Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Energetic metabolism of *Chromobacterium violaceum*

Tânia B. Creczynski-Pasa[1] and Regina V. Antônio[2]

[1]Departamento de Ciências Farmacêuticas, Centro de Ciências da Saúde, and [2]Departamento de Bioquímica, Centro de Ciências Biológicas, Universidade Federal de Santa Catarina, Caixa Postal 476, 88040-900 Florianópolis, SC, Brasil
Corresponding author: T.B. Creczynski-Pasa
E-mail: taniac@hu.ufsc.br

Genet. Mol. Res. 3 (1): 162-166 (2004)
Received October 13, 2003
Accepted January 12, 2004
Published March 31, 2004

ABSTRACT. *Chromobacterium violaceum* is a free-living microorganism, normally exposed to diverse environmental conditions; it has a versatile energy-generating metabolism. This bacterium is capable of exploiting a wide range of energy resources by using appropriate oxidases and reductases. This allows C. *violaceum* to live in both aerobic and anaerobic conditions. In aerobic conditions, *C. violaceum* is able to grow in a minimal medium with simple sugars, such as glucose, fructose, galactose, and ribose; both Embden-Meyerhoff, tricarboxylic acid and glyoxylate cycles are used. The respiratory chain supplies energy, as well as substrates for other metabolic pathways. Under anaerobic conditions, *C. violaceum* metabolizes glucose, producing acetic and formic acid, but not lactic acid or ethanol. *C. violaceum* is also able to use amino acids and lipids as an energy supply.

Key words: *Chromobacterium violaceum*, Energetic metabolism, Aerobic metabolism, Anaerobic metabolism

INTRODUCTION

In all living organisms (bacteria, plants and animals), energy-rich substrates are converted into chemical energy as ATP. ATP is used by the cells to drive movement, for active transport, metabolic synthesis, and other processes requiring energy. Membrane-bound H^+-ATP synthases are the key enzymes of energy metabolism. They catalyze ATP synthesis from ADP and inorganic phosphate, coupled with transmembrane proton transport. Proton transport is driven by transmembrane pH and electric potential differences (Mitchell, 1974; Creczynski-Pasa and Gräber, 1994; Boyer, 1998). *Chromobacterium violaceum* has an F_0F_1-type ATP synthase, similar to the enzyme found in *E. coli* (Fischer and Gräber, 1999; Vasconcelos et al., 2003). However, *C. violaceum* also has alternative means to obtain energy. It is able to live under aerobic and anaerobic conditions as a free-living organism; this important characteristic enables this bacterium to survive under diverse environmental conditions. Furthermore, *C. violaceum* has a strong chemotactic capacity, and a strong capability to adapt to stress (Vasconcelos et al., 2003). All these processes require large amounts of energy. We present an overview of the energetic metabolism of *C. violaceum*, based on the information obtained from its genome analysis (Vasconcelos et al., 2003).

RESULTS AND DISCUSSION

As in all chemoheterotrophic bacteria, *C. violaceum* is able to grow in minimal medium that includes simple sugars, such as glucose, fructose, galactose, or ribose. However, *C. violaceum* is not able to synthesize glucose through gluconogenesis, since, based on genome analysis, it lacks the gene that codes for glucose-6-phosphatase (Vasconcelos et al., 2003). The Embden-Meyerhoff pathway, tricarboxylic acid and glyoxylate cycles, and the respiratory chain, supply cellular energy, as well as substrates for anabolic pathways under aerobic conditions. This bacterium has the capability to biosynthesize complex polysaccharides, such as cellulose, but not glycogen. *C. violaceum* mainly uses monosaccharides; however, it seems to be able to utilize cellulose and chitin, but not starch, as carbon sources, since it contains cellulases and chitinase, but lacks alpha amylase genes. In addition, *C. violaceum* is not able to utilize sucrose or lactose as energy resources, because these metabolic pathways are absent from its genome (Vasconcelos et al., 2003).

