Review

Biotechnological uses of Azotobacter vinelandii: Current state, limits and prospects

A. Díaz-Barrera* and E. Soto

Escuela de Ingeniería Bioquímica, Pontificia Universidad Católica de Valparaíso, Chile, Av. Brasil 2147, Casilla 4059, Valparaíso, Chile.

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Azotobacter vinelandii is a bacterium obligate aerobe able to fix nitrogen and grow under microaerophilic conditions. This microorganism has remarkable cellular machinery with the ability to biosynthesize three molecules of important biotechnological and biomedical applications: the extracellular polysaccharide alginate, the intracellular polyester poly-β-hydroxybutyrate (PHB) and siderophores compounds. Alginate and PHB have been produced in shake flasks and bioreactors (at laboratory scale) under different environmental and nutritional conditions. This mini-review gives a current overview on the application of wild-type and mutant’s strains of A. vinelandii in bioprocesses to produce alginate or PHB. Scales of production implemented and fermentation strategies used as well as the potential limitations of the use of A. vinelandii cells are analyzed. Based on an approach that integrates molecular and bioengineering techniques, new experimental strategies to enhance the productivity of alginate and PHB from A. vinelandii are proposed.

Key words: Azotobacter vinelandii, alginate, poly-β-hydroxybutyrate, siderophores, bioprocess, scales of production.

INTRODUCTION

Azotobacter vinelandii is a gram-negative bacterium, obligate aerobe capable of fixing nitrogen and to adapt its metabolism to diverse environmental conditions. A. vinelandii forms metabolically dormant cysts after exponential growth or upon induction with specific reagents (Sadoff, 1975). Several years ago, A. vinelandii was used as a model for biochemical, physiology and genetic studies, regarding mainly in biological nitrogen fixation studies (Dixon and Kahn, 2004). On this regard, the reduction of N₂ to ammonia (fixation of nitrogen) by nitrogenase enzyme complex is highly sensitive to oxygen, for which A. vinelandii have particular physiological mechanisms, such as a high respiration rate (Post et al., 1983) to protect its nitrogenase enzymes from oxygen inactivation.

The most important biotechnological application of A. vinelandii cells is their ability to biosynthesize at least three molecules that have important biotechnological and biomedical applications. Under determined nutritional and environmental conditions, this bacterium produces the intracellular polyester poly-β-hydroxybutyrate (PHB), the extracellular polysaccharide alginate and catechol compounds (siderophores). The chemical structure of these molecules is shown in Figure 1.

A. vinelandii excretes siderophores under iron limited conditions (Page and Von Tigerstrom, 1988), which bind iron in strong complexes that are then transported into the cell through highly specific transporters (Page et al., 2003). Recently, it has been found that A. vinelandii produces at least five different siderophores: the 2,3-dihydroxybenzoic acid and aminochelin (monocatechols), the azotochelin (bis(catechol)), protochelin (tris(catechol)) and the yellow-green fluorescent pyoverdine-like azotobactin (Kraepiel et al., 2009) (Figure 1a). Some works conducted in shake flasks have evaluated the production of siderophores by A. vinelandii and usually the influence of iron and oxygen stress has been studied (Cornish and Page, 1998; Tindale et al., 2000). However, less is known about optimal conditions of production at bioreactor scale.

*Corresponding author. E-mail: alvaro.diaz@ucv.cl.
Fax: (56-32) 2273803.

Abbreviations: PHB, poly-β-hydroxybutyrate; DOT, dissolved oxygen tension; OTR, oxygen transfer rate.
Figure 1. The chemical structures of the molecules produced by *A. vinelandii*. (a) Siderophores compounds, (b) alginate and (c) poly-β-hydroxybutyrate (PHB).

(Fekete et al., 1983). It is known that the main biotechnological applications of siderophores are as drug delivery agents (Möllmann et al., 2009), antimicrobial agents (Upadhyay and Srivastava, 2008) and soil remediation (Braud et al., 2009). Therefore, studies at greater scale would be necessary in order to increase siderophore productivity and evaluate its potential biotechnological/biomedical application.

