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BRIEF REPORT

Control of a pyrimidine ribonucleotide salvage pathway in *Pseudomonas oleovorans*

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Abstract

The control of a pyrimidine ribonucleotide salvage pathway in the bacterium *Pseudomonas oleovorans* ATCC 8062 was studied. This bacterium is important for its ability to synthesize polyesters as well as for its increasing clinical significance in humans. The pyrimidine salvage pathway enzymes pyrimidine nucleotide *N*-ribosidase and cytosine deaminase were investigated in *P. oleovorans* ATCC 8062 under selected culture conditions. Initially, the effect of carbon source on the two pyrimidine salvage enzymes in ATCC 8062 cells was examined and it was observed that cell growth on the carbon source succinate generally produced higher enzyme activities than did glucose or glycerol as a carbon source when ammonium sulfate served as the nitrogen source. Using succinate as a carbon source, growth on dihydrouracil as nitrogen source caused a 1.9-fold increase in the pyrimidine nucleotide *N*-ribosidase activity and a 4.8-fold increase in cytosine deaminase activity compared to the ammonium sulfate-grown cells. Growth of ATCC 8062 cells on cytosine or dihydrothymine as a nitrogen source elevated deaminase activity by more than double that observed for ammonium sulfate-grown cells. The findings indicated a relationship between this pyrimidine salvage pathway and the pyrimidine reductive catabolic pathway since growth on dihydrouracil appeared to increase the degradation of the pyrimidine ribonucleotide monophosphates to uracil. The uracil produced could be degraded by the pyrimidine base reductive catabolic pathway to β-alanine as a source of nitrogen. This investigation could prove helpful to future work examining the metabolic relationship between pyrimidine salvage pathways and pyrimidine reductive catabolism in pseudomonads.

Keywords Pyrimidine salvage · Regulation · Pyrimidine ribonucleotide *N*-ribosidase · Cytosine deaminase · *Pseudomonas oleovorans*

Introduction

The gram-negative bacterium *Pseudomonas oleovorans* has been shown to produce polyhydroxyalkanoates that can be used to produce bioplastics of commercial interest (Brandl et al. 1988; Hazenberg and Witholt 1997; Prieto et al. 1999; Allen et al. 2010; Santhanam and Sasidharan 2010). A recent study has shown the bacterium to cause sepsis in humans indicating that it may be of clinical significance (Gautam et al. 2015). With *P. oleovorans* generating interest relative to its ability to be used for bioplastics and its increasing clinical significance, its nucleic acid metabolism has been previously investigated where the regulation of pyrimidine biosynthesis in *P. oleovorans* ATCC 8062 was explored (Haugaard and West 2002). The pyrimidine biosynthetic pathway enzyme activities aspartate transcarbamoylase (EC 2.1.3.2), dihydroorotase (EC 3.5.2.3), dihydroorotate dehydrogenase (EC 1.3.3.1), orotate phosphoribosyltransferase (EC 2.4.2.10) and orotidine 5'-monophosphate (OMP) decarboxylase (EC 4.1.1.23) were detected in the ATCC 8062 cells (Haugaard and West 2002). Interestingly, growth in a uracil-containing succinate minimal medium induced the aspartate transcarbamoylase and dihydroorotase activities in the ATCC 8062 cells (Haugaard and West 2002). At the enzyme activity level, aspartate transcarbamoylase was strongly inhibited by pyrophosphate, UDP, UTP, GTP and ATP (Haugaard and West 2002).

Although regulation of pyrimidine biosynthesis in *P. oleovorans* has been studied, much less is known regarding

