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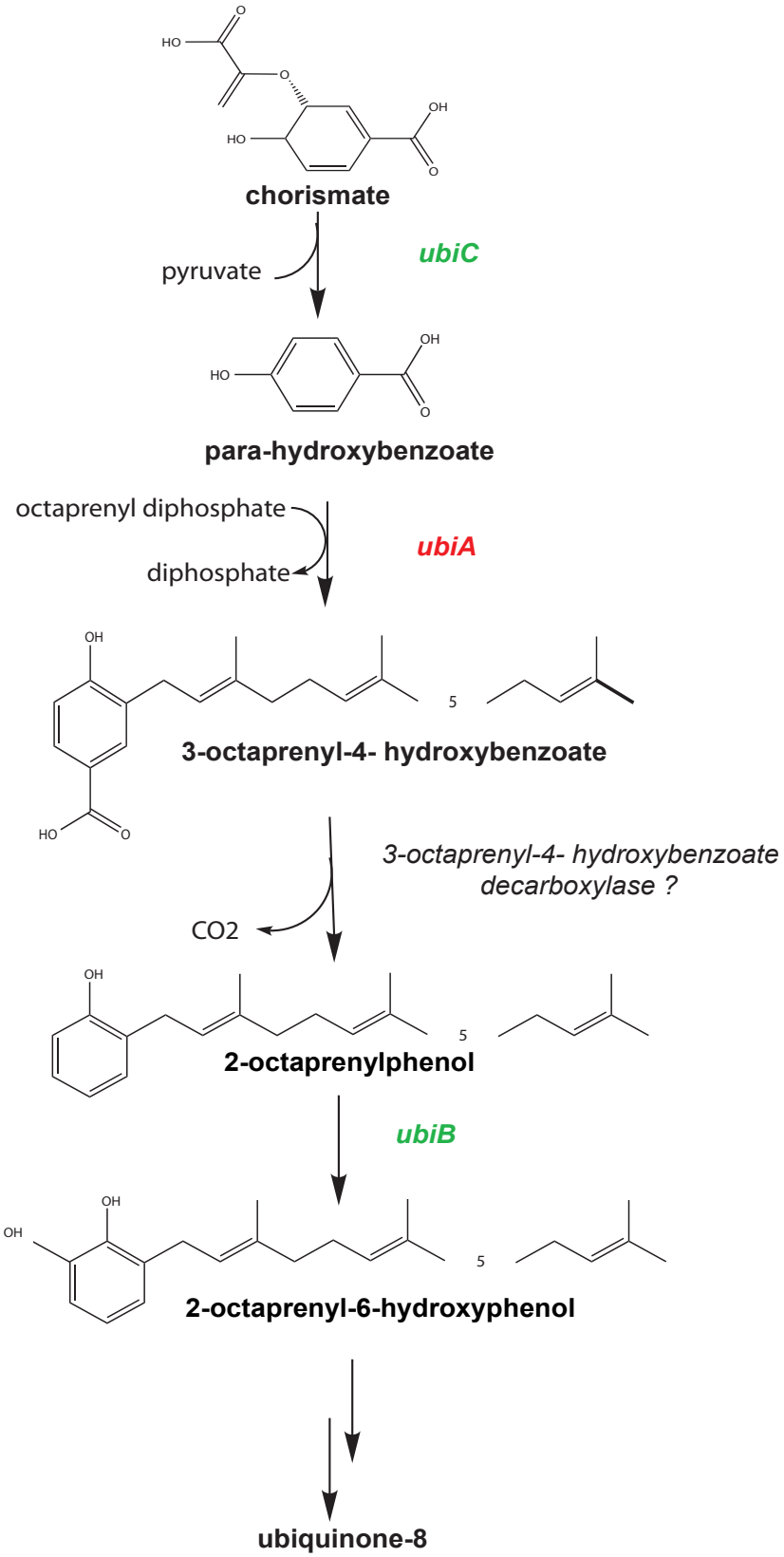
Supplementary Annexe 1 : Double Band (DB) mutants: essential or non essential genes?

