Yeast-Based Virus-like Particles

Subjects: Mycology

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Virus-like particles (VLPs) are empty, nanoscale structures morphologically resembling viruses. Internal cavity, noninfectious, and particulate nature with a high density of repeating epitopes, make them an ideal platform for vaccine development and drug delivery.

yeast virus-like particles (VLPs) subunit drug delivery

1. Introduction to Virus-Like Particles

Virus-like particles (VLPs), ghost viruses, or dummy viruses lacking genetic material are nanostructures first observed in sera samples from hepatitis patients in 1968 [1]. VLPs can exist naturally (in a virally infected host) and can be generated in the laboratory. The size of VLPs may vary from 20 nm to 200 nm or more [2]. The size of VLPs depends on the virus species and viral proteins used for developing these particles [2]. It is essential to mention that VLPs can be formed using the capsid, envelope, or core viral proteins [2]. These particles are usually formed naturally by folding viral proteins under appropriate conditions, including the optimum pH, salt concentration, temperature, and so on [3][4][5]. The VLPs form when monomeric proteins fold into a pentameric form, also called capsomers, which are then assembled to form VLPs [5]. VLPs can exist either non-enveloped, as seen in the case of HPV VLPs, or enveloped with a lipid membrane (eVLPs), such as SARS coronavirus VLPs [6][7]. The shape of VLPs also differs from icosahedral to rod-shaped [8]. These particles may be composed of a single protein or can be a fusion of two different proteins (chimeric VLPs) [9][10].

The feature that makes VLPs important in vaccine development is their high density of epitopes, particulate nature, and lack of genetic material that restricts their replication and makes them safe for the host [1][11][12][13]. In addition to their use in vaccine development, VLPs are also widely investigated to deliver drugs and other small molecules inside the host system. This property is attributed to the internal cavity in VLPs. Several studies also showed the feasibility of using VLPs in photo imaging [12][13]. Different applications of VLPs are shown in **Figure 1**.

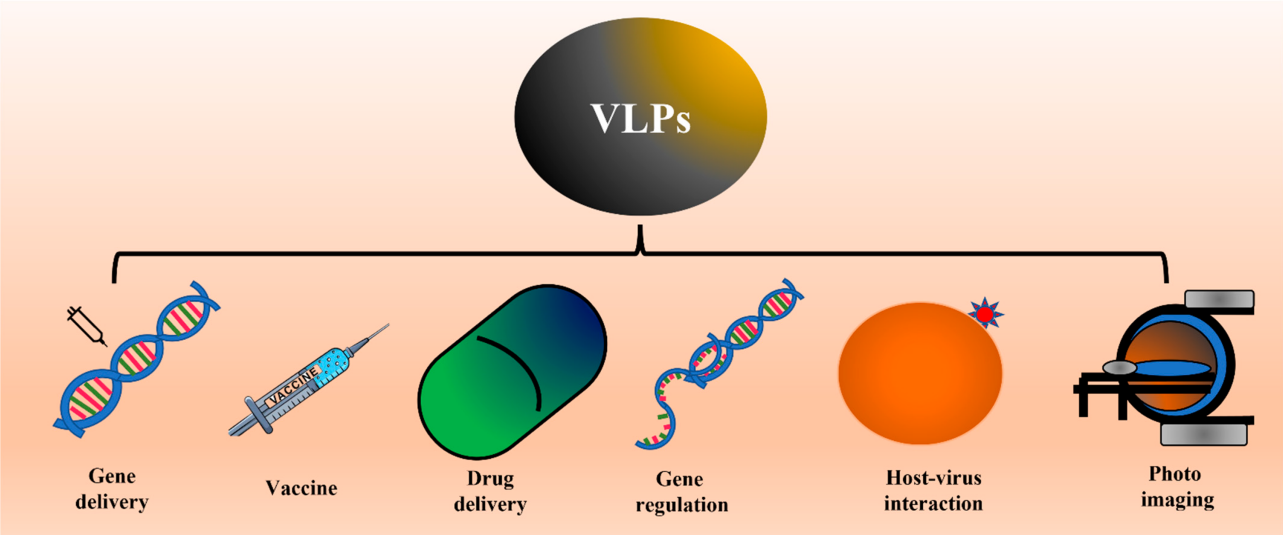

Figure 1. Schematic showing the different applications of VLPs. Several studies have already shown proof of concept for applications like vaccines, drug delivery, and photo imaging. The researchers propose the possible use of VLPs to study virus-host interaction or internalization.

