LINDA PRISCILA GUAMAN BAUTISTA

Clonagem e super expressão dos genes do catabolismo de xilose em *Burkholderia sacchari* e avaliação de seu efeito na repressão catabólica e produção de polihidroxibutirato a partir de açucares hemicelulósicos

Tese apresentada ao programa de Pós-Graduação em Microbiologia do Instituto de Ciências Biomédicas da Universidade de São Paulo, para obtenção do Título de Doutor em Ciências.

Area de Concentracao: Microbiologia

Orientadora: Prof. Dra. Luiziana Ferreira da Silva

Versão corrigida. A versão original eletrônica, encontra-se disponível tanto na Biblioteca do ICB quanto na Biblioteca Digital de Teses e Dissertações da USP (BDTD).

Sao Paulo
2016

RESUMO

Guamán LP. Clonagem e superexpressão dos genes de metabolismo de xilose em *Burkholderia sacchari* e avaliação do efeito na repressao catabólica e no acúmulo de Polihidroxibutirato usando açúcares lignocelulósicos. Tese (Doutorado em Microbiologia). São Paulo: Instituto de Ciências Biomédicas, Universidade de São Paulo; 2016.

Apesar das muitas vantagens que os Polihidroxialcanoatos apresentam, uma das principais razões que limitam seu uso a grande escala, é o alto custo da fonte de carbono usada como substrato, por tanto, o uso de fontes de carbono mais baratas obtidas de resíduos lignocelulósicos são uma alternativa para reduzir os custos de produção, além de serem completamente renováveis. No Brasil, a quantidade de bagaço de cana de açúcar disponível é de 20.8×10^6 por ano, o que representa, 10×10^6 toneladas de xilose disponíveis, que são geralmente queimadas ou descartadas. A pesar de ser o segundo açúcar mais abundante da natureza, o uso de xilose ainda representa uma barreira técnica, devido ao fenômeno de repressão catabólica quando a glicose está presente, e também devido ao ineficiente consumo de xilose em várias linhagens. Usamos como modelo de estudo Burkholderia sacchari, uma bactéria isolada no Brasil, que consome xilose, e acumula ate um 80% de P(3HB) como massa seca, com o objetivo de melhorar a velocidade especifica máxima de crescimento ($0.14\ h^{-1}$) e o teor de acumulo de P(3HB), a traves da expressão de genes metabólicos, reguladores e de transporte de xilose. Primeiro, nós descrevemos a organização dos genes responsáveis da assimilação da xilose, e avaliamos a superexpressão dos dois primeiros genes metabólicos xylAB. Nós demonstramos que a superexpressão destes genes melhorou a velocidade específica máxima de crescimento assim como o acúmulo de P(3HB), atingindo o mais alto fator de conversão de xilose a P(3HB) (0.35 g/g), com um incremento na velocidade a $0.203\ h^{-1}$. Depois, a foram realizados ensaios em misturas de açúcares para avaliar a repressão catabólica (CCR) na presença de xilose, glicose e arabinose. Foi possível identificar uma forte CCR de glicose sob xilose, e uma CCR mais relaxada na mistura de glicose sob arabinose. A super expressão dos genes xylE-xylAB permitiu abolir a repressão catabólica melhorando assim o acúmulo de P(3HB) ate 66%. Finalmente, e devido a que uma das maiores limitações para a aplicação de biologia sintética e engenharia metabólica em B. sacchari é a falta de ferramentas moleculares apropriadas, nos decidimos construir um set de plasmídeos adaptando o sistema BglBrick usando origens de replicação compatíveis com B. sacchari. Foram avaliados também, dois diferentes promotores e se identificaram os melhores níveis de cada um dos indutores. Usando este set de plasmídeos, nos reportamos que a superexpressão de XylR, o fator de regulação transcricional do operon de xilose, nos permitiu atingir a maior velocidade máxima específica de crescimento para B. sacchari, $0.25h^{-1}$, quando usada xylose como fonte de carbono, o fator de conversão de xilose a P(3HB) e o teor de acúmulo foram também incrementadas. Em resumo, nos reportamos os diferentes níveis de repressão catabólica em B. sacchari em misturas de xilose, glicose e arabinose, e também, concluímos que a superexpressão dos genes xylAB e xylR melhoram a velocidade específica de crescimento, o fator de conversão e o teor de acumulo de P(3HB) em B. sacchari usando xilose como fonte de carbono

Palavras chave - Xilose. *Burkholderia sacchari*. Poli-3-Hidroxibutirato. Lignocellulose.

