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REVIEW

Pseudomonas putida in Industrial Biotechnology: Recent Advances and Future Directions

Pseudomonas putida en la biotecnología industrial: Avances recientes y orientaciones futuras

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ABSTRACT

Gram-negative, rod-shaped Pseudomonas putida bacteria may be found in a variety of biological environments. This ubiquity can be attributed to its extraordinarily adaptable metabolism, ability to endure physicochemical stress, and propensity to survive in unfavorable conditions. These traits have led to an increase in interest in microorganisms for industrial usage, also associated with studying has advanced quickly in recent years. Strong motivators in this regard include use of inexpensive waste streams and sustainable feedstocks for manufacturing with added value compounds along with continual advancement of Systematic biology of this bacterium and genetic strain engineering. Here, provide a summary of current developments and future directions *P. putida* is used as a cell factory in genetic engineering, computer systems, and synthetic biology.

Keywords: Pseudomonas Putida; KT2440; EDEMP Cycle; Metabolic Engineering; Synthetic Biology; Lignin; Microbial Cell Factory; Biotransformation; PHA; Bioeconomy; Synthetic Biology; Bacterial Chassis.

RESUMEN

La bacteria Pseudomonas putida, gramnegativa y con forma de bastoncillo, puede encontrarse en una gran variedad de entornos biológicos. Esta ubicuidad puede atribuirse a su metabolismo extraordinariamente adaptable, su capacidad para soportar estrés fisicoquímico y su propensión a sobrevivir en condiciones desfavorables. Estos rasgos han provocado un aumento del interés por los microorganismos de uso industrial, cuyo estudio también ha avanzado rápidamente en los últimos años. Entre las principales motivaciones a este respecto se encuentran el uso de flujos de residuos baratos y materias primas sostenibles para la fabricación de compuestos con valor añadido, junto con el avance continuo de la biología sistemática de esta bacteria y la ingeniería genética de cepas. Aquí se ofrece un resumen de los avances actuales y las direcciones futuras P. putida se utiliza como fábrica celular en ingeniería genética, sistemas informáticos y biología sintética.

Palabras clave: Pseudomonas Putida; KT2440; Ciclo EDEMP; Ingeniería Metabólica; Biología Sintética; Lignina; Fábrica Celular Microbiana; Biotransformación; PHA; Bioeconomía; Biología Sintética; Chasis Bacteriano.

INTRODUCTION

A desirable host for biotechnological purposes is Pseudomonas putida because it has a fast growth rate, a low food requirement, an exceptionally diverse metabolism, and a strong capacity into generate redox power. These only some of qualities such make Pseudomonas putida such an attractive candidate.

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In addition, species is tolerant to a broad operational process window, it includes a broad pH value range, high temperatures, and contains substantial amounts of hazardous chemicals alongside organic solvents. Because of their wide metabolic flexibility, members of this species can adapt to a variety of environments and dietary conditions. *P. putida* strains may often be found in soil and water.[1]

Beyond its original use for a breakdown of numerous hazardous substances, *P. putida* is presently successfully used for production of bio-based polymers and a variety about compounds. This bacterium may now be further designed into a flexible cell factory for bio-industrial usage to sequence its genomic repertoire and genome-wide pathway modeling. Here, genetic repertoire and phenotypic characteristics of the many *P. putida* species to some extent, offer a wide variety of various potential industrial applications. Presented here emphasizes current developments in systems metabolic engineering, biology, and systems key elements of cellular physiology of *P. putida*.[2]

The metabolism of *P. putida* prioritizes stress tolerance over biomass growth in this way. Furthermore, a completely biochemical regulatory layer that is driven by demand and not transcriptional regulation is applied to key pathways that provide the fundamental cellular precursors, enabling cells to respond quickly to changing environmental conditions. Conversely, transcriptional control is used to control peripheral, substrate-specific pathways. This bacterium's metabolic and physiological characteristics make them an effective receiver of pathways intended for, among others complex biotransformation, valorization of lignocellulosic wastes, and generation of surfactants.[3]

The opportunistic and undemanding dietary traits, fast development, and resilience to oxidative stress and toxic substances are all reflections of variability of *P. putida's* natural habitat. Over past five decades, *P. putida's* genetics, biochemistry, and physiology have made steady progress. This research was spurred by discovery of *P. putida's* promise in 1960s for biodegradation of xenobiotics. This resulted in, among other things, whole genomic repertoire's decryption, and creation of in silico simulations and data mapping of genome-scale metabolic models.[4]

Bacteria that encourage plant development, bioremediation tools, and hosts for industrial biomanufacturing have all been found to be representative members of species. This includes a manufacturing of both general and specialized chemicals, and natural products like rhamnolipids, terpenoids, polyketides, non-ribosomal peptides, and biopolymers. Due to its biosafety status, most well-known and extensively researched for biotechnological purposes, *P. putida* Strain KT2440, a TOL-plasmid-free variant, received extra attention.[5]

Describe an latest advancements and upcoming directions using *P. putida* and cell factory, systems also synthetic biology, and genetic engineering are discussed. The paper[6] examined a current state, difficulties, and potential for divergent biosynthesis of a variety of products using *P. putida* an organism to convert waste-derived feedstocks.

