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THE PROSPECT OF USING BAKERDREAM YEAST (SACCHAROMYCES CEREVISIAE) IN THE PRODUCTION O ENZYMES

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Annotation. The oligopeptide was isolated, purified and the amino acid composition was studied from the yeast Saccharomyces cerevisiae. Using the data obtained, enzymes were analyzed from the database closer in terms of molecular weight and analysis of the amino acid composition of the resulting oligopeptide. Among the enzymes of the Saccharomyces cerevisiae strain S288C, the most convenient enzymes for preparing the conjugate for ELISA are PHO3, PHO5, PHM8, PHO11, PHO12, putative acid phosphatase DIA3, PAH. phosphatidate phosphatase, glucan-1.3-beta-glucosidase EXG1, glutathione peroxidase URE2, cytochrome c peroxidase LPP1, GTT1, T-protein catalase CTT1. GPP1 has the lowest MolProbity and Clash scores, indicating slightly better structural quality. PHM8 and CPP1 have the highest number of lysine residues, which may offer more sites for conjugation compared to GTT1 and LPP1. GTT1 has the lowest collision score and MolProbity, indicating fewer steric clashes and potentially higher structural fidelity. PHM8: Contains a large number of lysine residues and good structural integrity, making it suitable for stable conjugation with BSA. This can improve stability, solubility and prolong half-life, which is useful for applications such as drug delivery and therapy. CCP1 is also a strong candidate with slightly more lysines and better structural quality, but its structural score is slightly higher than PHM8.

Key words: enzyme immunoassay, conjugate, Saccharomyces cerevisiae, polipetides, dialysis, electrophoresis, ultrasonic extraction.

INTRODUCTION

After the discovery of enzyme immunoassay by Eva Engvall and P. Perlman, the method has become widespread in medical diagnostics. Enzyme immunoassay is cost-effective and has a high level of accuracy, and the industrialization of test systems is at a high level. The enzyme horseradish peroxidase is used in the technology of conjugate formation in test systems. Given the development and prospects of biotechnology, the use of biotechnological methods in obtaining

conjugates is of interest, including the use of active enzymes in conjugate technology - enzymes of microorganisms [2,4,18].

In experiments, such work was rarely carried out, experiments allow in the future to create continuous testing systems. The use of Saccharomyces cerevisiae yeast enzymes to obtain conjugates and study the activity of the resulting conjugate is an urgent problem [21].

Studies of the structure and functions of proteins suggest the presence of highly purified preparations. Obtaining a protein in a homogeneous state is a rather complex task. As a rule, the biological material that is the source of the isolation contains a large number of proteins, their complexes with each other and other biopolymers. Similar properties of the components of this mixture require the use of various methods and their various combinations when isolating individual proteins. Difficulties in obtaining pure protein are also associated with the lability of proteins and the risk of their denaturation, which narrows the range of possible isolation methods.

When isolating enzymes from yeast, extraction, salting out, dialysis, extraction and purification of total polipetides are of great importance [5,20].

Based on the above, the aim of the study was to isolate, purify and predict promising enzymes for conjugation of polipetides of Saccharomyces cerevisiae yeast.

MATERIAL AND METHODS

The ultrasound method was used to isolate Saccharomyces cerevisiae yeast. Extraction was carried out by improving the quality of extraction with ultrasound; the propagation of ultrasound pressure waves and the resulting cavitation phenomena were explained. The main effect of ultrasound on a continuous liquid medium is the superposition of acoustic pressure (Pa) in addition to the hydrostatic pressure already acting on the medium.

Salting out of proteins with ammonium sulfate. The precipitation of proteins when neutral salts are added to their solutions - NaCl, KCl, Na_2SO_4, $(NH_4)_2SO_4$, $MgSO_4$ in high concentrations is called salting out. Salting out is based on a decrease in the degree of hydration of protein molecules due to the binding of water molecules with salt ions. Deprived (completely or partially) of a hydration shell, protein molecules aggregate, stick together and precipitate.

Isolation and purification of polipetides. To isolate polipetides from dry yeast, extraction was performed with 0.2 N sodium hydroxide, 0.05 Tris HCl (pH 8.5), 2% Na_2CO_3, 0.2% NaOH. with periodic stirring on a magnetic stirrer at 1000 rpm for 3 hours. For complete destruction of cells, the homogenate was treated with ultrasound at an oscillation frequency of 34 kHz. for 5-15 min. The obtained extracts were centrifuged in a refrigerated centrifuge at 8000 rpm for 30 min. The transparent supernatant was treated with 80% ammonium sulfate to precipitate the protein, the solution was kept in the refrigerator for 24 hours to form a protein precipitate. The precipitate was separated by centrifugation and dissolved with an extractant. The desalted protein solution obtained after dialysis was lyophilized under high vacuum and low temperature (-35^0C). The resulting lyophilized proteins of the studied samples were weighed.

Protein isolation and purification. 5 g of the obtained defatted soybean powder was extracted with 0.2N sodium hydroxide (NaOH). The extraction was carried out at a ratio of 1:10, defatted soybean powder to the volume of 0.2N sodium hydroxide. The extraction was carried out

with constant stirring on a magnetic stirrer for 1 hour. The obtained extract was centrifuged in a refrigerated centrifuge RS-6. Equilibrated centrifuge tubes were centrifuged at 3000 rpm for 20 minutes. Then the supernatant was collected, the sediment was removed from the centrifuge tubes. In the obtained supernatant solution, dry ammonium sulfate ($(NH_4)_2SO_4$) was carried out at 60% precipitation, namely, grams of ammonium sulfate were added to 100 ml of the solution. The resulting solution (suspension) was left in the refrigerator for 16 hours. Then it was centrifuged at 6000 rpm for 30 minutes. The sediment was collected, dissolved in a minimum volume of water and dialyzed in running water for 24 hours. In the alkaline extract after centrifugation, the protein content in 10 studied samples was quantitatively determined using the Kalkar spectrophotometric method [4,12,20].

