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Strategy to Accelerate the Biopharmaceuticals Production Using *Komogataella phaffii* (*Pichia pastoris*)

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Abstract

Revenue generation has been the primary goals for nations as well as the industries worldwide. Biopharmaceutical verticals have become potential revenue generators as they are the industrial workhorses and the economic engines that drive the upcoming biopharmaceutical industries with advances in DNA recombinant technology. A breakthrough in the indusrial biotechnology for the production of biopharmaceuticals using Pichia pastoris is awaited. Ablynx NV, Recombinant proteins (RP), HSA-pFSH beta, antibodies, borosin, N-glycoprotein, antimicrobial peptides, interferon alpha-2b, human interferon (hIFN gamma), recombinant human insulin along with C-peptide, growth hormones, monoclonal antibodies (mAbs), antimicrobial peptides, clavanina, a modified MO are some of the classical examples of the biopharmaceuticals that can be produced using Pichia pastoris as the host. The ease and fast growth rates, like the bamboos in the forest, makes Pichia pastoris as a preferred choice compared to many other fungal and bacterial strains to be used as promising host. Moreover, tunability of culture medium, sustainability of the yeast strain even under nutrient starved conditions, ease of tuning of the metabolic pathways via synthetic biological schemes facilitate Pichia pastoris to yield unconventional drugs and hormones for the alleviation of suffering of mankind.

Keywords: *Pichia pastoris*; yeast; support; dispersion; biopharmaceuticals; acceleration; production

INTRODUCTION

A web of science search with the keywords, namely, *Pichia pastoris*, yields 10, 908 results, indicating the fertility of the research area. The development of the field during the past decade (2014-2023) is shown in Figure 1.

The afore mentioned number shrinks down to 64, three orders of magitude lower, when the keywords, namely, *Pichia pastoris* and biopharmaceuticals, were crossed together [1-30]. The potential of *Pichia pastoris* is not utilized to its fullest. The manufacturing of the recombinant therapeutic proteins is rapidly growing. Yeasts are well-known for the production of heterologous proteins. Advantages with yeasts include – rapid growth even on inexpensive and sustainable supports that are even deficient of any nutrients. Moreover their growth is easily tunable at the genetic level. Though *Saccharomyces cerevisiae* is used as a model strain for the production of biopharmaceuticals, there are wide opportunities with the choice of fungal strains, namely, Pichia pastoris, *Kluyveromyces lactis*, *Hensunula polymorpha* and *Yarrowia lipolytica*, that have been less explored [11]. Enormous effrots were made (in the glycoengineering of the N and O-type glycosylation leading to the removal or suppression of the yeast specific glycans) so as to improve the glycosylation pathways. Glycosylation is vital for the folding process of a majority of

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recombinant proteins. Glycosylation has a great impact on the pharmacokinetics and pharmacodynamics of the therapeutic proteins. As a result of the advances made in the glycosylation pathways of the yeast cells, the conventional challenges encountered in the direction of the synthesis of biopharmaceuticals (recombinant proteins) are surmounted and the application of yeast for biopharmaceuticals is gaining the past glory as well as the pre-eminence [12].

ADVANCES IN THE PRODUCTION OF BIOPHARMACEUTICALS USING *PICHIA PASTORIS*

Komogataella phaffii is the new name for *Pichia pastoris* [1]. Among several biopharmaceuticals, the recombinant proteins standout. In particular, the interferons secreted by autocrine and paracrine glands have high market value and can generate rich revenue for the upcoming pharmaceutical industry. Clinical trials with interferons are in progress. Such biopharmaceuticals improved the well-being of the patients suffering with several diseases. Among various approved biopharmaceuticals, the therapeutic proteins, antibodies and their derivatives standout. DNA recombinant technology facilitated the boom in the biopharmaceutical market, owing to the production of highly selective proteins for therapy and also with the potential of targeted drug delivery. Ever since the introduction of Isaacs and Lindemann in 1957, interferons served as a ray of hope that were approved for human clinical trials during 1980's [2]. The interferons are the proteins that were secreted by the autocrine and the paracrine glands. They are clinically effective against viral, malignant and multiple sclerosis diseases. Their activity is related to their ability to regulate several biochemical pathways. Pedro and coworkers produced a state of the art review on interferons with emphasis on their revenue generation potential highlighting various strategies for development in the manufacturing of interferons (upstream stage, downstream stage, formulation stage and delivery stage) [13]. The performance of the biopharmaceuticals belonging to the class of borsins (lentinulins and dendriothelins) was enhanced (with respect to their proteolytic stability, membrane permeability and target specificity) via backbone N-methylation as well as by macrocyclization. Such homologous peptide based natural products with biopharmaceutical as well as biopesticide activity were produced by engineering the yeast, namely, *Pichia pastoris*, to coexpress OphMA hybrid proteins and OphP in the same yeast. The recombinant peptides (borosins thus produced exhibited nematotoxic activity as well against the famous plant pathogen, namely, *Meloidogyme incognita* apart from being a promising biopharmaceutical [14]. Fermentation using *Pichia pastoris* is widely used for the production of biopharmaceuticals as shown in Table 1.