Out of the 206 DB mutants obtained, 121 have clear orthologues (Best Bidirectional Hit and same gene name) with *E. coli* and *P. aeruginosa* genes. Of them, 51 are described as essential in at least one of the following references in *E. coli* (Baba *et al*., 2006; Gerdes *et al*., 2003) and at least one of the following references in *P. aeruginosa* PA01 and PA14 (Jacobs *et al*., 2003, Liberati *et al*., 2006), 23 are described as essential in either all *E. coli* references or in all *P. aeruginosa* references. All the data on gene essentiality used above are inferred from mutant libraries obtained on a rich medium (i.e. LB) and no conditional essential genes (i.e. metabolic genes involved in biosynthesis of amino acids, cofactors, etc) are supposed to be present in these lists of essential genes. Our library was obtained on minimal medium and a number of the metabolic genes are supposed to be required for life under these conditions. Fortunately, mutants of the Keio collection were tested for growth on minimal medium resulting in conditional essential genes identification. Out the 46 remaining DB mutants, we have found 25 genes where the *E. coli* orthologue mutants are severely impaired for growth on minimal medium (OD <0.1). In addition, for the 22 remaining DB mutants, it should be noticed that some of them are described as essential in the literature (alanyl-tRNA synthetase, loss of function of *thiG*, *thiD* and *thiC* genes resulting respectively in thiazole, pyrimydine and thiamine auxotrophy). In some other cases, the discrepancies could result from the use of different experimental conditions or highlight metabolic differences between *E. coli* and *A. baylyi* ADP1. For example, (1) the gene *dctA* encodes an aerobic C4-dicarboxylate transport protein and is expected to be essential for growth with succinate as the sole carbon source, (2) three acetolactate synthase isozymes are present in *E. coli* K-12 while only one complex (*ilvH*, *ilvI*) seems to be present in *A. baylyi* ADP1. In total, 105 DB mutants (87%) show strong evidence for essentiality or conditional essentiality of the corresponding genes. Supplementary Table 2 shows information on the essentiality of the 121 genes including the 16 genes were no evidence for essentiality were found.

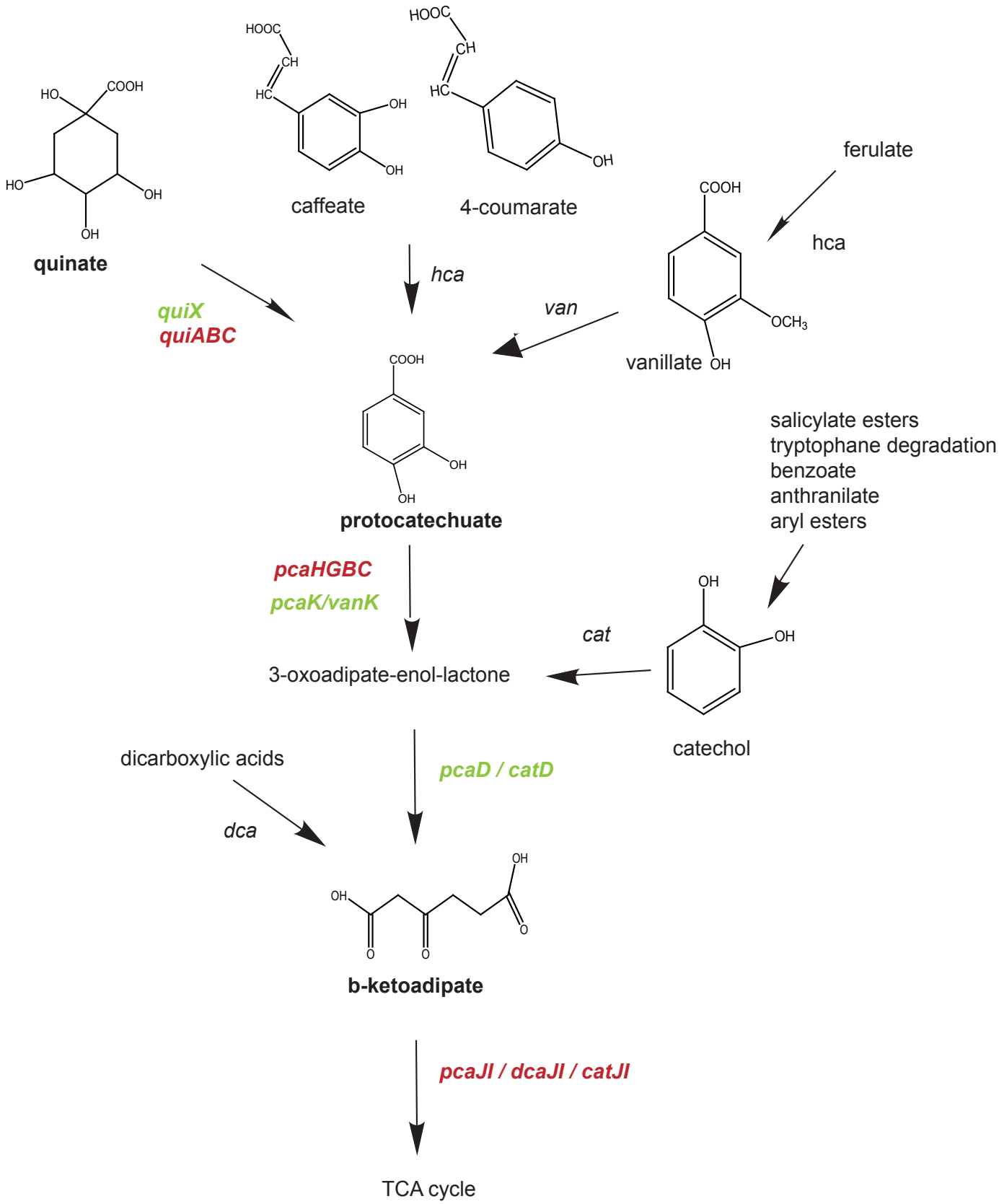
Bacteria mutant collections	Number of essential genes	Orthologous genes in *Acinetobacter baylyi* ADP1			
		Number of genes	Essential in the compared bacteria	Essential also in *A.* ADP1	Dispensable in *A.* ADP1
Escherischia coli					
Baba *et al.,* 2006 (Keio collection)	303	1177	213	189 (89%)	24
Gerdes *et al.,* 2003	620	1177	285	186 (65%)	99
Hashimoto *et al.,* 2005 (PEC database)	302	1177	186	164 (88%)	22
Pseudomonas aeruginosa					
Liberati *et al.,* 2006	335	1655	209	167 (80%)	42
Jacobs *et al.,* 2003	678	1655	316	215 (68%)	101
Bacillus subtilis					
Kobayashi *et al.,* 2003	271	704	172	149 (87%)	23
Corynebacterium glutamicum					
Suzuki *et al.,* 2007	658	655	208	153 (71%)	55