Electrophoresis of the proteins obtained from yeast in polyacrylamide gel. The principle of this method is that under the influence of an electric field, proteins migrate in the gel formed by the copolymerization of acrylamide and N,N-methylenebisacrylamide. The scope of this method: electrophoretic fractionation of proteins. Zonal electrophoresis in polyacrylamide gel is the best method for electrophoretic fractionation of protein mixtures. Coomassie Brilliant Blue R-250 was used to stain the protein after electrophoresis in a polyacrylamide gel. A Coomassie solution (0.5%) was prepared using a mixture of methanol: soda: acetic acid (10:30:1). The dye was washed with a solution of 7% acetic acid. The washed gels were stored in the same solution that was used to wash it in the presence of glycerol, as a result of which the gel becomes elastic. The resulting gel was photographed and then dehydrated between two tightly stretched pieces of cellophane [6,15,17].

RESEARCH RESULTS

We plan to develop a method for isolating and purifying polipetides from dry yeast Saccharomyces cerevisiae.

The object of the study was dry yeast Saccharomyces cerevisiae (Bakerdream). The protein content in the studied objects was determined after preliminary mineralization of the sample with sulfuric acid with subsequent determination of protein nitrogen with Nessler's reagent. For this purpose, a spectrophotometer, analytical scales with an accuracy of 0.0001 g, filter paper (white tape), conical funnels, 50 ml measuring flasks, sodium hydroxide, Rochelle salt, Nessler's reagent, distilled water, concentrated sulfuric acid, concentrated hydrogen peroxide were used. Weighed portions of the prepared samples were taken into heat-resistant flasks, concentrated sulfuric acid H_2SO_4 (ρ1.84 g/cm) was added and decomposed on a sand bath or hotplate, avoiding vigorous boiling. The end of the mineralization process was the receipt of an absolutely transparent colorless solution. The protein content in the prepared samples was determined by the colorimetric method with Nessler's reagent at a wavelength of 400 nm. on a V-5000 Metash spectrophotometer. The results obtained with a twofold repetition of the experiment are presented in Table 1.

Table#1

Results of protein quantitative analysis

Object of study	Weight, gr.	Aliquot, ml	400 nm	protein,%	nitrogen, %

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Yeast	0.287	0.2	0.578	44.8	7.16

Next, optimal conditions for protein extraction were developed. Extraction was carried out with 0.05 Tris HCl (pH 8.5), 2% Na_2CO_3, 0.2% NaOH. The hydromodule was selected (1:5, 1:10, 1:15), and the extraction time was 60-120-180-240 minutes, for complete destruction of cells, the homogenate was treated with ultrasound at an oscillation frequency of 34 kHz.

Optimal extraction conditions. Extraction was performed with 0.2% NaOH for 3 hours, 37^0C, at a water ratio of 1:5, then sonication for 5 minutes, which ensured the maximum protein yield.

After extraction, centrifugation was performed for 30 minutes at 8000 rpm. The next stage of purification involved fractionation with dry ammonium sulfate. To remove high-molecular proteins, ammonium sulfate was used at 20-40-60% precipitation. The results showed that 20% salting out of the extract with ammonium sulfate is the most optimal for removing ballast (high-molecular) proteins.

Then, ammonium sulfate was used at 80% precipitation to obtain low-molecular polipetides. The 80% sediment extract was centrifuged and the resulting low-molecular oligopeptide precipitate was desalted on a Sephadex G25 column. Gel filtration on Sephadex G25 was performed with the collection of 4 ml of eluate. The concentration of the obtained desalted protein fractions was determined on a Cary 60 spectrophotometer (Germany) at a wavelength of 280 and 260 nm. The resulting desalted protein fraction was collected and lyophilized under high vacuum (-87^0C) on a CHRIST Alpha 2-4 LSCbasic device (Germany).

The yield of purified (polipetides) was 5.7%.

To identify the functional groups, IR spectral analysis was obtained for polipetides. The geometry of the compounds under study was constructed using the MaSK program and preliminarily optimized by the B3LYP1 / 6-31G (d, p) method. Calculations were performed in the Firefly 8.0.1 software package. IR spectra of the studied polipetides in KBr tablets were recorded (4000-400 cm-1) on a Perkin-Elmer System 2000 FTIR spectrophotometer. IR spectra are expressed as a dependence of frequencies on optical density (Absorbance).

The results are shown in Fig. 1-2.

