Microaerophilies of campylobacters and related organisms

Han, Yeong-Hwan

Department of Biology, Dongguk University, Kyongju, Korea

INTRODUCTION

Since some species of the genus *Campylobacter* and related organisms were recognized as important pathogens in humans and animals, many medical and veterinary microbiologists have concerned with these organisms (Penner, 1988; Skirrow and Benjamin, 1980; Smibert, 1984). *C. jejuni* and *C. coli* cause gastroenteritis in humans (Butzler and Skirrow, 1979; Dekeyser *et al.*, 1972). *C. fetus* causes abortion in cattle and sheep, sometimes, causes blood infections in humans (Florent, 1959). *C. hyointestinalis* have been isolaed from pigs with proliferative ileitis (Gebhart *et al.*, 1983; Gebhart *et al.*, 1985). *Helicobacter pylori* (previously called, *C. pylori*) have been recognized as causative agents of gastric and duodenal ulcers in humans (Goodwin *et al.*, 1989; Marshall, 1986; Marshall *et al.*, 1984).

The general characteristics of campylobacters and related organims (e.g., species of the genera *Helicobacter* and *Wolinella, Bacteroides ureolyticus,* and *Bacteroides gracilis*) are as follows: slender, non-sporeforming, gram-negative, vibroid bacteria (helical- or spiral- shaped; except that *B. ureolyticus* and *B. gracilis* are straight-rod), 0.2-0.5 μm in width and 0.5 μm in length. (Smibert, 1984; Penner, 1988). The species of genus *Campylobacter* and related organisms are chemoorganotrophs;however, they neither oxidize nor ferment carbohydrates and instead obtain energy from amino acids, the salts of tricarboxylic acids (TCA) cycle intermediates, the salts of organic acids, or, in some species, H_2.

With regard to their oxygen responses for growth, they all are microaeophilic. I.e., they are capable of oxygen-dependent growth (respiring with oxygen as a terminal electron acceptor) but can not grow in the presence of a level of oxygen equivalent to that present in an air atmosphere (21% oxygen). This review will take interests in how these microorganisms response to oxygen for growth and what repiratory types they have.

BACTERIAL RESPONSES TO OXYGEN

Living organisms can be divided into the following four categories on the basis of their relationship to oxygen:

aerobe: An organism that is capable of using oxygen as a terminal electron acceptor, can tolerate a level of oxygen equivalent to or higher than that present in an air atmosphere, and has a strictly respiratory type of metabolism. Some aerobes may also be capable of growing anaerobically with electron acceptors other than oxygen. Examples of aerobes include the genus *Pseudomonas* (Palleroni, 1984) and the genus *Neisseria* (Vedros, 1984).

anaerobe: An organism that is incapable of oxygen-dependent growth and can not grow in the presence of an oxygen concentration equivalent to that present in an air atmosphere (21% oxygen). Some anaerobes may have a fermentative type of metabolism;others may carry out anaerobic respiration in which a terminal electron acceptor other than oxygen is used. The genus *Clostridium* (Cato *et al.*, 1986) and genus *Bacteroides* (Holdeman *et al.*, 1984) are examples of this group.

facultative: An organism that can grow well both in the absence of oxygen and in the presence

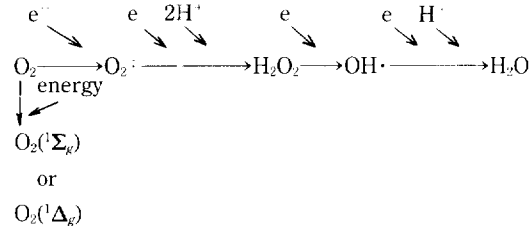
of a level of oxygen equivalent to that in an air atmosphere. Some are capable of growing aerobically by respiring with oxygen and of growing anaerobically by fermentation; examples are the genus *Enterobacter* (Richard, 1984) and the genus *Vibrio* (Baumann *et al.*, 1984). Other facultatives have a strictly fermentative type of metabolism and do not respire with oxygen. Oxygen may be taken up, but not for energy-yielding respiration; in fact, oxygen uptake usually leads to formation of hydrogen peroxide (H_2O_2). Examples include the genus *Streptococcus* (Hardie, 1986) and the genus *Meniscus* (Irgens, 1984). Although streptococci lack catalase, they possess peroxidases, thereby enabling the organisms to grow in the presence of oxygen.

microaerophile: An organism that is capable of oxygen-dependent growth but can not grow in the presence of a level of oxygen equivalent to that present in an air atmosphere (21% oxygen). Oxygen-dependent growth occurs only at low oxygen levels. In addition to being able to respire with oxygen, some microaerophiles may be capable of respiring anaerobically with electron acceptors other than oxygen. Examples include the genus *Campylobacter* (Smibert, 1984) and the genus *Spirillum* (Krieg, 1984).