The high epitope density and particulate nature of VLPs make them ideal systems for mounting immune responses. It is essential to mention that VLPs can mount both humoral and cellular immune responses [14][15][16][17][18][19]. Because of their natural resemblance to viruses, VLPs act as pathogen-associated structural patterns (PASP) and are easily recognized and taken up by host immune cells [14][15]. Additionally, their structural properties help activate immune cells like dendritic cells [20]. Due to their ability to mount both humoral and cellular immune responses, VLPs appear to be a better choice for vaccine delivery than purified proteins [21].

Due to their importance in vaccine development and drug delivery, efforts were made to express and purify VLPs from different biological systems and identify the most suitable hosts for producing VLPs commercially. To this end, VLPs are successfully expressed and purified from both the prokaryotic system (for example, *Escherichia coli*) [9] as well as from the eukaryotic system, including yeast (for example, *S. cerevisiae*) [22], insect cell lines [23][24], mammalian cell lines [14][25], and plants [26] (as shown in **Figure 2**).

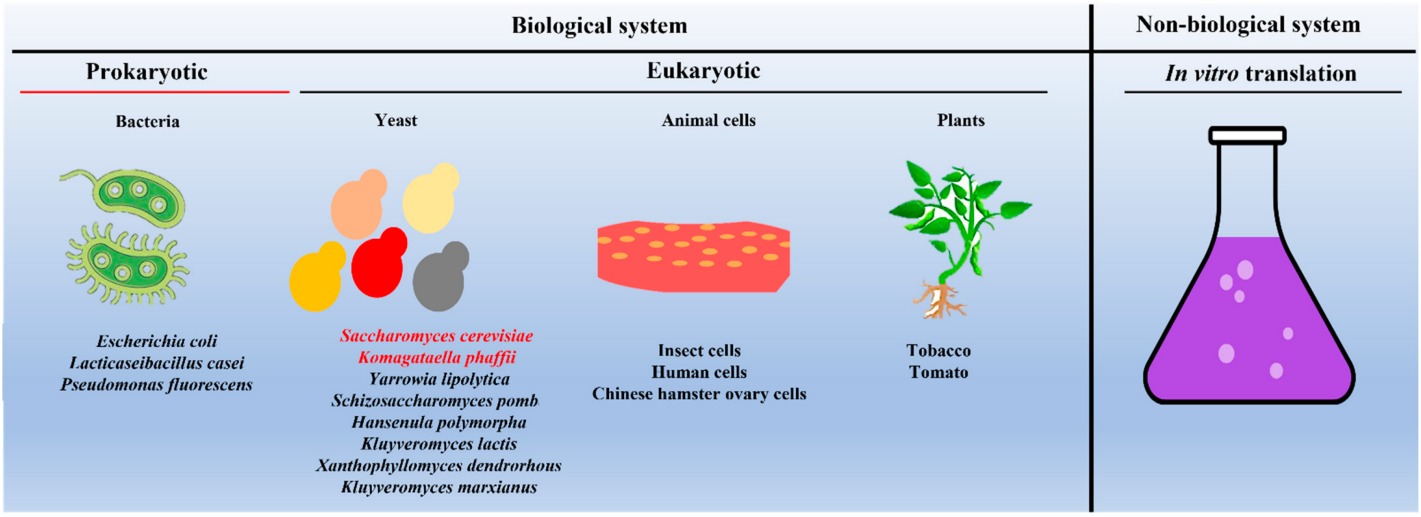

Figure 2. Schematic showing different systems used as hosts for expressing and purifying VLPs. Note: *Saccharomyces cerevisiae* and *Komagataella phaffii* (in red font) remain the most commonly used yeast species. In the case of in vitro production of VLPs, one can use the protein translational machinery of either bacteria (prokaryotes) or yeast (eukaryotes).