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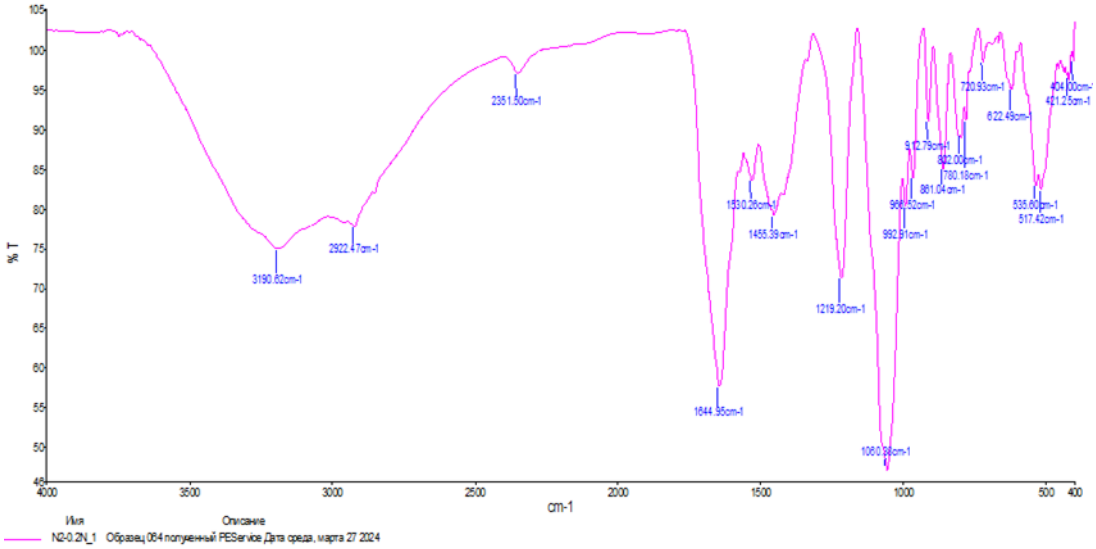

Fig. 1. Oligopeptide obtained with 0.2n NaOH extractant

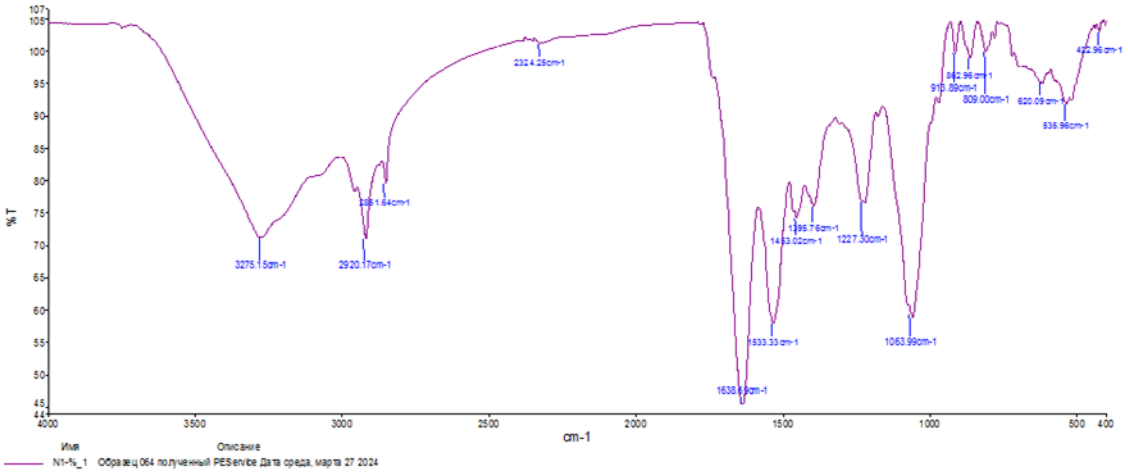

Fig. 2. Oligopeptide obtained with 0.2% NaOH extractant

IR spectrum (KBr, νmax, cm-1): 3196 (NH), 3034 (CH_3), 1646 (C=O), 1403 signals are characteristic of aromatic rings, 1240, 1049 (-C-O-C-), showed absorption lines characteristic of groups 704 aromatic rings (C-H), 602 cm-1 (C-SH). IR spectrum (KBr, νmax, cm-1): 3275 (NH), 2920, 2852 (CH_2), 1639 (C=O), 1533, 1453 signals are characteristic of aromatic rings, 1396 (CH_3), 1227, 1064 (-C-O-C-), 863, 809 showed absorption lines characteristic of aromatic ring groups (C-H), 620, 536 cm-1 (C-SH, -C-S-C-)

Characterization of purified polipetides was carried out by electrophoresis in 10% PAAG. Study of electrophoretic mobility of polipetides. Fractions were analyzed by electrophoresis in 10% polyacrylamide gel. The standard Novex™ Sharp pre-stained Protein Ladder (Invitrogen) was used as markers. Electrophoresis was carried out under denaturing conditions. Samples were applied in 20 µl to each well. During sample migration in the stacking gel (6%), the voltage was maintained at 50 V. When the front moved in the separating gel (10%), the voltage was increased to 80 V. Electrophoresis was carried out until bromophenol blue was released from the gel. The

gels were stained with 0.25% Coomassie G250 solution (Sigma, USA) in a mixture of water: ethanol: acetic acid: water (1:1:2). (Fig. 3)

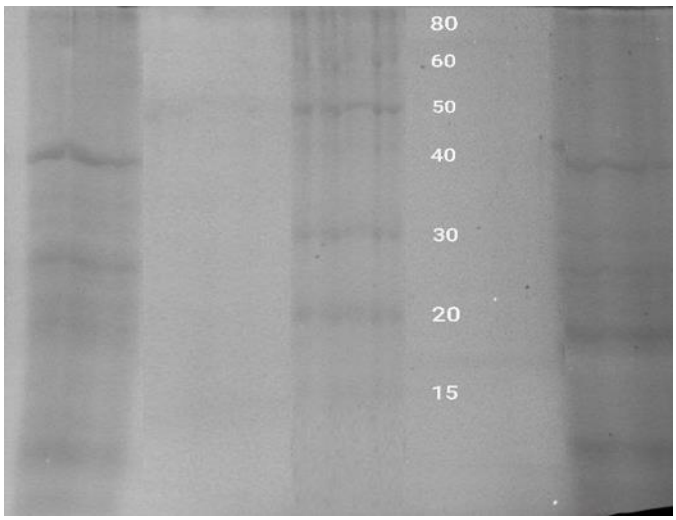

Fig. 3. Electropherogram of yeast polipetides

0.2% NaOH 2) Markers 3) 0.2N NaOH

According to electrophoresis, the purified polipetides have an identical fractional composition with a molecular weight of 8 kDa to 40 kDa (Fig. 4).