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the control of the pyrimidine salvage pathways in this bacterium. It is known that pyrimidine salvage pathway differs in *P. oleovorans* ATCC 8062 from other related pseudomonads such as *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* and this may be related to taxonomic differences between the species (Yamamoto et al. 2000; Beck and O'Donovan 2008; Saha et al. 2010). While phylogenetically analyzing a pseudomonad isolate, it was determined that the isolate was a subspecies of *P. oleovorans* (Saha et al. 2010). Based on DNA-DNA relatedness, chemotaxonomic as well as molecular profiles of *P. pseudoalcaligenes* ATCC 17740 and *P. oleovorans* ATCC 8062, it was concluded that *P. pseudoalcaligenes* ATCC 17740 should be classified as a subspecies of *P. oleovorans* ATCC 8062 (Saha et al. 2010). While *P. oleovorans* ATCC 8062 cells contains a pyrimidine ribonucleotide *N*-ribosidase (EC 3.2.2.10) and cytosine deaminase activities (EC 3.5.4.1), cells of *P. pseudoalcaligenes* ATCC 17440 contain ribonucleoside hydrolase (EC 3.2.2.8) and cytosine deaminase activities (West 1991a) indicating pyrimidine salvage pathway differences between these strains (Fig. 1). The pyrimidine ribonucleotide *N*-ribosidase activity has been purified to homogeneity from *P. oleovorans* ATCC 8062 cells and the purified enzyme can degrade CMP or UMP to cytosine or uracil, respectively, along with ribose-5-phosphate (Yu 2005). It has also been shown (Sakai et al. 1971a, 1971b, 1976) that ATCC 8062 cells contain cytosine deaminase activity that deaminates cytosine to uracil (Fig. 1). The focus of this research was to investigate the regulation of the pyrimidine ribonucleotide *N*-ribosidase and cytosine deaminase activities in *P. oleovorans* relative to cell growth on selected carbon or nitrogen source to determine whether their enzyme activities were influenced. The findings from this work should reveal new information regarding whether this novel pyrimidine salvage pathway in *P. oleovorans* is regulated in response to growth conditions.

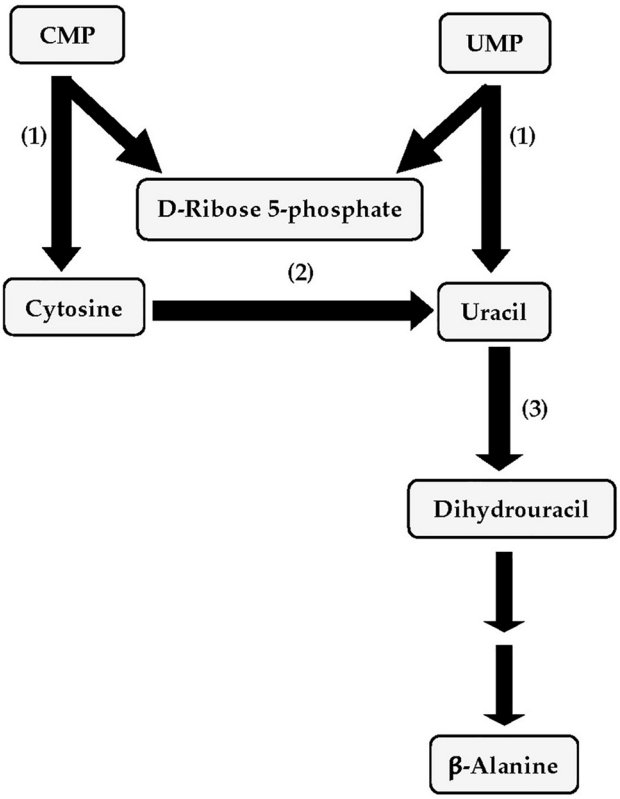

Fig. 1 Degradation of pyrimidine ribonucleotide monophosphates in *P. oleovorans* by the enzymes (1) pyrimidine nucleotide *N*-ribosidase; (2) cytosine deaminase; and (3) dihydropyrimidine dehydrogenase

Materials and methods

Strain and culture conditions

The strain utilized in this study was *Pseudomonas oleovorans* ATCC 8062 (Lee and Chandler 1941). The strain was grown in a minimal medium modified as previously described (West 1989). When added to the modified minimal medium as a carbon source, succinate, glucose or glycerol was added at a concentration of 0.4% (v/v). In those experiments in which uracil, cytosine, 5-methylcytosine, uridine, cytidine, dihydrouracil, dihydrothymine, β-alanine or β-aminoisobutyric acid served as the nitrogen source at a concentration of 0.2% (w/v), 0.4% (w/v) ammonium sulfate was not present in the medium. To learn which compounds supported the growth of the pseudomonad strain as a sole nitrogen or carbon source, approximately 10^6 washed bacterial cells were used to inoculate each respective medium (5 mL). Subsequently, these cultures were shaken (200 rpm) for 120 h at 30 °C. To serve as a control, minimal medium cultures and cultures lacking a nitrogen or carbon source, were also inoculated and shaken for 120 h at 30 °C. Growth was followed spectrophotometrically at 600 nm. Bacterial cell concentration (colonies mL^{-1}) was estimated from a previously determined A_{600} versus cell concentration calibration curve. Microscopic examination of the cells did not indicate that variations in their shape had occurred during the growth period.

