

## Rešitve nalog

1.

Za iskanje člankov uporabimo pubmed, kjer vstavimo xylanase v iskalno vrstico in dobimo

a) 7833 rezultatov (Slika1)

The screenshot shows the PubMed search interface. At the top, the PubMed logo is on the left, and a search bar contains the text 'xylanase'. To the right of the search bar are buttons for 'Advanced', 'Create alert', and 'Create RSS', and a 'Search' button. Below the search bar are buttons for 'Save', 'Email', and 'Send to', and a 'Sort by: Best match' dropdown menu. The main content area shows '7,833 results' and a pagination control for 'Page 1 of 784'. On the left side, there are filters for 'MY CUSTOM FILTERS', 'RESULTS BY YEAR' (with a bar chart showing an increasing trend from 1955 to 2026), 'PUBLICATION DATE' (with radio buttons for 1 year, 5 years, 10 years, and Custom Range), and 'TEXT AVAILABILITY'. The search results list two articles:

- Expression of xylanase enzymes from thermophilic microorganisms in fungal hosts.**  
1  
Cite Bergquist P, Te'o V, Gibbs M, Cziferszky A, de Faria FP, Azevedo M, Nevalainen H. *Extremophiles*. 2002 Jun;6(3):177-84. doi: 10.1007/s00792-001-0252-5. Epub 2002 Mar 8. PMID: 12072952 Review.  
Bulk production of **xylanases** from thermophilic microorganisms is a prerequisite for their use in industrial processes. ...The *K. lactis* system has been tested with two thermophilic **xylanases** and secretes gram amounts of largely pure **xylanase A** from *Dictyoglom* ...
- Xylanase from the psychrophilic yeast *Cryptococcus adeliae*.**  
2  
Cite Petrescu I, Lamotte-Brasseur J, Chessa JP, Ntarima P, Claeysens M, Devreese B, Marino G, Gerday C. *Extremophiles*. 2000 Jun;4(3):137-44. doi: 10.1007/s007920070028. PMID: 10879558  
A **xylanase** belonging to family 10 is produced by *Cryptococcus adeliae*, an Antarctic yeast that exhibits optimal growth at low temperature. The mature glycosylated **xylanase** secreted by *C. adeliae* is composed of 338 amino acid residues and 26 +/- 3 osidic residues, an ...
- Identification, heterologous expression, and characterisation of beta-1,3-**

Slika 1: Iskanje v Pubmedu člankov, ki se navezujejo na ksilanazo.

Potem pa v iskanje dodamo še avtorja in to tako da gremo pod advanced in ponovno vpišemo xylanase potem pa izberemo term »Author » in zraven dopišemo »Yingying Zheng«. (Slika2)

PubMed Advanced Search Builder



Add terms to the query box

Author

AND

Show Index

Query box

Search

Slika 2: Prikazuje vpis v advanced kjer združimo xylanase in Yingying Zheng.

b) Kot rezultat iskanja dobimo 3 rezultate (Slika 3)

Search results for: (xylanase) AND (Yingying Zheng[Author])

Advanced Create alert Create RSS Search User Guide

Save Email Send to Sort by: Best match Display options

MY CUSTOM FILTERS  
Edit custom filters

RESULTS BY YEAR

Year	Count
2013	1
2014	1
2015	1

PUBLICATION DATE

1 year  
5 years  
10 years  
Custom Range

TEXT AVAILABILITY

3 results

1  **Structural perspectives of an engineered beta-1,4-xylanase with enhanced thermostability.**  
Cite: Chen CC, Luo H, Han X, Lv P, Ko TP, Peng W, Huang CH, Wang K, Gao J, **Zheng Y**, Yang Y, Zhang J, Yao B, Guo RT. J Biotechnol. 2014 Nov 10;189:175-82. doi: 10.1016/j.jbiotec.2014.08.030. Epub 2014 Sep 3. PMID: 25193708  
The glycoside hydrolase 10 (GH10) **xylanase** from Streptomyces sp. 9 (XynAS9) can operate in a broad range of pH and temperature, and thus is a potential candidate for commercial applications. Recently, we engineered XynAS9 via mutating several residues in accordance with th ...

2  **Preliminary X-ray diffraction analysis of thermostable beta-1,4-xylanase from Streptomyces sp. S9.**  
Cite: Lv P, Zhang L, Luo H, Chen CC, Huang CH, Peng W, Wang K, Ko TP, **Zheng Y**, Zhang J, Yao B, Guo RT. Acta Crystallogr F Struct Biol Commun. 2014 Jan;70(Pt 1):105-7. doi: 10.1107/S2053230X13033335. Epub 2013 Dec 24. PMID: 24419629 **Free PMC article.**  
**Xylanase**, which catalyzes the random hydrolysis of internal xylosidic linkages, is a critical enzyme participating in **xylan** decomposition and has been widely applied in industrial utilizations. **Xylanase**

Slika 3: Rezultat našega iskanja so trije članki, za katere veljajo postavljeni pogoji

2.

Za iskanje v PDB smo morali iti pod »Advanced search« kjer smo izbrali atribut »UniProt Molecule Name« in potem vpisali xylanase. (Slika 4)

**Advanced Search Query Builder** ▾

Choose a search tool or combine multiple tools with **AND** logic.

Structure Attributes Sequence Similarity Sequence Motif 3D Similarity 3D Motif ?

Structure Attributes ▾

Select an **attribute** for searching (e.g., *Organism*, *Experimental Method*, *Resolution*). Add more conditions to narrow results. Examples: Homo sapiens, BRCA1, Insulin.

UniProt Molecule Name is xylanase NOT

AND ▾ Attribute Group

Include CSM Search

Slika 4: Advanced search z uniprot molecule name.