Supplementary Table III Summary of gene essentiality data of *A. baylyi* ADP1 and others mutant collections (*E. coli*, *P. aeruginosa*, *B. subtilis* and *C. glutamicum*).

Column 1: name of bacterial mutant collection of the reference row bacteria
Column 2: number of essential genes according the reference cited in column 1 .
Column 3: number of orthologous genes between *A. baylyi* ADP1 and the reference row bacteria
Column 4: number of essential genes in the reference row bacteria having orthologues in *A. baylyi* ADP1 with essentiality data (dispensable/essential)
Column 5: number of essential genes (column 4) that is also essential in *A. baylyi* ADP1; the percentage of genes essential in both collections is noted between brackets
Column 6: number of essential genes (column 4) that is dispensable in *A. baylyi* ADP1



Supplementary Figure 1 First steps of ubiquinone biosynthesis.
Essential genes are in red and dispensable genes are in green



Supplementary Figure 2 Degradation pathways for aromatic compounds *via* protocatechuate in *Acinetobacter baylyi* ADP1

Supplementary Annexe 2 : Specificity and sensitivity of the growth phenotyping approach

Quinate dissimilation

The quinate dissimilation, is composed of 13 genes corresponding to 10 enzymatic activities and two transporters (Supplementary Figure 2).

Our profiling for growth on quinate has shown the essentiality of (1) *quiA, quiB and quiC* which are involved in the three steps of degradation from quinate to protocatechuate. It should be notice that *quiB* is not complemented by *aroQ* even if these share to same enzymatic activity due to a different cellular localization (periplasm *vs* cytoplasm). (2) *pqqACDE* which are involved in pyrroloquinoline quinone (PQQ) biosynthesis confirming the requirement of PQQ cofactor for QuiA dehydrogenase activity (Elsemore and Ornston, 1994); (3) four genes of the *pca* operon (*pcaH*, *pcaG*, *pcaB* and *pcaC*) involved in the degradation of protocatechuate to 3-ketoadipate (4) three genes of the *pca* operon (*pcaI*, *pcaJ*, *pcaF)* involved in 3-ketoadipate degradation. The dispensability of (1) *quiX* (quinate porin) (Elsemore and Ornston, 1995); (2) *pcaD* which could be explained by the presence of *catD*; (3) *pcaK* for which an isofunctional genes vanK exist (D'Argenio *et al.*, 1999), (4) *pqqB* which was expected to be essential and is the only discrepancy we found in this degradation pathway.