Apart from whole cells, several studies have shown that VLPs can be produced by in vitro protein translation systems [27]. The advantages and disadvantages of in vitro protein translation-based VLP generation are discussed by others [27][28][29]. In **Table 1**, the advantages and disadvantages of different systems used for the expression and purification of VLPs are compared.

Table 1. A comparison of the different model systems used as a host for the production of VLPs [28][30].

Feature	Bacteria	Insect Cells	Mammalian Cells	Plant	Yeast
Production cost	Low	High	High	Moderate	Low
Growth media	Simple	Complex	Complex	Simple	Simple
Growth	Fast	Slow	Slow	Very slow	Fast
Growth duration	Very small	Small	Small	Long	Small
Indoor/Outdoor	Indoor	Indoor	Indoor	Outdoor/polyhouse	Indoor
Scale-up	Easy	Very difficult	Very difficult	Difficult	Easy
Secretion	No	Yes	Yes	NA	Yes
Enveloped/non-enveloped	Non-enveloped	Enveloped/Non-enveloped	Enveloped/Non-enveloped	NA	Non-enveloped/Enveloped possible

Feature	Bacteria	Insect Cells	Mammalian Cells	Plant	Yeast
Speed of transformant screening	Very fast	Slow	Slow	Very slow	Fast
Effect of seasonal variations	No	No	No	Yes	No

NA: clear information not available.

2. Yeast, Host to Produce VLPs on a Commercial Scale

As mentioned above, several biological systems have been evaluated over the years for a suitable host to produce VLPs on a commercial scale. An ideal host for the commercial purification of VLPs should be nonpathogenic, easy to handle, able to grow on economic media, able to express the protein of interest in the maximum amount, allow proper folding of a large amount of expressed protein, be genetically responsive, and be easily scaled up to industrial measures [30]. The secretion of the expressed protein(s) and VLPs (both enveloped and non-enveloped) into the medium will be another beneficial facet. Looking at these attributes, a yeast-based system appears as an ideal host for the commercial production of VLPs.

In the past, several yeasts were successfully used to express and purify clinically relevant proteins. Yeast species, *S. cerevisiae* and *K. phaffii* (formerly known as *Pichia pastoris*), fall under GARS (Generally Recognized as Safe), which is another significant advantage of using yeast-based systems for the development of VLPs [31]. Unlike the bacterial system, the yeast-based system does not suffer from endotoxin problems [32], and the solubility of expressed proteins and folding are much better in yeast compared to bacteria [33]. Furthermore, utilizing mammalian and insect cells for VLP production is expensive due to the high cost of media and poor scalability [34]. In contrast, yeast can quickly grow on simple media [35]. A yeast species, *K. phaffii*, can be grown to a high cell density on a commercial scale, which is not feasible with animal cells. The rapid growth of yeast cells (unlike animal cells, yeast cells grow faster with a doubling time of around 90–120 min, whereas animal cells have a doubling time of 16–18 h or more) is another advantage [36]. The growth of plants is slow and may take several weeks, months, or even years to reach the desired maturity. Another issue is the varying expression levels in different organs or tissues and more batch-to-batch variation. Other concerns include the possibility of escape into the natural environment [37]. The seasonal variation may severely impact plant growth, so the expression of the protein of interest remains an important consideration. Due to several advantages (as mentioned), yeast has been extensively used for expressing and purifying VLPs, especially *S. cerevisiae* (**Table 2**) and *K. phaffii* (**Table 3**).

Table 2. Studies in which *S. cerevisiae* yeast was used to generate VLPs.