Dobimo 6 rezultatov. (Slika 5)

RCSB PDB Deposit Search Visualize Analyze Download Learn About Careers COVID-19 Help Contact us MyPDB ▾

**Search Results** Structures Macromolecules Assemblies Ligands View Results As Tabular Reports

Group Results by No Grouping ▾

**Refinements**

**Structure Determination Methodology**  
 experimental (6)

**Scientific Name of Source Organism**  
 Dickeya chrysanthemi (2)  
 Aegilops speltoides subsp. speltoides (1)  
 Bacillus sp. (in: firmicutes) (1)  
 Bacteroides thetaiotaomicron VPI-5482 (1)  
 Rhodothermus marinus (1)

**Taxonomy**  
 Bacteria (5)  
 Eukaryota (1)

**Experimental Method**  
 X-RAY DIFFRACTION (6)

**Polymer Entity Type**

This query matches **6 Structures**. Query Summary

1 to 6 of 6 Structures | Page 1 of 1 | Per page 25 | Sort by ↓ Score

Download Multiple Files:  All  Selected

**1NOF | pdb\_00001nof** Download File View File

**THE FIRST CRYSTALLOGRAPHIC STRUCTURE OF A XYLANASE FROM GLYCOSYL HYDROLASE FAMILY 5: IMPLICATIONS FOR CATALYSIS**

Larson, S.B., Day, J., McPherson, A., Barba De La Rosa, A.P., Keen, N.T.

(2003) *Biochemistry* **42**: 8411-8422

**Released** 2003-09-16  
**Method** X-RAY DIFFRACTION 1.42 Å  
**Organisms** Dickeya chrysanthemi  
**Macromolecule** xylanase (protein)  
**Unique Ligands** ACT

**2V24 | pdb\_00002v24** Download File View File

Slika 5: Rezultati pridobljeni iz Slike 4.

Potem pa na levi strani (z rdečo podčrtano na Sliki 5) izberemo »Eukaryota« kot zahteva naloga, da iščemo le evkariontske proteine. Dobimo le en zadetek. (Slika 6)

Slika 6: Pridobljen rezultat ko vklopimo filter za evkarionte.

a) Iz dobljenega rezultata (rdeči kvadrat na Sliki 6) vidimo, da je koda proteina 5AY7

Da najdemo strukturno podoben protein gremo v PDBefold in tam vpišemo PDB kodo. (Slika 7)

c3) 14 Apr 2014

Slika 7: Rdeča puščica prikazuje mesto vpisa kode v PDBefold.

Potem kliknemo »Submit your query«. Iz dobljenih rezultatov vidimo, da je prvi na seznamu 5AY7, kar je ista struktura, ki smo jo vnesli. Izberemo naslednjo možnost, kot vidimo na Sliki 8 sta naslednja dva zadetka obadva 5D4Y eden je edino A eden pa B. Ni pomembno katerega izberemo oba sta isti protein z isto Q vrednostjo.

in Europe  
Bringing Structure to Biology

Structure Alignment Results.

Query: pdb entry 5ay7, chain [A] : 315 residues.  
A PSYCHROPHILIC GLYCOSIDE HYDROLASE FAMILY 10 ENDO-BETA-1,4-XYLANASE

Examined 198545 entries, (581769 chains). Displaying Matches 1-20 of 328.

Back to query | next | last page | Sort by: Q-score | arrange by SCOP family | match | 1 | jump

#	Scoring			RMSD	N <sub>align</sub>	N <sub>g</sub>	% <sub>seq</sub>	Query	Target (PDB entry)				Title
	Q	P	Z						% <sub>ase</sub>	Match	% <sub>ase</sub>	N <sub>res</sub>	
1	1.00	88.1	28.3	0.00	315	0	100	100	5ay7:A	100	315		A PSYCHROPHILIC GLYCOSIDE HYDROLASE FAMILY 10 ENDO-BETA-1,4-XYLANASE
2	0.97	58.9	21.4	0.31	311	1	100	100	5d4y:B	91	312		A PSYCHROPHILIC GLYCOSIDE HYDROLASE FAMILY 10 ENDO-BETA-1,4-XYLANASE
3	0.97	51.4	21.6	0.38	312	1	100	100	5d4y:A	95	314		A PSYCHROPHILIC GLYCOSIDE HYDROLASE FAMILY 10 ENDO-BETA-1,4-XYLANASE
4	0.95	50.7	21.4	0.39	315	1	100	100	5ay7:B	95	326		A PSYCHROPHILIC GLYCOSIDE HYDROLASE FAMILY 10 ENDO-BETA-1,4-XYLANASE
5	0.93	43.3	19.7	0.66	310	2	59	90	7ezo:A	90	312		GH10 DOMAIN OF BIFUNCTIONAL ENDOXYLANASE AND ARABINOFURANOSIDASE OF BI0569
6	0.80	35.8	17.8	1.03	304	5	44	90	3msg:B	86	328		ENZYME-SUBSTRATE INTERACTIONS OF IXT6, THE INTRACELLULAR XYLANASE OF G. STEAROTHERMOPHILUS.
7	0.80	35.8	17.8	1.03	304	5	44	90	3mau:B	86	328		ENZYME-SUBSTRATE INTERACTIONS OF IXT6, THE INTRACELLULAR XYLANASE OF G. STEAROTHERMOPHILUS.
8	0.80	36.0	17.9	1.03	304	5	44	90	2q8x:B	86	328		THE HIGH-RESOLUTION CRYSTAL STRUCTURE OF IXT6, A THERMOPHILIC, INTRACELLULAR XYLANASE FROM G. STEAROTHERMOPHILUS
9	0.80	36.5	18.0	1.03	304	5	44	90	1n82:B	86	328		THE HIGH-RESOLUTION CRYSTAL STRUCTURE OF IXT6, A THERMOPHILIC, INTRACELLULAR XYLANASE FROM G. STEAROTHERMOPHILUS