Glucose assimilation

The glucose dissimilation has been biochemically described by Juni, (1978) and during the genome annotation process genes have been associated with these enzymatic activities (Barbe *et al.*, 2004). This pathway involves two transporters (glucose and gluconate) and 5 genes corresponding to 5 enzymatic activities (Supplementary Figure 3).

Mutants were obtained for all of these and five of them (*edd*/ACIAD0542, *eda*/ACIAD0543, *gntT*/ACIAD0544, *gntK*/ACIAD0545, *gcd*/ACIAD2983) are unable to grow on glucose. The mutants of glucose porin (ACIAD2984, which flanks the glucose dehydrogenase gene) and the gluconolactonase gene are not impaired for growth on glucose. The glucose dehydrogenase (*gcd*) is PQQ-dependent and the five mutants of *pqq* genes, involved in the biosynthesis of PQQ are, as expected, unable to grow on glucose. Moreover the *gap*/ACIAD0546 is essential and further analyses are required to understand its role in glucose degradation.

References

Elsemore DA and Ornston LN (1994) The *pca-pob* supraoperonic cluster of *Acinetobacter calcoaceticus* contains *quiA*, the structural gene for quinate-shikimate dehydrogenase. *J Bacteriol* **176**: 7659-7666

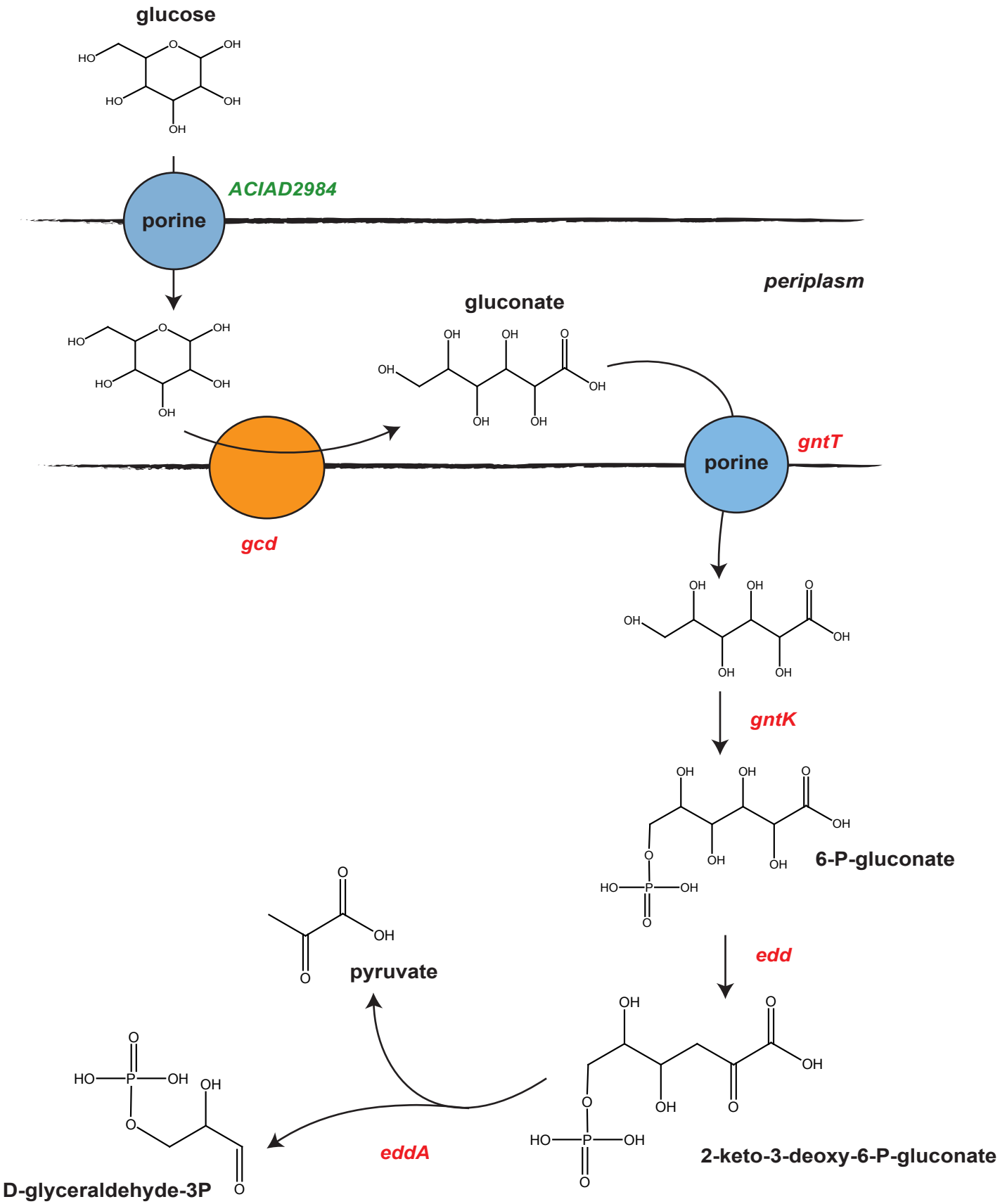
Elsemore DA and Ornston LN (1995) Unusual ancestry of dehydratases associated with quinate catabolism in *Acinetobacter calcoaceticus. J Bacteriol* **177**: 5971-5978