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S. No	Protein Antigen	Virus	Protein Localization	Promoter	References
1	VP2	Human parvovirus 4	IC	Hybrid GAL10-PYK1promoter	[38]
2	Capsid protein	Hepatitis E virus	IC	GAL promoter	[39]
3	Capsid protein	Porcine circovirus type 2	EC	GPD, TEF2	[40]
4	Nucleocapsid protein	Sendai virus	IC	GAL7	[41]
5	VP2, VP1	Human bocaviruses	IC		[42]
6	Surface antigen	Hepatitis B virus	IC	GAL	[43][44][45]
7	p55(gag)	HIV-1	EC		[46]
8	VP1	Human polyomaviruses	IC	GAL	[47]
9	L1	HPV 16	IC	GAL10	[48][49]
10	Nucleocapsid protein	Tioman virus	IC	GAL10	[50]
11	L-HDAg and surface antigen	Hepatitis delta virus	IC	GAD	[51]
12	Capsid protein	Porcine circovirus type 2	IC	GAL10	[52]
13	Capsid protein	Enterovirus 71	IC	GAL10	[53]
14	VP1	Human and non-human polyomaviruses	IC	GAL	[54]
15	Capsid protein	Adeno-associated virus	IC	GAL1	[55]
16	Nucleocapsid protein	Human parainfluenza virus 4	IC	GAL7	[56]
17 *	Coat protein	Cacteriophage Qbeta virus	IC	GAL	[57]
18	Capsid protein	Nervous necrosis virus	IC	GAL	[58]
19	VP1,2	Parvovirus B19	IC	ADH2/GAPDH	[59][60]
20	VP1	Bird polyomaviruses	IC	GAL	[61]
21	P1	Enterovirus 71 and Coxsackievirus A16	IC	GAL1	[62]

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S. No	Protein Antigen	Virus	Protein Localization	Promoter	References
22	Capsid protein	Porcine circovirus type 2	IC	GAL1	[63]
23	VP2,6,7	Rotavirus	IC	PGK1, TEF1	[64][65]
24	VP2	Human parvovirus 4	IC	GAL1-10	[66]
25	Nucleocapsid protein	Human parainfluenza virus 2	IC	GAL	[67]
26	Nucleocapsid protein	Menangle virus	IC	GAL7	[68]
27	Gag	HIV-1	IC	GAP	[69]
28	VP2	Porcine parvovirus	IC	GAL1-10	[70]
29	P1, CD3	Coxsackievirus A16	IC	GAL1	[71]
30	Capsid protein	Porcine circovirus type 2	IC	GAL	[72]
31	VP1,2	Hepatitis B/Polyomavirus	IC	GAL7	[73]
32	VP1	Hamster polyomavirus	IC		[74]
33	L1/L1 + L2	Cottontail rabbit papillomavirus	IC	GAL1-10	[75]
34	L1	HPV 11	IC	GAL	[76][77][78]
35	Coat protein	Potyvirus (Johnsongrass mosaic virus)	IC	ADC1	[79]
36	P1, CD3	Poliovirus type I	IC		[80]
37		HIV-1	IC		[81]
38	L1	HPV 16	IC	GAL	[82]
39	L1	HPV 6,11 16	IC	GAL	[83]
40	VP1 with Puumala hantavirus nucleocapsid protein segments	Hamster polyomavirus	IC	Hybrid GAL10-PYK1	[84]
41	M protein	Hepatitis B virus	IC	GAL10/CYC1	[85]

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S. No	Protein Antigen	Virus	Protein Localization	Promoter	References
42	VP1,2	Goose hemorrhagic polyomavirus	IC	GAL	[86]
43	CEA/VP1	Hamster polyomavirus	IC	GAL	[87]
44	E7 oncoprotein of HPV16	Hepatitis B virus	IC		[88]
45	Capsid protein	Red-spotted grouper nervous necrosis virus	IC	GAL10	[89]
46	L1	HPV 58	IC	GAL10	[90]
47	C69R variant of surface antigen	Hepatitis B virus	IC	GAL10/CYC1	[91]
48	VP1	Human polyomavirus 2	IC	GAL	[92]