TOXIC FORMS OF OXYGEN

Aerobes, microaerophiles, and many facultative organisms can respire with molecular oxygen (O_2). Molecular oxygen is an excellent terminal electron acceptor because E'o of the O_2/H_2O half-cell system is very high (+0.8 Volt at pH 7.0). However, it has two unpaired electrons with parallel spin, each in a separate orbital. Thus when O_2 oxidizes another compound it must either (i) accept a pair of electrons of opposite spin, or (ii) one of its unpaired electrons must undergo a spin reversal before pairing, allowing it to accept two electrons of antiparallel spin which reduce the O_2 to H_2O_2. This is why many direct two-electron spontaneous oxidations require a high energy of activation to start the reaction (Byczkowski and Gessner, 1988). This activation is unlikely to occur at ordinary physiological temperatures in the absence of an appropriate enzyme. As an alternative to accepting two electrons, molecular oxygen can accept a single electron, provided it has a spin opposite to that of its unpaired electrons. An O_2 molecule that accepts a single electron becomes reduced to asuperoxide radical ($O_2^{\cdot-}$). This is a free radical, which by definition is a molecule or atom that possesses one unpaired electron. Molecular oxygen can also accept three electrons to form a hydroxyl radical (OH · a free radical) and a hydroxyl ion (OH^-). In actuality, this occurs by prior formation of H_2O_2 (due to reduction of O_2 by two electrons), which then accepts one electron. Molecular oxygen can also accept four electrons to form two molecules of water (H_2O). The mechanism by which this occurs is largely conjectural, but it requires enzymatic catalysis, usually by an enzyme called cytochrome oxidase, which is composed of functionally different subunits. In addition, an O_2 molecule can absorb energy in such a way as to change the direction of spin of one of its two unpaired electrons. This results in either of two energized forms of a form of O_2, both of which are called singlet oxygen. In one form, the sigma form, $O_2(^1\Sigma_g)$, both electrons remain unpaired in their separate orbitals. In the other and more common form, the delta form, $O_2(^1\Delta_g)$, the electron pairs with the other electron so that one orbital contains both electrons and the other contains none.

The relationship between O_2 and its derivatives can be summarized as follows:



Of the various derivatives of O_2, it is H_2O_2, $O_2(^1\Delta_g)$ and OH^- that are considered to be the most damaging to living organisms. These toxic derivatives can be formed as the by-products of cellular metabolism, as well as from spontaneous autooxidation or photochemical generation in the environment. Superoxide radicals seem to function mainly as reducing agents in the production of hydroxyl radicals and are usually not considered to be toxic *per se*.

Hydrogen peroxide is a stronger oxidizing agent than molecular oxygen because it has no unpaired electrons and there is no restriction on the direction of spin of an electron that is accepted. It can spontaneously oxidize many biological substances and may even cause strands breaks in DNA when added exogenously to cells (Steiner *et al.*, 1984). Hydrogen peroxide is produced metabolically mainly in the course of oxidase reactions, such as those catalyzed by xanthine oxidase or amino acid oxidases. Many oxidase reactions generate O_2^- as an intermediate, and these radicals in turn collide with one another and spontaneously dismutate to form H_2O_2:

$$O_2^- + O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$$

However, other oxidase reactions generate H_2O_2 directly without forming O_2^- as an intermediate. Both O_2^- and H_2O_2 can also be generated nonenzymatically during the autooxidation of various reduced flavins, quinones, thiols, and iron-sulfur proteins. For example, autooxidation of ferredoxin or cysteine generates superoxide radicals (Misra and Fridovich, 1971; Carlsson *et al.*, 1978).

Compared to ground state O_2, $O_2(^1\Delta_g)$ is extremely reactive. The reactivity of ground state O_2, which contains two unpaired electrons, is severely limited by the spin conversion rule, as mentioned previously. Oxygen in the $^1\Delta_g$ state has no unpaired electrons (they are both in the same orbital) and, in addition, has 23 kcal/mol more energy than ground state O_2. Singlet oxygen rapidly oxidizes a large variety of biological molecules and can cause strand breakage in DNA (Di Mascio *et al.*, 1989). $O_2(^1\Delta_g)$ is generated mainly by photochemical reactions but may also be generated by nonphotochemical reactions. For instance, polymorphonuclearleucocytes generate $O_2(^1\Delta_g)$ by a peroxidase-dependent mechanism.

Hydroxyl radicals are the most reactive form of oxygen and act as very strong oxidizing agents. They can damage almost any biological molecule and can cause strand breakage in DNA (Brawn and Fridovich, 1980). In biological systems they arise mainly from an iron-catalyzed Haber-Weiss reaction:

$$O_2^- + H_2O_2 \xrightarrow{\text{Fe chelates}} OH^- + OH\cdot + O_2$$

Thus if one has superoxide radicals, some will dismutate to form hydrogen peroxide, which will then react with O_2^- to form hydroxyl radicals. The main function of superoxide radicals to reduce the iron from the ferric to the ferrous form. If some other source of reduced iron occurs in a biological system, superoxide radicals are not needed for the generation of hydroxyl radicals:

$$H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + OH^- + OH\cdot$$

Enzymes that protect against toxic forms of oxygen. The enzyme superoxide dismutase (SOD) catalyzes the dismutation of superoxide radicals and causes the reaction to occur approximately a billion times faster. This is because the enzyme makes it unnecessary for superoxide radicals to collide directly with each other. This eliminates the problem of electrostatic repulsion between the negatively charged radicals.

Hydrogen peroxide can be destroyed by either catalase or by peroxidases:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

$$2H_2O_2 + AH_2 \xrightarrow{\text{peroxidase}} 2H_2O + A$$

(reduced electron donor)

It should be noted that elimination of either O_2^- or H_2O_2 will inhibit the production of $OH\cdot$ by the iron-catalyzed Haber-Weiss reaction.

MICROAEROPHILY

Many bacterial species have been identified as microaerophiles. These include *Spirillum volutans, Campylobacter species, Treponema pallidum subsp. pallidum, Aquaspirillum magnetotacticum, Gallionella ferruginea,* and the N_2 fixer, *Azospirillum lipoferum.* A more complete listing has been presented by Krieg and Hoffman (1986).