The second key enzyme of the aerobic metabolism is cytochrome oxidase, which is a member of a large superfamily of proteins (Calhoun et al., 1994). Phylogenetic and evolutionary analysis of this family indicated three basic types of cytochrome oxidase, which are: SoxM (or aa3, also found in mitochondria), SoxB and FixN (orcbb3) (Castresana, 2001). Whereas SoxM and SoxB work under normal aerobic conditions, the FixN oxidases are involved in respiration at very low oxygen pressure (microaerobic conditions) (Castresana, 2001). Genes homologous to SoxM and FixN, as well as, the cytochrome bd oxidase, have been found in the *C. violaceum* genome. This enzyme is a terminal oxidase, with a high affinity for oxygen, but it is completely unrelated to the cytochrome oxidase superfamily. The cytochrome bd seems to play an important role in oxidative stress, although it is also able to create an electrochemical membrane gradient, available for energetic requirements (Castresana, 2001).

There is an operon for HCN synthase (*hcn*A, *hcn*B and *hcn*C) in the *C. violaceum* genome, encoding a formate dehydrogenase and two amino acid oxidases, respectively, similar

to those found in *Pseudomonas aeruginosa*, which are involved in cianidric acid synthesis. Previous work on cyanide (HCN) synthesis in bacteria show that, *in vivo*, the four electrons produced by HCN synthase are transferred to oxygen, probably throughout the respiratory chain. These reactions occur at low levels of oxygen. Analysis of enzyme sequences has shown that each subunit is similar to known enzymes involved in electron transport (Laville et al., 1998).

Likewise, lipid metabolism in *C. violaceum*, with the degradation and biosynthesis of various types of lipids (triacylglycerol, phospholipids, and lipopolysaccharides), provides another energy resource. The beta-oxidation of fatty acids leads to the production of reduced nucleotides, and acetyl-CoA leads to energy production.

Extracellular proteases enable the bacterium to utilize the resultant amino acids as substrates for endogenous protein biosynthesis, as well as for carbon and nitrogen resources. The presence of several deaminases, as well as transaminases, results in the production of keto acids from amino acids, which can be converted to acetyl-CoA, or tricarboxylic cycle intermediates, and eventually to an energy resource.

Chromobacterium violaceum also anaerobically metabolizes glucose, with production of mixed organic acids, such as acetic acid and formic acid. CO_2 and H_2 are produced from formic acid, but not from lactate or ethanol. This alternative way of yielding energy, where organic molecules serve as both electron donors and acceptors, is known as fermentation. In the absence of oxygen, the microorganisms decrease pyruvate dehydrogenase activity, and use the pyruvate or one of its derivatives as an electron and a hydrogen acceptor in the reoxidation of NADH. Also, under anaerobic conditions, *C. violaceum* is able to obtain large amounts of energy using nitrate or fumarate as final electron acceptors in the respiratory chain. All components of anaerobic fumarate reductase are present, as well as all the necessary elements for reduction of nitrate to nitrite, similar to the situation in *E. coli*. In *C. violaceum*, most of the enzymes involved in the N cycle, including nitrate reductase, nitrite reductase, as well as the nitric oxide reductase responsible for N_2O production from nitric oxide, are present (Vascocelos et al., 2003; Moura et al., 2003). However, it lacks nitrous oxide reductase, the enzyme that converts nitrous oxide to N_2 (Vasconcelos et al., 2003).

The genes involved in the fermentation of mixed acids (pyruvate-formate lyase) are also present in *C. violaceum*. Alcoholic fermentation of glucose can also be performed by the Entner-Doudoroff pathway. Although *C. violaceum* has alcohol dehydrogenase, it is not able to produce ethanol because the pyruvate produced by this pathway, as well as that produced by the Embden-Meyerhoff pathway, cannot be converted to ethanol, since *C. violaceum* lacks the pyruvate decarboxylase gene (Vasconcelos et al., 2003).

Chromobacterium violaceum is able to synthesize all the amino acids it needs; however, the most notable characteristic of this bacterium is related to the metabolism of a specific amino acid, tryptophan, which is involved in the production of a chemically well-characterized pigment named violacein (Bromberg and Durán, 2001). Violacein is synthesized only when *C. violaceum* grows in aerobic conditions; however, the function of this pigment in this bacterium is unknown. There is some speculation that violacein is a kind of storage form of tryptophan, but there is no proof for such a hypothesis. There are some indications that violacein has antibiotic, antichagasic (Momen and Hoshino, 2000), antitumoral (Melo et al., 2000; Duran and Menck, 2001) and antileishmanial (Leon et al., 2001) activity. During the last few years, a new property of violacein has been characterized; it appears that this molecule has antioxidant properties

(Azevedo et al., 2000; Konzen et al., 2003). Violacein is involved with oxygen metabolism, but no direct relationship with energy metabolism has been found.

