Properties of a cryptic lysyl oxidase from haloarchaeon *Haloterrigena turkmenica*

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B ackground: Lysyl oxidases (LOX) have been extensively studied in mammals, whereas properties and functions of recently found homologues in prokaryotic genomes remain enigmatic. **Methods:** LOX open reading frame was cloned from *Haloterrigena turkmenica* in an *E. coli* expression vector. Recombinant *Haloterrigena turkmenica* lysyl oxidase (HTU-LOX) proteins were purified using metal affinity chromatography under denaturing conditions followed by refolding. Amine oxidase activity has been measured fluorometrically as hydrogen peroxide release coupled with the oxidation of 10-acetyl-3,7-dihydroxyphenoxazine in the presence of horseradish peroxidase. Rabbit polyclonal antibodies were obtained and used in western blotting. **Results:** Cultured *H. turkmenica* has no detectable amine oxidase activity. HTU-LOX may be expressed in *E. coli* with a high protein yield. The full-length protein gives no catalytic activity. For this reason, we hypothesized that the hydrophobic N-terminal region may interfere with proper folding and its removal may be beneficial. Indeed, truncated His-tagged HTU-LOX lacking the N-terminal hydrophobic signal peptide purified under denaturing conditions can be successfully refolded into an active enzyme, and a larger N-terminal truncation further increases the amine oxidase activity. Refolding is optimal in the presence of Cu^{2+} at pH 6.2 and is not sensitive to salt. HTU-LOX is sensitive to LOX inhibitor 3-aminopropionitrile. HTU-LOX deaminates usual substrates of mammalian LOX such as lysine-containing polypeptides and polymers. The major difference between HTU-LOX and mammalian LOX is a relaxed specificity of the former. HTU-LOX readily oxidizes various primary amines including such compounds as taurine and glycine, benzylamine being a poor substrate. Of note, HTU-LOX is also active towards several aminoglycoside antibiotics and polymyxin. Western blotting indicates that epitopes for the anti-HTU-LOX polyclonal antibodies coincide with a high molecular weight protein in *H. turkmenica* cells. **Conclusion:** *H. turkmenica* contains a lysyl oxidase gene that was heterologously expressed yielding an

active recombinant enzyme with important biochemical features conserved between all known LOXes, for example, the sensitivity to 3-aminopropionitrile. However, the native function in the host appears to be cryptic. **Significance:** This is the first report on some properties of a lysyl oxidase from Archaea and an interesting example of evolution of enzymatic properties after hypothetical horizontal transfers between distant taxa.

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3

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10   Short title: *Archaeal Lysyl Oxidase*

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16

17   **ABSTRACT**

18   **Background:** Lysyl oxidases (LOX) have been extensively studied in mammals, whereas

19   properties and functions of recently found homologues in prokaryotic genomes remain

20   enigmatic. **Methods:** LOX open reading frame was cloned from *Haloterrigena turkmenica* in an

21   *E. coli* expression vector. Recombinant *Haloterrigena turkmenica* lysyl oxidase *(*HTU-LOX)

22   proteins were purified using metal affinity chromatography under denaturing conditions followed

23   by refolding. Amine oxidase activity has been measured fluorometrically as hydrogen peroxide

24   release coupled with the oxidation of 10-acetyl-3,7-dihydroxyphenoxazine in the presence of

25   horseradish peroxidase. Rabbit polyclonal antibodies were obtained and used in western blotting.

26   **Results:** Cultured *H. turkmenica* has no detectable amine oxidase activity. HTU-LOX may be

27   expressed in *E. coli* with a high protein yield. The full-length protein gives no catalytic activity.

28   For this reason, we hypothesized that the hydrophobic N-terminal region may interfere with

29   proper folding and its removal may be beneficial. Indeed, truncated His-tagged HTU-LOX

30   lacking the N-terminal hydrophobic signal peptide purified under denaturing conditions can be

31 successfully refolded into an active enzyme, and a larger N-terminal truncation further increases

32 the amine oxidase activity. Refolding is optimal in the presence of Cu^{2+} at pH 6.2 and is not

33 sensitive to salt. HTU-LOX is sensitive to LOX inhibitor 3-aminopropionitrile. HTU-LOX

34 deaminates usual substrates of mammalian LOX such as lysine-containing polypeptides and

35 polymers. The major difference between HTU-LOX and mammalian LOX is a relaxed

36 specificity of the former. HTU-LOX readily oxidizes various primary amines including such