The paper[7] discussed recent developments in cost-effective manufacturing of biosurfactants, along with creation of biosurfactants into medication delivery mechanisms and use in synthesis of nanomaterials. Which combines Adaptive Laboratory Evolution (ALE) with Atmospheric Pressure Room Temperature Plasma (ARTP)-induced mutation, enhanced Pseudomonas putida KT2440's capacity to use Block Development Officer (BDO). The paper[8] provided a summary of genetic biosensing components discovered in non-model animals and current attempts. They next look at challenges that prohibit these methods from being used to non-model bacteria on a large scale. The paper[9] developed on microalgae growing in wastewater, including different pressures that affect an production of high-quality components and quality and quantity of microalgae. Prospects of biotechnology have also been considered in this review. The paper[10] presented a most recent findings on Baeyer-Villiger Monooxygenases (BVMOs) and sketches prospects for commercial application of these unique oxidative biocatalysts. The paper[11] specified algorithm-based software and model systems, and this might be solved. A unique summary of current developments a dedicated biodegradation prediction system, Quantitative Structure-Activity Relationship (QSAR) modeling, and an algorithm have also been given. The paper[12] suggested carrier-free immobilization technique is Cross-Linked Enzyme Aggregate (CLEA) technology. The dependability, simplicity, co-immobilization of a wide range of enzymes, and use of crude enzyme extract make this method particularly desirable. The paper[13] included information on three significant plants, a microbe, and plant-microbe-metal current revisions.

They have also evaluated current advancements in advantageous plant-microbe interactions and their use in control of metal-induced abiotic stress in plants. The paper[14] presented at most recent Bacterial Locomotion and Signal Transduction (BLAST XIV) conference, they have compiled a summary of current developments and research trends in this area below. The paper[15] envisaged that advancements in fermentation and downstream processing methods, creation of novel recombinant strains, and mass production by transgenic plants would lower cost of Polyhydroxyalkanoates (PHA) manufacturing and make them competitive with traditional polymers.

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DEVELOPMENT

Core Carbon And Energy Metabolism Regulation

In contaminated environments, *P. putida* is commonly found, which attests to microbe's amazing capacity for adaption to challenging circumstances. Its unusual cyclic core metabolism, which redox demand controls, do essential now enabling great endurance demonstrated. In cytoplasm or periplasm, glucose is either absorbed after entering a periplasmic region. The latter oxidation route results in formation of Gluconate (GLN), followed by 2-ketogluconate (2KG).

Both acids are capable of entering a cytoplasm where they may then be phosphorylated to become 6-phospho-2-ketogluconate (2K6PG) and 6-phosphogluconate (6PG). As a result, are three distinct entrance points for glucose into main metabolic process, which come together at level 6PG. *P. Putida* may avoid an ATP-demanding direct glucose absorption via an ABC transporter (GtsABCD) by using oxidation routes, and it can also partially decouple ATP synthesis from NADH synthesis. Periplasmic glucose undergoes two electron releases in each stage of oxidation to 2KG and GLN, which are connected to ATP synthesis through synthesizing ATP, as show in figure 1 typical Pseudomonas putida carbon flow pattern throughout core carbon metabolism.

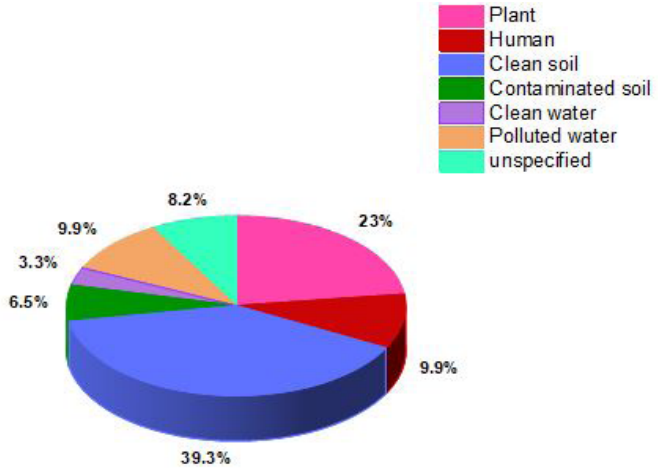

Figure 1. *P. putida* strains: isolation, source, and distribution

The oxidation route largely contributes to providing ATP, whereas Cells grown on glucose create an excess of ATP. In *P. putida*, a 6-phosphofructo-1-kinase (Pfk) glycolytic enzyme is lost, resulting in a dysfunctional Pathway Emden-Meyerhof-Parnas (EMP). Route Entner-Doudoroff (ED) proceeds to catabolize key Pyruvate (PYR) and glyceraldehyde-3-P(G3P), two C3 intermediates, which are produced almost completely from intermediate 6PG.