Alginates are polysaccharides produced by *Pseudomonas* and *Azotobacter* species (Remminghorst and Rehm, 2006a). Most knowledge about alginate biosynthesis have been particularly well characterized in the opportunistic human pathogen *Pseudomonas aeruginosa*, mainly because alginate plays an important role as a virulence factor during cystic fibrosis (Ramsey and Wozniak, 2005). In contrast to *P. aeruginosa*, *A. vinelandii* is a nonpathogenic bacterium, which has been used for the development of biotechnological process to produce alginate. Alginate is a linear polysaccharide composed of variable amounts of (1-4)-β-D-mannuronic acid and its C-5-epimer α-L-guluronic acid (Figure 1b), which has a wide range of applications such as thickener, stabilizer, gelling agent and emulsifier in food, as well as textile and pharmaceutical industries. Recent biotechnological advances show a promising potential application of alginate in the medical field; for they can be used in the control release...
of medical drugs (Yao et al., 2009). It is well-known that their properties depend on the concentration, the molecular weight and, to a lesser extent, on the relative content of the two monomers (mannuronic and guluronic) in alginate molecule (Galindo et al., 2007). Strategies for the biotechnological production of high-quality and/or tailor-made bacterial alginates have been performed by means of the evaluation of the influences of different nutritional and operational conditions (Sabra et al., 2001; Trujillo-Roldán et al., 2004; Díaz-Barrera et al., 2009). Studies at bioreactor laboratory scale (1 or 2 l) have shown the influence of dissolved oxygen tension (DOT) on alginate production (Peña et al., 2000; Sabra et al., 2000; Trujillo-Roldán et al., 2004). In this context, alginate could play a decisive role in protecting the nitrogenase (Sabra et al., 2000) due to higher DOT (20%); alginate forms a capsule on the cell surface, suggesting a possible role as an effective barrier for oxygen.

A. vinelandii also produces the intracellular polyester PHB (polymer of the polyhydroxylalcanoates family), which is a biodegradable and biocompatible thermoplastic used as substitute for bulk plastics such as polyethylene and polypropylene. PHB is polyester that the bacteria accumulate intracellularly as both a carbon and energy reserve material. The production of PHB by the bacteria accumulate intracellularly as both a carbon and energy reserve material. The production of PHB by fermentation has been normally operated as a two-stage fed-batch process, in which an initial growth phase in nutritionally enriched medium yields sufficient biomass, followed by a PHB formation phase (Chen and Page, 1997). Different strategies have to be addressed in order to make the process economically attractive. In the case of PHB production, as well as alginate production, the construction of recombinant strains to produce more or higher quality polymers and the implementation of different fermentation procedures would be necessary to evaluate the possible development of an attractive bioprocess with scaling-up potential. This possibility, together with the availability of the complete sequence of A. vinelandii genome (Setubal et al., 2009) have made of this bacterium an interesting model of study, from both technological and scientific points of view.

A SEARCH OF ARTICLES PUBLISHED

In order to quantify the studies performed on alginate, PHB and siderophores produced by A. vinelandii, a search of research articles in the SCOPUS web was carried out (Figure 2). Figure 2a shows the progress of total publications in the last 20 years from 1989 to 2009. The results clearly indicate a higher number of publications regarding alginate (119 in total) as compared to those obtained for PHB (34 publications in total) and siderophores (27 publications in total) production using A. vinelandii cells.

An analysis of the publications (from 1989 to 2009) shows that siderophore production by A. vinelandii has not been studied with the purpose of developing a bioprocess to greater scale. A sustained increase in the number of publications regarding alginate from A. vinelandii was observed from 1989 to 2000; however, the publications showed a decrease from 2003 onwards. This evolution of alginate publications could be explained by the fact that from 2001 onwards, two research groups (W. Sabra and W-D. Deckwer, Germany/F. Clementi and E. Parente, Italy) have not published works with respect to alginate and A. vinelandii cells. In particular, Figure 2b shows the evolution of the number of publications regarding alginate, PHB and siderophores production by fermentation (in shake flasks and bioreactors) using A. vinelandii. It is interesting to note that although the total publications of A. vinelandii and alginate showed a decrease from 2003 (Figure 2a), an increase about twofold in the publications of alginate production by fermentation has been observed between 2004 and 2009 (Figure 2b). Considering that alginate and PHB have been described as the main products of industrial interest from A. vinelandii and that there are a higher number of works regarding alginate and PHB production (compared to siderophores) with the purpose of improving the bioprocess, the next sections will be focused on analyzing the biosynthesis pathways, fermentation procedures and scales of production used for the production the these biopolymers.