37 compounds as taurine and glycine, benzylamine being a poor substrate. Of note, HTU-LOX is

38 also active towards several aminoglycoside antibiotics and polymyxin. Western blotting

39 indicates that epitopes for the anti-HTU-LOX polyclonal antibodies coincide with a high

40 molecular weight protein in *H. turkmenica* cells. **Conclusion:** *H. turkmenica* contains a lysyl

41 oxidase gene that was heterologously expressed yielding an active recombinant enzyme with

42 important biochemical features conserved between all known LOXes, for example, the

43 sensitivity to 3-aminopropionitrile. However, the native function in the host appears to be

44 cryptic. **Significance:** This is the first report on some properties of a lysyl oxidase from Archaea

45 and an interesting example of evolution of enzymatic properties after hypothetical horizontal

46 transfers between distant taxa.

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49 **INTRODUCTION**

50 Lysyl oxidase is an amine oxidase that is well characterized in mammals. The human genome

51 contains five lysyl oxidase isoforms (LOX and LOXL1-4), all of them possess the highly

52 conserved C-terminal catalytic domain, the N-terminal signal peptide, and the accessory

53 segments in between. Catalytic domain of LOX is unique among other mammalian amine

54 oxidases because of its ability to oxidatively deaminate various amines including ε-amino groups

55 of lysine residues in peptides and proteins. LOX activity initiates cross-link formation between

56 certain proteins, including elastin, collagen, and fibronectin, and this process is important for

57 maturation and remodeling of the extracellular matrix (*Lucero and Kagan, 2006)*. Most animal

58 genomes sequenced to date contain from one to five LOX genes, only placozoans, nematodes

59 and ctenophores seem to lack any LOX genes. (*Grau-Bove et al, 2015*). There are LOX genes in

60 some fungal genomes, whereas plants are unknown to possess it. LOX genes are also absent

61 from the vast majority of prokaryotic genomes. Therefore, the presence of true homologues of

62   animal LOX in just several species of Eubacteria and Archaea is of significant interest; it reflects

63   the unique history of this enzyme, that most parsimoniously can be explained by multiple

64   horizontal transfer events (HGT) (*Grau-Bove et al, 2015*). Among Eubacteria, LOX genes are

65   frequent in Actinomycetes (especially Streptomyces), some Deltaproteobacteria, occasionally –

66   in other eubacteria, and very rarely – among Archaea. This aspect is exciting not only from the

67   phylogenetic point of view, but also because of potential biotechnological applications, i.e. the

68   fact that distantly related enzymes may have useful properties *(Noda-García et al, 2013)*.

69   It is interesting to note that, in contrast to eukaryotic lysyl oxidases, several LOX homologues

70   identified in prokaryotes exhibit a simple architecture even without a signal peptide (*Grau-Bove*

71   *et al, 2015*). On the other hand, some prokaryotic LOXes are more complex. Specifically, LOX

72   from *Sorangium cellulosum (*WP_012233967.1*)* possesses a unique Cys-rich C-terminal non-

73   catalytic domain, which is presumably highly disulfide cross-linked.

74   The few lysyl oxidase homologues from Archaea that have been sequenced are clustered in two

75   independent groups. This suggests that the two major phyla, Thaumarchaeotes and

76   Euryarchaeotes, may had acquired LOX genes in two independent HGT events (*Grau-Bove et al,*

77   *2015*). Indeed, HGT is widespread in Archaea (*Papke et al, 2004, Papke et al, 2015*).

78   *Haloterrigena turkmenica* was isolated from Turkmenistani sulfate saline soil by Zvyagintseva

79   and Tarasov and described in 1987 as *Halococcus turkmenicus* (*Zvyagintseva and Tarasov,*

80   *1987*). In 1999 it was proposed to rename it to *Haloterrigena* (*Ventosa et al, 1999*). *H.*

81   *turkmenica* belongs to the family Halobacteriaceae typus Euryarchaeota and is a fairly fast

82   growing chemoorganotrophic extreme halophile that requires at least 2 M NaCl with optimal

83   temperature around 45°C. The complete genome of this archaeon has been sequenced. It consists

84   of 5,440 kbp (including plasmid 6), and it was annotated as encoding 5,287 proteins and 63

85   ncRNAs *(Saunders et al, 2010)*.

86   Here, we attempted for the first time a study on the properties of lysyl oxidase from this

87   haloarchaeon.