The Majority of former enters lower catabolism. However, in an amphibolic design resembling an EDEMP cycle and gluconeogenic EMP pathway, a sizeable fraction (between 10 and 20% under conditions of balanced growth) is converted back into hexoses.

The amount of recycling and percentage of glucose that has been phosphorylated by Glucokinase (GLK) have a major impact on NADPH production, which is connected to process of Glucose-6-P 1-Dehydrogenase (G6PDH) catalyzes. The ability of *P. putida* to switch NADPH synthesis at expense of ATP is a crucial component of its resistance to oxidative stress.

It has been demonstrated that trait is crucial for this bacterium's capacity to evolve novel catabolic pathways and most value in redox-demanding biocatalytic activities. Notably, development of an EMP-based functional linear glycolysis, which gives cells new, custom-made features, allowed de novo reformation about core carbon metabolism of KT2440 to be proven, as show in table 1.

Recent developments in systems biology of pseudomonas

Understanding underlying regulatory and metabolic networks, particularly intricate connections between recently introduced and natural biochemical pathways, is a significant problem in synthetic biology and metabolic engineering. Understanding relationships between many cellular components, genome, transcriptome, proteome, metabolome, and fluxome are a few examples, at various functional and hierarchical levels is a crucial requirement for breeding better cell factories. Recent systems biology research, utilizing and merging multiple systems-level data in online databases using omics technology has significantly improved understanding of *P. putida* have demonstrated benefit in helping to create complex along with effective factory cells.

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Table 1. *P. putida* strain isolation, sourcing, and distribution

Source	Distribution
Plant	22,6
Human	9,7
Clean soil	38,7
Contaminated soil	6,4
Clean water	3,2
Polluted water	9,7
unspecified	8,1

Metabolic Reconstructions and Genomics

The *P. putida* KT2440 complete genome sequence, which was first published in 2002 and updated in 2016, has provided information on organism's many transport and metabolic systems. In addition, research on *P. putida's* pangenome revealed 3386 conserved genes that are part of metabolism of proline, arginine, and Entner-Doudoroff (ED) and Pentose Phosphate (PP) route genes are all part of basic genome, degradation of fragrant compounds, a sizable number of nutrient transporters. In addition to 85 % of its coding regions being shared with Pseudomonas aeruginosa, *P. putida* also differs from *P. aeruginosa* in that it lacks important exotoxins and virulence factors as show in figure 2 typical Pseudomonas putida carbon flow pattern throughout core carbon metabolism.

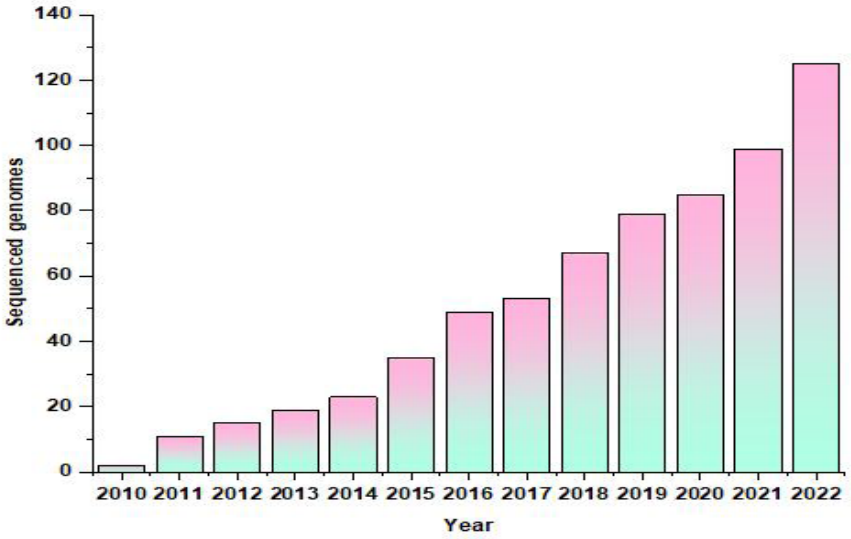

Figure 2. Pseudomonas putida glucose-grown core carbon metabolism

As shown in figure 2, there have been a significant number of published *P. putida* genome sequences since 2010. Access to 88 draught and 28 complete *P. putida* genomes are now available in the Pseudomonas genome database, enabling a discovery of novel traits for use in industry, as show in table 2.