BIOSYNTHESIS OF ALGINATE AND PHB IN A. vinelandii

In this section, a brief review and information update regarding alginate and PHB biosynthesis pathways are presented. A more detailed analysis of alginate biosynthesis pathways and their regulation is given in recent reviews (Remminghorst and Rehm, 2006a; Galindo et al., 2007).

Alginic biosynthesis

The alginate biosynthesis pathway and the knowledge about the genetics of alginate biosynthesis have been widely studied (Loret et al., 1996; Muhammadi and Ahmed, 2007). The alginate biosynthesis pathway can be divided into four different stages (Remminghorst and Rehm, 2006a): (1) Synthesis of precursor substrate (GDP-mannuronic acid) at cytoplasmic level, (2) polymerization and cytoplasmic membrane transfer, (3) periplasmic transfer and modification and (4) export through the outer membrane. Figure 3 shows the biosynthesis of alginate and PHB in A. vinelandii cells. It is known that alginate is synthesized from fructose-6-phosphate, which is converted by means of four enzymatic steps involving three enzymes to GDP-mannuronic acid (alginic precursor) (Figure 3a). The transfer of GDP-mannuronic acid that happens through the cytoplasmic membrane and the
Figure 2. Number of research papers appearing with *A. vinelandii* and ‘alginate’, or ‘PHB’, or ‘siderophores’ in the Scopus database from 1989 to 2009 (database searched 22.10.09). (a) Total publications, (b) publications regarding alginate, PHB and siderophores production in shake flasks and bioreactor.

polymerization of GDP-mannuronic acid (polymannuronic acid) are carried out by a mannuronate polymerase, which is presumably located as a cytoplasmic membrane complex (polymerase complex) (Figure 3b). It has been suggested that AlgG, AlgK, AlgX and AlgL are periplasmic proteins forming a scaffold in the periplasm surrounding the nascent alginate chain. Moreover, the evidence shows that Alg8 and Alg44 are also catalytic subunits of the alginate polymerase (Mejía-Ruiz et al., 1997; Remminghorst and Rehm, 2006b).
recently, Remminghorst and Rehm (2006b) demonstrated that it is possible to increase in at least 10 times the alginate production of *P. aeruginosa* (a bacterium that also produces alginate) by the incorporation of additional copies of Alg8, suggesting that Alg8 (part of polymerase complex) might be a rate-limiting step of alginate production.

At periplasmic level, polymannuronic acid undergoes chemical modifications comprising acetylation (genes involved *algI, algJ, algF*) and control of molecular weight by alginate-lyase (*AlgE7*) (Kennedy et al., 1992; Ertesvåg et al., 1995; Svanem et al., 2001) and recently, three new *A. vinelandii* alginate lyases have been characterized (Gimmestad et al., 2009). Polymannuronic acid is exported through the outer membrane where the epimerization of manuronate residues occurs (Figure 3b). *A. vinelandii* have a periplasmic mannuronan C5-epimerase (*AlgL*), the secreted bifunctional manuronan C5-epimerase and alginate lyase *AlgE7* (Kennedy et al., 1992; Ertesvåg et al., 1995; Svanem et al., 2001) and recently, three new *A. vinelandii* alginate lyases have been characterized (Gimmestad et al., 2009). Polymannuronic acid is exported through the outer membrane where the epimerization of manuronate residues occurs (Figure 3b). *A. vinelandii* have a periplasmic mannuronan C5-epimerase (*AlgG*) and additionally, the genome of *A. vinelandii* encodes a
family of seven extracellular Ca$^{2+}$ dependent epimerases (AlgE1 - 7) (Ertésvágh et al., 1995; Steigedal et al., 2008). The possibility of manipulating the acetylation degree of polymer, the epimerization pattern and the molecular weight of alginate produced by A. vinelandii will allow the production of alginates with different properties.