Preparation of cell extracts

Batch cultures (60 mL) of *P. oleovorans* were grown by shaking in 250 mL flasks at 30 °C and harvested when a concentration of about 10^8 cells mL^{-1} is achieved during the exponential phase of growth. The cells were washed with 0.85% NaCl and collected by centrifugation at 7,155 × g for 20 min at 4 °C. The cell pellet was resuspended in 50 mM Tris–HCl buffer pH 7.30 (3 mL) and the cells ultrasonically

disrupted in an ice bath using 30 s bursts for a total of 4 min. Subsequently, the extract was centrifuged at 12,100 × *g* for 30 min at 4 °C and the cell-free extract was dialyzed against Tris–hydrochloride buffer pH 7.3 for 16 h at 4 °C (West 1991a).

Enzyme and protein assays

Enzyme assays were performed at 30 °C. The activity of the enzyme pyrimidine nucleotide *N*-ribosidase was measured using the difference in the molar extinction coefficients ($\Delta\varepsilon$) between UMP and uracil at 290 nm under alkaline conditions (Sakai et al. 1971b; Yu 2004). The difference in the molar extinction coefficients ($\Delta\varepsilon$) between UMP and uracil at 290 nm and pH 12 was $\Delta\varepsilon = 4.8 \times 10^3$ M^{-1} cm^{-1}. The assay mixture (1 mL) contained 50 mM Tris–HCl buffer, pH 7.3, 60 mM $MgCl_2$, 5 mM UMP and cell extract. At various time intervals, assay mixture (0.1 mL) was removed and was added to 1 N NaOH (0.9 mL) to halt the reaction. The background absorbance was 0.2–0.3. A unit of pyrimidine ribonucleotide *N*-ribosidase activity is expressed as nmol UMP hydrolyzed to uracil min^{-1}. The cytosine deaminase assay utilized was a continuous assay based on the difference in the molar extinction coefficients ($\Delta\varepsilon$) between cytosine and uracil at 285 nm and pH 7.30 being $\Delta\varepsilon = 1.04 \times 10^3$ $M^{-1} \cdot cm^{-1}$ (West et al. 1982; West 1992). The background absorbance was 0.3–0.4. The assay mix (1 mL) contained 50 mM Tris–HCl buffer (pH 7.30), cell extract and 0.50 mM cytosine. The change in absorbance of the mix was followed at 285 nm against a control assay mix (lacking cytosine) for a period of 10 min. A unit of cytosine deaminase activity is expressed as nmol cytosine deaminated to uracil min^{-1}. Protein was measured according to Bradford (1976) using lysozyme as the standard protein. Specific activity for both enzymes was expressed as nmol uracil formed·min^{-1} (mg protein)$^{-1}$.

Statistical analysis

Specific activity was defined as nmol substrate utilized or product formed·min^{-1} (mg protein)$^{-1}$ at 30 °C with each being the mean of three independent determinations. During the statistical analysis, the Student's *t* test was used to compare individual pairs of pyrimidine nucleotide *N*-ribosidase and cytosine deaminase activities shown in Tables 2 and 3.