Fig. 4. The process of lyophilization of the oligopeptide Saccharomyces cerevisiae

Isolation of free amino acids. Precipitation of proteins and peptides of the aqueous extract of the samples was carried out in centrifuge cups. For this purpose, 1 ml (exact volume) of 20% TCA was added to 1 ml of the studied sample. After 10 min, the sediment was separated by centrifugation at 8000 rpm for 15 minutes. After separating 0.1 ml of the supernatant, it was lyophilized. The hydrolysate was evaporated, the dry residue was dissolved in a mixture of triethylamine-acetonitrile-water (1:7:1) and dried. This operation was repeated twice to neutralize the acid. Phenylthiocarbamyl derivatives (PTC) of amino acids were obtained by reaction with phenylthioisocyanate according to the method of Steven A., Cohen Daviel.

Identification of amino acid derivatives was carried out by HPLC (Fig. 5).

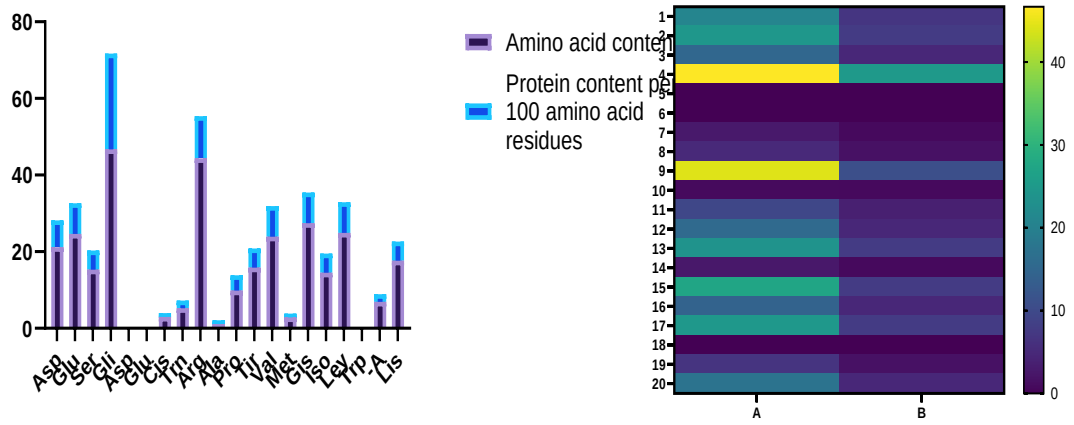

Fig. 5. Identification of amino acids by HPLC

HPLC conditions: Agilent Technologies 1200 chromatograph with DAD detector, 75x4.6 mm Discovery HS C18 column. Solution A: 0.14 M CH_3COONa + 0.05% TEA pH 6.4, B: CH_3CN. Flow rate 1.2 ml/min, absorption 269 nm. Gradient %B/min: 1-6%/0-2.5 min; 6-30%/2.51-40 min; 30-60%/40.1-45 min; 60-60%/45.1-50 min; 60-0%/50.1-55 min. The results obtained are presented in Table 2.

Table#2

Amino acid composition of the studied polipeptide

#	Nomenclature of amino acids	Amino acid content mg/g	FASTA nomenclature	Protein content per 100 amino acid residues
1	Asp	21.18344	D	7
2	Glu	24.64502	E	8
3	Ser	15.31665	S	5
4	Gli	46.67304	G	25
5	Asp	0	N	0
6	Glu	0	Q	0
7	Cis	2.940984	C	1
8	Trn	5.240469	T	2
9	Arg	44.39112	R	11
10	Ala	1.088789	A	1
11	Pro	9.861871	P	4

12	Tir	15.83588	Y	5
13	Val	23.9325	V	8
14	Met	2.827883	M	1
15	Gis	27.41822	H	8
6	Iso	14.51658	I	5
17	Ley	24.88968	L	8
18	Trp	0	W	0
19	-A	6.89199	F	2
10	Lis	17.61042	K	5
	Total	**305.2645**		

According to the data in Table 2, in 305, 2645 oligopeptide samples, the highest content was found for amino acids (mg/g) gli and arg, less content was determined for amino acids asp, glu, val, gis, ley, ser, tir, iso, lis.

Enzyme-linked immunosorbent assay is a convenient, cost-effective method of medical and biological research. It is important to create cheap and effective conjugates when organizing ELISA. Including analysis of S. cerevisiae yeast enzymes to obtain conjugates [11].