**Poly-β-hydroxybutyrate (PHB) biosynthesis**

Under unbalanced growth conditions, A. vinelandii produces PHB, a polymer of the polyhydroxyalcanoates family of polyesters. This polymer is accumulated when bacterial growth is limited by depletion of nitrogen, phosphorous or oxygen and when there is an excess amount of a carbon source present (Anderson and Dawes, 1990). It is known that the PHB accumulated serves as a store of carbon and energy and as an electron sink into which excess reducing power can be channeled (Senior and Dawes, 1973). The PHB biosynthesis is a NADPH-dependent pathway (Li et al., 2009). In A. vinelandii, the precursor of PHB biosynthesis is acetyl-coenzyme-A (acetyl-CoA) formed from catabolising the carbohydrate through the Entner–Doudoroff pathway (Still and Wang, 1964; Beale and Foster, 1996). It is well known that under oxygen limitation and excess of carbon source, NADH increases, inhibiting the citrate synthase and isocitrate dehydrogenase activities of the tricarboxylic acid cycle and hence acetyl-CoA levels rise, starting the PHB synthesis (Senior and Dawes, 1973). PHB is synthesized by three biosynthetic enzymes from acetyl-CoA. The condensation of two molecules of acetyl-CoA to form acetoacetyl-CoA is catalyzed by a β-Ketothiolase. Acetoacetyl-CoA reductase produce β-hydroxybutyryl-CoA from acetoacetyl-CoA and NADPH$_2$ and the polymerization of β-hydroxybutyryl-CoA with the release of CoASH is catalyzed by PHB synthase (Verlinden et al., 2007) (Figure 3a). The PHB synthesis and its regulation in A. vinelandii cells can be revised with more detail in a recent article (Galindo et al., 2007).

**Use of mutant’s strains to produce alginate and PHB**

From a productivity perspective, A. vinelandii cells can potentially be used for the production of alginate or PHB. However, it is clear that the synthesis of alginate constitutes a waste of substrate when seeking to optimize PHB production and the synthesis of PHB is undesirable when optimizing alginate production. The difficulty to separate the synthesis of PHB and alginate in a wild-type organism has been pointed out (Martínez et al., 1997). A strategy used by different groups has been blocking the synthesis of either alginate or PHB by mutation. In the case of alginate, different studies have been performed using A. vinelandii strains unable to produce PHB (Peña et al., 2002; Segura et al., 2003; Mejía et al., 2010). The main objective, when such strains are used, has been to increase the alginate production as compared to the use of a wild-type strain. However, the use of mutant A. vinelandii strains impaired in PHB has shown opposite results. By comparing mutant decrease in PHB accumulation (named AT268) with the wild-type, Peña et al. (2002) showed that in batch cultures conducted in a bioreactor (at 3% of DOT), alginate production generated by mutants was lower (2.6 g l$^{-1}$) than the one obtained with the wild-type (3.5 g l$^{-1}$). Thus, those authors suggested that regulation of the carbon flux is very complex and that carbon source consumed is not necessarily diverted to alginate biosynthesis. It is now clear that alginate and PHB synthesis are under a very complex genetic control (Galindo et al., 2007). Nevertheless, it is possible to change this performance by implementing a different cultivation strategy. In fact, it has been recently shown that the cultivation of the strain AT6 (impaired in PHB) in a two stage fermentation process is potentially useful for the production of alginate, improving the productivity of the polymer (Mejía et al., 2010). In this work, a high efficiency of conversion of sucrose to alginate (0.74 g g$^{-1}$) and a high alginate concentration (9.5 g l$^{-1}$) as compared with the wild-type strain (4.8 g l$^{-1}$) was obtained, which made the process more competitive. In order to compare the alginate production using different mutant’s strains of A. vinelandii, different results are shown in Table 1. To establish a comparable basis of analysis, the alginate yields ($Y_{p/x}$ and $Y_{p/s}$) and alginate production were normalized, that is, the values obtained using mutant strain were divided by the values using wild-type strain of A. vinelandii. As expected, the highest normalized alginate yields and alginate concentration values (above 1) were obtained using the AT6 strain, which was impaired in PHB production.