Although the physiological basis of microaerophily is poorly understood, there are several hypotheses for explaining microaerophily, as summarized by Krieg and Hoffman (1986). However, it is likely that no single hypothesis applies to all microaerophiles. Some possibilities are as follows. (i) Microaerophilic bacteria are more susceptible to exogenous toxic forms of oxygen in culture media than are other oxygen-dependent bacteria such as aerobes and facultatives (Hoffman *et al.*, 1979). The enhanced aerotolerance of *Campylobacter jejuni* by the addition of ferrated norepinephrine and a combination of sodium metabisulfite, sodium pyruvate, and iron salts (FBP) was attributed to the superoxide-scavenging ability of FBP and the ability of pyruvate to destroy H_2O_2. Also, the microaerophile *Spirillum volutans* is extremely sensitive to exogenous hydrogen peroxide in its culture medium (Padgett *et al.*, 1981). (ii) Microaerophiles might be expected to be deficient in catalase and/or SOD activity, since this could render an organism less able to destroy toxic forms of oxygen and thus decrease its aerotolerance. For instance, *Spirillum volutans* is highly susceptible to exogenous H_2O_2, and this organism lacks catalase which catalyzes the destruction of H_2O_2 (Padgett *et al.*, 1981). The addition of catalase to the culture medium increased the aerotolerance of *S. volutans,* and a combination of catalase and SOD acted in a synergistic manner to increase aerotolerance. Moreover, Padgett and Krieg (1986) found that a variant strain of *S. volutans* that was aerotolerant possessed approximately three times the peroxidase activity of the wild type *S. volutans.* However, some microaerophiles such as *C. jejuni* do not lack (iii) Low redox-potential substances in a cell could be potential targets for inactivation by toxic derivatives of oxygen. For instance, the oxygen-labile nitrogenase complex in nitrogen-fixing bacteria such as *Azospirillum lipoferum* can limit growth at oxygen levels higher than 1% (v/v). Bacteria that do not fix nitrogen may contain other oxygen-labile substance; for instance, *C. jejuni* contains a low redox-potential cytochrome c oxidase (Hoffman and Goodman, 1982). Lascelles and Calder (1985) reported that *C. fetus* possesses an oxygen-labile pyruvate:ferredoxin oxidoreductase, and this might be a target for inactivation by O_2 or H_2O_2. Moreover, the autooxidation of the reduced ferredoxin resulting from pyruvate oxidation in *C. fetus* might generate O_2^-. (iv) Microaerophiles may generate excessive amounts of toxic derivatives of oxygen. For instance, the microaerophilic nature of *Campylobacter sputorum* may be due to its ability to produce O_2^- and H_2O_2 and an absence or low activities of protective enzymes (Niekus *et al.*, 1977; Stouthamer *et al.*, 1979). (v) Low respiratory rates might be a factor in microaerophily. If oxygen is not used up rapidly at the cell surface, it might reach oxygen-labile enzymes within the cell. The importance of respiratory protection is emphasized by the discovery of certain microaerophilic mutants of the normally aerobic N_2-fixer *Azotobacter chroococcum* (Ramos and Robson, 1985).

RESPIRATORY SYSTEMS OF CAMPYLOBACTERS AND RELATED ORGANISMS

Respiration differs from fermentation in two respects: (i) electrons are passed along a series of enzymes or compounds (electron transport system), each component of which is alternately reduced and re-oxidized in a cyclic fashion (electron transport system); and (ii) an exogenous terminal electron acceptor must be present in the medium or environment. An electron transport system consists of flavoprotein dehydrogenases, quinones,

and nonheme-iron proteins, together with various cytochrome pigments. Bacterial respiratory systems are associated with the cytoplasmic membrane and have properties similar to those of the respiratory system of eucaryotic mitochondria. This is not surprising, since mitochondria evolved from bacteria, according to the endosymbiont theory of the origin of mitochondria. Substrates that are oxidizable by various bacterial respiratory systems include a broad range of compounds, such as ammonia, sulfide, nitrite, ferrous iron, and H_2. The cytochromes and terminal oxidases of a respiratory system are usually membrane-bound entities, play a major role in electron transport, and are very important in generating energy in the form of a proton motive force. Many organisms use O_2 as the terminal electron acceptor in a respiratory chain; however, some organisms are able to use nitrate, sulfate, sulfur, and fumarate instead of O_2, in which instance the process is more specifically termed 'anaerobic respiration'.

Among the campylobacters and related bacteria that use H_2 and formate as electron donors for respiration, formate dehydrogenase and hydrogenase, respectively, catalyze the removal of electrons from these donors. These enzymes are involved in the electron transport system of *C. fetus* (Carlone and Lascelles, 1982), *C. jejuni* (Harvey and Lascelles, 1980; Hoffman and Goodman, 19 82), *C. sputorum* biovar bubulus (Niekus *et al.*, 1980a, 1980b), *C. mucosalis* (Lawson *et al.*, 1981). They are also involved in anaerobic respiration by *W. succinogenes,* for which fumarate serves as the terminal electron acceptor (Jacobs and Wolin, 1963b; Kröger *et al.*, 1979; Kröger *et al.*, 1980; Unden *et al.*, 1982; Wolin *et al.*, 1961).

In *W. succinogenes,* acid-extractable and covalently-bound FAD, FMN, and an iron-sulfur protein also are associated with the anaerobic respiratory system (Kröger and Innerhofer, 1976a; Kröger *et al.*, 1980). The iron-sulfur protein is sensitive to 4-chloromercuriphenyl sulfonate (Kröger and Innerhofer, 1976b; Kröger *et al.*, 1979).