In recent years, particular interest has been attracted by the search for new sources of enzymes, the use of biotechnological methods for separating protein products to obtain conjugates. In this regard, it is important to conduct a meta-analysis of data on some enzymes to study the selection of enzymes and their biological properties [6]. The study of microorganism enzyme systems in conjugate technology, the investigation of their properties and their use for conjugate technology have a number of advantages. For example, fungi - microorganisms have the property of rapid reproduction in a short period of time, in addition, they have many types of enzymatic activity systems. Therefore, the database was analyzed for information on enzymes and their molecular weights optimal for ELISA of the Saccharomyces cerevisiae S288 strain [8,14,19]. Saccharomyces cerevisiae is a eukaryotic microorganism, genetically more complex than bacteria. A yeast cell contains 3.5 times more DNA than an Escherichia coli cell. At the same time, yeast as an experimental object has many technical advantages over prokaryotes and viruses, allowing for the rapid development of yeast molecular genetics [3,4, 7,16]. Their ability to rapidly reproduce, the ability to manipulate individual yeast cells, the ease of transferring several cultures to selective media and isolating mutants simultaneously, the fact that yeast has been studied in detail as a genetic system, and, perhaps most importantly, the system of genetic transformation of yeast, including versatility. Unlike many microorganisms, the presence of multiple genetic markers in the genotype of a yeast cell allows it to remain viable. Yeast is not a pathogen, so working with it does not require precautions [1,6,9,20].

In 1996, the S. cerevisiae genome became the first fully edited eukaryotic genome. The S. cerevisiae genome is approximately 12.2 Mb and contains 6,275 genes compactly distributed

across 16 chromosomes. Only about 5,800 of these genes are functional. In 2011, the genome assembly and genome annotation were made available by the Saccharomyces Genome Database (SGD) [5]. Strain S288c was isolated in 1938 by Emil Mrak from a rotting fig found in Merced, California. Using the unique reproductive cycle of strain S288c, Robert Mortimer genetically crossed the strain isolated from the fig with other yeast strains isolated by scientists. As a result, he created a new strain called S288c, which was later used as the parent strain for the isolation of most of the mutant strains currently used in research. In addition, this strain was subsequently used to sequence the genome of S. Cerevisiae [9,10,14,17].

Major Strains of Saccharomyces cerevisiae

1. Wild-Type Strains

Saccharomyces boulardii: Previously used as a probiotic to treat diarrhea caused by bacteria. Clinical studies have shown that this species and S. cerevisiae are genetically identical. Saccharomyces uvaruium: Used in beer fermentation. Due to a recent reclassification, this species is now considered another wild-type strain.

2. Laboratory Strains,

S288c: This strain was isolated by genetic selection in the 1950s by Robert C. Mortimer. It was used as a parent strain in the isolation of mutants. Strain S288c was used in 1996 to completely sequence the genome of S. cerevisiae. However, under nitrogen- and growth factor-deficient environmental conditions, its reduced sporulation rate prompted scientists to select alternative strains for the study.

A634A: Used in cell cycle studies. It is also closely related to S288c due to a cross between S288c and another unknown strain.

BY4716: Since it is virtually identical to S288c, it is often used as a reference strain.

CEN.PK: In Europe, it is used as a secondary standard strain along with S288c in fungal genome studies. In addition, it can grow well on a variety of carbon sources as well as under anaerobic conditions. It is used in growth rate and product formation studies.

∑1278b: This strain is unique in that it contains unique genes involved in nitrogen metabolism. Studies of this strain under nitrogen deficiency have yielded good results, under these conditions the cells elongate, form unique patterns, physically approach each other, and form unique buds when they join.

SK1: Because this strain produces a lot of spores, it is used in meiosis studies.

W303: S288c is closely related to S288c due to crosses with an unknown strain that is used in genetic and biochemical analysis.

Among the above-listed enzymes of the Saccharomyces cerevisiae S288C strain, the most convenient enzymes for preparing a conjugate for ELISA are the following: PHO3, PHO5, PHO11, PHO12, PHM8 putative acid phosphatase DIA3, PAH, phosphatidate phosphatase, glucan-1.3-beta-glucosidase EXG1, glutathione peroxidase URE2, LPP1, GTT1, cytochrome-c-peroxidase CCP1, T-protein catalase CTT1 (Table 3).

Table#3

Main enzymes of strain S288c [(www.ncbi.nlm.nih.gov)]

№	Gene	Number of nucleotides in a gene	Protein composition Number of amino acid residues

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1.	PHO5	1381	475
2.	PHO3	1381	466
3.	PHM8	962	317
4.	PHO11	1381	471
5.	PHO12	1381	471
6.	DET1	961	333
7.	YER134C	481	218
8.	DIA3	1381	82
9.	PAH1	2581	862
10.	LTP1	781	160
11.	LPP1	781	274
12.	BGL2	901	313
13.	EXG1	1321	505
14	URE2	1021	412
15.	CCP1	1021	361
16.	TSA1	541	196
17.	TSA2	541	195
18.	GPX2	481	162
19.	GPX1	481	168
20.	GTT1	661	234
21.	CTT1	1681	567

In further studies, from the obtained polipeptide with a molecular weight from 8 kDa to 40 kDa, the enzymes PHM8, GTT1, LPP1, CCP1 closest to the molecular weight were analyzed by Molprobity, Clash Scores indicators as candidates for obtaining conjugates. PHM8 Amino acid composition: The protein consists of 321 amino acids, lysine is present at 28 positions, which is 8.7% of the total composition. The molecular weight of the protein is 37676.86 daltons. The theoretical pI of the protein is 5.23, indicating that it is overall neutrally charged at this pH.

Lysine locations: Both lysine residues are located at external positions: 5, 16, 17, 44, 53, 66, 83, 113, 115, 138, 142, 152, 153, 154, 157, 263, 266, 275, 279) and internal positions: 151, 160, 169, 177, 204, 214, 268 protein molecules.

