Acetone carboxylase (Acx) is a key enzyme involved in the biodegradation of acetone by bacteria. Except for the Helicobacteraceae family, genome analyses revealed that bacteria that possess an Acx, such as Cupriavidus metallidurans strain CH34, are associated with soil. The Acx of CH34 forms the heterohexameric complex αβγ and can carboxylate only acetone and 2-butanone in an ATP-dependent reaction to acetoacetate and 3-keto-2-methylbutyrate, respectively.

Acetone is a toxic compound found in air, water, and soil, both naturally as a pollutant (5, 17) and also as a result of being produced by mammals and bacteria (8, 11, 12, 23). Acetone can also be degraded by various bacteria using a CO₂-dependent pathway including acetone carboxylase as a key enzyme, a property of increasing interest for bioremediation purposes (1–3, 6, 7, 9, 10, 15, 16, 18–22). Acetone carboxylase (Acx) is a member of a protein family that also contains acetophenone carboxylase and ATP-dependent hydantoinases/oxoprolinases. While the members of this family share several similar characteristics, they differ with respect to the substrates, the products of ATP hydrolysis, and structural properties (16).

Genome analyses revealed a lot of bacterial species that possess the acetone carboxylase and thus are potentially able to detoxify acetone (see Fig. S1 in the supplemental material). Most of these bacteria, such as Cupriavidus metallidurans, were found in soil or in contact with soil (e.g., by plant symbiosis) and belong to Proteobacteria and especially Betaproteobacteria. In the Alphaproteobacteria class, the Rhizobiales and the Rhodobacterales orders were found to contain an Acx. In the Deltaproteobacteria class, only one species (Geobacter uranireducens R4), up to now, was discovered to contain an Acx, which had around 30% amino acid (aa) sequence identity with the CH34 Acx depending on the subunit. The only pathogenic species that possess the enzyme are those belonging to the Helicobacteraceae family (Epsilonproteobacteria) which are found in the mammalian stomach (4).

In general, similar gene organizations for the Acx operon were found, with three genes, acxA, acxB, and acxC, encoding the three acetone carboxylase subunits (β, α, and γ subunits, respectively) and one regulator, acxR, which was identified as a σ₅₄- or σ₇₀-specific transcriptional regulator and can be divergently transcribed (19). The only known paralogous enzyme with similar biochemical function is acetophenone carboxylase from Aromatoleum aromaticum (16). This enzyme consists of a hetero-octa-mer of four subunits whose corresponding genes apcABCDE are clustered as an operon. Acetone carboxylase does not contain a parologue of ApcE.

Acetone carboxylase induction in Cupriavidus metallidurans. A recent study focused on acetone metabolism in C. metallidurans CH34, a betaproteobacterium found in industrial biotopes highly contaminated with metals (14), showing an overexpression of the acetone carboxylase when grown in spacelight conditions (13). As observed in Rhodobacter capsulatus and Xanthobacter autotrophicus, the C. metallidurans Acx subunits were induced at a high level (19% ± 4% of the total proteins) when acetone was present in the culture (Fig. 1) (19, 20). An acxR knockout mutant was constructed in this study. This mutant, in which no acetone carboxylase was produced (Fig. 1), was unable to grow with acetone or isopropanol. High expression of this enzyme may compensate for a low turnover number for catalysis, allowing a reasonable rate of acetone carboxylation to support growth with a relatively low doubling time (4 to 20 h for X. autotrophicus, R. capsulatus, and C. metallidurans CH34) (19).

Acetone carboxylase purification and characterization. The partial characterization of acetone carboxylase was conducted in X. autotrophicus strain Py2, Rhodococcus rhodochrous strain B276, R. capsulatus strain B10, Alicyclus denitrificans strain K601, two species of Paracoccus, and very recently Aromatoleum aromaticum, showing high structural similarities (1, 6, 7, 15, 16, 18–20).