Isoprenoid quinones have taxonomic significance in bacterial classification. Isoprenoid quinones are divided into two groups: naphthoquinones, which include menaquinones (vitamin K_2) and phylloquinones, and benzoquinones, which include ubiquinones (coenzyme Q). Both menaquinones and ubiquinones occur frequently in bacteria and are located in the bacterial cytoplasmic membrane where they function in electron transport systems (Collins and Jones, 1981). Members of the genus *Campylobacter* possess menaquinone-6 (MK-6; 2-methyl-3-hexaprenyl-1,4-naphthoquinone) and methyl-substituted menaquinone (*MK-6; 2, [5 or 8]-dimethyl-3-hexaprenyl-1,4-naphthoquinone), which is designated thermoplasmaquinone (Carlone and Anet, 1983; Collins *et al.*, 1984). The *MK-6 has been suggested as a useful chemical marker of *Campylobacter* species (Moss *et al.*, 1984). However, *W. succinogenes* has both menaquinones, and *C. pylori* has no *MK-6 (Collins and Fernandez, 1984; Goodwin *et al.*, 1986; Kröger and Innerhofer, 1976a). In *W. succinogenes,* electron transport between low-potential cytochrome *b* and menaquinone is inhibited by 2-(n-nonyl)-4-hydroxyquinoline *N*-oxide (Kröger and Innerhofer, 1976b).

Bacteria that can respire with O_2 as the terminal electron acceptor have cytochromes which function in a manner similar to that of the cytochromes of eucaryotic mitochondria. The types of cytochromes in bacterial electron transport systems vary widely, and there are no apparent requirements for any particular cytochrome type. Moreover, the concentrations of cytochromes vary, particularly with growth conditions (Smith, 1978). Some bacterial species synthesize branched electron transport systems, which may include as many as three different terminal oxidases.

The four major types of cytochromes are the *a-, b-, c-,* and *d*-types; cytochrome *d* is found only in bacteria. Cytochrome a contains heme a with a formyl side chain as the prosthetic group. In cytochrome b protoheme is the prosthetic group, and there are no covalent linkages to the protein. Cytochrome *c* has covalent linkages between heme side chains and the protein, and the prosthetic

group of cytochrome *d* is a chlorin (iron-dihydroporphyrin). The heme prosthetic groups of cytochromes have the typical absorption spectra of hemochromes in the reduced form, with sharp α- and β-absorption bands in the visible region of the absorption spectrum and a strong band in the near ultraviolet (the Soret band). Cytochromes designated by a primed letter, e.g., *c'*, or by some other nomenclature such as cytochromes *o* or P-450 (which are *b*-type cytochromes), are not hemochromes and show different kinds of absorption spectra.

CYTOCHROMES OF CAMPYLOBACTERS AND RELATED ORGANISMS

Relatively little information is available about the respiratory systems of most campylobacters and related bacteria, but studies have been made of the respiratory systems of *C. jejuni, C. fetus* subsp. *fetus, C. sputorum* biovar bubulus, and *C. mucosalis.* The respiratory system of *W. succinogenes* has also been studied, but only as ananaerobic respiratory system (with fumarate as the terminal electron acceptor). The proposed models of the electron transport systems of these organisms is illustrated in Fig. 1.

C. fetus subsp. *fetus* was reported by Harvey and Lascelles (1980) to contain membrane-bound cytochrome *c*, cytochrome *b*, and a CO-binding cytochrome, and also soluble cytochrome *c* and CO-binding cytochrome. They suggested that the respiratory chain contains low- and high-potential forms of cytochromes *b* and *c*. Lascelles and Calder (1985) reported that the cytochrome *c'* of *C. fetus* subsp. *fetus* was the major CO-reactive pigment in both the membrane and soluble fractions of cell extracts. They also reported amembrane-associated high-potential cytochrome-oxidase system, which included cytochrome *c* (reducible by succinate and by ascorbate-TMPD) and a cyanide-sensitive terminal oxidase. Little information is available about the role of soluble cytochrome *c* in *C. fetus* subsp. *fetus.* Harvey and Lascelles (1980)

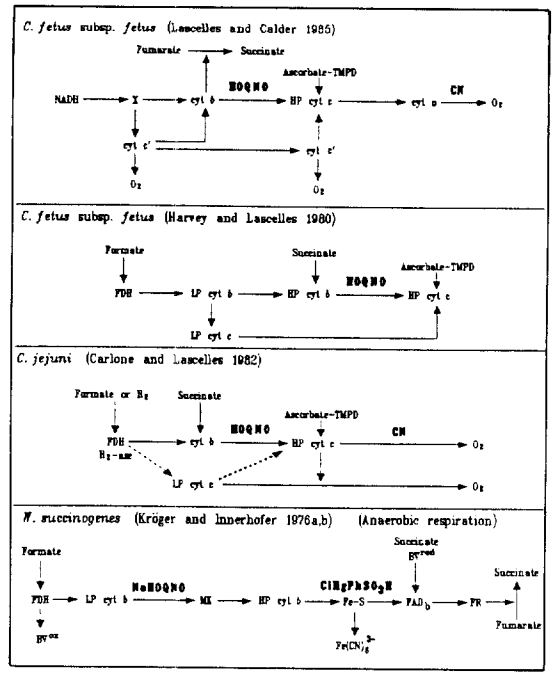


Fig. 1. Proposed models of respiratory systems in *C. fetus* subsp. *fetus, C. jejuni,* and *W. succinogenes.* Abbreviations: TMPD, N,N,N',N'-tetramethyl-*p*-phenylenediamine; LP and HP are low- and high-potential, respectively; X, unknown intermediate; cyt *c'*, CO-binding cytochrome c; FDH, formate dehydrogenase; MK, menaquinone; Fe-S, iron-sulfur protein; FADb, membrane-bound FAD; FR, fumarate reductase; BV^{ox} and BV^{red}, oxidized- and reduced-benzyl viologen. Compounds in bold face type are inhibitors: HOQNO, 2-heptyl-4-hydroxyquinoline-*N*-oxide; CN, cyanide; No-HOQNO, 2-(n-nonyl)-4-hydroxyquinoline-*N*-oxide; $ClHgPhSO_3H$, 4-chloromercuriphenyl sulfonate.

and Lascelles and Calder (1985) suggested that the soluble pigment might be responsible for cyanide-insensitive NADPH oxidase activity in the cytoplasm.