88

89   **EXPERIMENTAL PROCEDURES**

90   *Materials and strains.* A fresh stock of *Haloterrigena turkmenica VKMB-1734* was purchased

91   from the All-Russian Collection of Microorganisms (G.K. Skryabin Institute of Biochemistry

92   and Physiology of Microorganisms, Pushchino, Moscow Region, Russia). Capreomycin was

93   from S.P. Incomed (Moscow, Russia), amikacin from OAO Sintez (Kurgan, Russia), substance P

94   was custom synthesized at Syneuro (Moscow, Russia), hexylamine and 3-aminopropionitrile

95   fumarate from Alfa Aesar (USA), all other amine substrates were from Sigma (USA).

96   *Cultivation of H. turkmenica.* Various haloarchaeal media with NaCl around 200 g/l such as

97   INMI medium-3, DSMZ-372 are suitable. Care should be taken to adjust pH since *H. turkmenica*

98   does not grow in acidic media. We found that a simpler medium (hereafter referred to as **IAO**) is

99   a better choice: casamino acids, 5 g/l; yeast extract, 5 g/l; NaCl, 220 g/l; pH 7.6 – autoclaved and

100   supplemented with $MgSO_4$, 5 mM; $CuCl_2$, 10 μM. Solid IAO medium may be used for growing

101   single colonies, however, only with high quality agar (some batches inhibit growth). Also, *H.*

102   *turkmenica* can be easily adapted to a defined medium, hereafter referred to as **MHTU,** an

103   enriched version of HMM (*Mosin and Ignatov, 2014*)): L-alanine, 0.4 g/l; L-arginine, 0.4 g/l; D-

104   asparagine, 0.2 g/l; L-aspartic acid, 0.4 g/l; L-cysteine, 0.1 g/l; L-glutamic acid, 1.5 g/l; L-histidine, 0.7

105   g/l; L-isoleucine, 0.5 g/l; L-leucine, 0.8 g/l; D,L-lysine, 2 g/l; D,L-methionine, 0.4 g/l; L-phenylalanine,

106   0.3 g/l; L-proline, 0.4 g/l; D,L-serine, 0.6 g/l; L-threonine, 1 g/l; L-tyrosine, 0.2 g/l; D,.L-tryptophan,

107   0.5 g/l; L-valine, 1 g/l; AMP, 0.1 g/l; NaCl, 220 g/l; $MgSO_4 \cdot 7H_2O$, 20 g/l; KCl, 2 g/l; NH_4Cl, 0.5 g/l;

108   KNO_3, 0.1 g/l; KH_2PO_4, 0.1 g/l; K_2HPO_4, 0.1 g/l; Na_3·citrate, 0.8 g/l; $MnSO_4 \cdot 2H_2O$, 0.0003 g/l;

109    $CaCl_2 \cdot 6H_2O$, 0.1 g/l; $ZnSO_4 \cdot 7H_2O$, 0.05 mg/l; $FeSO_4 \cdot 7H_2O$, 0.05 g/l; $CuCl_2$, 10 μM; glycerol, 1 g/l; D-

110   leucine-OH, 0.1 g/l; norleucine, 0.1 g/l; thymine, 0.1 g/l; uracil, 0.1 g/l; pH 7.5.

111   *Isolation of Halorubrum sp. VKK1262.* Commercial salt from Upper-Kama deposit

112   (Uralmedprom, Berezniki, Russia) was mixed with IAO medium without NaCl (200 g/l) and

113   filtered through a 0.2 μm GSWP filter (Millipore, USA). Filters were incubated on IAO medium

114   plates prepared with Noble agar (Difco, USA) at 37°C for one week and colored colonies were

115   restreaked several times on fresh plates. 16S RNA sequence was analyzed using PCR with

116   primers Arch16S-f2 and Arch16S-r934 and Sanger sequencing of the amplicons in both

117   directions.