Table 2. Glucose-grown core carbon metabolism in pseudomonas putida

Year	Sequenced genomes
2010	2
2011	11
2012	15
2013	19
2014	23
2015	35

2016	49
2017	53
2018	67
2019	79
2020	85
2021	99
2022	125

Proteomics and Transcriptomics

The transcriptional level of genes in *P. putida* is closely controlled, as evidenced by a large number of open-reading frames (450 in all), 24 distinct RNA polymerase sigma subunits, and transcriptional factors. In response to alterations in an environment (ferulic acid, citrate, fructose, succinate, serine, and glycerol), transcriptome profiles of *P. putida* KT2440 were studied and profiles during a transition between different carbon sources. Recent transcriptome investigations under imipenem, oxidative, and osmotic stress elevated heavy metal concentrations have further added to understanding mechanisms governing *P. putida's* stress response. Notably, solvent exposure led to a general stress response that led to development of stress-resistance proteins and molecular chaperons, which is beneficial for biocatalysts in a hydrophobic environment.

When producing nitrogen-limited mcl-PHAs, the transcriptional response was studied using carbon sources are oleic acid (-oxidation process) and gluconate. Several genes were proposed as potential target organisms' development toward increased production efficiency. Furthermore, by analyzing lab-created using a metabolic and transcriptional regulatory basis for ethylene glycol metabolism in *P. putida KT2440* was discovered using genome re-sequencing and qRT-PCR. Another intriguing recent work investigated every starvation's effects on *P. putida* KT2440's metabolism and transcription by simulating periodic glucose scarcity during large-scale heterogeneously mixed fermentation. Furthermore, using qRT-PCR with genome re-sequencing to characterize laboratory-developed *P. putida* KT2440, in *P. putida*, ethylene glycol metabolism's metabolic and transcriptional regulatory underpinnings were found. Another intriguing recent work investigated a *P. putida* KT2440's metabolic and transcriptional response to starvation by simulating periodic glucose scarcity during large-scale heterogeneously mixed fermentation.

Fluxomics and Metabolics

Combining isotope labeling, intracellular central metabolic intermediates quenched, extracted, and analyzed by LC-MS, in vitro enzyme testing, has been used to demonstrate cyclic activity of EDEMP pathway in *P. putida*. In recent times, investigations with parallel labeling yielded a flux map of *P. putida* KT2440 in high resolution growing on glucose.

By that point, an unresolvable cyclic network utilizing conventional flux techniques severely restricted GC-MS-based flux research in *P. putida*. By using an expanded technique that included incorporated labeling data from glycogen, lipopolysaccharides, and peptidoglycan, labeling patterns for G6P and F6P were derived, so avoiding this problem. Systems biology research on *P. putida* and related strains has advanced thanks to a unique approach.

Integration of several omics

For a more comprehensive view, some research has merged several omics techniques. The effects of various nutritional circumstances in *P. putida* KT2442 on PHA and biomass production were examined using transcriptomics, proteomics, and metabolomics integration.

For conditions of single nutrient limitation as opposed to nutritional co-limiting, a markedly distinct pattern of cellular rewiring was observed. A mix of metabolic, transcriptomic, and metabolic constraint-based flow measurements was used to explore Carbon Catabolite Repression (CCR). It has been established that CCR controls a core cells that were grown using succinate and glucose as carbon sources' metabolic fluxes and that this control aids in organization and improvement of development and metabolism.

Later studies also demonstrated that CCR and ensuing metabolic rearrangements were advantageous for growth in a complete LB medium. Another illustration research examined *P. putida KT2440's* cellular responses at transcriptome, during a chemostat culture, proteomic and metabolic levels. Every appealing C4 alcohol used in biofuel, n-butanol, was either used alone or in combination with glucose. Carbon portioning was found based on 13C-fluxome analysis: when both substrates were eaten, While n-butanol drove TCA cycle, glucose went through ED and PP pathways. Additionally, using transcriptome and proteomic analysis, a hitherto unidentified *n-butanol* breakdown pathway was found.

Recent Developments In Genetic Engineering

The creation of dependable *P. putida* production strains is made easier by a current abundance of genetic and genomic engineering tools. The platform for Standard European Vector Architecture (SEVA) offers a library of modular vectors recently been shown as an invaluable tool for creating *P. putida* recombinant strains. Fully synthesized versions of transposon vectors based on Tn5 and Tn7 are one long-standing molecular biology resource for *P. putida* genome analysis and manipulation. The transposon can be inserted into genome either randomly (using, for example, mini-Tn5 vectors) or at a specified position (using, for example, mini-Tn7 vectors).