C. jejuni has been reported to contain high- and low-potential forms of cytochrome *b* and *c* (Carlone and Lascelles, 1982). These forms were thought to transfer electrons to a complex terminal oxidase system. The predominant CO-binding pigment in *C. jejuni* appeared to be a form of cytochrome *c*. The compound 2-heptyl-4-hydroxyquino-

line *N*-oxide inhibited the electron transport system between cytochrome *b* and cytochrome *c*.

C. sputorum biovar bubulus has been reported to contain cytochromes *b*, cytochrome *c*, and a CO-binding pigment (Niekus *et al.*, 1977; Niekus *et al.*, 1980a; Niekus *et al.*, 1980b).

C. mucosalis possesses high- (−135 mV) and low-potential (−300 mV) cytochrome *c*, and also CO-binding cytochrome (Elkurdi *et al.*, 1982; Goodhew *et al.*, 1988; Lawson *et al.*, 1981). Elkurdi *et al.* (1982) found soluble cytochrome c (+86 mV) and possibly cytochrome *b* in the soluble fraction.

W. succinogenes has been reported to possess high-potential cytochromes *b* (−20 mV) and *c* (+70 mV), and also low-potential cytochromes *b* (−200 mV) and *c* (−160 mV). Soluble cytochrome *c* was also present. Anaerobic respiration (with fumarate as an electron acceptor) required cytochrome *b* and other electron transport components but not cytochrome *c* (Jacobs and Wolin, 1963b; K rtner *et al.*, 1990; Kröger and Innerhofer, 1976a; Kröger and Innerhofer, 1976b; Kröger *et al.*, 1979; Kröger *et al.*, 1980; Unden *et al.*, 1982; Wolin *et al.*, 1961). Jacobs and Wolin (1963a, 1963b) and Wolin *et al.* (1961), reported the presence of cytochrome *b* and *c*; this finding, and the demonstration of the ability of the organism to utilize O_2 under a microaerobic atmosphere, suggested that electrons might be transferred from the oxidizable substrate (formate or H_2) to O_2 via cytochrome *b* and *c*.

W. recta, W. curva, B. ureolyticus, and *B. gracilis* have been reported to contain soluble cytochrome *c* and CO-binding cytochrome *c* as well as membrane-bound cytochrome *b*, *c*, and CO-binding cytochrome *c* (Han *et al.*, 1992; Jackson and Goodman, 1978).

TERMINAL OXIDASES

According to Jurtshuk *et al.* (1975), a bacterial terminal oxidase is the final enzyme complex of the electron transport chain and transfers electrons from the respiratory chain directly to O_2, the terminal electron acceptor. The complex is usually referred to as the cytochrome *c* oxidase or, more simply, the cytochrome oxidase. To characterize the terminal oxidase of the electron transport system of a bacterium, two methods are usually used. (i) The competitive binding of carbon monoxide. This is based on specific binding of carbon monoxide at the same site at which molecular oxygen is activated prior to its binding. (ii) Inhibition by cyanide of a cytochrome oxidase reaction, or a TMPD oxidase reaction. Sensitivity of the reaction to cyanide at concentrations of 5×10^{-4} M is indicative of a functional terminal oxidase consisting of a high-potential cytochrome c.

Campylobacter species seem to have multiple terminal oxidase systems, which differ in their sensitivity to cyanide, but none of these has been characterized (Carlone and Lascelles, 1982; Harvey and Lascelles, 1980; Hoffman and Goodman, 1982; Niekus *et al.*, 1980b). Although the terminal oxidase(s) of *C. fetus* subsp. *fetus* has not been identified, there is spectroscopic evidence of a cytochrome *o* type of pigment (Lascelles and Calder, 1985). Harvey and Lascelles (1980) suggested that the cytochrome *c'* of *C. fetus* subsp. *fetus* might be a functional terminal oxidase. Carlone and Lascelles (1982) reported spectrophotometric evidence for the occurrence of cytochrome *o* in *C. jejuni;* however, Hoffman and Goodman (1982) were unable to detect cytochrome *o*. No conventional cytochrome oxidases have been detected in *C. sputorum* biovar bubulus (Niekus *et al.*, 1980a, 1980b), although there was cytochrome c peroxidase activity. However, both cytochrome oxidase and cytochrome peroxidase are usually considered to play a role in the terminal electron transfer from cytochrome *c* to O_2, and small amounts of cytochrome oxidase in *C. sputorum* might be masked by the large peaks of the CO-binding cytochrome *c* in reduced plus CO minus reduced difference spectrum.

No spectrophotometric evidences for cytochrome oxidases (cytochrome a/a_3, cytochrome *d*, or cytochrome *o*) in *W. recta, W. curva,* and *B. ureoly-*

ticus have been reported, although there were cytochrome *c* oxidase activities (except that *B. gracilis* has no cytochrome *c* oxidase activity; Han *et al.*, 1992).

CONCLUDING REMARKS

An explosive burst of interest for the genus *Campylobacater* and its relatives (the genera *Wolinella* and *Helicobacater*) has continued to present, since some of these species were recognized as important pathogens in humans and animals. Although they are chemoorganotrophs, they neither oxidise nor ferment carbohydrates as carbon and energy sources. Due to the limited number of physiological and biochemnical tests, it has been very difficult to characterize them by using conventional tests for clinical and veterinary laboratories.