Slika 8: Dobljeni rezultati kjer vidimo, da je prvi zadetek 5AY7, drugi in tretji pa sta 5D4Y.

b) Odgovor vidimo v prvem stolpcu, da je Q vrednost 0.97

Da ugotovimo če je članek podoben prej najdenim člankom moramo odpreti protein 5D4Y tako da kliknemo na kodo v vrstici »Match«. Odpre se nam slika proteina in zraven lahko najdemo »Primary publication«, kjer nam navaja kje je bil članek prvo objavljen (Slika 9). Če ta zadetek primerjamo z zadetki ki smo jih dobili pri 1. b delu naloge, ugotovimo da je ena izmed opciji isti članek.

c) Naš članek je ena izmed opciji. Yingying Zheng se nahaja na prvem mestu. (Slika 10)

**pdb\_00005d4y** X-ray diffraction 2.5Å Released 24 Feb 2016 [10.2210/pdb5d4y/pdb](https://doi.org/10.2210/pdb5d4y/pdb)

Summary | Model Quality | Complexes | Macromolecules | Ligands and Environments | Domains | Text Annotations (AI) | Citations | Other Resources

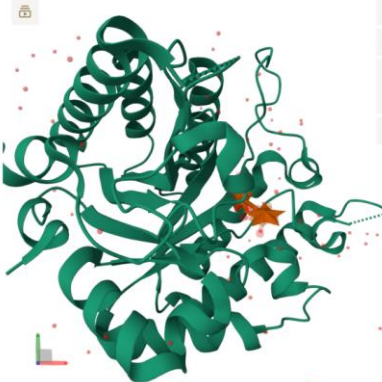
Entry title: A psychrophilic glycoside hydrolase family 10 endo-beta-1,4-xylanase

Entry authors: Zheng, Y., Guo, R.T.

Source organism: [environmental samples](#)

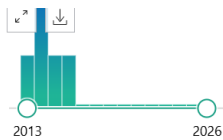
Primary publication: Structural insight into potential cold adaptation mechanism through a psychrophilic glycoside hydrolase family 10 endo-β-1,4-xylanase. Zheng Y, Li Y, Liu W, Chen CC, Ko TP. *J. Struct. Biol.* 193 206-211 (2016) PMID 26719223 DOI 10.1016/j.jsb.2015.12.010 Also associated with: [pdb\\_00005ay7](#)

PDB model quality summary: Model geometry Fit model/data Percentile-sliders comparing the quality scores of a model with other models in the archive.



Preferred complex   
Macromolecules (2)   
Ligands (0)   
Domains (2)   
Modifications (0)

Slika 9: Pridobljene informacije o proteinu 5D4Y, med njimi tudi mesto publikacije.



PUBLICATION DATE

- 1 year
- 5 years
- 10 years
- Custom Range

TEXT AVAILABILITY

- Abstract
- Free full text
- Full text

ARTICLE ATTRIBUTE

- Associated data

ARTICLE TYPE

- Books and Documents
- Clinical Trial
- Meta-Analysis
- Randomized Controlled

Page 1

- 1 **Structural perspectives of an engineered beta-1,4-xylanase with enhanced thermostability.**  
 Cite Chen CC, Luo H, Han X, Lv P, Ko TP, Peng W, Huang CH, Wang K, Gao J, **Zheng Y**, Yang Y, Zhang J, Yao B, Guo RT.  
 J Biotechnol. 2014 Nov 10;189:175-82. doi: 10.1016/j.jbiotec.2014.08.030. Epub 2014 Sep 3. PMID: 25193708  
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- 2 **Preliminary X-ray diffraction analysis of thermostable beta-1,4-xylanase from Streptomyces sp. S9.**  
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 Acta Crystallogr F Struct Biol Commun. 2014 Jan;70(Pt 1):105-7. doi: 10.1107/S2053230X13033335. Epub 2013 Dec 24. PMID: 24419629 **Free PMC article.**  
**Xylanase**, which catalyzes the random hydrolysis of internal xylosidic linkages, is a critical enzyme participating in **xylan** decomposition and has been widely applied in industrial utilizations. **Xylanase** isolated from the extremophilic Streptomyces sp. S9 (Xyn ...
- 3 **Structural insight into potential cold adaptation mechanism through a psychrophilic glycoside hydrolase family 10 endo-beta-1,4-xylanase.**  
 Cite **Zheng Y**, Li Y, Liu W, Chen CC, Ko TP, He M, Xu Z, Liu M, Luo H, Guo RT, Yao B, Ma Y.  
 J Struct Biol. 2016 Mar;193(3):206-211. doi: 10.1016/j.jsb.2015.12.010. Epub 2015 Dec 21. PMID: 26719223  
 The cold-adapted **xylanases** can catalyze at low temperature and hold great potential in food industry applications. ...Structural comparison with a thermophilic GH10 **xylanase** highlighting various parameters that may explain the cold adaptation features were analyzed. ...

*Slika 10: To so pridobljeni članki iz 1. b dela naloge, kjer lahko vidimo da se naslov, ki smo ga dobili od 5D4Y sklada z naslovom tretjega članka. Opazimo pa tudi da je Yingying Zheng napisan na prvem mestu, vendar je zapisano prvo priimek in potem kratica (vendar program že sam označi ime, ker smo vstavili filter pri iskanju).*

3.

Informacije o encimih bo najlažje najti v PDB, ker imamo PDB id za oba.