Transposon vectors based on Tn5 have been used to create random mutant libraries and to randomly insert entire gene clusters, followed by screening for improved phenotypes, due to randomness of insertion. To maximize a production of secondary metabolites, a collection of translational couplers and promoters was built using Tn7-based transposon vectors. Additionally, regulated gene expressions using artificial promoters are necessary for industrial operations.

In addition to a previously described library of constitutive promoters for chromosomal expression, a further intriguing investigation showed capability of *P. putida* in a broad range of chromosomal expression. Some inducible promoters, both synthetic and organic, were also discovered in *P. putida*, including those previously discussed.

Recent research has focused on development and testing of KT2440 strain of *P. putida* RoxS/RoxR-based auto-inducible promoters that are reliant on cell density. These are promoter systems that don't require induction particularly intriguing as industrial operations in which protein production without addition is of an inducer sought to reduce metabolic demand throughout each step of exponential growth.

A substantial preference for integrating biosynthetic gene clusters has also been shown by *rrn* operons in *P. putida*, perhaps as a result of their native promoters, steady integration, and strong expression. Finally, it has recently been proven that modifying a new control layer for defined pathways by targeted proteolysis through metabolic pathways.

Pseudomonas Putida as an Industrial Bacterial Host: Innately Advantageous Characteristics

A microbial host must satisfy many performance standards and quality specifications for industrial sector, including simplicity of handling, consistency, and repeatable construction behavior, also inherent resilience to facilitate procedure creation. In actuality, *P. putida* comes with a lot of favorable traits by default.

Due to an enormous amount of study, there is first and foremost a wealth of fundamental understanding of *P. putida*, which is a must for any additional metabolic engineering to have a streamlined workflow, following are lessons regarding *P. putida* learned:

(a) Every bacterium grows quickly, produces a lot of biomass, secretes few to no by-products, and requires little maintenance.

(b) The hexose metabolism caused by EDEMP overload, *P. putida* naturally maintains an excess of ATP synthesis and high NADPH regeneration rates. Metabolic pathways can also be rewired to feed an EDEMP cycle in a top-down manner into facilitate NADPH overproduction of additional gluconeogenic substrates, such glycerol.

(c) P. putida's extensive regulatory system gives it high flexibility it needs to respond fast to steadily changing settings, which is particularly important in a sizable bioreactor with heterologous microenvironments.

(d) The organism may catabolize a variety of carbon sources. In addition, effectively identified a *P. putida* substrate spectrum broadened to include sucrose consumption. As a result, it is possible to produce value-added chemicals from inexpensive, renewable feedstocks with a high degree of impurities, such as glycerol, a byproduct of manufacturing of biodiesel, and aromatic compounds generated from lignin. One further way of acquiring external nutrients has been identified as production of Outer Membrane Vesicles (OMVs).

(e) An effective regulation mechanism, a high level of resistance to chemical and physical stress has been associated with changes in cell membrane phospholipid head group composition, secretion mechanisms, and trans-isomerization of cell membrane, cadmium, arsenic, solvents, and oxidative stress.

(f) In addition, *P. putida* has a naturally high GC content (61-63%), which enables a heterologous expression of GC-rich microorganisms' genes that include gene clusters for biosynthesis of secondary metabolites, including actino- and myxobacteria. *P. putida* is a great host for industrial biotechnology due to all of these characteristics.

Pseudomonas putida as an industrial bacterial host: development of custom synthetic characteristics

The reasons already outlined, *P. putida* is known as a possible microbe chassis for bioindustry. Its inherent capacity is already being increased by some research teams. Table 3 and Figure 3 demonstrate enhanced traits increased availability of ATP and NAD(P)H, reduced genome size of streamlined *P. putida* strains, superior growth characteristics, and increased resilience to oxidative stress. *P. putida* is a strictly aerobic bacteria by nature since it lacks fermentative pathways and cannot utilize other electron acceptors. This issue can be resolved, which will improve *P. putida's* performance in significant bioreactors. Nitrate/nitrite respiration and artificial fermentation pathways were introduced to strain KT2440, increasing its ability to survive in anoxic

environments.

Table 3. Top-down methods for creating more efficient *P. putida* strains

Strain	Genome reduction	Deletion	Characteristics
407,1-Δ2	~ 4,5 %	ΔPP_3534-PP_3733; ΔPP_4290-PP_4308	Growth comparable to or superior to wild-type strain
407.3-Δ2	~ 7,5 %	ΔPP_3534-PP_3733; ΔPP_3533-PP_3360	Similar to or superior to wild-type strain's growth
EM42	~ 4,4 %	Δflagellar operon, ΔendA-1, ΔendA-2, Δprophages, ΔTn7, ΔTn4652, ΔhsdRMS	Superior to strain KT2440 in terms of growth characteristics, energy charge, NADPH level, genomic stability, and plasmid structural stability.
EM383	~ 4,4 %	ΔrecA	EM42 derivative with increased genomic stability
SEM10	~ 4,9 %	Δß-lact(-like) genes ΔpvdD ΔbenABCD	Derivative of EM42; increased biosafety, decreased autofluorescence, and facilitated use of 3-methylbenzoate as an inducer
EM371	~ 4,8 %	Δflagellum Δfimbriae Δsurface adherence proteins ΔEPS ΔO-antigen side chain ΔTn7 Δprophage 4	Superior to strain KT2440 in terms of cell surface's accessibility, stability of genome, and UV resistance.
KTU-U13	~ 4,2 %	Δgenomic islands	Similar growth, improved plasmid stability, and maybe higher heterologous protein expression