Results and discussion

It has been noted previously that succinate, glucose or glycerol can serve as a carbon source to support growth of *P. oleovorans* ATCC 8042 (Lee and Chandler 1941; Haugaard and West 2002). The ability of pyrimidine-related compounds as possible sources of nitrogen was studied when succinate served as the carbon source (Table 1). After 120 h at 30 °C, it appeared that the dihydropyrimidine bases, dihydrouracil and dihydrothymine, supported the highest level of growth of ATCC 8062 cells (Table 1). The pyrimidine bases uracil, thymine, cytosine and 5-methylcytosine supported similar levels of ATCC 8062 cell growth after 120 h at 30 °C as nitrogen sources (Table 1). In contrast, the pyrimidine biosynthetic pathway intermediate, orotic acid, was unable to support the growth of ATCC 8062 as a nitrogen source while the pyrimidine ribonucleoside cytidine supported the

Table 1 Bacterial cell concentrations of *P. oleovorans* ATCC 8062 grown on selected nitrogen sources using succinate as the carbon source based on absorption measurements at 600 nm

Nitrogen source	Bacterial cells $mL^{-1} \times 10^6$
Orotic acid	0.8 (0.1)
Cytidine	5.3 (0.0)
Uridine	1.1 (0.1)
Cytosine	4.6 (0.1)
5-Methylcytosine	5.7 (0.1)
Uracil	6.4 (0.1)
Dihydrouracil	9.1 (0.3)
β-Alanine	7.3 (0.3)
Thymine	5.1 (0.1)
Dihydrothymine	9.9 (0.1)
β-Aminoisobutyric acid	7.3 (0.2)

The minimal medium contained 0.4% (v/v) succinate as the carbon source and 0.2% (w/v) nitrogen source. Liquid medium cultures were grown for 120 h at 30 °C. Results represent the mean of 3 separate determinations. The number in parentheses indicates the standard deviation. After 120 h at 30 °C, control cultures of minimal medium lacking a carbon source or minimal medium lacking a nitrogen source contained 5.90×10^4 cells mL^{-1} or 1.40×10^5 cells mL^{-1}, respectively

Table 2 Effect on synthesis of pyrimidine ribonucleotide *N*-ribosidase and cytosine deaminase activities in *P. oleovorans* ATCC 8062 relative to carbon source

Carbon source	Specific activity [nmol min^{-1} (mg protein)$^{-1}$]	
	Pyrimidine ribonucleotide *N*-ribosidase	Cytosine deaminase
Succinate	7.5 (1.7)[a]	24.1 (0.8)[a]
Glucose	4.0 (0.3)[b]	21.8 (0.8)[b]
Glycerol	10.1 (2.1)[a]	21.6 (1.1)[b]

The minimal medium contained 0.4% (v/v) carbon source and 0.4% (w/v) ammonium sulfate as the nitrogen source. Each value is the mean of three independent determinations (SD). Superscripts in the same column of data that have a common letter show no statistical significance between them while those that do not have a common letter are statistically different ($P < 0.01$) using Student's *t* test. Individual pairs of data in each column were analyzed statistically to determine if statistical significance existed between each pair

Table 3 Effect of nitrogen source on pyrimidine ribonucleotide *N*-ribosidase and cytosine deaminase activities in succinate-grown *P. oleovorans* ATCC 8062 cells

Nitrogen source	Specific activity [nmol min^{-1} (mg protein)$^{-1}$]	
	Pyrimidine ribonucleotide *N*-ribosidase	Cytosine deaminase
Ammonium sulfate	7.5 (1.7)[a]	24.1 (0.8)[a]
Cytidine	3.1 (0.8)[b]	28.4 (1.0)[b]
5-Methylcytosine	2.6 (0.2)[b]	29.3 (1.1)[b]
Cytosine	5.3 (1.1)[a]	60.6 (0.9)[c]
Uracil	2.0 (0.8)[b]	33.7 (1.0)[d]
Thymine	6.9 (0.6)[a]	36.6 (0.3)[d]
Dihydrouracil	14.2 (3.5)[c]	116.5 (2.8)[e]
Dihydrothymine	2.1 (1.5)[b]	56.4 (1.7)[c]
β-Alanine	2.4 (0.8)[b]	28.4 (1.3)[b]
β-Aminoisobutyric acid	5.0 (0.8)[a]	15.9 (1.0)[f]

The minimal medium contained 0.4% (v/v) succinate as the carbon source and 0.2% (w/v) nitrogen source except for 0.4% (w/v) ammonium sulfate. Each value is the mean of three independent determinations (SD). Superscripts in the same column of data that have a common letter show no statistical significance between them while those that do not have a common letter are statistically different ($P < 0.01$) using Student's *t* test. Individual pairs of data in each column were analyzed statistically to determine if statistical significance existed between each pair

growth of ATCC 8062 cells as a nitrogen source unlike the pyrimidine ribonucleoside uridine (Table 1). The products of the pyrimidine reductive catabolic pathway, β-alanine and β-aminoisobutyric acid, were both able to support the growth of the ATCC 8062 cells as a nitrogen source (Table 1). The growth data indicate that the compounds associated with the pyrimidine reductive catabolic pathway all supported growth of *P. oleovorans* ATCC 8042.