Biological Assembly 1

5AY7 | pdb\_00005ay7

A psychrophilic glycoside hydrolase family 10 endo-beta-1,4-xylanase

PDB DOI: <https://doi.org/10.2210/pdb5AY7/pdb>

Classification: HYDROLASE

Organism(s): *Aegilops speltoides* subsp. *speltoides*

Expression System: *Escherichia coli* 'BL21-Gold(DE3)pLysS AG

Mutation(s): No

Deposited: 2015-08-10 Released: 2016-02-24

Deposition Author(s): Zheng, Y., Li, Y., Liu, W., Guo, R.T.

Funding Organization(s): Ministry of Science and Technology of China

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

Resolution: 2.15 Å

R-Value Free: 0.257 (Depositor), 0.267 (DCC)

R-Value Work: 0.192 (Depositor), 0.202 (DCC)

R-Value Observed: 0.195 (Depositor)

wwPDB Validation

Metric	Percentile Ranks	Value
Rfree		0.267
Clashscore		5
Ramachandran outliers		1.1%
Sidechain outliers		5.2%
RSRZ outliers		1.2%

Slika 11: Ko vpišemo kodo 5AY7 v iskalnik dobimo ven to sliko. Iz slike lahko razberemo, da pod »Organism(s)« piše »*Aegilops speltoides* subsp. *speltoides*«.

Biological Assembly 1

5D4Y | pdb\_00005d4y

A psychrophilic glycoside hydrolase family 10 endo-beta-1,4-xylanase

PDB DOI: <https://doi.org/10.2210/pdb5D4Y/pdb>

Classification: HYDROLASE

Organism(s): environmental samples

Expression System: *Escherichia coli* 'BL21-Gold(DE3)pLysS AG

Mutation(s): No

Deposited: 2015-08-10 Released: 2016-02-24

Deposition Author(s): Zheng, Y., Guo, R.T.

Funding Organization(s): Ministry of Science and Technology of China

Experimental Data Snapshot

Method: X-RAY DIFFRACTION

Resolution: 2.50 Å

R-Value Free: 0.285 (Depositor), 0.296 (DCC)

wwPDB Validation

Metric	Percentile Ranks	Value
Rfree		0.296
Clashscore		10

Slika 12: Ko vpišemo kodo 5D4Y v iskalnik dobimo ven to sliko. Iz slike lahko razberemo, da pod »Organism(s)« piše »environmental samples«.

a) Encim 5AY7 je pridobljen iz »*Aegilops speltoides* subsp. *Speltoides*« (Slika 11). Encim 5D4Y pa je pridobljen iz »environmental samples« (Slika 12).

b) Pri obeh je ekspresijski sistem »*Escherichia coli* 'BL21-Gold(DE3)pLysS AG« (Slika 11 in 12). Zato vemo, da bo primeren ekspresijski sistem bakterijski.

Slabosti so; ni kompleksnih posttranslacijskih modifikacij (npr. glikozilacije), težave z napačnim zvižanjem proteinov, tvorjenje netopnih agregatov, narobna tvorba disulfidnih vezi.

4.

Da najdemo uniprot kodo moramo v UniProt, kjer v »Advanced Search« vpišemo »xylanase« in potem dodamo še en filter, ki ga nastavimo na »Organism [OS]« in tam potem poiščemo »saccharolyticum«. Tukaj lahko pride do dveh možnosti, če že v iskalniku izberemo »Thermoanaerobacterium saccharolyticum« potem dobimo samo en protein, ki je bil pregledan (Slika 13 in 14). Če pa v iskalnik vstavimo namesto »Thermoanaerobacterium saccharolyticum« samo »saccharolyticum« potem dobimo rezultate pod Slikama 15 in 16.

**Advanced Search**

Searching in: UniProtKB

All: xylanase Remove

AND Organism [OS]: Thermoanaerobacterium saccharolyticum [28896] Remove

[Add Field](#) Cancel Search

Type \* in the search box to search for all values for the selected field.

Slika 13: V uniprot vstavimo »xylanase« in »Thermoanaerobacterium saccharolyticum«.

**Status**

- Reviewed (Swiss-Prot) (1)
- Unreviewed (TrEMBL) (2)

**Taxonomy**

28896 ×

Filter by taxonomy

**Group by**

- Taxonomy
- Keywords
- Gene Ontology
- Enzyme Class

**Proteins with**

- Active site (2)
- Biophysicochemical properties (1)
- Catalytic activity (3)
- Chain (1)

**The unreviewed UniProtKB/TrEMBL database will be reduced in size in release 2026\_02 (first half of 2026).**

- Entries to be retained in UniProtKB:
  - Entries from reference proteomes
  - All reviewed (Swiss-Prot) entries
  - Selected unreviewed (TrEMBL) entries with experimental or biologically important data
- Entries to be removed: Unreviewed (TrEMBL) entries that are not part of a reference proteome

Entries removed from unreviewed UniProtKB/TrEMBL will remain accessible in the UniParc sequence archive. Please read our [help page](#), [view affected entries and proteomes](#), or [contact us](#) with any questions.

### UniProtKB 3 results

or expand search to taxon ID "28896" to include lower taxonomic ranks

Tools Download (3) Add View: Cards Table Customize columns Share

Entry	Entry Name	Protein Names	Gene Names	Organism	Length
<input type="checkbox"/> P36917	XYNA_THESA	Endo-1,4-beta-xylanase A[...]	xynA	Thermoanaerobacterium saccharolyticum	1,157 AA
<input type="checkbox"/> D2X5N2	D2X5N2_THESA	Beta-xylanase[...]		Thermoanaerobacterium saccharolyticum	1,429 AA
<input type="checkbox"/> E5KBL2	E5KBL2_THESA	Beta-xylanase[...]	xynFCB	Thermoanaerobacterium saccharolyticum	413 AA

Slika 14: Z iskalnikom iz Slike 13 dobimo tri možnosti, vendar je samo ena od njih »Reviewed« zato je ta prava odločitev.