Additionally, *P. putida* was grown under anoxic conditions in an anodic compartment about Bio Electrochemical System (BES) that balances intracellular redox also energy parameters with use of extracellular electron sink for anode and redox mediators. A great place to begin without synthesis of sugar acids with a high yield every requirement being oxygen is *P. putida's* innovative electro-biotechnology.

The morphology of *P. putida* has been engineered in an intriguing way to change its transition from planktonic life to one based on biofilms, which can be more resilient to severe reaction conditions during biotransformation. The ability of whole-cell catalyst use of spatial layout considerably helps scrub up extracellular products is a special trait of Pseudomonas species.

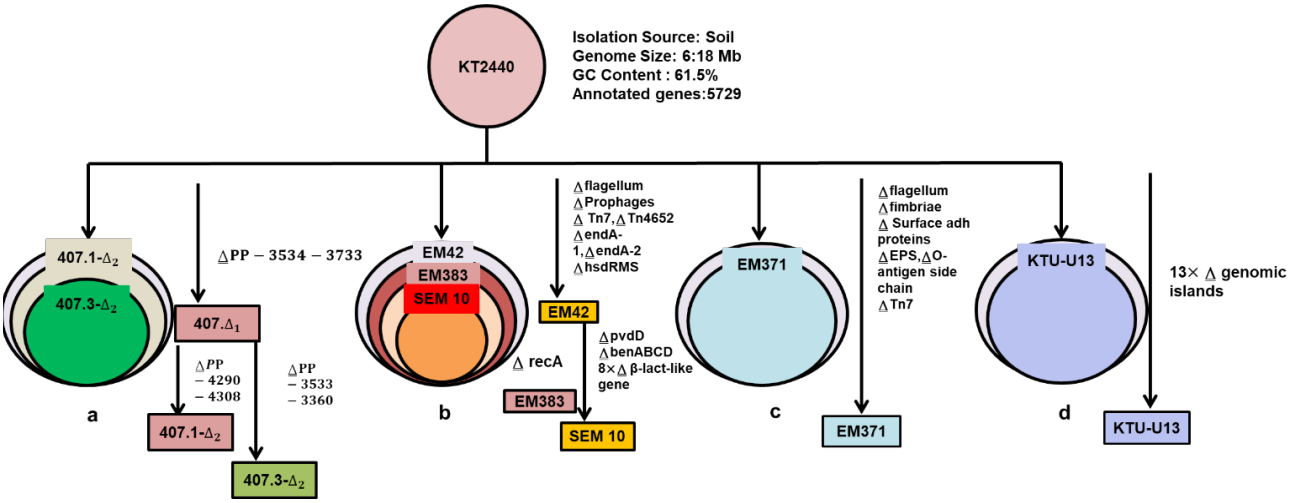

Figure 3. To date, *P. putida KT2440 genome*-reduced strains have been created; (a) random large-scale deletions using a *mini-Tn5* transposon; (b) Large target deletions with *homologous* recombination-based *I-SceI* method; (c) large target deletions performed with *I-SceI* technique; (d) An *upp*-based counter-selection technique was used to target *13 genomic islands* for substantial deletion

Industrial Application and BioProduction

P. putida was examined and created for use in bioremediation, as a biocontrol agent and bacterium that encourages plant development, which is not unexpected given biotechnological applications of this bacteria. As

shown in Figure, *P. putida* has also demonstrated that it is a superior bacterial host for production of polymers, bulk chemicals, medicines, and expensive specialties. In addition, application range of *P. putida* has expanded significantly over past several years.

Polyhydroxyalkanoates

P. putida spontaneously produces endo polymeric *mcl-PHAs* under specified circumstances, such as carbon surplus under nutrient constraint *(N, O_2, P, S)*, a molecule that stores energy and carbon. One of most popular product categories investigated in Pseudomonas species is family of PHA polyesters.

Due to their capacity to biodegrade and competitive material qualities including biocompatibility, safety, insolubility, and thermostability, PHAs provide a great replacement for plastics made from petroleum. Precursor feeding, culture conditions, and strain engineering may all be used to modify a structural makeup of PHAs. These procedures may potentially have negative environmental effects, excessive prices, or polymer breakdown.