118   *Gene cloning.* The DNA used as a template for PCR was isolated from the cell culture using a ZR

119   Fungal / Bacterial DNA MicroPrep kit (Zymo Research, USA) according to the manufacturer's

120   instructions. For PCR, in an equal volume of 25 μl, primers (sequences in Supplement) at a

121   concentration of 0.8 μM, PCR buffer 5x Phusion GC reaction buffer, 2 μl 2.5 mM

122   deoxyribonucleotide solution, 0.2 μl of Phusion DNA polymerase, and *H. turkmenica* genomic

123   DNA as a template were used. The cycling parameters were as follows: 1. Hot start 98ºC for 2 min;

124   2. Denaturation at 98ºC, 30 s; 3. Annealing at 55ºC, 1 min; 4. Elongation at 72ºC, 2 min. 30 cycles

125   between steps 4 and 2. 5. Final elongation at 72ºC for 7 min. Purified polynucleotide fragments

126   HTU-AA and HTU-QV (AA and QV stand for corresponding dipeptides in the HTU-LOX

127   sequence) were digested with *Bam*H I and *Hin*d III restriction enzymes and ligated into the

128   corresponding sites of the pQE-30 vector (Qiagen, USA), followed by transformation of the *E. coli*

129   strain XL1-Blue by electroporation. Colonies screening was performed by PCR, and the sequence

130   was confirmed by Sanger sequencing.

131   *Protein expression.* The XL-1 Blue transformants HTU-AA and HTU-QV were grown in LB

132   medium containing ampicillin on an orbital shaker at 37ºC until $OD_{600} = 0.7$ was reached, followed

133   by induction of expression with 0.5 mM IPTG for 3 hours. The cells were then harvested by

134   centrifugation and stored at -70ºC. His-tagged proteins were purified under denaturing conditions (8

135   M urea) on the metal-chelating sorbent Ni-NTA agarose (*Korneenko and Pestov, 1997*). Typical

136   yields of the purified proteins HTU-QV and HTU-AA were around 25-27 mg per liter of culture.

137   The resulting proteins in 8 M urea pH 6.3 buffered with 0.5 M imidazole, 0.1 M sodium phosphate,

138   and 20 mM Tris were dialyzed against different buffers (optimization briefly described in Results).

139

140   *Activity assays.* Determination of substrate specificity was performed using a fluorometric method

141   suitable for various amine oxidases as the release of hydrogen peroxide coupled to the oxidation of

142   10-acetyl-3,7-dihydroxyphenoxazine (Biotium, Germany), also known as Amplex red, in the

143   presence of horseradish peroxidase (*Palamakumbura and Trackman, 2002*). The fluorescence of the

144   reaction product (resorufin) was assayed with a Microplate analyzer "Fusion" (Perkin Elmer, USA)

145   at excitation and emission of 535 and 620 nm, respectively. More specifically, the reaction was

146   carried out in 0.1 M borate buffer pH 8.3 in the presence of 1 U / ml horseradish peroxidase at

147   37°C, 100 μl volume and 0.5-5 μg purified protein (up to 50 μg protein for crude lysates). For the

148   negative control, 0.1 mM 3-aminopropionitrile was added to block any lysyl oxidase activity.

149   Calibration has been done with known amount of hydrogen peroxide, and data were fitted to

150   Michaelis-Menten equation using Prism software package (GraphPad, USA). Sheep LOX was

151   isolated from aorta as described before for the purpose of comparison with HTU-LOX (*Pestov et al,*

152   *2014*).

153   *Immunization* of rabbits was carried out with purified folded protein HTU-QV. Initially, rabbits

154   were subcutaneously injected with 100 µg protein as an emulsion in Freund's complete adjuvant.

155   The first booster injection was made with the same quantity of the antigen in incomplete Freund's

156   adjuvant 5 weeks after the first immunization, and the second booster injection – with 250 µg

157   antigen and no adjuvants 6 weeks later. One week after the second booster injection sera were

158   collected and stored with the preservative sodium azide at 4ºC. Immunization of rabbits has been

159   approved by Animal Care and Use Review Board of Shemyakin-Ovchinnikov Institute of

160   Bioorganic Chemistry, protocol No 15/2011.

161   *Affinity purification of antibodies* has been performed on a small-scale essentially as before (*Pestov*

162   *et al, 2004*) using purified HTU-QV protein electrophoresed using SDS-PAGE and blotted on a

163   PVDF membrane: the HTU-QV band on PVDF was blocked in TBST buffer containing 5% bovine

164   serum albumin and 5% non-fat milk, then serum was added and incubated for 3 h. After several

165   washes with TBST bound antibodies were eluted with 0.1 M sodium citrate (pH 2.0) for 10 min at

166   followed by immediate neutralization with unbuffered tris and addition of 0.1% bovine serum

167   albumin and 0.02% sodium azide.