**Advanced Search** ✕

Searching in  
UniProtKB

All  
xylanase Remove

AND **Organism [OS]** Remove  
saccharolyticum

Add Field Cancel Search

Type \* in the search box to search for all values for the selected field.

Slika 15: Če v uniprot iskalnik vstavimo »xylanase« in samo »saccharolyticum« dobimo rezultate na Sliki 16.

**Status**

- Reviewed (Swiss-Prot) (3)
- Unreviewed (TrEMBL) (26)

**Taxonomy**

"saccharolyticum" ✕

Filter by taxonomy

**Group by**

- Taxonomy
- Keywords
- Gene Ontology
- Enzyme Class

**Proteins with**

- 3D structure (2)
- Active site (13)
- Biophysicochemical properties (2)
- Catalytic activity (17)
- Chain (7)

- Selected unreviewed (TrEMBL) entries with experimental or biologically important data
- ✕ Entries to be removed: Unreviewed (TrEMBL) entries that are not part of a reference proteome

Entries removed from unreviewed UniProtKB/TrEMBL will remain accessible in the UniParc sequence archive. Please read our [help page](#), [view affected entries and proteomes](#), or [contact us](#) with any questions.

**UniProtKB 29 results**

Tools Download (29) Add View: Cards Table Customize columns Share

Entry	Entry Name	Protein Names	Gene Names	Organism	Length
<input type="checkbox"/> P36917	XYNA_THESA	Endo-1,4-beta-xylanase A[...]	xynA	Thermoanaerobacterium saccharolyticum	1,157 AA
<input type="checkbox"/> P23556	XYNA_CALSA	Endo-1,4-beta-xylanase A[...]	xynA	Caldicellulosiruptor saccharolyticus (Caldocellum saccharolyticum)	342 AA
<input type="checkbox"/> P23557	XYN4_CALSA	Putative endo-1,4-beta-xylanase[...]		Caldicellulosiruptor saccharolyticus (Caldocellum saccharolyticum)	312 AA
<input type="checkbox"/> D2X5N2	D2X5N2_THESA	Beta-xylanase[...]		Thermoanaerobacterium saccharolyticum	1,429 AA
<input type="checkbox"/> ESKBL2	E5KBL2_THESA	Beta-xylanase[...]	xynFCB	Thermoanaerobacterium saccharolyticum	413 AA

Slika 16: Tukaj dobimo 29 rezultatov, vendar so samo trije od njih »Reviewed«. Da najdemo pravega pa moramo upoštevati, da so vzeli termoanaerobno bakterijo, tako da je pravilna odločitev prvi protein.

a) Pri obeh možnostih pridemo do istega rezultata in to je P36917.

Da najdemo mesto mutacije na aktivnem mestu moramo prvo odpreti protein in potem iti pod »Function« del z »Features« kjer nam pišejo tri aaktivna mesta na ostankih 495, 537 in 600. Potem pa pogledamo še mutacije pod »Phenotypes & Variants« kjer vidimo da nastanejo tri pogoste mutacije na 537, 600 in 602. Če kliknemo na njih nam pokaže kateri aminokislinski ostanki se zamenjajo.

b) Mutacija se zgodi na aktivnem mestu 537 in 600, mutacija na 495 ni nikjer zabeležena zato sklepamo, da se ne zgodi ali pa še ni bila odkrita.

c) Pri 537 pride do mutacije, kjer se zamenja D → N, pri 600 pa se zamenja E → Q.

5.

Naloga nas spomni na program Blast oziroma natančneje Blastp. V katerega vstavimo dobljeno zaporedje in potem poiščemo podobne proteine. (Slika 17)

BLAST® » blastp suite Home Recent Results Saved Strategies Help

blastn **blastp** blastx tblastn tblastx Standard Protein BLAST

BLASTP programs search protein databases using a protein query. more... Reset page Bookmark

**Enter Query Sequence**

Enter accession number(s), gi(s), or FASTA sequence(s)  Query subrange

ASRHIVEGM  
IDTQYEPNKTVTRAEFTAMILRLNLIKEEQYSGEFSVDVNSGDWYANAIEAAYK  
AGIEGD  
GKNAKPNDSITREEMTORVANCQDITFLQEANRGD

Or, upload file  Nobena datoteka ni izbrana

Job Title

Enter a descriptive title for your BLAST search

Align two or more sequences

**Choose Search Set**

Database

Organism

Optional

**Program Selection**

Algorithm  blastp (protein-protein BLAST)  
 PSI-BLAST (Position-Specific Iterated BLAST)  
Choose a BLAST algorithm

Search database ClusteredNR using Blastp (protein-protein BLAST)  
 Show results in a new window

Slika 17: Odpremo blastp in vstavimo FASTA zaporedje ki nam je podano pod 5 nalogo.

Naloga nas sprašuje če je to zaporedje podobno tistemu od XynA, ki ga opazimo na drugem mestu med zadetki. (Slika 18)

a) Opazimo tudi, da je E-value enaka 0, kar pomeni da je verjetnost da to zaporedje naključno najdemo v bazi zelo zelo mala oziroma 0. Zato vemo da je ta encim podoben XynA.

b) Odstotek identičnosti vidimo da je 99.62%, torej je večina zaporedja istega.