ABSTRACT

Guamán LP. Cloning and overexpression of xylose catabolism genes of Burkholderia sacchari and evaluation of the impact on catabolic repression and Polyhydroxybutyrate production using hemicellulosic sugars. [Ph.D. thesis (Microbiology). Microbiology] São Paulo University, São: Paulo Instituto de Ciências Biomédicas, Universidade de São Paulo; 2016

Despite the many advantages of polyhydroxyalkanoates, their higher production costs when compared with petroleum-based polymers still represent a barrier for make them competitive. One of the major reasons is the high cost associated at the carbon source used as substrate, therefore, the use of low-cost carbon sources obtained from lignocellulose residues, is an alternative to reduce production costs, besides of being completely renewable. In Brazil, the amount of sugarcane bagasse available is around 20.8×10^6 tonnes $year^{-1}$ which means 10×10^6 tonnes $year^{-1}$ of xylose available, mostly burned or discarded. Despite of being the second most abundant sugar in nature, xylose utilization still represents a technical barrier, because of carbon catabolite repression when in presence of glucose, and due to inefficient xylose uptake in several strains. In this research we study *Burkholderia sacchari*, a bacterium isolated in Brazil, which consumes xylose and accumulate up to 80% of the cell mass as P(3HB), with the aim of improving its specific growth rate (0.14 h^{-1}) and P(3HB) yield, through overexpression of xylose catabolic, transport and regulator genes. First, we described the organization of the genes responsible for xylose assimilation, and we tested the first two metabolic genes (*xylAB)* demonstrating through its overexpression, that it is possible to improve *B. sacchari* ability to growth and use xylose as a sole carbon source and production of P(3HB). The highest conversion rate of xylose to P(3HB) (0.35 g/g) was achieved and also, the highest titer (11.3 g L^{-1}) at a specific growth rate of 0.203 h^{-1}. Then a series of sugar mixtures assays were performed to assess carbon catabolite repression (CCR) in mixtures of xylose, glucose, and arabinose. We identified strong CCR over xylose when glucose is supplied, and a relaxed CCR in glucose arabinose mixtures. *xylE-xylAB* genes were overexpressed to abolish CCR allowing us also improve P(3HB) accumulation up to 62%. Finally, and because one of the major limitations for applying metabolic engineering or synthetic biology approaches in *B. sacchari* is the lack of appropriate molecular tools, we decided to construct a set of inducible vectors adapting the existing Bgll Brick system, using compatible replication origins to *B. sacchari*, two different promoters were assessed, and the best induction levels were described. Using this set of plasmids we reported that XylR (transcriptional xylose regulator) overexpression allowed us to achieve the highest growth rate reported to *B. sacchari* when cultivated in xylose as the sole carbon source ($0.25h^{-1}$), improving also P(3HB) conversion factor and yield. In summary, we report *B. sacchari* has different levels of CCR in mixtures of xylose, glucose and arabinose; and also, we conclude that overexpression of *xylAB* and *xylR* genes improve growth rate, conversion factor and yield when P(3HB) is produced using xylose as carbon source in *B. sacchari.*

Keywords - Xylose. *Burkholderia sacchari*. Polyhydroxybutyrate. Lignocellulose.

1 INTRODUCTION

Polyhydroxyalkanoates (PHA's), are intracellular granules of polyester, accumulated as carbon and energy storage materials, synthesized by many microbial strains under unbalanced growth conditions such as the presence of excess carbon source and limitation of at least one essential nutrient. Its biodegradability, biocompatibility and similar physical properties to synthetic polymers make them an environmentally friendly alternative to petrochemical based plastics (1).

Since PHA's first description in 1926 by Lemoigne (2), several advances towards PHA's large scale production have been conducted, however, currently, the high production costs make PHA's more expensive than conventional plastics. One of the main reasons, is the high cost associated to carbon source used. According to Nonato and coworkers, it can account for up to 29% of its overall production cost, even when integrated to sugarcane mills (3). Several attempts including use of inexpensive lignocellulosic biomass have been conducted in recent years (4). In Brazil sugarcane production doubled the last decade, increasing at the same level bagasse availability by around 208 million tonnes (5). It is estimated around 30% (on a dry weight basis) of the harvested sugarcane corresponds to lignocellulosic byproducts (6).