When we compare the versatility of the energy metabolism of *C. violaceum* with that of five other bacteria from other genera (Table 1), *C. violaceum* has six enzymes involved with energy metabolism in a general way, while the other bacteria lack one to four of these enzymes. The catabolic activity results in ATP synthesis, or in the production of reduced nucleotides, which should be aerobically or anaerobically reoxidized, through one of the mechanisms present in *C. violaceum*, depending on environmental conditions.

Table 1. Versatility of the energy metabolism of *Chromobacterium violaceum.*

Enzyme	*Chromobacterium violaceum*	*Pseudomonas aeruginosa*	*Ralstonia solanarum*	*Escherichia coli*	*Neisseria meningitidis*	*Xylella fastidiosa*
Sox-type cytochrome oxidase	+	+	+	+	–	+
FixN-type cytochrome oxidase	+	+	+	–	+	–
Cytochrome bd oxidase	+	+	+	+	–	–
Nitrate reductase	+	+	+	+	–	–
Fumarate reductase	+	–	–	+	–	–
HCN synthase	+	+	–	–	–	–

CONCLUSION

The analysis *of C. violaceum* DNA allowed us to conclude that this bacterium has an efficient apparatus for energy production, under both aerobic and anaerobic conditions. All the genes that are necessary for the glycolysis and tricarboxylic acid cycle were found in its genome, as well as the main proteins involved in electron transport (dehydrogenases, quinones and cytochromes). This apparatus allows a high production of energy under aerobic conditions, since complete degradation of glucose is possible. Besides the energy required for other functions, *C. violaceum* seems to spend considerable energy on motility, assuring its survival under variable environmental conditions, such as a lack of food, and other types of stress.

ACKNOWLEDGMENTS

The authors thank MCT/CNPq (Brazilian National Genome Project Consortium) and FUNCITEC for their support.

REFERENCES

Azevedo, M.B.M., Justo, G.Z., Rettori, D., Rodriguez, J.A., Haun, M. and **Duran, N.** (2000). Antioxidant activity of an inclusion complex of violacein and β-cyclodextrin. *Proc. Int' I. Symp. Control. Rel. Bioact. Mater. 27*: 6332.

Boyer, P.D. (1998). Energy, life, and ATP. *Biosci. Rep. 18*: 97-117.

Bromberg, N. and **Durán, N.J.** (2001). Violacein transformation by peroxidases and oxidases: implications on its biological properties. *Mol. Catal. B: Enzymatic. 11*: 463-467.

Calhoun, M.W., Thomas, J.W. and **Gennis, R.B.** (1994). The cytochrome-oxidase superfamily of redox-driven proton pumps. *Trends Biochem. Sci. 19*: 325-330.

Castresana, J. (2001). Comparative genomics and bioenergetics. *Biochim. Biophys. Acta 1506*: 147-162.

Creczynski-Pasa, T.B. and **Gräber, P.** (1994). ADP binding and ATP synthesis by reconstituted H^+-ATPase from chloroplasts. *FEBS Lett. 350*: 195-198.

Duran, N. and **Menck, C.F.M.** (2001). *Chromobacterium violaceum*: a review of pharmacological and industrial perspective. *Crit. Rev. Microbiol. 27*: 201-222.

Fischer, S. and **Gräber, P.** (1999). Comparison of ΔpH- and $\Delta\phi$-driven ATP synthesis catalyzed by the H^+-ATPases from *Escherichia coli* or chloroplasts reconstituted into liposomes. *FEBS Lett. 457*: 327-332.

Konzen, M., Fernandez, H., Vieira, T.O., Cordova, C.A.S., Antônio, R.V., De Marco, D. and **Creczynski-Pasa, T.B.** (2003). Antioxidant properties of violacein. *XXXII Annual Meeting of SBBq*, Caxambu, MG, Brazil, 05-17 to 05-20, pp. 243.