Clusters producing significant alignments Download Select columns Show 100

select all 100 clusters selected [GenPept](#) [Graphics](#) [Distance tree of results](#) [Multiple alignment](#) [MSA Viewer](#)

	Cluster Composition	Cluster Ancestor	Cluster Representative Sequence	Max Score	Total Score	Query Cover	E value	Per Ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	6 member(s), 5 organism(s)	firmicutes	endo-1.4-beta-xylanase [Thermoanaerobacterium xylanolyticum]	2202	2202	99%	0.0	92.23%	1232	WP_013788491.1
<input checked="" type="checkbox"/>	1 member(s), 1 organism(s)	firmicutes	XynA [Thermoanaerobacterium saccharolyticum]	2132	2346	99%	0.0	99.62%	1429	ADB23440.1
<input checked="" type="checkbox"/>	3 member(s), 3 organism(s)	firmicutes	MULTISPECIES: endo-1.4-beta-xylanase [Thermoanaerobacte...	2023	2228	99%	0.0	93.12%	1432	WP_014758337.1
<input checked="" type="checkbox"/>	1 member(s), 1 organism(s)	firmicutes	endo-1.4-beta-xylanase_partial [Thermoanaerobacterium aotea...	2018	2018	89%	0.0	93.12%	1187	WP_084214950.1
<input checked="" type="checkbox"/>	3 member(s), 3 organism(s)	firmicutes	endo-1.4-beta-xylanase [Thermoanaerobacterium thermosacch...	1930	2119	100%	0.0	89.99%	1429	WP_398503082.1
<input checked="" type="checkbox"/>	1 member(s), 1 organism(s)	firmicutes	endoxylanase precursor [Thermoanaerobacterium sp.]	1886	2079	99%	0.0	87.67%	1348	AAC43719.1
<input checked="" type="checkbox"/>	3 member(s), 3 organism(s)	firmicutes	endo-1.4-beta-xylanase [Thermoanaerobacterium sp. CMT556...	1827	1959	99%	0.0	91.92%	1077	WP_303264344.1
<input checked="" type="checkbox"/>	1 member(s), 1 organism(s)	firmicutes	endo-1.4-beta-xylanase [Thermoanaerobacterium sp. RB11TD]	1803	1999	100%	0.0	84.70%	1440	WP_096232049.1
<input checked="" type="checkbox"/>	1 member(s), 1 organism(s)	firmicutes	endo-1.4-beta-xylanase [Thermoanaerobacterium thermosacch...	1633	1993	99%	0.0	82.16%	1087	WP_253666370.1
<input checked="" type="checkbox"/>	1 member(s), 1 organism(s)	firmicutes	endo-1.4-beta-xylanase [Thermoanaerobacter mathranii]	1457	1457	88%	0.0	67.78%	1269	WP_431467670.1
<input checked="" type="checkbox"/>	6 member(s), 5 organism(s)	firmicutes	endo-1.4-beta-xylanase_partial [Thermoanaerobacter pentosa...	1439	1577	99%	0.0	67.14%	1448	WP_307681577.1
<input checked="" type="checkbox"/>	1 member(s), 1 organism(s)	firmicutes	endo-1.4-beta-xylanase [Thermoanaerobacterium thermosacch...	1437	1998	99%	0.0	82.95%	1282	WP_013297490.1
<input checked="" type="checkbox"/>	2 member(s), 1 organism(s)	firmicutes	endo-1.4-beta-xylanase [Caldanaerobius polysaccharolyticus]	1400	1672	89%	0.0	71.63%	1320	WP_051585735.1
<input checked="" type="checkbox"/>	1 member(s), 1 organism(s)	firmicutes	endo-1.4-beta-xylanase [Clostridium sp. 19966]	1087	1087	88%	0.0	53.07%	1121	WP_315673356.1
<input checked="" type="checkbox"/>	3 member(s), 1 organism(s)	firmicutes	endo-1.4-beta-xylanase [Clostridium thermanum]	1061	1061	88%	0.0	51.31%	1381	WP_163192232.1
<input checked="" type="checkbox"/>	1 member(s), 1 organism(s)	firmicutes	endo-1.4-beta-xylanase [Clostridium sp. 1001275H_160808_H3]	969	1080	89%	0.0	48.91%	1169	WP_195263722.1

Slika 18: Dobljen rezultat z blastanjem, kjer lahko vidimo vse možne podobne proteine.

c) Za poravnavo dveh zaporedji uporabljamo ali Needle ali Water, ker sta ta dva encima skoraj da identična je boljša poravnava Needle. Needle je globalna poravnava, ki poravna celoti obeh zaporedji in zato se uporablja ko sta zaporedji podobno dolgi (ti dve sta) in ko sta tudi si med seboj dokaj podobni (tudi sta). Water pa je lokalna poravnava, ki poravna zaporedje z delom zaporedja, ki najbolj ustreza (npr. eno domeno), zato ni najbolj primerna za te dve zaporedji, ki sta si skoraj identični

Da odkrijemo score poravnave moramo odpreti Needle, kam moramo prilepiti obe zaporedji. Prvo zaporedje je podano že v nalogi, drugo zaporedje pa dobimo če kliknemo pri »Accession« na kodo ADB23440.1. In potem kliknemo na »FASTA« (Slika 19), dobimo celo zaporedje tega proteina (Slika 20), ki ga kopiramo.

The screenshot shows the GenBank entry for XynA [Thermoanaerobacterium saccharolyticum] (GenBank: ADB23440.1). The 'FASTA' link is highlighted with a red arrow. The entry includes details such as LOCUS, DEFINITION, ACCESSION, VERSION, DBSOURCE, KEYWORDS, SOURCE, ORGANISM, REFERENCE, and FEATURES. The right sidebar contains options for 'Analyze this sequence', 'Related information', and 'Recent activity'.