The amount of sugarcane bagasse estimated to be used only for hydrolysis process is around 7.0×10^6 tonnes year (5) which means 35×10^3 tons per year of xylose available, mostly burned or discarded (7). *Burkholderia sacchari* LFM 101 (8,9), is able to accumulate up to 75% of cell dry weight (CDW) as PHA (10), this bacteria can utilize a variety of carbon sources including xylose, and even hemicellulosic hydrolysates from sugarcane bagasse (11–13) to produce not only PHA's but also other high-value chemicals like xylitol and xylonic acid (9,14). Despite of its capability to use xylose as carbon source, its slow growth rate ($0.16\ h^{-1}$) associated with xylose consumption, represent a barrier for using this bacteria as a chassis to produce PHA's or other high value chemicals on industrial scale. Therefore, it is highly relevant to understand the particular features of xylose metabolism in *B. sacchari* and use this information to overcome metabolic roadblocks and exploit its potential for renewable bioproduction.

Within this context, the aim of this research was to improve xylose uptake in *B. sacchari*, through (1) Genome analysis and organization of operon(s) involved in xylose metabolism in B. sacchari. (2) Overexpression of key genes related to xylose metabolism, transport, or regulation, (3) Evaluate gene overexpression impact in growth rate and P(3HB) production. (4) Evaluate carbon catabolite repression in glucose, arabinose and xylose mixtures. (5) Finally, we also wanted to contribute to future synthetic biology approaches constructing a set of plasmids with replications origins compatible with *B. sacchari*, testing also two different promoters and appropriate induction levels.

2 CONCLUSIONS

- xylAB overexpression in *B. sacchari* leads to biomass improvement by 32% from 12.78 to 16.89 g L^{-1} indicating significantly improved xylose catabolism and cell growth. The enhanced utilization of xylose most strikingly improved the production of P(3HB) to 11.29 g L^{-1}, which represents a 34% improvement compared to the parental strain (8.4 g L^{-1}). Xylose consumption was significantly increased, achieving the highest yield of xylose to P(3HB) reported to date (0.35 g/g), representing a 35% improvement. LFM1402 accumulated 67% P3HB of cell dry weight using xylose. The specific growth rate reached a μmax of 0.20 h^{-1} and P(3HB) productivity increased 25,5% when compared to wild type (LFM 101).

- Overexpression of xylFGH and xylE didn't not improve growth rate in *B. sacchari*. However, expression of appropriate levels of the transcriptional regulator (*xylR*) further enhance growth rate (67%), P(3HB) production (34%), and yield (80%). this research represents the first approach of improving growth rate and PH3B production using of synthetic biology in *B. sacchari.* This work emphasizes the relevance of having tools allowing precise and tunable control of expression in non-model organisms.

- Unlike glucose-xylose mixture, growth profile in arabinose-xylose does not present strong diauxic growth, leading to growth rate of 0.44 h^{-1}. In both mixtures, sugars were totally depleted after 16 h.

- *xylE xylAB* overexpression successfully overcome glucose repression over arabinose and xylose. All three sugars were consumed simultaneously, although glucose is always the preferred carbon source, being depleted at hour 14, when 63% of arabinose and 45 % of xylose was already consumed. By hour 23 all sugars were consumed with a growth rate of 0.35 h-1, whereas, *gatC xylAB* overexpression didn't not resulted in any improvement showing a similar behavior than control strain.

REFERENCES*

1. Steinbüchel A, Aerts K, Babel W, Follner C, Liebergesell M, Madkour MH, et al. Considerations on the structure and biochemistry of bacterial polyhydroxyalkanoic acid inclusions. Can J Microbiol (Internet). 1995;41 Suppl 1:94–105. Available from: http://www.ncbi.nlm.nih.gov/pubmed/7606669
2. M. Lemoigne. Produits de deshydratation et de polymersation de la acideoxybutyrique. Bull Soc Chim Biol. 1926;770–82.
3. Nonato RV, Mantelatto PE, Rossell CEV. Integrated production of biodegradable plastic, sugar and ethanol. Applied Microbiology and Biotechnology. 2001;57(1-2):1–5.
4. Ramsay JA, Hassan M-CA, Ramsay BA. Hemicellulose as a potential substrate for production of poly(β-hydroxyalkanoates). Can J Microbiol. 1995 Dec 15;41(13):262–6.
5. Hofsetz K, Silva MA. Brazilian sugarcane bagasse: Energy and non-energy consumption. Biomass and Bioenergy. 2012 Nov;46:564–73.
6. Alonso Pippo W, Luengo CA, Alonsoamador Morales Alberteris L, Garzone P, Cornacchia G. Energy Recovery from Sugarcane-Trash in the Light of 2nd Generation Biofuels. Part 1: Current Situation and Environmental Aspects. Waste Biomass Valor. 2011 Feb;2(1):1–16.
7. Raicher G. Análise econômica da produção de polímeros biodegradáveis no contexto de uma biorefinaria a partir de cana-de-açucar (Doctoral dissertation). 2011.
8. Bramer CO, Vandamme P, Silva LF. Burkholderia sacchari sp. nov., a polyhydroxyalkanoate-accumulating bacterium isolated from soil of a sugar-cane plantation in Brazil. International Journal of Systematic and Evolutionary Microbiology. 2001;v. 5.
9. Raposo RS, de Almeida MCMD, de Oliveira M da CMA, da Fonseca MM, Cesário MT. A Burkholderia sacchari cell factory: production of poly-3-hydroxybutyrate, xylitol and xylonic acid from xylose-rich sugar mixtures. N Biotechnol. 2016 Oct 5;34:12–22.
10. Gomez JGC, Rodrigues MFA, Alli RCP, Torres BB, Netto CLB, Oliveira MS, et al. Evaluation of soil gram-negative bacteria yielding polyhydroxyalkanoic acids from carbohydrates and propionic acid. Appl Microbiol Biotechnol. 1996 Jul 24;45(6):785–91.
11. Lopes MSG. Produção de plásticos biodegradáveis utilizando hidrolisado hemicelulósico de bagaço de cana-de-açúcar. 2010; 43:45-9.
12. Lopes MSG, Rocha RCS, Zanotto SP. Screening of bacteria to produce polyhydroxyalkanoates from xylose. World Journal of Microbiology & Biotechnology,. 2009;1751–6.
13. Silva L, Taciro M, Michelin Ramos M, Carter J, Pradella J, Gomez J. Process development of poly-3-hydroxybutyrate (P(3HB)) production by bacteria from xylose, glucose and sugar cane bagasse hydrolysate. J Ind Microbiol Biotechnol. 2004;31, 245–54.