With regard to responses to oxygen for growth, they show microaerophilic charateristics. I.e., they neither grow in the presence of a level of oxygen equivalent to that present in an air atmosphere (21% oxygen) nor grow under an anaerobic environment. They, like aerobic bacteria, respire with oxygen as a terminal electron acceptor and contain cytochrome *c* in their respiratory systems; however, they do not grow under aerobic conditions due to the toxic forms of oxygen (O_2^-, $OH^\cdot$, OH^-, or H_2O_2). Unless any possible electron acceptors (nitrate, nitrite, or fumarate) are supplied, they are incapable of growing under anaerobic conditions.

Regardless of species of the genus *Campylobacater* and its relatives, they usually contain cytochrome *b* and cytochrome *c* in their respiratory systems. Some species contain soluble cytochrome *c* as well as membrane-bound cytochrome *b* and *c*. The CO-binding cytochromes occurr in a few species. Some species possess cytochrome *o* as the terminal oxidase. Redox titration of membrane and soluble fraction of oxidase-positive and oxidase-negative bacteria might help to explain how *B. gracilis* is oxidase-negative and yet contains cytochrome *c*. The role or function of soluble cytochrome and CO-binding cytochrome is unknown. Yet in view of the fact that the cells produce large amounts of these water-soluble redox proteins, and expend energy while doing so, it is logical to assume that these proteins play some important role for the life of the cells. It is difficult to understand how they could function as components of the respiratory chain if they are not integral membrane proteins. It would be interesting to test these substances for alternative functions, such as nitrite reductase activity or an ability to quench toxic forms of oxygen such as superoxide radicals.

Microaerophilic characteristics of the genus *Campylobacter* and its relatives might be due to their susceptibilities to toxic forms of oxygen which are produced by their oxygen respiration. Because some species lack protective enzymes (SOD, catalase, or peroxidase) against the toxic oxygen derivatives, their DNA, low-potential enzymes, or lipid may be damaged by them. Although there are many possible explanations, it might be interesting to determine the effect of various oxygen concentrations on the kinetics of the terminal oxidase system in these bacteria. It is possible that this system might have a very low Km value, allowing it to scavenge very low levels of oxygen;if so, high levels of oxygen might actually inhibit the terminal oxidase system. It might be pertinent that Hoffman and Goodman (1982) reported a low-redox potential cytochrome *c* oxidase in *C. jejuni*, and that the partially purified cytochrome was inhibited by excess O_2 and H_2O_2.

Little information has, so far, been available about the types of respiratory systems in other *Campylobacter* species and related bacteria, except for *C. fetus* subsp. *fetus*, *C. jejuni*, and *W. succinogenes*.

REFERENCES

1. Baumann, P., A.L. Furniss, and J.V. Lee. 1984. Genus *Vibrio*, pp. 518-538. *In* N.R. Krieg, and J.G. Holt (ed.) Bergey's manual of systematic

bacteriology, vol. 1, The Williams and Wilkins Co., Inc., Baltimore, MD.

2. Brawn, K., and I. Fridovich. 1980. DNA strand scission by enzymatically generated oxygen radicals. *Arch. Biochem. Biophys.* **206**: 414-419.
3. Butzler, J.-P., and M.B. Skirrow. 1979. *Campylobacter* enteritis. *Clin. Gastroenterol.* **8**: 737-765.
4. Byczkowski, J.Z., and T. Gessner. 1988. Biological role of superoxide anion-radical. *Int. J. Biochem.* **20**: 569-580.
5. Carlone, G.M., and F.A.L. Anet. 1983. Detection of menaquinone-6 and a novel methyl substitute menaquinone-6 in *Campylobacter jejuni* and *Campylobacter fetus subsp. fetus. J. Gen. Microbiol.* **129**: 3385-3395.
6. Carlone, G.M., and J. Lascelles. 1982. Aerobic and anaerobic respiratory systems in *Campylobacter fetus* subsp. *jejuni* grown in atmospheres containing hydrogen. *J. Bacteriol.* **152**: 306-314.
7. Carlsson, J., G. Nyberg, and J. Wrethen. 1978. Hydrogen peroxide and superoxide radical formation in anaerobic broth media exposed to atmospheric oxygen. *Appl. Environ. Microbiol.* **36**: 223-229.
8. Cato, E.P., W.L. Lance, and S.M. Finegold. 1986. Genus *Clostridium*, pp. 1141-1200. *In* P. A. Sneath, N.S. Mair, M.E. Sharpe, and J.G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams and Wilkins Co., Baltimore, MD.
9. Collins, M.D., M. Costas, and R.J. Owen. 1984. Isoprenoid quinone composition of representatives of the genus *Campylobacter. Arch. Microbiol.* **137**: 168-170.
10. Collins, M.D., and F. Fernandez. 1984. Menaquinone-6 and thermoplasmaquinone-6 in *Wolinella succinogenes. FEMS Microbiol. Lett.* **22**: 273-276.
11. Collins, M.D., and D. Jones. 1981. Distribution of isoprenoid quinone structural types in bacteria and their taxonomic implications. *Microbiol. Rev.* **45**: 316-354.
12. Dekeyser, P., M. Gossuin-Detrain, J.-P. Butzler, and J. Sternon. 1972. Acute enteritis due to related vibrio: first positive stool cultures. *J. Infect. Dis.* **125**: 390-392.
13. Di Mascio, P., H. Wefers, H.-P. Do-Thi, M.V.M. Lafleur, and H. Sies. 1989. Singlet molecular oxygen causes loss of biological activity in plasmid and bacteriophage DNA and induces single-strand breaks. *Biochim. Biophys. Acta* **1007**: 151-157.
14. Elkurdi A.B., J.L. Leaver, and G.W. Pettigrew. 1982. The c-type cytochromes of *Campylobacter sputorum* ssp. *mucosalis. FEMS Microbiol. Lett.* **14**: 177-182.
15. Florent, A. 1959. Les deux vibriosis génitales: la vibriose due à *V. fetus venerealis* et la vibriose d'origine intestinale due à *V. fetus intestinalis. Meded. Veeartsenijsch. Rijksuniv. Gent* **3**: 1-60.
16. Gebhart, C.J., G.E. Ward, K. Chang, and H.J. Kurtz. 1983. *Campylobacter hyointestinalis* (new species) isolated from swine with lesions of proliferative ileitis. *Am. J. Vet. Res.* **44**: 361-367.
17. Gebhart, C.J., P. Edmonds, G.E. Ward, H.J. Kurtz, and D.J. Brenner. 1985. "*Campylobacter hyointestinalis*" sp. nov.:a new species of *Campylobacter* found in the intestines of pigs and other animals. *J. Clin. Microbiol.* **21**: 715-720.
18. Goodhew, C.F., A.B. Elkurdi, and G.W. Pettigrew. 1988. The microaerophilic respiration of *Campylobacter mucosalis. Biochim. Biophys. Acta* **933**: 114-123.
19. Goodwin, C.S., J.A. Armstrong, T. Chilvers, M. Peters, M.D. Collins, L. Sly, W. McConnell, and W.E.S. Harper. 1989. Transfer of *Campylobacter pylori* and *Campylobacter mustelae* to *Helicobacter* gen. nov. as *Helicobacter pylori* comb. nov. and *Helicobacter mustelae* comb. nov., respectively. *Int. J. Syst. Bacteriol.* **39**: 397-405.
20. Goodwin, C.S., M.D. Collins, and E. Blincow. 1986. The absence of thermoplasmaquinones in *Campylobacter pyloridis,* and its temperature and pH growth range. *Microbios Lett.* **32**: 137-140.
21. Han, Y.-H., R.M. Smibert, and N.R. Krieg. 1992. Cytochrome composition and oxygen-de-