Having identified which carbon and nitrogen sources support the growth of the ATCC 8062 cells, it was of interest to learn how growth on these carbon and nitrogen sources influenced the activities of the two pyrimidine salvage pathway enzymes, pyrimidine nucleotide *N*-ribosidase and cytosine deaminase. With respect to pyrimidine nucleotide *N*-ribosidase, its activity was slightly affected by carbon source (Table 2). With ammonium sulfate as a nitrogen source, it was observed that the *N*-ribosidase activity was 1.9-fold or 2.5-fold higher, respectively, when either succinate or glycerol as a carbon source compared to glucose as a carbon source (Table 2). On the other hand, growth of ATCC 8062 cells on succinate, glucose or glycerol as a carbon source had little effect upon cytosine deaminase activity when ammonium sulfate served as the nitrogen source (Table 2). The effect of carbon source on pyrimidine nucleotide *N*-ribosidase and cytosine deaminase was likely related to enzyme synthesis rather than activity. It has been shown in previous studies that carbon source can have a repressive effect on enzyme synthesis in pseudomonads (Rojo 2010). Catabolite repression of pyrimidine nucleotide *N*-ribosidase by glucose or succinate compared to glycerol would appear to be a factor while it was not for cytosine deaminase (Table 2). Other studies have investigated whether cytosine deaminase activity in pseudomonads were affected by carbon source. In *Pseudomonas lemonierri*, cytosine deaminase activity was 1.6-fold higher in cell grown on glycerol and ammonium sulfate compared to cells grown on glucose and ammonium sulfate (West 1988). In *Pseudomonas stutzeri* cells grown on glucose as a carbon source and ammonium sulfate as a nitrogen source, cytosine deaminase activity was more than double the activity of cells grown on glycerol and ammonium sulfate (West 1990). In *Pseudomonas mendocina*, cytosine deaminase activity was the same in the cells grown on glucose or glycerol as a carbon source and ammonium sulfate as a nitrogen source (West 1990). It is clear from these prior studies that catabolite repression of cytosine deaminase synthesis in related pseudomonads is variable and is dependent on the species being studied.

Having examined the effect of carbon source on the two pyrimidine salvage pathway enzymes in *P. oleovorans*, the influence of nitrogen source upon the levels of both enzymes was next explored. When succinate minimal medium cells of ATCC 8062 were grown on cytidine as a nitrogen source, pyrimidine nucleotide *N*-ribosidase activity was shown to decrease by about 60% compared to its activity on the succinate-grown cells using ammonium sulfate as a nitrogen source (Table 3). Growth of ATCC 8062 cells on the pyrimidine base 5-methylcytosine also produced a 65% drop in its activity compared to the succinate-grown cells using ammonium sulfate as a nitrogen source (Table 3). It was observed that growth on the pyrimidine base cytosine as a nitrogen source diminished the pyrimidine nucleotide *N*-ribosidase activity by 29% compared to the ammonium sulfate-grown cells (Table 3). Using uracil as a nitrogen source compared to ammonium sulfate, a reduction in *N*-ribosidase activity by 70% was observed (Table 3). In contrast, growth of ATCC 8062 on thymine as a nitrogen source had a slight effect on the activity of pyrimidine nucleotide *N*-ribosidase compared to its activity when ammonium sulfate served as a nitrogen source (Table 3). The pyrimidine reductive catabolic pathway intermediates and products dihydrouracil, dihydrothymine, β-alanine and β-aminoisobutyric acid were studied as nitrogen sources to determine if they could influence the activity of pyrimidine salvage pathway enzymes in ATCC 8062 succinate-grown cells. Using dihydrouracil as a nitrogen source, the *N*-ribosidase activity of the ATCC 8062 cells rose by 1.9-fold compared to its activity in ammonium sulfate-grown cells (Table 3). Interestingly, growth of ATCC 8062 cells on the dihydropyrimidine base dihydrothymine