S. No	Protein Antigen	Virus	Protein Localization	Promoter	References
1	Capsid protein	Red-spotted grouper nervous necrosis virus	IC	Pw42-2	[98]
2	ZS and S	Zika virus	IC	AOX1	[99]
3	112-608aa of the ORF2	Hepatitis E virus	EC	AOX1	[100]
4	P1 and CD3	Poliovirus type I	IC	AOX1	[101][102]
5	Chimeric HPV-HIV L1P18 protein	HPV and HIV	IC	GAP	[103]
6	NY-ESO-1 cancer testis antigen	Norovirus	EC	AOX1	[104]
7	Surface antigen	Hepatitis C virus	IC	AOX1	[105][106]
8	P1 and 3CD	Enterovirus 71	EC	AOX1	[107][108]
9	E domain III	Dengue Virus	IC	AOX1	[109][110]
10	P1 and 3CD	Coxsackievirus A16	EC	GAP	[111]
11	VP1	Norovirus	EC	AOX1	[112]
12	E antigen	Dengue virus	IC	AOX1	[113][114][115][116][117][118]
13	prME	Japanese encephalitis virus	EC	AOX1	[119]
14	E antigen	Dengue virus	EC	GAP	[120]

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S. No	Protein Antigen	Virus	Protein Localization	Promoter	References
15	Matrix protein	Nipah virus	IC	AOX1	[121]
16	P1 and 3CD	Enterovirus D68	IC	AOX1	[122]
17	prM and E protein	Tick-borne encephalitis virus	EC	GAP	[123]
18	P1 and 3CD	Coxsackievirus A10	IC	AOX1	[124]
19	Surface antigen	Hepatitis B virus	IC	AOX1	[125][126][127][128][129][130][131]
20	prM and E protein	Dengue virus	IC	GAP	[132]
21	L1	HPV16 and 18	IC	AOX1	[133][134][135]
22	L1	HPV 52		AOX1	[136]
23	Capsid protein	Cowpea chlorotic mottle virus	EC	AOX1	[137]
24	L1	HPV 58	IC	AOX1	[138]
25	Envelope protein domain III (EDIII), hepatitis B surface antigen	Dengue virus	IC	AOX1	[139]
26	VP2	Infectious bursal disease virus	IC	AOX1	[140]
27	P1 and 3CD	Coxsackievirus A16	IC	AOX1	[141]
28	Core protein	Hepatitis B virus	IC	AOX1	[142][143]
29	E protein	Dengue virus	IC	AOX1	[144]
30	L2	Grapevine fanleaf virus	EC	AOX1	[145]
31	Capsid protein (VP60)	Rabbit hemorrhagic disease virus	IC	AOX1	[146]
32	HBc-influenza virus LAH domain	Hepatitis B/Influenza H3N2 virus	IC	AOX1	[147]
33	CoreE1E2 Protein	Hepatitis C virus	EC	AOX1	[148]
34	P1 and 3CD	Coxsackievirus A6	IC	AOX1	[149]
35	L1, L2	HPV 16	IC	AOX1	[150]

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S. No	Protein Antigen	Virus	Protein Localization	Promoter	References
36	prM/Env	Japanese encephalitis virus	IC	AOX1	[151]
37	Den2E-HBsAg	Dengue/Hepatitis B virus	IC	AOX1	[152]
38	prM and E protein	Dengue virus	IC	GAP	[153]
39	Polyprotein	Chikungunya virus	EC	AOX1	[154]
40	L1	HPV 16			[155]
41	Major capsid protein	Iridovirus	EC	AOX1	[156]
42	Surface antigen	Hepatitis B virus			[157]
43	VP1	Rabbit hemorrhagic disease virus	IC	AOX1	[158]
44	L1	Bovine papillomavirus 1,2,4			[159]
45	L1	HPV 16	IC	AOX1	[160]
46	Capsid protein	Norovirus	IC	AOX1	[161]
47	L1	HPV 16	EC	PGK1	[162]
48	Core protein	Hepatitis B virus			[163]
49	VP1	Calicivirus virus	EC	AOX1	[164]
50	Core protein	Hepatitis C virus	IC	AOX1	[165][166][167][168]

IC: intracellular; EC: extracellular.

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