In the case of PHB, few reports have analyzed the production using a mutant of A. vinelandii impaired in the alginate production (Martinez et al., 1997; Segura et al., 2003). Segura et al. (2003) evaluated (in shake flasks cultures) a strain that can not produce alginate, affected in the algA gene. The evidence shows an increase in PHB accumulation (between 3 and 10 times), thus, a blockade at the first enzymatic step in alginate biosynthesis (AlgA) improves PHB accumulation. However, the performance of this strain at bioreactor scale has not been evaluated. A summary of normalized PHB yields values ($Y_{p/x}$ and $Y_{p/s}$) using mutant’s strains of A. vinelandii are shown in Table 2. In these cases, the strategy used has been the construction of mutant’s strains of A. vinelandii with improved ability to produce PHB (Noguez et al., 2008; Pyla et al., 2009); however, it is interesting note that these strains have not been evaluated at bioreactor scale.

**SCALES OF PRODUCTION AND FERMENTATION STRATEGIES USED**

Biotechnological process on a large-scale requires the
Table 1. Normalized maximal alginate concentration and alginate yields produced with different mutant’s strains of *A. vinelandii*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>( Y_{p/s} ) normalized</th>
<th>( Y_{p/x} ) normalized</th>
<th>Alginate(_{\text{max}}) normalized</th>
<th>Culture condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT6</td>
<td>3.75</td>
<td>2.73</td>
<td>0.46</td>
<td>Two stage</td>
<td>Mejía et al., 2010</td>
</tr>
<tr>
<td></td>
<td>2.18</td>
<td>1.19</td>
<td>1.98</td>
<td>fermentation</td>
<td></td>
</tr>
<tr>
<td>AT6</td>
<td>1.15</td>
<td>2.68</td>
<td>1.16</td>
<td>Flasks</td>
<td>Segura et al., 2003</td>
</tr>
<tr>
<td></td>
<td>1.37</td>
<td>4.18</td>
<td>1.42</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SML2</td>
<td>0.94</td>
<td>0.94</td>
<td>0.94</td>
<td>Batch bioreactor</td>
<td>Trujillo-Roldán et al., 2004</td>
</tr>
<tr>
<td>SML2</td>
<td>nd</td>
<td>0.49</td>
<td>0.65</td>
<td>Batch bioreactor</td>
<td>Trujillo-Roldán et al., 2003</td>
</tr>
<tr>
<td>CNT26</td>
<td>nd</td>
<td>nd</td>
<td>0.74</td>
<td>Batchbioreactor</td>
<td>Peña et al., 2002</td>
</tr>
<tr>
<td>AT268</td>
<td>nd</td>
<td>nd</td>
<td>0.74</td>
<td>Batch bioreactor</td>
<td>Peña et al., 2002</td>
</tr>
<tr>
<td>DM</td>
<td>nd</td>
<td>nd</td>
<td>0.74</td>
<td>Batch bioreactor</td>
<td>Peña et al., 2002</td>
</tr>
<tr>
<td>LA21</td>
<td>0.09</td>
<td>0.044</td>
<td>0.042</td>
<td>Flasks</td>
<td>Martínez et al., 1997</td>
</tr>
</tbody>
</table>

nd: Not determined. The normalized alginate yields (\( Y_{p/s} \) and \( Y_{p/x} \)) and alginate\(_{\text{max}}\) corresponds to value obtained using mutant strain divided by the value obtained using wild-type strain (ATCC 9046) under the same culture conditions.

Table 2. Normalized PHB yields produced with different mutant’s strains of *A. vinelandii*.