pendent respiration-driven proton translocation in *Wolinella curva, Wolinella recta, Bacteroides ureolyticus,* and *Bacteroides gracilis. Can. J. Microbiol.* **38**: 104-110.

22. Hardie, J.M. 1986. Genus *Streptococcus,* pp. 1043-1071. In P.A. Sneath, N.S. Mair, M.E. Sharpe, and J.G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 2. The Williams and Wilkins Co., Baltimore, MD.
23. Harvey, S., and J. Lascelles. 1980. Respiratory systems and cytochromes in *Campylobacter fetus* subsp. *intestinalis. J. Bacteriol.* **144**: 917-922.
24. Hoffman, P.S., N.R. Krieg, and R.M. Smibert. 1979. Studies of the microaerophilic nature of *Campylobacter fetus* subsp. *jejuni.* I. Physiological aspects of enhanced aerotolerance. *Can. J. Microbiol.* **25**: 1-7.
25. Hoffman, P.S., and T.G. Goodman. 1982. Respiratory physiology and energy conservation efficiency of *Campylobacter jejuni. J. Bacteriol.* **150**: 319-326.
26. Holdeman, L.V., R.W. Kelley, and W.C. Moore. 1984. Genus *Bacteroides,* pp. 604-631. *In* N.R. Krieg and J.G. Holt(ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams and Wilkins Co., Baltimore, MD.
27. Irgens, R.L. 1984. Genus *Meniscus,* pp. 135-137. *In* N.R. Krieg and J.G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams and Wilkins Co., Baltimore, MD.
28. Jackson, F.L., and Y.E. Goodman. 1978. *Bacteroides ureolyticus,* a new species to accommodate strains previously identified as "Bacteroides corrodens, anaerobic". *Int. J. Syst. Bacteriol.* **28**: 197-200.
29. Jacobs, N.J., and M.J. Wolin. 1963a. Electron-transport system of *Vibrio succinogenes.* I. Enzymes and cytochromes of the electron-transport system. *Biochim. Biophys. Acta* **69**: 18-28.
30. Jacobs, N.J., and M.J. Wolin. 1963b. Electron-transport system of *Vibrio succinogenes.* II. Inhibition of electron transport by 2-heptyl-4-hydroxyquinoline *N*-oxide. *Biochim. Biophys. Acta* **69**: 29-39.
31. Jurtshuk, P.,Jr., T.J. Mueller, and W.C. Acord. 1975. Bacterial terminal oxidases. *Crit. Rev. Microbiol.* **3**: 399-468.
32. Körtner, C., F. Lauterbach, D. Tripier, G. Unden, and A. Kröger. 1990. *Wolinella succinogenes* fumarate reductase contains a dihaem cytochrome *b. Molec. Microbiol.* **4**: 855-860.
33. Krieg, N.R. 1984. Genus *Spirillum,* pp. 90-93. In N.R. Krieg and J.G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams and Wilkins Co., Baltimore, MD.
34. Krieg, N.R., and P.S. Hoffman. 1986. Microaerophily and oxygen toxicity. *Annu. Rev. Microbiol.* **40**: 107-130.
35. Kröger, A., E. Dorrer, and E. Winkler. 1980. The orientation of the substrate sites of formate dehydrogenase and fumarate reductase in the membrane of *Vibrio succinogenes. Biochim. Biophys. Acta* **589**: 118-136.
36. Kröger, A., and A. Innerhofer. 1976a. The function of menaquinone, covalently-bound FAD and iron-sulfur protein in the electron transport from formate to fumarate of *Vibrio succinogenes. Eur. J. Biochem.* **69**: 487-495.
37. Kröger, A., and A. Innerhofer. 1976b. The function of the b cytochromes in the electron transport from formate to fumarate of *Vibrio succinogenes. Eur. J. Biochem.* **69**: 497-506.
38. Kröger, A., E. Winkler, A. Innerhofer, H. Hackenberg, and H. Schägger. 1979. The formate dehydrogenase involved in electron transport from formate to fumarate in *Vibrio succinogenes. Eur. J. Biochem.* **94**: 465-475.
39. Lascelles, J., and K.M. Calder. 1985. Participation of cytochromes in some oxidation-reduction system in *Campylobacter fetus. J. Bacteriol.* **164**: 401-409.
40. Lawson, G.H.K., J.L. Leaver, G.W. Pettigrew, and A.C. Rowland. 1981. Some features of *Campylobacter sputorum* subspecies *mucosalis* subsp. nov., nom. rev. and their taxonomic significance. *Int. J. Syst. Bacteriol.* **31**: 385-391.
41. Marshall, B.J. 1986. *Campylobacter pyloridis* and gastritis. *J. Infect. Dis.* **153**: 650-657.
42. Marshall, B.J., H. Royce, D.I. Annear, C.S. Goodwin, J.W. Pearman, J.R. Warren, and J.A. Armstrong. 1984. Original isolation of *Campy-*