had the opposite effect on *N*-ribosidase activity (Table 3). It was observed that when succinate-grown ATCC 8062 cells were grown on dihydrothymine as a nitrogen, a 70% reduction in activity was observed compared to the activity in ammonium sulfate-grown cells (Table 3). This indicated that growth on the dihydropyrimidine bases had very different effects on the levels of the *N*-ribosidase activity in ATCC 8062 cells. Next, succinate-grown ATCC 8062 cells were grown on β-alanine as a nitrogen source and it was observed that the *N*-ribosidase activity decreased by 70% compared to its activity in ammonium sulfate-grown cells (Table 3). In contrast, succinate-grown ATCC 8062 cells using β-aminoisobutyric acid as a nitrogen source contained *N*-ribosidase activity that was slightly decreased by 33% compared to its activity in ammonium sulfate-grown cells (Table 3). The effect of nitrogen source on pyrimidine nucleotide *N*-ribosidase was probably related to enzyme synthesis rather than enzyme inhibition since allosteric control of this enzyme has not been reported (Yu 2005).

With respect to cytosine deaminase activity, growth of ATCC 8062 cells on cytidine as a nitrogen source produced a slight 1.2-fold increase in cytosine deaminase activity relative to its activity in ammonium sulfate-grown cells (Table 3). In *P. aeruginosa* glucose-grown cells, cytosine deaminase activity increased ninefold when cytidine served as a nitrogen source compared to ammonium sulfate as a nitrogen source (West 1996). The influence of the nitrogen source cytosine, uracil or thymine upon cytosine deaminase activity in ATCC 8062 succinate-grown cells was also investigated. Cytosine as a nitrogen source increased cytosine deaminase in ATCC 8062 succinate-grown cells by 2.5-fold compared to cells grown on ammonium sulfate as a nitrogen source (Table 3). This indicates growth on cytosine induces cytosine deaminase synthesis in ATCC 8062. With cytosine being the substrate of cytosine deaminase, it is probably not surprising that growth on cytosine as a nitrogen source elevated its enzyme activity since it has been witnessed for other pseudomonads. When *P. fluorescens* strain A126 was grown on glucose as a carbon source and cytosine as a nitrogen, cytosine deaminase activity was elevated by 3.4-fold compared to its activity in cells using ammonium sulfate as a nitrogen source (Chu and West 1990). In *P. pseudoalcaligenes* ATCC 17,440 succinate-grown cells, cytosine deaminase activity increased by 5.9-fold when cytosine served as the nitrogen source instead of ammonium sulfate (West 1991a). In *P. aeruginosa* glucose-grown cells, cytosine as a nitrogen source increased cytosine deaminase activity by more than fivefold compared to ammonium sulfate as a nitrogen source (West 1996). In ATCC 8062 succinate-grown cells, 5-methylcytosine increased deaminase activity slightly by 1.2-fold compared to its activity in cells using ammonium sulfate as a nitrogen source (Table 3). In *P. aeruginosa* glucose-grown cells, 5-methylcytosine as a nitrogen source increased cytosine deaminase activity by more than 20-fold compared to ammonium sulfate as a nitrogen source (West 1996). When uracil or thymine served as the nitrogen source in ATCC 8062 succinate-grown cells, cytosine deaminase increased by at least 1.4-fold relative to its activity in the succinate-grown ATCC 8062 cells using ammonium sulfate as the nitrogen source (Table 3). A prior study showed that cytosine deaminase activity in *P. pseudoalcaligenes* ATCC 17440 succinate-grown cells was elevated by 2.1-fold when uracil served as the nitrogen source compared to its activity in ammonium sulfate-grown cells (West 1991a). In *P. aeruginosa* glucose-grown cells, uracil or thymine as a nitrogen source increased cytosine deaminase activity by about 19-fold or 16-fold, respectively, compared to ammonium sulfate as a nitrogen source (West 1996). Relative to dihydropyrimidine bases as nitrogen sources, it was observed that growth on dihydrouracil as a nitrogen source by succinate-grown ATCC 8062 caused nearly a fivefold increase in cytosine deaminase activity compared to its activity in ammonium sulfate-grown cells (Table 3). This was the largest increase in cytosine deaminase activity observed in ATCC 8062 cells relative to the nitrogen sources tested. When dihydrothymine served as the nitrogen source for the succinate-grown ATCC 8062 cells, cytosine deaminase activity was shown to increase by 2.3-fold compared to its activity in the ammonium sulfate-grown cells (Table 3). The findings showed that growth of ATCC 8062 cells on pyrimidine or dihydropyrimidine bases as nitrogen sources produced a greater elevation in cytosine deaminase activity than it does for the pyrimidine ribonucleotide *N*-ribosidase activity (Table 3). With no report of allosteric regulation of cytosine deaminase activity, it can be concluded that regulation of its synthesis is occurring. It should be noted that cytosine deaminase activity could not be detected in the *P. pseudoalcaligenes* ATCC 17440 succinate-grown cells when either dihydropyrimidine base served as the nitrogen source (West 1991a). This indicated further metabolic differences between ATCC 17440 and ATCC 8062 despite both strains being reclassified taxonomically as *P. oleovorans* (West 1991a; Saha et al. 2010). The effect of the pyrimidine reductive pathway catabolic products β-alanine or β-aminoisobutyric acid as a nitrogen source on cytosine deaminase activity in succinate-grown *P. oleovorans* ATCC 8062 cells was next explored. Compared to the cytosine deaminase activity in the succinate-grown ATCC 8062 cells using ammonium sulfate as a nitrogen source, β-alanine as a nitrogen source produced a slight 1.2-fold increase in cytosine deaminase activity while β-aminoisobutyric acid as a nitrogen source caused a 34% drop in cytosine deaminase activity (Table 3). It is evident that β-alanine as a nitrogen source in succinate-grown ATCC 8062 cells had an opposite effect on the levels of *N*-ribosidase and deaminase activities while β-aminoisobutyric acid as a nitrogen source had exactly the