<table>
<thead>
<tr>
<th>Strain</th>
<th>( Y_{p/s} ) normalized</th>
<th>( Y_{p/x} ) normalized</th>
<th>Culture condition</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \triangle \text{arrF} )</td>
<td>nd</td>
<td>250</td>
<td>Flasks</td>
<td>Pyla et al., 2009</td>
</tr>
<tr>
<td>ptsN-</td>
<td>nd</td>
<td>2.08</td>
<td>Flasks</td>
<td>Noguez et al., 2008</td>
</tr>
<tr>
<td>AJ1678</td>
<td>nd</td>
<td>3.4</td>
<td>Plates</td>
<td>Segura and Espin, 2004</td>
</tr>
<tr>
<td>JG41</td>
<td>9.67</td>
<td>nd</td>
<td>Flasks</td>
<td>Segura et al., 2003</td>
</tr>
<tr>
<td></td>
<td>1.61</td>
<td>nd</td>
<td></td>
<td></td>
</tr>
<tr>
<td>UWD</td>
<td>nd</td>
<td>2.49</td>
<td>Plates</td>
<td>Page et al., 2001</td>
</tr>
<tr>
<td>LA21</td>
<td>3.4</td>
<td>nd</td>
<td>Flasks</td>
<td>Martínez et al., 1997</td>
</tr>
<tr>
<td>UWD</td>
<td>2.7</td>
<td>nd</td>
<td>Flasks</td>
<td>Page and Knosp, 1989</td>
</tr>
</tbody>
</table>

nd: Not determined. The normalized PHB yields (\( Y_{p/s} \) and \( Y_{p/x} \)) corresponds to value obtained using mutant strain divided by the value obtained using wild-type strain under the same culture conditions.

development of a suitable technology and economically favorable conditions for the production. Studies of scale-up are necessary to ensure that the optimal production (in the small scale) can be maintained at larger scale (Diaz and Acevedo, 1999; Schmidt, 2005). Although there is an extensive literature about scale-up of bioprocess, there is no common applicable scale-up strategy. Moreover, scale-up of bioprocess is even more complicated when the oxygen demand is high (such as the case of *A. vinelandii* cells), or when the rheological properties of the broth offer a high resistance to the mass transfer, as in alginate production.

There are very few reports of aspects referring to the scale-up of the process for alginate production (Trujillo-Roldán et al., 2001; Reyes et al., 2003) and to our knowledge; no reports are available regarding scale-up strategies for PHB production using *A. vinelandii* cells. In general, the production of alginate at greater scale (at bioreactor laboratory scale) has been realized without considering a scale-up criterion. The available literature shows works regarding scale-up alginate production only from shake flasks to stirred tank of laboratory (1 or 14 l) (Trujillo-Roldán et al., 2001; Reyes et al., 2003; Peña et al., 2008). Studies at pilot scale have not been performed. A typical difficulty found has been the considerable decrease in alginate molecular weight and the viscosity of the broths when the bioprocess is translated to laboratory bioreactor conducted at pH and DOT constant (Peña et al., 1997; Peña et al., 2000; Seáñez et al., 2001). A rigorous and interesting work of scale-up of alginate production by *A. vinelandii* was developed by Reyes et al. (2003). Utilizing as scale-up criterion the initial power drawn, Reyes et al. (2003) demonstrated that this criterion (initial PV\(^{-1}\)) did not permit to reproduce both alginate concentration and viscosity profiles of shake flasks in a stirred fermentor of 1 l. Recently, Peña et al. (2008) demonstrated that by simulating the evolution of the actual power input observed in shake flasks in a stirred fermentor, it was possible to produce alginates with a very similar molecular weight (1,800 kDa) and concen-
Figure 4. Influence of the scale of production on maximum alginate concentration and alginate yields obtained in *A. vinelandii* cultures using wild-type and mutant’s strains. ■, Alginate concentration; ▲, alginate yield per cell basis (Y\textsubscript{p/x}); ●, carbon source yield on alginate (Y\textsubscript{p/s}). Numbers on each symbol indicate the values analyzed.

From an industrial perspective, one potential problem that *A. vinelandii* cultures have is that an optimal alginate production can be obtained when the DOT is accurately controlled in a microaerophilic range (Peña et al., 2000; Sabra et al., 2000). *A. vinelandii* cultures conducted at

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...
low DOT (between 0 and 1%) showed a decrease in alginate production, which was linked to an increase in intracellular storage of PHB (Horan et al., 1983; Diaz-Barrera et al., 2007); whereas at high DOT (about 10%), A. vinelandii cells use the carbon source mainly for biomass production (Parente et al., 1998).