○

*De acordo com: International Committee of Medical Journal Editors. [Internet]. Uniform requirements for manuscripts submitted to biomedical journals. [2011 Jul 15]. Available from: http://www.nlm.nih.gov/bsd/uniform_requirements.htlm

14. Mendonça TT, Gomez JGC, Buffoni E, Sánchez Rodriguez RJ, Schripsema J, Lopes MSG, et al. Exploring the potential of Burkholderia sacchari to produce polyhydroxyalkanoates. J Appl Microbiol. 2014 Apr;116(4):815–29.

15. Young FK, Kastner JR, May SW. Microbial Production of Poly-beta-Hydroxybutyric Acid from d-Xylose and Lactose by Pseudomonas cepacia. Appl Environ Microbiol. 1994 Nov;60(11):4195–8.

16. Mekonnen T, Mussone P, Khalil H, Bressler D. Progress in bio-based plastics and plasticizing modifications. J Mater Chem A. 2013;1(43):13379.

17. Pathak S, Sneha C, Mathew BB. Bioplastics: Its Timeline Based Scenario & Challenges. Journal of Polymer and Biopolymer Physics Chemistry (Internet). 2014 Jan 23; Available from: http://pubs.sciepub.com/jpbpc/2/4/5/

18. EU EU. Green Paper on a European Strategy on Plastic Waste in the Environment. (Internet). Green Paper on a European Strategy on Plastic Waste in the Environment. 2013 (cited 2016 Oct 28). Available from: http://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:52013DC0123>

19. Li WC, Tse HF, Fok L. Plastic waste in the marine environment: A review of sources, occurrence and effects. Sci Total Environ. 2016 Oct 1;566-567:333–49.

20. Shah AA, Hasan F, Hameed A, Ahmed S. Biological degradation of plastics: a comprehensive review. Biotechnol Adv. 2008 Jun;26(3):246–65.

21. Jambeck JR, Geyer R, Wilcox C, Siegler TR, Perryman M, Andrady A, et al. Marine pollution. Plastic waste inputs from land into the ocean. Science. 2015 Feb 13;347(6223):768–71.

22. Hahn-Hägerdal B, Karhumaa K, Jeppsson M, Gorwa-Grauslund MF. Metabolic engineering for pentose utilization in Saccharomyces cerevisiae. Adv Biochem Eng Biotechnol. 2007;108:147–77.

23. Thompson RC, Swan SH, Moore CJ, vom Saal FS. Our plastic age. Philos Trans R Soc Lond, B, Biol Sci. 2009 Jul 27;364(1526):1973–6.

24. Lopes MSG. Engineering biological systems toward a sustainable bioeconomy. J Ind Microbiol Biotechnol. 2015 Jun;42(6):813–38.

25. Silva LF, Taciro MK, Raicher G, Piccoli RAM, Mendonça TT, Lopes MSG, et al. Perspectives on the production of polyhydroxyalkanoates in biorefineries associated with the production of sugar and ethanol. Int J Biol Macromol. 2014 Nov;71:2–7.