168   *Western blotting. H. turkmenica* cells were centrifuged and the pellets were lyzed in 10 mM tris-

169   HCl, 1 mM $MgCl_2$, pH 7.5 containing 0.5 mM tris(2-carboxyethyl)phosphine (Sigma, USA),

170   Complete protease inhibitor cocktail (Roche, Switzerland) and 1 u/ml Benzonase (EMD Millipore,

171   USA) for 15 min at 37°C followed by centrifugation for 10 min at 15000 g. The supernatants were

172   mixed with Laemmli sample loading buffer without mercaptoethanol and analyzed by

173   electrophoresis in 8% SDS PAGE gels. Following electrophoresis, the protein samples and colored

174   protein weight markers (Spectra Multicolor Brad Range, Thermo, USA) were transferred from

175   polyacrylamide gel onto a PVDF membrane (GE Healthcare, USA). The membrane was washed for

176   5 min with 2% SDS, then blocked in TBST buffer containing 5% non-fat milk, 0.02% sodium azide

177   and 10% w/w Bløk blocker (EMD Millipore, USA), overnight at 4ºC. On the next stage, the

178   membrane was incubated in 10 ml of TBST buffer solution with 0.1% non-fat dry milk and primary

179   rabbit antibodies (1:10000) for one hour at room temperature, rinsed out with TBST buffer 10 times

180 for 5 minutes each, followed by incubation with secondary antibodies (HRP-conjugated anti-rabbit

181 antibodies, Biotium, Germany, 1:50000) in 10 ml TBST buffer with 0.1% non-fat dry milk for one

182 hour, and rinsed out again in the same way. Chemiluminescence was recorded using Femto

183 Maximum Sensitivity Western Blotting Detection Reagent (Thermo, USA) and Carestream Kodak

184 Biomax Light film (Sigma, USA).

185 **RESULTS**

186 We initially attempted to produce the full-length HTU-LOX protein in *E. coli* but found that it

187 precipitates as inclusion bodies without any detectable amine oxidase activity (Supplement), and

188 all attempts at its refolding were unsuccessful (results not shown). For this reason, we proceeded

189 to truncations without the N-terminal peptide (hydrophobic segments are common sources of

190 problematic expression in *E. coli*) with subsequent purification under denaturing conditions and

191 refolding. The purity of the resulting eluate was checked by SDS PAGE. Fig. 1 illustrates the

192 expression and purification of HTU-LOX exemplified by HTU-QV variant. Of note is the fact of

193 its anomalously slow electrophoretic mobility that corresponds to an apparent molecular weight

194 of 34 kDa, whereas the theoretical value of the His-tagged HTU-QV is 24.3 kDa. Since HTU-

195 LOX is a rather acidic protein (theoretical pI 4.58 for the His-tagged HTU-QV and 4.09 for the

196 full-length protein), this peculiarity is common among acidic proteins (*García-Ortega et al,*

197 *2005*).

198 Refolding of the purified his-tagged proteins HTU-AA (Δ1-34) and HTU-QV (Δ1-91) was

199 achieved using dialysis against different buffers and results in good amine oxidase activity. We

200 investigated a variety of factors that may improve the formation of catalytically active proteins

201 HTU-AA and HTU-QV: buffer type and concentration (Tris, phosphate buffered saline, acetate,

202 etc.), the ionic strength of the solution (concentration of NaCl), temperature, metal ions (Cu, Fe,

203 Zn, Ni, Co, Mn) in different concentrations, pH of the solution (5.0 – 8.0), as well as the dialysis

204 with a gradual decrease in the concentration of the denaturing agent (urea). Optimal pH is around

205 6.2 (Fig. 2A). Since it is known that mammalian LOX requires the presence of a copper ion in

206 the catalytic domain in order to achieve the formation of the lysyl-tyrosine quinone (LTQ) in the

207 catalytic center, we expected similar results of for HTU-LOX. Indeed, only Cu^{2+} increases

208 activity (Fig. 2B,C), whereas a mixture of different ions gives an inhibition (Fig. 2C). It is

209 interesting to note that refolding efficiency is only slightly affected by NaCl concentration,

210   contrary to the expectations from the fact that *H. turkmenica* is an extreme halophile that requires

211   at least 2 M NaCl (Fig. 2B). In the case of NaCl similar results were obtained in folding by

212   dilution experiments, demonstrating also that 1 M and 2 M NaCl cannot improve activity any

213   further (results not shown).