Shanley MS, Harrison A, Parales RE, Kowalchuk G, Mitchell DJ, Ornston LN (1994) Unusual G + C content and codon usage in *catIJF*, a segment of the *ben-cat* supra-operonic cluster in the *Acinetobacter calcoaceticus* chromosome. *Gene* **138**: 59-65

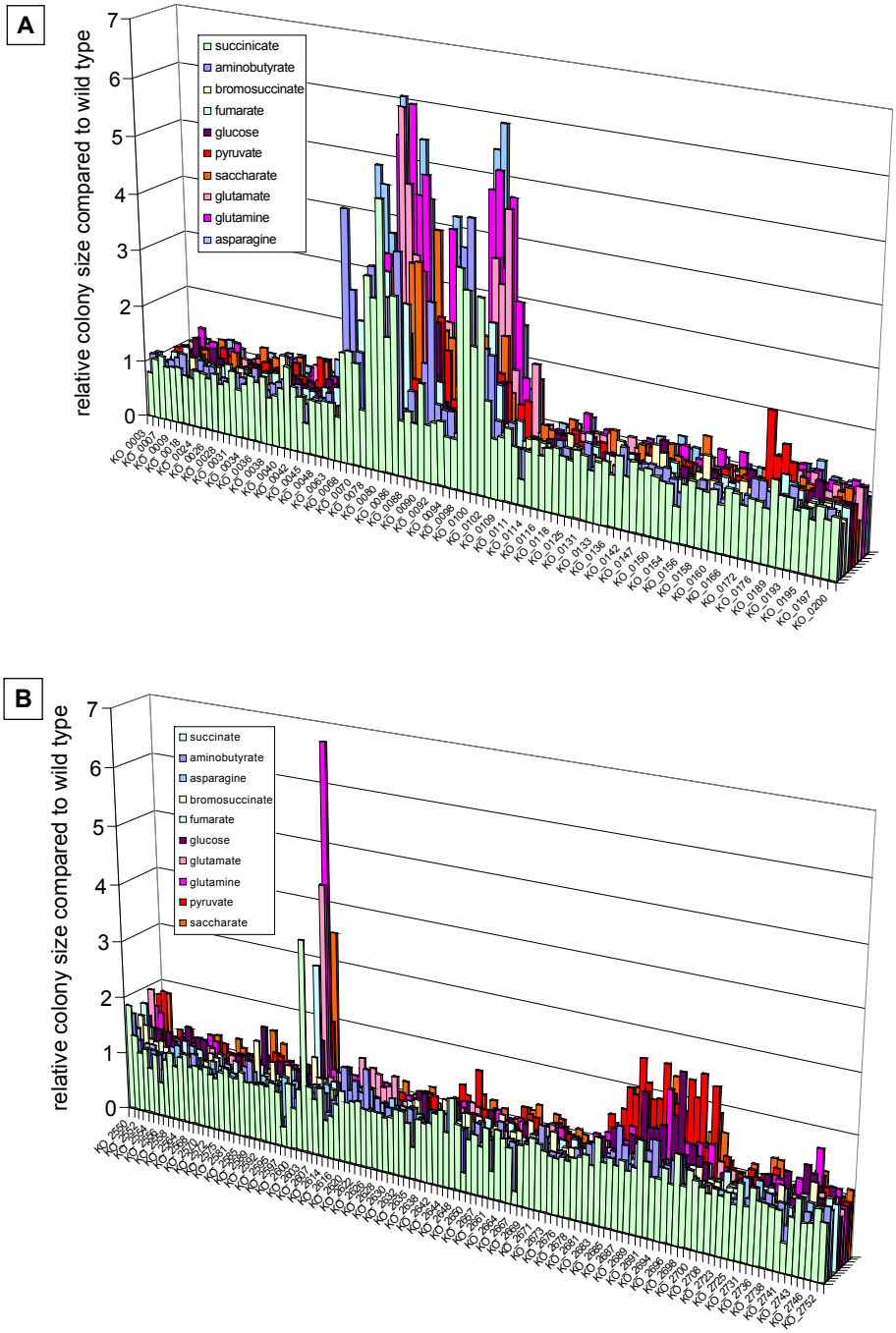
D'Argenio DA, Segura A, Coco WM, Bunz PV, Ornston LN (1999) The physiological contribution of *Acinetobacter pcaK*, a transporter system that acts upon protocatechuate, can be masked by overlapping specificity of *vanK*. *J Bacteriol* **181**: 3505-3515