MolProbity Results:

- MolProbity score: 1.13, which is an indicator of the overall quality of the structure (lower scores are better).
- Clash score: 1.14, indicating few steric clashes within the protein (Fig. 6).
- Ramachandran plot analysis (Fig. 7):

- 95.61% of residues are in favorable regions and only 0.94% have extreme values, indicating good backbone bend angles.
- 0.33%, which generally indicates good side chain conformation.
- C-Beta Deviations: No deviations were observed (0%).
- Bad Links and Angles: Few bad angles (13 out of 3668), showing good geometry overall.
- Cis-peptides: One cis-proline was observed (A204 LYS-A205 PRO).

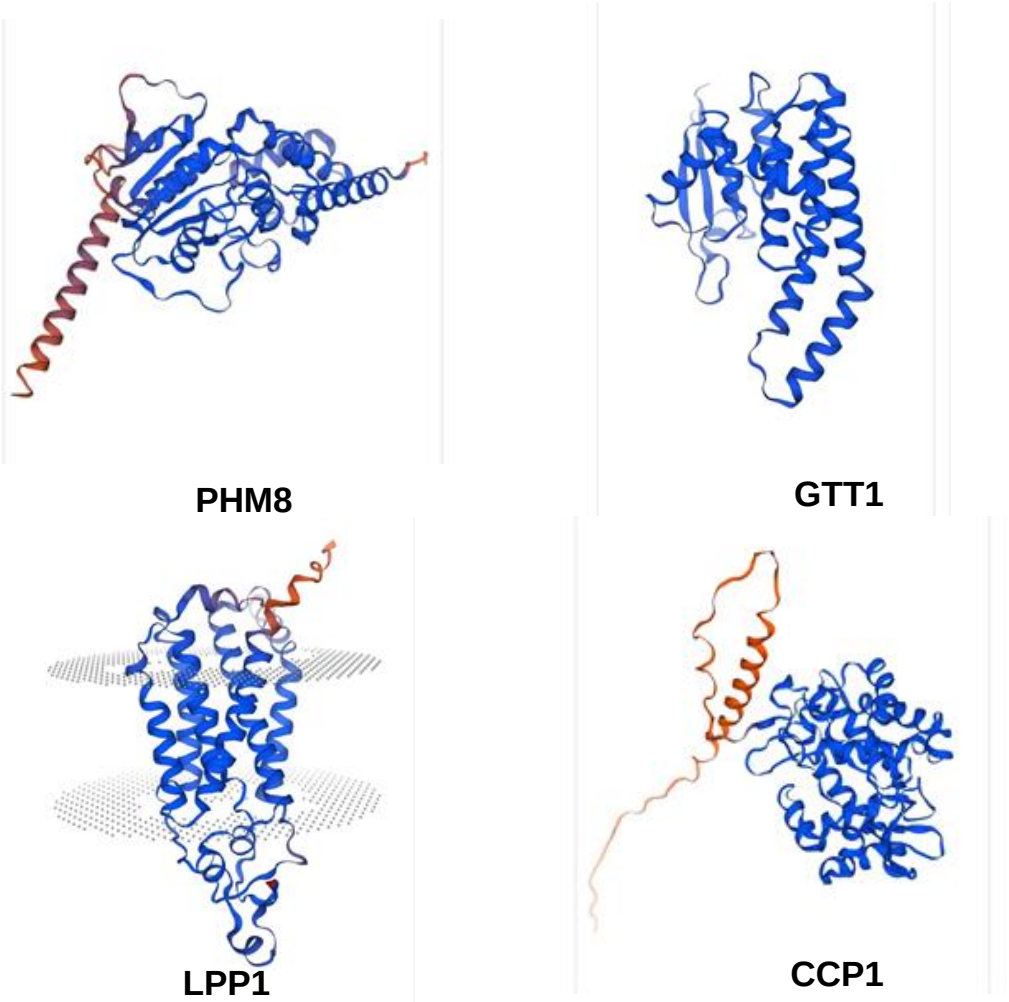

Fig. 6. Conformational structures of the enzymes PHM8, GTT1, LPP1, CCP1

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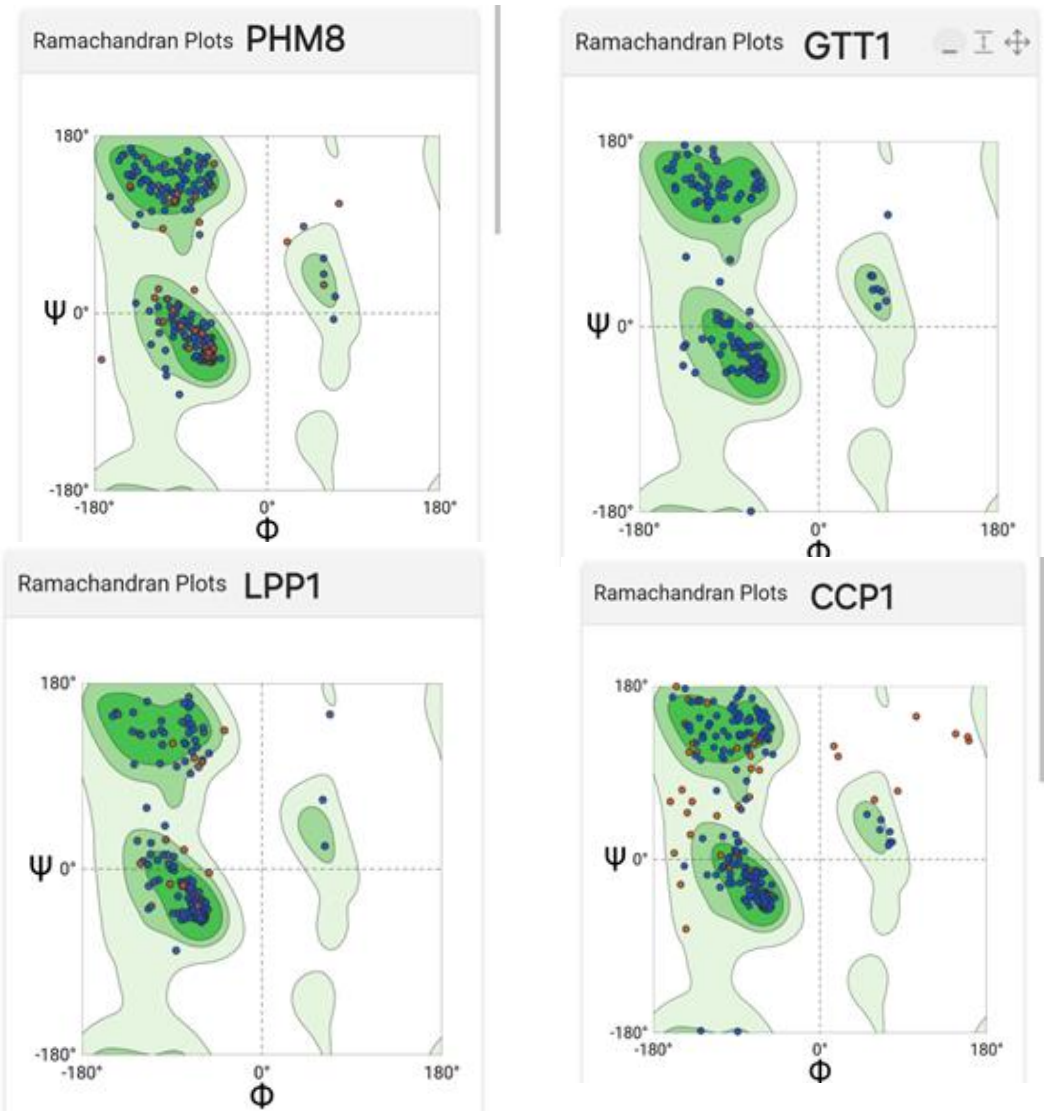

Fig. 7. Ramachandra map for enzymes PHM8, GTT1, LPP1, CCP1

The PHM8 protein is well folded and has a low Molprobity value, indicating a good overall structural quality. The presence of lysines both inside and outside the molecule may indicate functional significance related to interactions or modifications. The theoretical pI suggests that the protein may have specific charge properties under physiological conditions. The amino acid composition and molecular weight provide key biochemical data necessary for understanding its function and properties.

GTT1 - the protein consists of 234 amino acids. Lysine (Lys, K) is present with a frequency of 19 residues and accounts for 8.1% of the total amino acid composition. The molecular weight of the protein is 26794.80 Daltons. The theoretical isoelectric point of the protein is 6.18, indicating that it is slightly acidic under standard conditions.

Location of Lysine Residues:

Lysine residues are located at specific sites in the protein sequence in the external and internal parts of the molecule. Specific positions include:

- Outside: 7, 36, 48, 49, 68, 69, 142, 146, 157, 169, 197, 209, 212.

- Inside: 70, 126, 128, 178, 224, 226.

MolProbity Results

MolProbity score of 0.92; lower scores indicate better structure quality.

- Clash score: 1.06 measures steric clashes between atoms; lower is better (Figure 6).
- Ramachandran plot analysis (Figure 7):
- 97.41% percentage of residues in favorable regions of the Ramachandran plot.
- 0.00% percentage of residues with unusual side chain conformation.
- C-Beta Deviations: 2 number of deviations from the ideal position of the C-beta atom.)
- Bad Bonds: 0/1945 no bad bonds were detected in the structure.
- Bad Angles: 13/3668 few bad bond angles were detected.

Additional design features:

- Cis-Prolines: 2 cases of cis-peptide bonds involving proline residues were identified.

The GTT1 protein is well structured and has a predominantly favorable conformation, as indicated by the high percentage of residues in the favorable region of the Ramachandran plot (97.41%) and the low collision score (1.06). The presence of lysine residues both inside and outside the molecule suggests its potential role in interactions and structural stabilization. The overall MolProbity score of 0.92 indicates a good overall quality of the structure with no significant deviations from the ideal structural parameters.

LPP1 protein consists of 274 amino acids. Molecular weight: The molecular weight of the protein is 31586.27 daltons. The theoretical pI is 8.63, indicating that the protein is basic under standard conditions.

Location of lysine residues:

- Lysine residues are distributed both outside and inside the protein molecule:
- Outside: 9, 11, 15, 94, 98, 171, 246.
- Inside: 56, 89, 93, 136, 156, 175, 183.