26. Hazer B, Steinbüchel A. Increased diversification of polyhydroxyalkanoates by modification reactions for industrial and medical applications. Appl Microbiol Biotechnol. 2007 Feb;74(1):1–12.

27. Schlegel HG, Gottschalk G, Von Bartha R. Formation and Utilization of Poly-β-Hydroxybutyric Acid by Knallgas Bacteria (Hydrogenomonas). Nature. 1961 Jul 29;191(4787):463–5.

28. Ciesielski S, Mozejko J, Przybyłek G. The influence of nitrogen limitation on mcl-PHA synthesis by two newly isolated strains of Pseudomonas sp. J Ind Microbiol Biotechnol. 2010 May;37(5):511–20.

29. Akaraonye E, Keshavarz T, Roy I. Production of polyhydroxyalkanoates: the future green materials of choice. J Chem Technol Biotechnol. 2010 Apr 23;85(6):732–43.

30. Możejko-Ciesielska J, Kiewisz R. Bacterial polyhydroxyalkanoates: Still fabulous? Microbiol Res. 2016 Nov;192:271–82.

31. Steimbuchel A. Economic Aspects of Biopolymer production. Biopolymers (Internet). 2003. p. 320. Available from: http://onlinelibrary.wiley.com/doi/10.1002/3527600035.bpola010/abstract

32. Lee S-M, Jellison T, Alper HS. Directed evolution of xylose isomerase for improved xylose catabolism and fermentation in the yeast Saccharomyces cerevisiae. Appl Environ Microbiol. 2012 Aug;78(16):5708–16.

33. Wallen LL, Davis EN. Biopolymers of activated sludge. Environ Sci Technol. 1972 Feb;6(2):161–4.

34. Wallen LL, Rohwedder WK. Poly-.beta.-hydroxyalkanoate from activated sludge. Environ Sci Technol. 1974 Jun;8(6):576–9.

35. Sudesh K, Abe H, Doi Y. Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. Prog Polym Sci. 2000 Dec;25(10):1503–55.

36. De Smet MJ, Eggink G, Witholt B, Kingma J, Wynberg H. Characterization of intracellular inclusions formed by Pseudomonas oleovorans during growth on octane. J Bacteriol. 1983 May;154(2):870–8.

37. Lageveen RG, Huisman GW, Preusting H, Ketelaar P, Eggink G, Witholt B. Formation of Polyesters by Pseudomonas oleovorans: Effect of Substrates on Formation and Composition of Poly-(R)-3-Hydroxyalkanoates and Poly-(R)-3-Hydroxyalkenoates. Appl Environ Microbiol. 1988 Dec;54(12):2924–32.

38. Brandl H, Knee EJ, Fuller RC, Gross RA, Lenz RW. Ability of the phototrophic bacterium Rhodospirillum rubrum to produce various poly (beta-hydroxyalkanoates): potential sources for biodegradable polyesters. Int J Biol Macromol. 1989 Feb;11(1):49–55.

39. Holmes PA. Applications of P(3HB) - a microbially produced biodegradable thermoplastic. Physics in Technology. 1985 Jan;16(1):32–6.

40. Byrom D. Polymer synthesis by microorganisms: technology and economics. Trends Biotechnol. 1987 Sep;5(9):246–50.

41. Silva L, Gomez J, Rocha R, Taciro M, Pradella J. Produção biotecnológica de poli-hidroxialcanoatos para a geração de polímeros biodegradáveis no Brasil. Química Nova. 2007;30 (7):1732–43.

42. Zhang Y-HP. Reviving the carbohydrate economy via multi-product lignocellulose biorefineries. J Ind Microbiol Biotechnol. 2008 May;35(5):367–75.

43. Mobley DP. Microbial synthesis of polymers and polymer precursors. Plastics from microbes (Internet). Munich; 1991. p. 288. Available from: http://onlinelibrary.wiley.com/doi/10.1002/adma.19950071218/abstract

44. Tanaka T, Iwata T. Physical Properties, Structure Analysis, and Enzymatic Degradation of Poly((*R*)-3-hydroxybutyrate-*co* -(*R*)-3-hydroxyvalerate) Films and Fibers. In: Khemani K, Scholz C, editors. Degradable Polymers and Materials: Principles and Practice (2nd Edition). Washington, DC: American Chemical Society; 2012. p. 171–85.