lobacter pyloridis from human gastric mucosa. *Microbios. Lett.* **25**: 83-88.

43. Misra, H.P., and I. Fridovich. 1971. The generation of superoxide radical during the autooxidation of ferredoxins. *J. Biol. Chem.* **246**: 6886-6990.
44. Moss, C.W., A. Kai, M.A. Lambert., C. Patton. 1984. Isoprenoid quinone content and cellular fatty acid composition of *Campylobacter species. J. Clin. Microbiol.* **19**: 772-776.
45. Niekus, H.G.D., W. de Vries, and A.H. Stouthamer. 1977. The effect of different dissolved oxygen tension on growth and enzyme activities of *Campylobacter sputorum* subspecies *sputorum. J. Gen. Microbiol.* **103**: 215-222.
46. Niekus, H.G.D., E. Van Doorn, W. de Vries, and A.H. Stouthamer. 1980a. Aerobic growth of *Campylobacter sputorum* subspecies *bubulus* with formate. *J. Gen. Microbiol.* **118**: 419-428.
47. Niekus, H.G.D., E. Van Doorn, and A.H. Stouthamer. 1980b. Oxygen consumption by *Campylobacter sputorum subspecies bubulus* with formate as substrate. *Arch. Microbiol.* **127**: 137-143.
48. Padgett, P.J., W.H. Cover, and N.R. Krieg. 1981. The microaerophile *Spirillum volutans*: Cultivation on complex liquid and solid media. *Appl. Environ. Microbiol.* **43**: 469-477.
49. Padgett, P.J., and N.R. Krieg. 1986. Factors relating to the aerotolerance of *Spirillum volutans. Can. J. Microbiol.* **32**: 548-552.
50. Palleroni, N.J. 1984. Genus Pseudomonas, pp. 141-199. *In* N.R. Krieg and J.G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams and Wilkins Co., Baltimore, MD.
51. Penner, J.L. 1988. The genus Campylobacter: a decade of progress. *Clin. Microbiol. Rev.* **1**: 157-172.
52. Ramos, J.L., and R.L. Robson. 1985. Isolation and properties of mutants of *Azotobacter chroococcum* defective in aerobic nitrogen fixation. *J. Gen. Microbiol.* **131**: 1449-1458.
53. Richard, C. 1984. Genus *Enterobacter,* pp. 465-469. *In* N.R. Krieg and J.G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams and Wilkins Co., Baltimore, MD.
54. Skirrow, M.B., and J. Benjamin. 1980. "1001" campylobacters: cultural characteristics of intestinal campylobacters from man and animals. *J. Hyg.* **85**: 427-442.
55. Smibert, R.M. 1984. Genus *Campylobacter,* pp. 111-118. *In* N.R. Krieg, and J.G. Holt (ed.) Bergey's manual of systematic bacteriology, vol. 1, The Williams and Wilkins Co., Inc., Baltimore, MD.
56. Smith, L. 1978. Bacterial cytochromes and their spectral characterization. *Meth. Enzymol.* LIII: 202-212.
57. Steiner, B.M., G.H. Wong, P. Sutgrave, and S. Graves. 1984. Oxygen toxicity in Treponema *pallidum*: deoxyribonucleic acid single-stranded breakage induced by low doses of hydrogen peroxide. *Can. J. Microbiol.* **30**: 1467-1476.
58. Stouthamer, A.H., W. De Vries, and H.G.D. Niekus. 1979. Microaerophily. *Antonie van Leeuwenhoek* **45**: 5-12.
59. Unden, G., R. Böcher, J. Knecht, and A. Kröger. 1982. Hydrogenase from *Vibrio succinogenes,* a nickel protein. *FEBS Lett.* **145**: 230-233.
60. Vedros, N.A. 1984. Genus *Neisseria,* pp. 290-296. *In* N.R. Krieg and J.G. Holt (ed.), Bergey's manual of systematic bacteriology, vol. 1. The Williams and Wilkins Co., Baltimore, MD.
61. Wolin, M.J., E.A. Wolin, and N.J. Jacobs. 1961. Cytochrome-producing anaerobic vibrio, *Vibrio succinogenes,* sp. n. *J. Bacteriol.* **81**: 911-917.