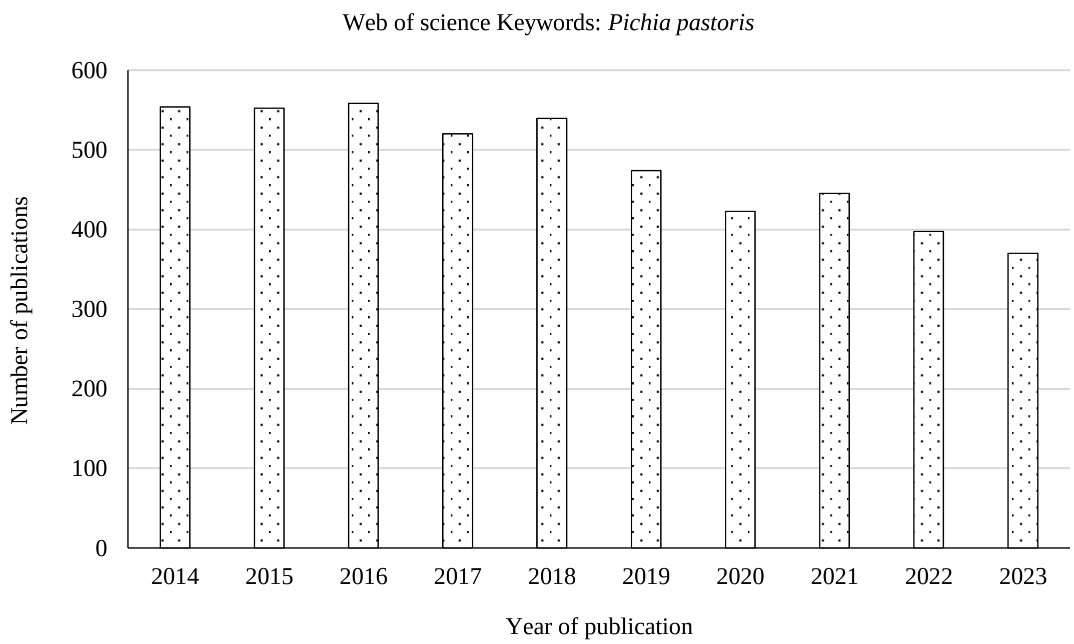

Figure 1. Development in the field of "*Pichia pastoris*" during the past decade (2014-2023).

Table 1. Biopharmaceuticals produced from fermentation process using *Pichia pastoris*.

S.N.	Biopharmaceutical produced	Reference
1	Ablynx NV, a subsidiary of Sanofi, [Trade name: Nanobody (R)]	4
2	Recombinant proteins (RP)	5
3	recombinant proteins (RP)	6
4	HSA-pFSH beta (recombinant protein, RP)	8
5	Recombinant Proteins (RP)	9
6	heterologous proteins, including industrial enzymes	10
7	pharmaceutical and therapeutic recombinant proteins	11
8	herapeutic proteins, antibodies, and their derivatives , nterferons	13
9	borosin peptide natural products, namely, lentinulins and dendrothelins, (showed biopesticide activity as well)	14
10	recombinant and homogeneous N-glycoprotein (*N*-GlcNAc)	15
11	Recombinant proteins	16
12	Antimicrobial peptides (AMPs)	17
13	IFN-alpha 2b protein and its variants	18
14	Interferon alpha-2b and granulocyte colony-stimulating factor	**19**
15	Recombinant human interferon (hIFN gamma)	20
16	recombinant human insulin along with C-peptide	21
17	Interferons (IFN alpha, beta, and gamma) and growth hormones	22
18	monoclonal antibodies (mAbs)	23
19	Antimicrobial peptides clavanin MO (clavMO), a modified version of Clavanin A	24
20	rabbit hemorrhagic disease virus (RHDV) capsid protein (VP1), a selfassembled multipurpose antigen/carrier (calici virus like particles)	25
21	Human glycoproteins	26

Atomic Level Mechanistic Aspects of the Action of Biopharmaceuticals – N-GluNAc Interacts with the Glycosite Asn297 [15]