45. Harper CA. Handbook of Plastic Processes (Internet). 2005. Available from: http://onlinelibrary.wiley.com/book/10.1002/0471786586

46. Lendlein A. Handbook of Biodegradable Polymers: Isolation, Synthesis, Characterization and Applications (Internet). Wiley; 2011. Available from: http://onlinelibrary.wiley.com/book/10.1002/9783527635818

47. Pachekoski WM, Agnelli JAM, Belem LP. Thermal, mechanical and morphological properties of poly (hydroxybutyrate) and polypropylene blends after processing. Mat Res. 2009 Jun;12(2):159–64.

48. Griffin G. Chemistry and Technology of Biodegradable Polymers (Internet). 1st ed. Springer Netherlands; 1994. Available from: http://www.springer.com/us/book/9780751400038

49. Lauzier C, Monasterios C, Saracovan I, Marchessault R, Ramsay B. Film formation and paper coating with poly(b-hydroxyalkanoate), a biodegradable latex. 1993 May 1; Available from: https://www.researchgate.net/publication/236511010_Film_formation_and_paper_coating_with_polyb-hydroxyalkanoate_a_biodegradable_latex

50. Scholz C. Poly(β-hydroxyalkanoates) as Potential Biomedical Materials: An Overview. In: Scholz C, Gross RA, editors. Polymers from Renewable Resources: Biopolyesters and Biocatalysis. Washington, DC: American Chemical Society; 2001. p. 328–34.

51. Galego N, Rozsa C, Sánchez R, Fung J, Analía Vázquez, Santo Tomás J. Characterization and application of poly(β-hydroxyalkanoates) family as composite biomaterials. Polym Test. 2000 Aug;19(5):485–92.

52. Thompson RC, Olsen Y, Mitchell RP, Davis A, Rowland SJ, John AWG, et al. Lost at sea: where is all the plastic? Science. 2004 May 7;304(5672):838.

53. Smith R. Polyhydroxyalkanoates. Biodegradable Polymers for Industrial Applications (Internet). Cambridge. MA: Science DIrect; 2005. p. 32. Available from: http://www.sciencedirect.com/science/book/9781855739345

54. Tokiwa Y, Calabia BP, Ugwu CU, Aiba S. Biodegradability of plastics. Int J Mol Sci. 2009 Aug 26;10(9):3722–42.

55. Chowdhury AA. (poly-beta-hydroxybutyric acid-splitting bacteria and an exoenzyme). Arch Mikrobiol. 1963 Dec 10;47:167–200.

56. Suyama T, Tokiwa Y, Ouichanpagdee P, Kanagawa T, Kamagata Y. Phylogenetic affiliation of soil bacteria that degrade aliphatic polyesters available commercially as biodegradable plastics. Appl Environ Microbiol. 1998 Dec;64(12):5008–11.

57. Bugnicourt E. Polyhydroxyalkanoate (PHA): Review of synthesis, characteristics, processing and potential applications in packaging. Express Polym Lett. 2014 Sep 1;8(11):791–808.

58. Peña C, Castillo T, García A, Millán M, Segura D. Biotechnological strategies to improve production of microbial poly-(3-hydroxybutyrate): a review of recent research work. Microb Biotechnol. 2014 Jul;7(4):278–93.

59. Stubbe J, Tian J, He A, Sinskey AJ, Lawrence AG, Liu P. Nontemplate-dependent polymerization processes: polyhydroxyalkanoate synthases as a paradigm. Annu Rev Biochem. 2005 Jan 1;74:433–80.

60. Huijberts GN, Eggink G, de Waard P, Huisman GW, Witholt B. Pseudomonas putida KT2442 cultivated on glucose accumulates poly(3-hydroxyalkanoates) consisting of saturated and unsaturated monomers. Appl Environ Microbiol. 1992 Feb;58(2):536–44.

61. Qi Q, Rehm BH. Polyhydroxybutyrate biosynthesis in Caulobacter crescentus: molecular characterization of the polyhydroxybutyrate synthase. Microbiology (Reading, Engl). 2001 Dec;147(Pt 12):3353–8.

62. Lee SY, Park SJ, Park JP, Lee Y, Lee SH. Economic Aspects of Biopolymer Production. In: Steinbüchel A, editor. Biopolymers Online: Biology • Chemistry •

Biotechnology • Applications. Weinheim, Germany: Wiley-VCH Verlag GmbH & Co. KGaA; 2005.

63. Gatenholm P, Tenkanen M, editors. Hemicelluloses: Science and Technology, Copyright, Foreword. Hemicelluloses: Science and Technology. Washington, DC: American Chemical Society; 2003. p. i–v.

64. Keenan TM, Nakas JP, Tanenbaum SW. Polyhydroxyalkanoate copolymers from forest biomass. J Ind Microbiol Biotechnol. 2006 Jul;33(7):616–26.