Slika 19: Informacije, ki se nam odprejo če kliknemo na ADB23440.1. Potem pa nadaljujemo tako, da kliknemo na FASTA.



Download

```
# Commandline: needle
# -auto
# -stdout
# -asequence emboss_needle-I20260512-094155-0410-10521866-p1m.asequence
# -bsequence emboss_needle-I20260512-094155-0410-10521866-p1m.bsequence
# -datafile EBLOSUM62
# -gapopen 10.0
# -gapextend 0.5
# -endopen 10.0
# -endextend 0.5
# -aformat3 pair
# -sprotein1
# -sprotein2
# Align_format: pair
# Report_file: stdout
#####

#=====
#
# Aligned_sequences: 2
# 1: EMB0SS_001
# 2: EMB0SS_001
# Matrix: EBLOSUM62
# Gap_penalty: 10.0
# Extend_penalty: 0.5
#
# Length: 1431
# Identity: 1148/1431 (80.2%)
# Similarity: 1154/1431 (80.6%)
# Gaps: 256/1431 (17.9%)
# Score: 5899.0
#
```

Slika 22: Dobljena poravnava, vidimo da sta zaporedji res skoraj identični (kar 80%), vendar nas zanima Score.

6.

Odpremo Weblogo in vanj prilepimo 4 zaporedja, ki so nam jih posredovali. (Slika 23)

### WebLogo 3: Create

Sequence Data Input:

Nobena datoteka ni izbrana URL:

>1  
MKRTYLSLIAAGVMSLSVSAWSLDGLVLPESGILVSVGQDQVDSVNDYASALGTIPAGVTN  
YVGIVNLDGLNSDADAGAGRNIIAELANAYPTSALVGVSMNGEVDVAASGRYNANIDTL  
LNTLAGYDRPVYLRWAYEVDGPWNGHSPSGIVTSFYVHDRIALGHQAKISLVWQVASY  
CPTPGGQLDQWVPGSEYDWMVGLSYFAPDCNWDRIWFAAOCFARSKGKPLFLNDSTPQRY

Download to local drive

Title:

Output Format:

Sequence type:

Logo-size:

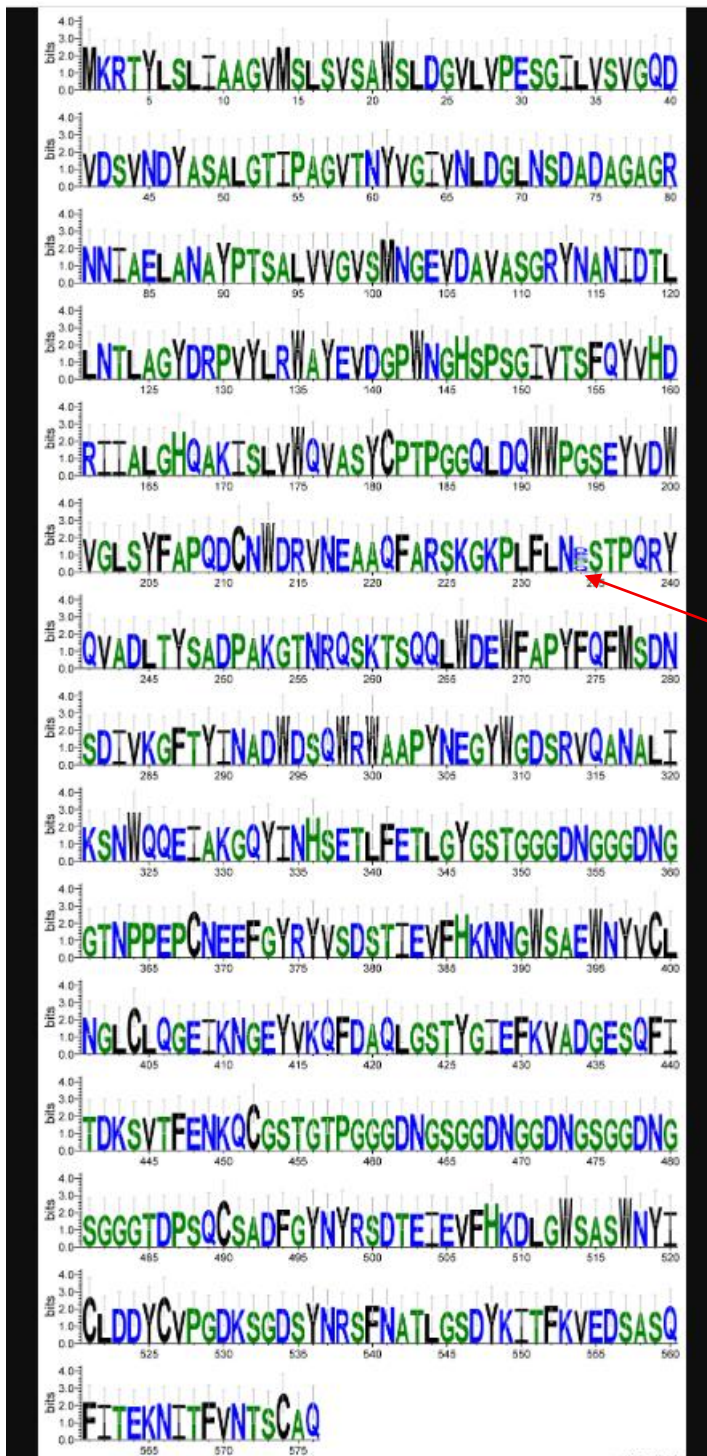
Error bars:

Show Sequence Ends labels:

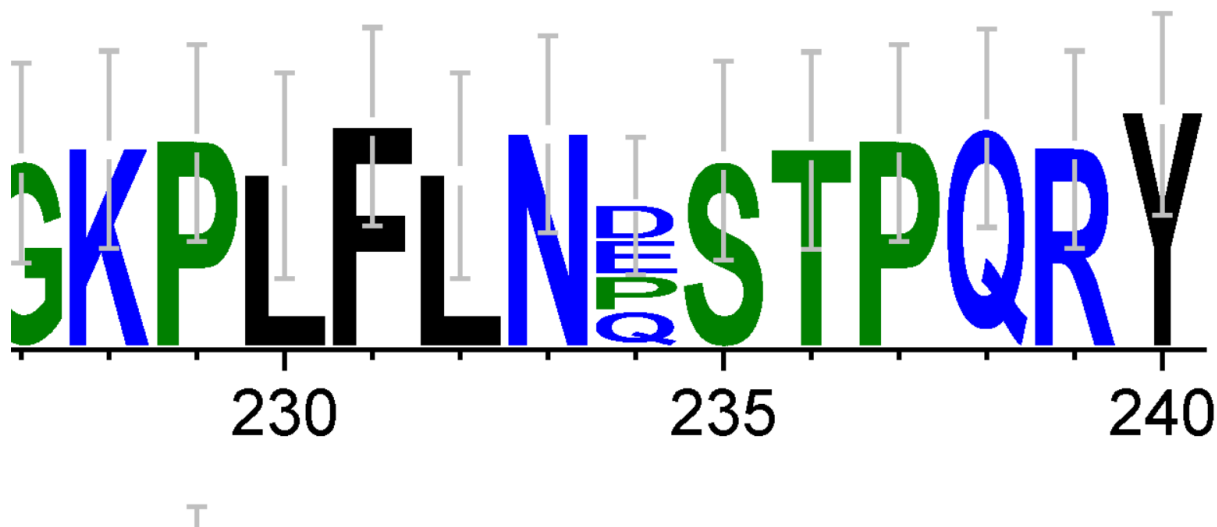
Version fingerprint:

X-axis:  | a|b|c|

Slika 23: V WebLogo vstavimo vsa štiri zaporedja, da dobimo prikaz vseh aminokislinskih ostankov in kje se zaporedja razlikujejo.



Slika 24: Vizualni prikaz vseh štirih zaporedji v WebLogu. Rdeča puščica prikazuje edino mesto v zaporedju kjer opazimo da je več črk na istem aminokislinskem zaporedju.



Slika 25: Ko približamo opazimo da pride do spremembe ostankov na mestu 234.

a) Na Sliki 25 vidimo, da edina razlika med zaporedji je na aminokislinskem ostanku 234.

Da bi ugotovili funkcijo tega ostanka gremo na UniProt, kjer vpišemo v iskalnik D5MP61. Potem gremo pod »Function« in spodaj pod »Features«, opazimo da je 234 aminokislinski ostanek aktivno mesto proteina. (Slika 26)

b) Aktivno mesto

UniProt BLAST Align Peptide search ID mapping SPARQL UniProtKB Advanced List Search Help

Entry Variant viewer Feature viewer Genomic coordinates Publications External links History

**Temperature dependence**  
Optimum temperature is 37 degrees Celsius. Inactive above 60 degrees Celsius. 1 Publication

**Features**  
Showing features for active site:

Download

133 50 100 150 200 250 300 350 400 450 500 550 602

L R M A Y E V D G P M N G H S P S G I V T S F Q Y V H D R I

±	TYPE	ID	POSITION(S)	DESCRIPTION
+	Active site		138	Proton donor PROSITE-ProRule Annotation
+	Active site		234	Nucleophile PROSITE-ProRule Annotation

**Gene Ontology**

GO annotations GO-CAM models New

Gene Ontology (GO) annotations organized by slimming set.

Slika 26: Ko vpišemo uniprot kodo v iskalnik se nam odpre ta protein

Če pogledamo v Weblogo vidimo, da je aminokislinski ostanek v zaporedjih E, D, Q ali P (Slika 25). Če kliknemo na aktivno mesto v uniprotu vidimo da je na tistem mestu E. Če gremo pod »Phenotypes & Variants« vidimo da se na 234 zgodita dve mutaciji in to v D in Q. (Slika 27)

c) Če je v zaporedju na mestu 234 E potem je katalitična aktivnost normalna oziroma nespremenjena. Če se tam nahaja D potem to povzroči nizko katalitično aktivnost. Q na mestu 234 pa povzroči popolno izgubo katalitične aktivnosti (Slika 27). Tudi če zamenjam E s P se bo

izgubila katalitična aktivnost, saj E (glutamat) ima negativen naboj med tem ko P (prolin) nima naboja. To pomeni da se izgubijo pomembne elektrostatske interakcije, kar povzroči izgubo katalitičnega mesta.

d)

±	TYPE	ID	POSITION(S)	DESCRIPTION
+	Mutagenesis		138	Very low catalytic activity. <a href="#">1 Publication</a>
+	Mutagenesis		138	Complete loss of catalytic activity. <a href="#">1 Publication</a>
-	Mutagenesis		234	Very low catalytic activity. <a href="#">1 Publication</a>
-	Mutagenesis		234	Complete loss of catalytic activity. <a href="#">1 Publication</a>
+	Mutagenesis		268	Catalytic activity reduced by 30%. <a href="#">1 Publication</a>

Sequence: E → D

Sequence: E → Q

Slika 27: Vidimo vse možne mutacije, mi se osredotočimo na tiste na aminokislinskem ostanku 234. Zgodita se dve mutaciji E → D in E → Q.

d) Sprememba v P bo spremenila strukturo, saj kot sem pojasnil že v c) delu se spremeni naboj kar podre interakcije, kar vodi do spremembe strukture proteina.