Recently, a programmed *P. putida* KT2440 that has a cell lysis mechanism that can respond to osmotic pressure conditions was demonstrated to recover about 94 % of produced mcl-PHA after 3 hours of cell disruption. When combined, recent accomplishments will open door to further cutting back on process expenses at level of downstream processing and raw materials choices.

Alginates

Dehydration has been considered a universal cue for production of these polysaccharides by *P. putida*, despite fact that it has received less research. Alginate is a popular food and cosmetic ingredient with a wide range of medicinal uses. However, up until this point, research on alginate synthesis has mostly focused on pathogenic *P. aeruginosa*, and there hasn't been any connection between these efforts and any commercial applications.

Cis,cis-muconic acid

P. putida's genome has an arsenal of oxidoreductases, mono- and dioxygenases that allow every bacterium to break down a range of aromatic chemicals included in renewable feedstock lignin. The *ketoadipate* (-KA) route is used to channel aromatic compounds through catabolic funneling, resulting in a small number of core intermediates that are then further broken down into intermediates of TCA cycle.

The cis,cis-Muconic Acid (MA) in this route is quite a promising intermediate because utilized a synthesis's raw ingredient of chemicals with high value-added bulk chemicals in polymer processes including caprolactam, terephthalic, and adipic acid to create muconic homo- and copolymers.

Adipic acid and nylon 66

Through hydrogenating MA to produce a precursor to commercial nylon-66, adipic acid, and whole lignin to bio-based nylon value chain was shown in figure 4, a combined chemical and biological process is shown. Using recent restricted life cycle analysis about *P. putida* a whole-cell biocatalyst determined an viability of softwood lignin-derived aromatics for bio-based manufacture of adipic acid and possible offset credits for bioethanol biorefineries were pushed if lignin in their effluent wasn't being burned anymore, but instead transformed into a value-added product called adipic acid.

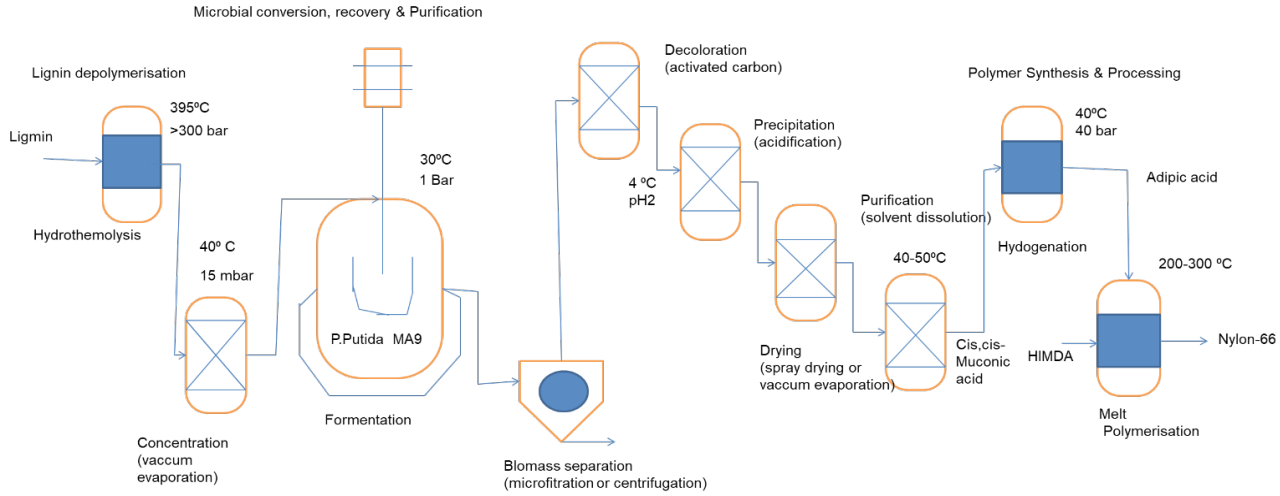

Figure 4. The production of nylon 66 involved a cascade biochemical and chemical process using metabolically altered P. putida

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2,5-Furan dicarboxylic acid

Important platform chemistry Using mono- and polysaccharides from pre-treated biomass, hydroxymethyl furfuraldehyde (HMF) is produced. The fundamental substance 2,5-Furandicarboxylic Acid (FDCA), which may be used to make plasticizers, polyamides, and polyesters, is produced when HMF is subjected to further catalytic oxidation. Ethylene glycol and FDCA are copolymers that form polyethylene furanoate (PEF), which is one polymer of special importance. It is an attractive substitute for packaging made of Polyethylene Terephthalate (PET) because of to material's excellent thermal and barrier properties. By combining Red-mediated recombination using CRISPR/Cas9 system, co-integration of chromosomes hmfH and hmfT1 in P. putida S12 was recently established. A subsidiary of Corbion, a business involved in commercial manufacturing of PEF derived from FDCA, filed a patent application that is quite promising and deals with production of FDCA from HMF utilizing *P. putida*.