65. Xu F, Sun JX, Liu CF, Sun RC. Comparative study of alkali- and acidic organic solvent-soluble hemicellulosic polysaccharides from sugarcane bagasse. Carbohydr Res. 2006 Feb 6;341(2):253–61.

66. Nunes A. Steam explosion pretreatment and enzymatic hydrolysis of eucalyptus wood. Bioresour Technol. 1996 Aug;57(2):107–10.

67. Kambourova M, Mandeva R, Fiume I, Maurelli L, Rossi M, Morana A. Hydrolysis of xylan at high temperature by co-action of the xylanase from Anoxybacillus flavithermus BC and the beta-xylosidase/alpha-arabinosidase from Sulfolobus solfataricus Oalpha. J Appl Microbiol. 2007 Jun;102(6):1586–93.

68. Brienzo M, Siqueira AF, Milagres MF. Search for optimum conditions of sugarcane bagasse hemicellulose extraction. Biochemical Engineering Journal. 2009;46(2), 199–204.

69. Bothast RJ, Nichols NN, Dien BS. Fermentations with new recombinant organisms. Biotechnol Prog. 1999 Oct;15(5):867–75.

70. Bertrand JL, Ramsay BA, Ramsay JA, Chavarie C. Biosynthesis of Poly-beta-Hydroxyalkanoates from Pentoses by Pseudomonas pseudoflava. Appl Environ Microbiol. 1990 Oct;56(10):3133–8.

71. Cesário MT, Raposo RS, de Almeida MCMD, van Keulen F, Ferreira BS, da Fonseca MMR. Enhanced bioproduction of poly-3-hydroxybutyrate from wheat straw lignocellulosic hydrolysates. N Biotechnol. 2014 Jan 25;31(1):104–13.

72. Companhia Nacional de Abastecimento C. Acompanhamento da safra brasileira: Cana-de-Açúcar. Safra 2014/2015. Tercer levantamento. Dez 2015. Pag 18 (Internet). Acompanhamento da safra brasileira: Cana-de-Açúcar. Safra 2014/2015. Tercer levantamento. Dez 2015. Pag 18. (cited 2016 Sep 18). Available from: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/15_12_17_09_03_29_boletim_cana_portugues_-_3o_lev_-_15-16.pdf

73. Macedo IC, Seabra JEA, Silva JEAR. Green house gases emissions in the production and use of ethanol from sugarcane in Brazil: The 2005/2006 averages and a prediction for 2020. Biomass and Bioenergy. 2008 Jul;32(7):582–95.

74. Lee J. Biological conversion of lignocellulosic biomass to ethanol. J Biotechnol. 1997 Jul 23;56(1):1–24.

75. Chandel AK, Kapoor RK, Singh A, Kuhad RC. Detoxification of sugarcane bagasse hydrolysate improves ethanol production by Candida shehatae NCIM 3501. Bioresour Technol. 2007 Jul;98(10):1947–50.

76. Martinez A, Rodriguez ME, York SW, Preston JF, Ingram LO. Effects of Ca(OH)(2) treatments ("overliming") on the composition and toxicity of bagasse hemicellulose hydrolysates. Biotechnol Bioeng (Internet). 2000;69(5):526–36. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10898862

77. Tenenbaum DJ. Food vs. fuel: diversion of crops could cause more hunger. Environ Health Perspect. 2008 Jun;116(6):A254–7.

78. Oliveria N.A, Construindo OsRecursos do Amanhã. In: Núcleo de Estudos Avançados em Meio Ambiente (NEAMA), editor. Instrumentos Econômicos para Gestão Ambiental: Lições das Experiências Nacional e Internacional. 2003. p. 328.

79. Goldemberg J. The Brazilian biofuels industry. Biotechnol Biofuels. 2008 May 1;1(1):6.

80. Birgit K. Lignocellulosic Hydrolysates for the Production of Polyhydroxyalkanoates. Microorganisms in Biorefineries (Internet). Springer-Verlag Berlin Heidelberg; 2015. p. 79–104. Available from: http://www.springer.com/gp/book/9783662452080#aboutBook

81. Silva LF, Gomez JG, Oliveira MS, Torres BB. Propionic acid metabolism and poly-3-hydroxybutyrate-co-3-hydroxyvalerate (P(3HB)-co-3HV) production by Burkholderia sp. J Biotechnol. 2000 Jan 21;76(2-3):165–74.

82. Rocha R, Silva L, Taciro M, Pradella J. Production of P(3HB)-co-3HV with a broad range of 3HV content at high Y3HV/Prop values by B. sacchari IPT 189. World J Microbiol Biotechnol. 2008;24:427–31.