Laville, J., Blumer, C., Von Schroetter, C., Gaia, V., Defago, G., Keel, C. and **Haas, D.** (1998). Characterization of the hcnABC gene cluster encoding hydrogen cyanide synthase and anaerobic regulation by ANR in the strictly aerobic biocontrol agent *Pseudomonas fluorescens* CHA0. *J. Bacteriol. 180*: 3187-3196.

Leon L.L., Miranda, C.C., De Souza, A.O. and **Duran, N.** (2001). Antileishmanial activity of the violacein extracted form *Chromobacterium violaceum*. *Antimicrob. Agents Chemoth. 48*: 449-450.

Melo, P.S., Maria, S.S., Vidal, B.C., Haun, M. and **Duran, N.** (2000). Violacein cytotoxicity and induction of apoptosis in V79 cells. *In Vitro Cell Dev. Biol. Anim. 36*: 539-543.

Mitchell, P. (1974). A chemiosmotic molecular mechanism for proton translocating adenosine triphosphatases. *FEBS Lett. 43*: 189-194.

Momen, A.Z.M.R. and **Hoshino, T.** (2000). Biosynthesis of violacein: Intact incorporation of the tryptophan molecule on the oxindole side, with intramolecular rearrangement of the indole ring on the 5-hydroxyindole side. *Biosci. Biotechnol. Biochem. 64*: 539-549.

Moura, I., Cabrito, I., Almeida, G., Cunha, C., Romao, M.J. and **Moura, J.J.G.** (2003). Molecular aspects of denitrification/nitrate dissimilation. *J. Inorg. Biochem. 96*: 195-195.

Vasconcelos, A.T.R., Almeida, D.F., Almeida, F.C., Almeida, L.G.P., Almeida, R., Alves-Gomes, J.A., Andrade, E.M., Antônio, R.V., Araripe, J., Araújo, M.F.F., Astolfi-Filho, S., Azevedo, V., Baptista, A.J., Bataus, L.A.M., Batista, J.S., Beló, A., van den Berg, C., Blamey, J., Bogo, M., Bonatto, S., Bordignon, J., Brito, C.A., Brocchi, M., Burity, H.A., Camargo, A.A., Cardoso, D.D.P., Carneiro, N.P., Carraro, D.M., Carvalho, C.M.B., Cascardo, J.C.M., Cavada, B.S., Chueire, L.M.O., Creczynski-Pasa, T.B., Duran, N., Fagundes, N., Falcão, C.L., Fantinatti, F., Farias, I.P., Felipe, M.S.S., Ferrari, L.P., Ferro, J.A., Ferro, M.I.T., Franco, G.R., Freitas, N.S.A., Furlan, L.R., Gazzinelli, R.T., Gomes, E.A., Gonçalves, P.R., Grangeiro, T.B., Grattapaglia, D., Grisard, E.C., Guimarães, C.T., Hanna, E.S., Hungria, M., Jardim, S.N., Laurino, J., Leoi, L.C.T., Lima, L.F.A., Loureiro, M.F., Lyra, M.C.C.P., Macedo, M., Madeira, H.M.F., Manfio, G.P., Maranhão, A.Q., Martins, W.S., di Mauro, S.M.Z., Medeiros, S.R.B., Meissner, R.V., Moreira, M.A.M., Nascimento, F.F., Nicolas, M.F., Oliveira, J.G., Oliveira, S.C., Paixão, R.F.C., Parente, J.A., Pedrosa, F.O., Pena, S.D.J., Pereira, J.O., Pereira, M., Pinto, L.S.R.C., Pinto, L.S., Porto, J.I.R., Potrich, D.P., Ramalho-Neto, C.E., Reis, A.M.M., Rigo, L.U., Rondinelli, E., Santos, E.B.P., Santos, F.R., Schneider, M.P.C., Seuanez, H.N., Silva, A.M.R., Silva, A.L.C., Silva, D.W., Silva, R., Simões, I.D.C., Simon, D., Soares, C.M.A., Soares, R.B.A., Souza, E.M., Souza, K.R.L., Souza, R.C., Steffens, M.B.R., Steindel, M., Teixeira, S.R., Urmenyi, T., Vettore, A., Wassem, R., Zaha, A. and **Simpson, A.J.G.** (2003). Complete genome sequence of *Chromobacterium violaceum* reveals remarkable and exploitable bacterial adaptability. *Proc. Natl. Acad. Sci. USA 100*: 11660-11665.