Juni E (1978) Genetics and physiology of *Acinetobacter*. *Annu Rev Microbiol* **32**: 349-371

Barbe V, Vallenet D, Fonknechten N, Kreimeyer A, Oztas S, Labarre L, Cruveiller S, Robert C, Duprat S, Wincker P, Ornston LN, Weissenbach J, Marliere P, Cohen GN, Medigue C (2004) Unique features revealed by the genome sequence of *Acinetobacter sp*. ADP1, a versatile and naturally transformation competent bacterium. *Nucleic Acids Res* **32**: 5766-5779



Supplementary Figure 3 Glucose assimilation pathway in *Acinetobacter baylyi* ADP1



Supplementary Figure 4 Regions of *A. baylyi* ADP1 genome enriched in large size colonies. The relative size of mutant colonies, grown on solid media using several carbon sources, is reported according to the chromosomal location of their corresponding deleted gene.

Gene	Organisms	Primer sequence
metA _PacI	***E. coli***	5-gggccttaattaaATGCCGATTCGTGTGCCGGACG
metA _NotI	***E. coli***	5'-ataagaatgcggccgcTTAATCCAGCGTTGGATTCATG
metX _PacI	ADP1	5-gggccttaattaaGTGTCTTTTCCATCTGACTCAGTGG
metX _NotI	ADP1	5'-ataagaatgcggccgcTCAGTTGATCTCCACTTGAGG
metW _PacI	ADP1	5'-gggccttaattaaCTGATGCGTATTGACCAACAATTG
metW _NotI	ADP1	5'-ataagaatgcggccgcTCATAGAGCGCTCACTCGG
metX/metW _PacI	ADP1	5'-gggccttaattaaGTGTCTTTTCCATCTGACTCAGTGG
metX/metW _NotI	ADP1	5'-ataagaatgcggccgcTCATAGAGCGCTCACTCGG
metY _PacI	ADP1	5'-gggccttaattaaATGACTCATCGCTACAAAGATG
metY _NotI	ADP1	5'-ataagaatgcggccgcTCAAACTGTGGCCAATGCTTG
ACIAD3524_PacI	ADP1	5'-gggccttaattaaTTGCGGTATAAATATCCCCATAGAG
ACIAD3524_NotI	ADP1	5'-ataagaatgcggccgcTTAAACAGCAGCACAGTTTTTTTCAG

Supplementary Table VII Primers used for the amplification of the genes involved in methionine biosynthesis