83. Lopes MSG, Gosset G, Rocha RCS, Gomez JGC, Ferreira da Silva L. P(3HB) biosynthesis in catabolite repression mutant of Burkholderia sacchari. Curr Microbiol. 2011 Oct;63(4):319–26.

84. McMillan JD. Xylose fermentation to ethanol. A review. Golden, CO (United States): National Renewable Energy Laboratory (NREL); 1993.

85. Bailey JE. Toward a science of metabolic engineering. Science. 1991 Jun 21;252(5013):1668–75.

86. Jeffries TW, Shi NQ. Genetic engineering for improved xylose fermentation by yeasts. Adv Biochem Eng Biotechnol (Internet). 1999;65:117–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/10533434

87. Jeffries TW. Utilization of xylose by bacteria, yeasts, and fungi. Pentoses and Lignin. Berlin/Heidelberg: Springer-Verlag; 1983. p. 1–32.

88. Webb SR, Lee H. Regulation of D-xylose utilization by hexoses in pentose-fermenting yeasts. Biotechnol Adv. 1990;8(4):685–97.

89. Jeffries T. Effects of culture conditions on the fermentation of xylose to ethanol by Candida shehatae. Biotechnol Bioeng Symp (Internet). 1985; Available from: https://www.researchgate.net/profile/Thomas_Jeffries/publication/252397433_Effects_of_Culture_Conditions_on_the_Fermentation_of_Xylose_to_Ethanol_by_Candida_shehatae/links/0c96052ec0af16232a000000.pdf

90. Kang HY, Song S, Park C. Priority of pentose utilization at the level of transcription: arabinose, xylose, and ribose operons. Mol Cells (Internet). 1998;8(3):318–23. Available from: http://www.ncbi.nlm.nih.gov/pubmed/9666469

91. Sumiya M, Davis EO, Packman LC, McDonald TP, Henderson PJ. Molecular genetics of a receptor protein for D-xylose, encoded by the gene xylF, in Escherichia coli. Recept Channels. 1995;3(2):117–28.

92. Zhang Y-HP, Ding S-Y, Mielenz JR, Cui J-B, Elander RT, Laser M, et al. Fractionating recalcitrant lignocellulose at modest reaction conditions. Biotechnol Bioeng. 2007 Jun 1;97(2):214–23.

93. Hyvönen L, Koivistoinen P, Voirol F. Food Technological Evaluation of Xylitol. Advances in Food Research Volume 28. Elsevier; 1982. p. 373–83.

94. Lokman BC, van Santen P, Verdoes JC, Krüse J, Leer RJ, Posno M, et al. Organization and characterization of three genes involved in D-xylose catabolism in

Lactobacillus pentosus. Mol Gen Genet (Internet). 1991;230(1-2):161–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/1660563

95. Song S, Park C. Organization and regulation of the D-xylose operons in Escherichia coli K-12: XylR acts as a transcriptional activator. J Bacteriol. 1997 Nov;179(22):7025–32.

96. Underwood SA. Improving the performance of Escherichia coli KO11 during the fermentation of xylose to ethanol. (Doctoral dissertation). 2003.

97. Görke B, Stülke J. Carbon catabolite repression in bacteria: many ways to make the most out of nutrients. Nat Rev Microbiol. 2008 Aug;6(8):613–24.

98. Despalins A, Marsit S, Oberto J. Absynte: a web tool to analyze the evolution of orthologous archaeal and bacterial gene clusters. Bioinformatics. 2011 Oct 15;27(20):2905–6.

99. Stone GW, Sabik JF, Serruys PW, Simonton CA, Généreux P, Puskas J, et al. Everolimus-Eluting Stents or Bypass Surgery for Left Main Coronary Artery Disease. N Engl J Med. 2016 Oct 31;

100. Franco P, Bourdin H, Braun F, Briffod J, Pin I, Challamel M-J. (Overnight polysomnography versus respiratory polygraphy in the diagnosis of pediatric obstructive sleep apnea). Arch Pediatr. 2016 Oct 25; 8(4):738-41.

101. Konieczny I, Bury K, Wawrzycka A, Wegrzyn K. Iteron Plasmids. Microbiol Spectr. 2014 Dec;2(6).

102. Bentley GJ, Jiang W, Guamán LP, Xiao Y, Zhang F. Engineering Escherichia coli to produce branched-chain fatty acids in high percentages. Metab Eng. 2016 Jul 12;38:148–58.

103. Schleif R. AraC protein, regulation of the l-arabinose operon in Escherichia coli, and the light switch mechanism of AraC action. FEMS Microbiol Rev. 2010 Sep;34(5):779–96.