MolProbity Results

- MolProbity score: 1 is excellent, indicating high structural quality.
- Clash score: 0.22 is very low, indicating minimal steric clashes between atoms (Fig. 6).
- Ramachandran plot analysis (Fig. 7):
- 94.85% high percentage of residues in favorable regions of the Ramachandran plot.
- Outer: 0.37% low percentage of residues in outlying regions.
- Rotamer Deviations: 1.18% (minor deviations in side chain conformation.)
- C-Beta Deviations: 1 single deviation from ideal C-beta atom position.
- Bad Linkages: 0/2275 no bad linkages detected
- Bad Angles: 13/3087 few bad linkage angles detected.
- Cis-peptide bonds: not specified in the data provided.
- Specific Residual Issues:

According to MolProbity analysis, this protein exhibits excellent structural characteristics. Favorable regions of the Ramachandran plot have high percentages of residues, minimal clashes, and no significant bond or angle distortions. The distribution of lysine residues suggests potential roles in interactions or binding sites both inside and outside the protein. Overall, the protein appears well folded and structured, suitable for functional studies or further analysis related to its biological role.

The CCP1 protein consists of 361 amino acids. The molecular weight of the protein is 40,352.97 daltons. The theoretical pI is 5.95, indicating a slightly acidic environment under standard conditions. Lysine residue locations:

- Lysine residues are located both outside and inside the protein molecule:
- Outside: 17, 42, 62, 80, 89, 142, 164, 251, 280, 283, 294, 335, 345, 356.
- Inside: 97, 127, 143, 158, 191, 217, 246, 310, 316, 324, 331.

MolProbity analysis:

- Molprobity score: 1.32 is an average score, but higher than ideal, indicating some structural problems (Fig. 6).
- Clash score: 0.00 no steric clashes detected.
- Ramachandran plot analysis (Fig. 7):
- 91.64% most of the residues are in favorable regions of Ramachandran district.
- 3.34% some outliers in outlying regions indicate potential structural distortions.
- Rotamer deviations: 2.68% some deviations in side chain conformation.
- C-beta deviations: 3 deviations from ideal position of C-beta atom.
- Bad Links: 0/2935 No bad links detected.
- Bad Angles: 29/3979 Multiple bad link angles detected.

Additional design features:

- Cis-peptide bonds: 3 instances involving non-proline residues: (A1 MET-A2 THR), (A5 VAL-A6 ARG), (A7 LEU-A8 LEU).
- Twisted Peptide Bodies: 7 instances involving non-proline residues.
- Specific Residue Issues:
- A8 LEU, A2 THR, A47 SER, A55 GLY, A48 PRO, A57 ASN, A54 HIS, A9 PRO, A50 GLY, A11 LEU, A53 ASN, A5 VAL were isolated and these residues were identified in a specific structural context or may interact.

The CCP1 protein has a generally accepted structure with few notable deviations. The bulk of the deposits are located in favorable areas of the Ramachandran section, indicating a predominantly well-folded structure. However, the presence of edges and deviations in rotamer conformations indicate the presence of some local structural asymmetries. The absence of clashes and poor contacts is positive, but the abundance of poor angles and C-beta deviations indicate potential areas for structural improvement. Overall, further analysis may focus on understanding the functional implications of these structural features with respect to the biological role of the protein.

Possible conjugation candidates:

Based on the parameters for the number of lysine residues and the structural quality of the MolProbity and Clash Scores provided for protein analysis, a comparative analysis was performed to obtain conjugates with bovine serum albumin (BSA):

Analysis by specified parameters:

1. Number of lysine residues:

- PHM8: 28 lysine residues (8.7% of the total amino acids).
- GTT1: 19 lysine residues (8.1% of total amino acids).
- LPP1: 15 lysine residues (5.5% of total amino acids).
- CCP1: 25 lysine residues (7.2% of total amino acids).

PHM8 has the highest number of lysine residues, followed by CCP1 and GPP1. LPP1 has the lowest number of lysine residues among the four proteins.

2. Build Quality:
- PHM8: MolProbity Score: 1.13, Clash Score: 1.14.
- GTT1: MolProbity Score – 0.92, Clash Score – 1.06.
- LPP1: MolProbity Score - 1, Clash Score – 0.22.
- CCP1: MolProbity Score – 1.32, Clash Score – 0.00.

Conclusions: GTT1 has the lowest MolProbity and Clash scores, indicating slightly better structural quality. However, all proteins generally have good structural integrity.
- Lysine Accessibility: PHM8 and CCP1 have the highest number of lysine residues, which may offer more sites for conjugation compared to GPP1 and LPP1.
- Structural Integrity: GTT1 has the lowest Clash and MolProbity score, indicating fewer steric clashes and potentially higher structural fidelity. However, PHM8 follows it with a slightly higher score, but still within the acceptable limits.

PHM8: contains a high number of lysine residues and good structural integrity, making it suitable for stable conjugation with BSA. This may enhance stability, solubility, and prolong half-life, which is useful for applications such as drug delivery and therapeutics.
CCP1: also a strong candidate with slightly more lysines and better structural quality, but its structural score is slightly higher than PHM8.

CONCLUSION

Based on the presented parameters, PHM8 appears to be the most useful choice for conjugation to bovine serum albumin (BSA). It combines readily accessible lysine residues with good structural integrity, which is an important factor for successful conjugation and increased stability, solubility and bioavailability. However, CCP1 is also a strong contender due to comparable lysine content and structural features, albeit with a slightly higher structural score. Further detailed analysis and specific application requirements are needed to make an accurate choice.

COMPLIANCE WITH ETHICAL STANDARDS

Prior to the start of the study, all participants received an explanation of the procedure and the risks that would subsequently be encountered with their participation and provided informed consent to participate in this study.

CONFLICT OF INTEREST

The authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article.

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AUTHOR'S CONTRIBUTIONS

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