S., Sirsi, M. & Ramakrishnan, T. (1973).** Effect of age on the enzymes of tricarboxylic acid and related cycles in *Mycobacterium tuberculosis* H37Rv. *Am Rev Respir Dis* **108**, 689–690.
- Oren, A. & Gurevich, P. (1994).** Production of D-lactate, acetate, and pyruvate from glycerol in communities of halophilic archaea in the Dead sea and in saltern crystallizer ponds. *FEMS Microbiol Ecol* **14**, 147–156.
- Peterson, J. B. & LaRue, T. A. (1981).** Utilization of aldehydes and alcohols in soybean bacteroids. *Plant Physiol* **68**, 489–493.
- Peterson, J. B. & LaRue, T. A. (1982).** Soluble aldehyde dehydrogenase and metabolism of aldehydes by soybean bacteroids. *J Bacteriol* **151**, 1473–1484.
- Piekarska, K., Mol, E., van den Berg, M., Ardí, G., van den Burg, J., van Roermund, C., MacCallum, D., Odds, F. & Distel, B. (2006).** Peroxisomal fatty acid beta-oxidation is not essential for virulence of *Candida albicans*. *Eukaryot Cell* **5**, 1847–1856.
- Piekarska, K., Hardy, G., Mol, E., van den Burg, J., Strijbis, K., van Roermund, C., van den Berg, M. & Distel, B. (2008).** The activity of the glyoxylate cycle in peroxisomes of *Candida albicans* depends on a functional beta-oxidation pathway: evidence for reduced metabolite transport across the peroxisomal membrane. *Microbiology* **154**, 3061–3072.
- Prigneau, O., Porta, A., Poudrier, J. A., Colonna-Romano, S., Noël, T. & Maresca, B. (2003).** Genes involved in beta-oxidation, energy metabolism and glyoxylate cycle are induced by *Candida albicans* during macrophage infection. *Yeast* **20**, 723–730.
- Purohit, H. J., Cheema, S., Lal, S., Raut, C. P. & Kalia, V. C. (2007).** In search of drug targets for *Mycobacterium tuberculosis*. *Infect Disord Drug Targets* **7**, 245–250.

- Quan, T. J., Vanderlinden, J. J. & Tsuchiya, K. R. (1982). Evaluation of a qualitative isocitrate lyase assay for rapid presumptive identification of *Yersinia pestis* cultures. *J Clin Microbiol* **15**, 1178–1179.
- Ramírez, M. A. & Lorenz, M. C. (2007). Mutations in alternative carbon utilization pathways in *Candida albicans* attenuate virulence and confer pleiotropic phenotypes. *Eukaryot Cell* **6**, 280–290.
- Ramírez-Trujillo, J. A., Encarnación, S., Salazar, E., de los Santos, A. G., Dunn, M. F., Emerich, D. W., Calva, E. & Hernández-Lucas, I. (2007). Functional characterization of the *Sinorhizobium meliloti* acetate metabolism genes *aceA*, SMC00767, and *glcB*. *J Bacteriol* **189**, 5875–5884.
- Rauyaree, P., Choi, W., Fang, E., Blackmon, B. & Dean, R. A. (2001). Genes expressed during early stages of rice infection with the rice blast fungus *Magnaporthe grisea*. *Mol Plant Pathol* **2**, 347–354.
- Rehman, A. & Mcfadden, B. A. (1996). The consequences of replacing histidine 356 in isocitrate lyase from *Escherichia coli*. *Arch Biochem Biophys* **336**, 309–315.
- Rehman, A. & Mcfadden, B. A. (1997). Lysine 194 is functional in isocitrate lyase from *Escherichia coli*. *Curr Microbiol* **35**, 14–17.
- Rude, T. H., Toffaletti, D. L., Cox, G. M. & Perfect, J. R. (2002). Relationship of the glyoxylate pathway to the pathogenesis of *Cryptococcus neoformans*. *Infect Immun* **70**, 5684–5694.
- Samanich, K., Belisle, J. T. & Laal, S. (2001). Homogeneity of antibody responses in tuberculosis patients. *Infect Immun* **69**, 4600–4609.
- Schnappinger, D., Ehrh, S., Voskuil, M. I., Liu, Y., Mangan, J. A., Monahan, I. M., Dolganov, G., Efron, B., Butcher, P. D. & other authors (2003). Transcriptional adaptation of *Mycobacterium tuberculosis* within macrophages: insights into the phagosomal environment. *J Exp Med* **198**, 693–704.
- Schöbel, F., Ibrahim-Granet, O., Avé, P., Latgé, J. P., Brakhage, A. A. & Brock, M. (2007). *Aspergillus fumigatus* does not require fatty acid metabolism via isocitrate lyase for development of invasive aspergillosis. *Infect Immun* **75**, 1237–1244.
- Sebbane, F., Jarrett, C. O., Linkenhoker, J. R. & Hinnebusch, B. J. (2004). Evaluation of the role of constitutive isocitrate lyase activity in *Yersinia pestis* infection of the flea vector and mammalian host. *Infect Immun* **72**, 7334–7337.
- Segal, W. & Bloch, H. (1956). Biochemical differentiation of *Mycobacterium tuberculosis* grown in vivo and in vitro. *J Bacteriol* **72**, 132–141.
- Serrano, J. A. & Bonete, M. J. (2001). Sequencing, phylogenetic and transcriptional analysis of the glyoxylate bypass operon (*ace*) in the halophilic archaeon *Haloferax volcanii*. *Biochim Biophys Acta* **1520**, 154–162.