The mechanism of the therapeutic action of the biopharmaceuticals, with the specific example of glycoproteins is presented here. N-glycoproteins have huge market value as biopharmaceuticals. N-glycosylation of proteins results in optimal conformation, structrual stability, improved serum half-life, and biological efficiencies of the N-glycoprotein based biopharmaceuticals. Homogeneous and recombinant N-glycoproteins are much wanted as they have the well defined N-glycans with specific clinical therapeutic potential. However, high production costs, non-humanized N-glycan structure and the microheterogeneties related to the N-glycosylation are the major hurdles that need to be surmounted towards the commercial scale utilizaton of the recombinant, homogenous N-glycoproteins as the targeted biopharmaceuticals. The N-GluNAc interaction with the glycosite Asn297 was proved from the mass spectrometer studies while probing the molecular level therapeutic activity of the N-glycoproteins produced by *Pichia pastoris*. Thus glycoengineering of the yeast resulting in the production of homogeneous recombinant N-glyco protein (N-GlcNAc) is a promising strategy to combat several diseases. The recombinant protein (N-GluNAc) introduces an ENGase isoform (Endo-T) with a powerful hydrolytic activity towards the high mannose base N-glycans. The location of Endo-T in various subcellular components, namely, endoplasmic reticulum (ER), golgi, cell membrane was observed and has been attributed to the hydrolytic function of the biopharmaceutical towards the high mannose based N-glycans [15]. Love J Christopher and coworkers have reported a new design strategy for the synthesis of human growth harmone namely, interferon alpha-2b and granulocyte colony-stimulating factor and the potencies of these biopharmaceuticals were found to be on par with the reference products in the market [19].

Such molecular studies probing the therapeutic potential of various biopharmaceuticals is actively pursued using molecular docking and molecular dynamic simulation studies [27-32]. Use of unconventional yeast like *Hansenula polymorpha*, for biopharmaceuticals production is a new avenue [33]. Optimization of the growth medium of the yeasts for fermentation has also been found to play a role in the biopharmaceutical productivity [34]. Synthetic biological aspects of yeast strains are intensely studied for exploring the natural metabolic pathways in yeasts for producing biopharmaceuticals [35, 36]. A revolution is happening in the exploration of the analytical techiques for the characterization of the biopharmaceticals produced as well as the degradation products. Techniques like LC-MS and NMR are widely used [37]. Yeast cell engineering strategies to tackle the issue of carbon metabolism inefficiencies are widely studied [38]. Suppression of unwanted metabolic pathways to maximize protein productivity were suggested with *Saccharomyces cerevisiea* as the model yeast strain [39]. Use of other family of yeasts, namely, Trichoderma reesei, for the production of human interferons is reported [40]. Unconventional yeast strains like *Yarrowia lipolytica* have also been reported for bioactivity for synthesis of drugs [41]. Cultivation strategies of yeast (Pichia pastoris) improved the productivity of biopharmaceuticals [42].

FUTURE PERSPECTIVE

Dispersing the yeast, namely, *Pichia pastoris*, on a novel support like silica gel is surmised to accelerate the production of biopharmaceuticals via generation of orphaned yeast bud particles on the surface leading to their enhanced performance by full utilization of individual yeast particles [43]. Moreover, use of solar energy in the fermentation process using *Pichia pastoris* has never been attempted in literature and such a strategy is surmized to acclerate the biopharmaceutical production [44-47]. For effective light managment for fermentation under sun, the use of nanocellulose spheres encapsulated with sodium silicate is recommended owing to their possible light transparent features and such an idea has never been evaluated in scientific literature [48-50]. Thus yeast are workhorses and industrial engines for the production of natural therapeutics and vaccines [51]. Owing to the potential of the yeasts in general and *Pichia pastoris* in particular, the genomic and transcriptomic studies were actively investigated to utilize the potential of the biological objects to their fullest [52]. Among various microbial hosts, *Pichia pastoris*, is considered as promising owing to ease of high biomass productivity and correspondingly far higher yields of industrial biopharmaceuticals and enzymes [53].

CONCLUSION

Biopharmaceuticals industry is envisioned to play a key revenue generator role in near future owing to the advances in synthetic biology, biotechnology, DNA recombinant technology as well as the nanobiotechnology. Among various host microorganisms for the fermentative production of biopharmaceuticals, the yeast strain, namely, *Pichia pastoris*, stands out as exmplified by the diversity of the recobinant proteins, enzymes, antigens, vaccines, that could be produced. Some new ideas on the use of transparent nanospheres of cellulose as unconventional support to obtain orphaned nanoyeast particles dispersed on the support with effective solar energy harvesting were offered that could revolutionize the upcoming biopharmaceutical industry.

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