On the other hand, it is known that A. vinelandii have a high level of respiration (Post et al., 1983), which is important to be considered for their use in industrial bioprocess. Due to this fact, it is possible that biomass of A. vinelandii cannot be grown at sufficiently high densities in a typical fermentation strategy at bioreactor scale, which could be a problem when the purpose is to implement the production of alginate or PHB at industrial scale. Despite this potential difficulty, further research is required in order to understand the molecular and biochemical mechanisms involved in alginate and PHB production in A. vinelandii cells.

**PERSPECTIVES**

From a bioengineering perspective and in order to develop a large-scale bioprocess to produce alginate or PHB using A. vinelandii cells, it is evident that it is difficult to improve the production of one of these biopolymers, especially if a wild-type strain is used. So far, the findings obtained on alginate and PHB production from A. vinelandii cells have only been developed to laboratory scale and therefore, the development of an adequate and efficient bioprocess to greater scale needs further research. As it was indicated by Galindo et al. (2007), the implementation of a multidisciplinary approach integrating molecular and bioengineering strategies is fundamental for optimizing alginate and PHB production using A. vinelandii.

A molecular strategy implemented for some years has been to introduce the genes responsible for PHB biosynthesis into E. coli (Schubert et al., 1988; Lee, 1998). In particular, the PHB genes of Ralstonia eutropha (or Alcaligenes eutrophus), one of the most extensively studied microorganisms (Reinecke and Steinbüchel, 2009), have been inserted in E. coli (Anderson and Dawes, 1990). As a model organism, recombinant E. coli is regarded as a strong candidate for PHB production owing to the fact that it can use several inexpensive carbon sources, the simplicity of extraction and the ease for genetic manipulation (Kim, 2000; Naik et al., 2008; Li et al., 2009). A recent work developed on recombinant E. coli strain contains the pha biosynthetic genes from an Azotobacter sp. strain FA8 (Nikel et al., 2006). In this work, the PHB was efficiently produced from agroindustrial by-products by the recombinant E. coli strain grown aerobically in fed-batch cultures and in a laboratory scale bioreactor (5.6 l). This type of strategy becomes an interesting potential application of PHB genes from Azotobacter sp. in order to establish an adequate and efficient microbial production process using a recombinant microorganism. Certainly, there is a necessity to continue with the basic research regarding alginate and PHB production in A. vinelandii cultures. Also, more research at laboratory bioreactor and pilot scale is required. Based on the analyzed evidence, we believe in the possibility to develop two new strategies in order to increase the concentration of alginate or PHB:

1. In the case of PHB and considering the possibility of developing an efficient bioprocess to greater scale, we believe that a new recombinant strain could be built, likely, by the introduction of PHB genes from A. vinelandii, evaluating at least its performance at bioreactor laboratory and pilot scale. It is known that the PHB biosynthesis is a NADPH-dependent pathway (Li et al., 2009); hence, a recombinant that overproduces NADPH would be interesting to evaluate.

2. In the case of alginate produced by A. vinelandii, we propose the construction of a recombinant strain that overexpress the alg8 gene (a possibility not yet evaluated) and tests its performance at least in a bioreactor scale. In fact, evaluating the overexpression of alg8 into mutant strain AT6, which is impaired in PHB production, would be unquestionably interesting. This proposal is based on the fact that alginate production in shake flask cultures of P. aeruginosa was at least tenfold higher when additional copies of alg8 (as part of the polymerase complex) were inserted (Remminghorst and Rehm, 2006b).

There is no doubt that A. vinelandii is a remarkable cellular machinery with versatile genetic and metabolic components that has important implications for biotechnological exploitation. Taking into account the current findings, it is necessary to continue evaluating different mutant’s strains of A. vinelandii and to develop recombinant strains using genes of this microorganism and evaluating their performances at laboratory bioreactor level and at greater scale bioprocess. Overall, the implementation of a new fermentation process at bioreactor scale using mutant’s strains is required in order to enhance the productivity and molecular characteristics of alginate and PHB produced.

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