same effect on the *N*-ribosidase and deaminase activities (Table 3). The response of cytosine deaminase activity in ATCC 8062 succinate-grown cells to using pyrimidine and dihydropyrimidine bases as nitrogen sources appears to suggest a role for the enzyme in pyrimidine base catabolism. More specifically, growth of *P. oleovorans* ATCC 8062 cells on the pyrimidine base reductive pathway intermediate dihydrouracil as a nitrogen source has a role in increasing the levels of both pyrimidine nucleotide *N*-ribosidase and cytosine deaminase at the transcriptional level rather than at the level of enzyme activity. As has been observed in prior studies, growth on dihydrouracil as a nitrogen source appears to have a regulatory role with respect to elevating the levels of pyrimidine reductive pathway enzymes in pseudomonads (West 1991b; Xu and West 1992; Santiago and West 1999). In this study, growth on dihydrouracil as a nitrogen source increased the levels of the pyrimidine salvage pathway enzymes promoting a higher concentration of uracil available to the pyrimidine base reductive catabolic pathway for subsequent degradation to β-alanine and ammonium ions.

Conclusions

The findings of this study demonstrate that the synthesis of pyrimidine nucleotide *N*-ribosidase was more highly regulated by carbon source than was cytosine deaminase in *P. oleovorans* ATCC 8062. In contrast, cytosine deaminase synthesis was more highly regulated by nitrogen source than was pyrimidine nucleotide *N*-ribosidase synthesis. It was clear that dihydrouracil as a nitrogen source increased both pyrimidine nucleotide *N*-ribosidase and cytosine deaminase synthesis in the succinate-grown ATCC 8062 cells. There does seem to be a connection between the pyrimidine salvage pathway and pyrimidine base catabolism in *P. oleovorans*. With the increasing clinical significance of *P. oleovorans* or *P. pseudoalcaligenes* as human pathogens (Hage et al. 2013; Gautam et al. 2015), understanding the metabolic interconnection between pyrimidine base salvage and pyrimidine base catabolism may prove important from a biomedical perspective.

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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