- Sharma, V., Sharma, S., Höner zu Bentrup, K., McKinney, J. D., Russell, D. G., Jacobs, W. R., Jr & Sacchettini, J. C. (2000). Structure of isocitrate lyase, a persistence factor of *Mycobacterium tuberculosis*. *Nat Struct Biol* **7**, 663–668.
- Singh, V. K. & Ghosh, I. (2006). Kinetic modeling of tricarboxylic acid cycle and glyoxylate bypass in *Mycobacterium tuberculosis*, and its application to assessment of drug targets. *Theor Biol Med Model* **3**, 27.
- Smith, R. A. & Gunsalus, I. C. (1954). Isocitritase: a new tricarboxylic acid cleavage system. *J Am Chem Soc* **76**, 5002–5003.
- Solomon, P. S., Lee, R. C., Wilson, T. J. & Oliver, R. P. (2004). Pathogenicity of *Stagonospora nodorum* requires malate synthase. *Mol Microbiol* **53**, 1065–1073.
- Son, M. S., Matthews, W. J., Jr, Kang, Y., Nguyen, D. T. & Hoang, T. T. (2007). In vivo evidence of *Pseudomonas aeruginosa* nutrient acquisition and pathogenesis in the lungs of cystic fibrosis patients. *Infect Immun* **75**, 5313–5324.
- Srivastava, V., Jain, A., Srivastava, B. S. & Srivastava, R. (2008). Selection of genes of *Mycobacterium tuberculosis* upregulated during residence in lungs of infected mice. *Tuberculosis (Edinb)* **88**, 171–177.
- Stovall, I. & Cole, M. (1978). Organic acid metabolism by isolated *Rhizobium japonicum* bacteroids. *Plant Physiol* **61**, 787–790.
- Sturgill-Koszycki, S., Haddix, P. L. & Russell, D. G. (1997). The interaction between *Mycobacterium* and the macrophage analyzed by two-dimensional polyacrylamide gel electrophoresis. *Electrophoresis* **18**, 2558–2565.
- Tamir-Ariel, D., Navon, N. & Burdman, S. (2007). Identification of genes in *Xanthomonas campestris* pv. vesicatoria induced during its interaction with tomato. *J Bacteriol* **189**, 6359–6371.
- Tchawa Yimga, M., Leatham, M. P., Allen, J. H., Laux, D. C., Conway, T. & Cohen, P. S. (2006). Role of gluconeogenesis and the tricarboxylic acid cycle in the virulence of *Salmonella enterica* serovar Typhimurium in BALB/c mice. *Infect Immun* **74**, 1130–1140.
- Thirach, S., Cooper, C. R., Jr & Vanittanakom, N. (2008). Molecular analysis of the *Penicillium marneffeii* glyceraldehyde-3-phosphate dehydrogenase-encoding gene (*gpdA*) and differential expression of *gpdA* and the isocitrate lyase-encoding gene (*acuD*) upon internalization by murine macrophages. *J Med Microbiol* **57**, 1322–1328.
- Timm, J., Post, F. A., Bekker, L. G., Walther, G. B., Wainwright, H. C., Manganelli, R., Chan, W. T., Tsenova, L., Gold, B. & other authors (2003). Differential expression of iron-, carbon-, and oxygen-responsive mycobacterial genes in the lungs of chronically infected mice and tuberculosis patients. *Proc Natl Acad Sci U S A* **100**, 14321–14326.
- Tugarinov, V., Choy, W. Y., Orekhov, V. Y. & Kay, L. E. (2005). Solution NMR-derived global fold of a monomeric 82-kDa enzyme. *Proc Natl Acad Sci U S A* **102**, 622–627.
- Vereecke, D., Cornelis, K., Temmerman, W., Jaziri, M., Van Montagu, M., Holsters, M. & Goethals, K. (2002a). Chromosomal locus that affects pathogenicity of *Rhodococcus fascians*. *J Bacteriol* **184**, 1112–1120.
- Vereecke, D., Cornelis, K., Temmerman, W., Holsters, M. & Goethals, K. (2002b). Versatile persistence pathways for pathogens of animals and plants. *Trends Microbiol* **10**, 485–488.
- Wall, D. M., Duffy, P. S., Dupont, C., Prescott, J. F. & Meijer, W. G. (2005). Isocitrate lyase activity is required for virulence of the intracellular pathogen *Rhodococcus equi*. *Infect Immun* **73**, 6736–6741.
- Wanchu, A., Dong, Y., Sethi, S., Myneedu, V. P., Nadas, A., Liu, Z., Belisle, J. & Laal, S. (2008). Biomarkers for clinical and incipient tuberculosis: performance in a TB-endemic country. *PLoS One* **3**, e2071.
- Wang, Z. Y., Thornton, C. R., Kershaw, M. J., Debaio, L. & Talbot, N. J. (2003). The glyoxylate cycle is required for temporal regulation of virulence by the plant pathogenic fungus *Magnaporthe grisea*. *Mol Microbiol* **47**, 1601–1612.
- Wayne, L. G. & Lin, K. Y. (1982). Glyoxylate metabolism and adaptation of *Mycobacterium tuberculosis* to survival under anaerobic conditions. *Infect Immun* **37**, 1042–1049.
- Wong, P. P. & Evans, H. J. (1971). Poly- β -hydroxybutyrate utilization by soybean (*Glycine max* Merr) nodules and assessment of its role in maintenance of nitrogenase activity. *Plant Physiol* **47**, 750–755.