In this study, the Acx of CH34 was purified according to a two-step procedure consisting of anion DEAE-Sepharose chromatography followed by Sephacryl S300 molecular filtration (18, 19). The native molecular mass of the acetone carboxylase complex was determined by gel filtration and was estimated to be 388 ± 15 kDa, corresponding to an αβγ configuration (86, 76, and 19 kDa for the α, β, and γ subunits, respectively), as described previously in other bacteria (6, 7, 16, 18, 19). The absorption spectrum of acetone carboxylase between 250 and 350 nm exhibited a maximal peak at 287.2 nm, which is close to the value obtained in X. autotrophicus (281 nm) (18).

Enzymatic activity. Depending on the species, the properties of Acx enzymes differ with regard to the substrates and cofactors required to support the carboxylation reaction (1, 6, 16, 18–20).
The *C. metallidurans* enzyme showed poor stability and maximum activity in a pH range of 6.5 to 8.0.

Of all the tested high-energy compounds (ATP, ITP, UTP, or GTP), only Mg-ATP supported acetone carboxylation in CH34, resulting in acetoacetate formation (Fig. 2). Similar results were obtained in *X. autotrophicus*, *A. aromaticum*, and *R. capsulatus*, while in *R. rhodochrous*, no activity was observed with ATP (6, 16, 19). As observed in these bacteria, the acetone carboxylase reaction in CH34 showed that ATP is hydrolyzed into AMP and 2 inorganic phosphates.

NH$_4^+$ ions have also been shown to increase acetone carboxylase activity (19). Yet tests performed in the presence of NH$_4$Cl (100 mM) showed no significant increase in acetoacetate production by the Acx of CH34. In contrast, as observed in *X. autotrophicus*, potassium (40 mM) and CO$_2$ (KHCO$_3$) sources stimulated the CH34 acetone carboxylase activity.

The specific activities obtained with the purified enzyme of CH34 were 0.4 to 0.6 U/mg, compared to 0.08 to 0.240 U/mg for the other Acx enzymes (6, 16, 18, 19). Nevertheless, the comparison in terms of activity has to be taken cautiously due to the differences observed with the stability of the purified enzymes.

Among all the tested substrates, only acetone and 2-butanone were identified as substrates of the *C. metallidurans* Acx (Fig. 2). Interestingly, we showed that *C. metallidurans* CH34 was also able to grow in the presence of 2-butanone as the sole carbon source. Studies in *X. autotrophicus* and *A. aromaticum* also revealed that 2-butanone was the only alternative substrate of acetone carboxylase (16, 18). In *R. rhodochrous*, the acetone carboxylase was found to utilize a wider range of substrates, including 2-butanone, which was consumed at a rate identical to that of acetone, and also 2-pentanone, 3-pentanone, and 2-hexanone, which were degraded at rates that were 70, 40, and 42% of the rate of acetone, respectively (6).

We propose for *C. metallidurans* that 3-keto-2-methylbutyrate obtained by carboxylation of 2-butanone was then activated to coenzyme A (CoA) thioester and thiolytically cleaved to propionyl-CoA and acetyl-CoA, as observed in the leucine catabolism pathway.

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**FIG 1** Acetone carboxylase expression. SDS-PAGE of protein extracts (10 µg) from CH34 grown in the presence of 9 mM gluconate (1), 25 mM acetone (2), 25 mM isopropanol (3), and 25 mM n-propanol (4). (5) Protein extract (10 µg) from the acxR knockout mutant cultivated in the presence of acetone 25 mM. (6) Purified acetone carboxylase (5 µg). Molecular mass is indicated on the left.

**FIG 2** Comparison of the acetone and acetophenone carboxylases from various species. 1, information obtained from reference 16; 2, information obtained from references 18 and 19; 3, information obtained from reference 19; 4, information obtained from reference 6; 5, information obtained from reference 10. A superscript “a” indicates that the products of the enzymatic reaction were not identified in *X. autotrophicus* and *R. capsulatus*; for *A. aromaticum*, the nature of the product was suggested by the authors but not experimentally identified. A superscript “b” indicates that the product of the enzymatic reaction was not identified. ND, not determined. +, supports the Acx reaction at different levels; −, does not support the Acx reaction.
In conclusion, *C. metallidurans* CH34 is able to degrade acetone and, besides acetone, only 2-butanol using an ATP-dependent pathway including the Acx enzyme. The corresponding *acx* genes are located on the second chromosome or chromid.

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