Aromatics

Epoxidation of styrene in a water/octanol system with two liquid phases, region-selective biosynthesis of 3-methyl catechol, and biosynthesis of o-cresol from toluene all benefit from ability of organisms to tolerate and metabolize aromatic compounds. Pyruvate and lactate are other building blocks created from *p-coumarate* and benzoate. In addition to bioconversions and -transformations, *P. putida's* inherent resistance to aromatic chemicals has been used to create new aromatic compounds from intermediates in shikimate pathway. Cinnamic acid, phenol, and *p-hydroxy styrene* synthesis utilizing a biphasic fed-batch growing approach with solvent-tolerant *P. putida* S12 strain and water/1-decanol phase were described. Strains obtained from KT2440 were able to convert glucose into anthranilate, *para hydroxybenzoic acid*, and *p-coumaric acid*.

Biosurfactants

Rhamnolipids are low-toxic and biodegradable surfactants. By reducing surface tension, biosurfactants' action improves an solubility of hydrophobic compounds in water. Potential uses include food manufacturing, cosmetics, household goods, biocontrol, and soil remediation. After rhlAB(C) operon from *P. aeruginos* was heterologously integrated, and *P. putida* was able to create short-chain rhamnolipids, and Burkholderia glumae's rhl genes were expressed to make long-chain *rhamnolipids*.

Terpenoids

Terpenoids are among most prevalent and structurally varied groups of natural compounds, are produced in vast quantities thanks to *P. putida's* great tolerance to toxicity of intermediates or final products. The efficient de novo synthesis of monoterpenoid germanic acid, which has many documented antibacterial actions and functions as a flavor and aroma agent, may be shown by taking advantage of natural resistance. Comparing *P. putida* to Saccharomyces cerevisiae and *E. coli*, this study also showed that *P. putida* was noticeably more resistant to product concentrations that were more than six times higher.

Non-ribosomal peptides and polyketides

Polyketides (PKs) and Non-Ribosomal Peptides (NRPs) exhibit several groupings about natural products with often occurring therapeutically important actions. Both are created by condensation of basic amino acid or carboxylic building components. To create a finished product, generated polymers may be decorated and cycled. Both UV-protective pigment *flaviolin* and PKs 2,4-diacetyl phloroglucinol were effectively synthesized in *P. putida*. Prodigiosin, an antibiotic with anticancer and immunosuppressive properties, is an intriguing PK/NRP hybrid molecule. Prodigiosin transcription is prevented in *E. coli*. *P. putida*, however, is an exception to this rule.

Recombinant protein production

With correct folding and promising yields, antibodies might be expressed in *P. putida* KT2440. Additionally, *P. putida* entire cell enzymes are used in industry. A business that has reported producing chiral chemicals DSM uses *P. putida* ATCC 12633 isolated enzymes as a source of enzymes. The production of 5-cyanopentanamide, *D-p-hydroxyphenyl glycine*, along with 5-methyl pyrazine-2-carboxylic acid is made possible by whole-cell *P. putida* biocatalysts. Additionally, Commercial bacteria of strain BIRD-1 are available that promote plant development.

CONCLUSION

Bacterial chassis (platform) than on historical precedence when it comes to microbial hosts for biotechnological purposes that best satisfy a specified process parameter. This is mostly due to fact that well-studied and characterized microorganisms may be modified more readily to increase output, and at same time, their behavior is more predictable during industrial scale-up.

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Recent advances in systems biology and synthetic biology techniques are helping to change how businesses choose their microbial hosts. For *P. putida*, this is especially true. Long recognized for its flexible metabolism and low nutrient needs, recent developments in omics technologies now make it possible to discover novel appealing traits of genome-sequencing representatives, to regulate transcriptional and translational levels of gene expression, also to decode each central core and energy metabolism by flow analysis.

Findings from this research, combined with advancements in genetic engineering techniques, do especially important for completing comprehensive improving commercial strains and increasing knowledge of *P. putida*. Findings from this research, together with advancements in genetic engineering methods, are especially important for completing their comprehensive comprehension an *P. putida* and facilitating every creation about improved industrial strains.

It ought to emphasize such capacity to produce value-added chemicals made using different feedstocks is a better trait to pass into an imagined circular bioeconomy. Future options include redesigning a biochemical portfolio to incorporate full synthetic processes (bio-bricks) to produce items that are novel such as halogenated substances and boron-containing compounds, to nature.

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CONFLICT OF INTEREST

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