214   A slow decrease of the denaturant (urea) concentration was found to lack any advantages over

215   the stepwise approach with immediate transfer into a buffer without urea. This was confirmed by

216   refolding by dilution (results not shown). Ultimately, a simple refolding procedure may be

217   considered as optimal:

218   • Dialysis against 40 mM sodium acetate, pH 6.2 with 1 mM $CuSO_4$ at 4ºC for 3 hours;

219   • Dialysis against 40 mM sodium acetate, pH 6.2 without copper at 4ºC overnight.

220

221   Interestingly, under any conditions used, the amine oxidase activity of the protein HTU-QV AA

222   (typical activity for HTU-QV with 1 mM taurine at pH 8.3 was approximately 0.014 µmole/min

223   hydrogen peroxide per·mg protein) was about fifteen times higher than that of HTU-AA.

224   Therefore, the segment of HTU-LOX sequence from Ala^{35} to Gln^{92} may function as an inhibitory

225   (pro)peptide or just interferes during refolding.

226   Refolded proteins HTU-QV and HTU-AA exhibit activity against a wide variety of primary

227   amines (Table 1): histamine, methylamine, lysine, cadaverine, tyramine, etc. Even glycine, β-

228   alanine are efficiently oxidized, in contrast with mammalian LOX. HTU-LOX readily oxidizes

229   some amine-containing antibiotics: polymyxin and aminoglycosides such as capreomycin and

230   amikacin. This is a unique property of lysyl oxidases, since other amine oxidases either do not

231   deaminate aminoglycosides or even are inhibited by them, as in the case of *E. coli* amine oxidase

232   (*Elovaara et al, 2015*). Regarding various proteins, lysine-containing peptides, and polymers

233   (e.g., poly-L-lysine, poly-allylamine, lysozyme, and substance P as an example of a Lys-

234   containing peptide), the HTU-LOX behaves almost like LOX from the aorta. Taurine is one of

235   the best substrates for HTU-LOX. It is also capable of oxidizing glycine, β-alanine, and γ-

236   aminobutyric acid. The only amine that HTU-LOX oxidizes much worse than mammalian LOX

237   is benzylamine. Importantly, the HTU-LOX demonstrated good sensitivity to the classical

238   inhibitor of all LOXes – 3-aminopropionitrile (BAPN). Also, HTU-LOX is somewhat different

239   from the mammalian enzyme in terms of pH dependence. In contrast to the latter, HTU-LOX

240   activity does not exhibit a steep decline from its maximum around 8.3, and even displays a

241   certain degree of bimodality retaining some activity even below 7 (Fig. 3).

242   We also attempted to study the HTU-LOX protein in the host – the archaeal halophile *H.*

243   *turkmenica*. For this purpose, we raised polyclonal antibodies against the truncated HTU-LOX

244   (variant QV). The full-size HTU-LOX theoretically contains 308 amino acids with a molecular

245   weight of 33829 Da, whereas the full-length protein expressed in *E. coli* has electrophoretic

246   mobility corresponding to 52 kDa (Suppl. Fig.). However, this apparently large discrepancy

247   should be regarded as normal, since the anomalous mobility has been observed for purified

248   recombinant HTU-LOX (Fig. 1). Western blotting indicates that HTU-LOX may be present in *H.*

249   *turkmenica* cells (Fig. 4, lanes 1-2, compare to negative controls in lanes 3-6, where lanes 4-6

250   show non-specific binding of unrelated antibodies plus secondary antibodies, and lanes 3 and 6

251   provide an additional negative control – non-specific binding of antibodies to proteins from

252   *Halorubrum,* a totally different haloarchaeal species). It should be emphasized that specific

253   bands were detected only at a high sensitivity, meaning that the normal expression level of the

254   protein in cultured *H. turkmenica* is quite low, and detection of the full-length, unprocessed

255   HTU-LOX was obscured by non-specific bands (Fig. 4, lanes 4-5). Most interestingly, only a

256   very high molecular weight band specific to anti-HTU-LOX antibodies of about 210 kDa has

257   been reliably detected. Importantly, this band is certainly absent from the *Halorubrum* sample

258   (Fig. 4, lane 3), where any HTU-LOX should be absent a priori. Prolonged incubation of the

259   cells in saturated salt in the medium results in a marked decrease in the intensity of this band

260   (Suppl. Fig.). Therefore, we can hypothesize that HTU-LOX in *H. turkmenica* predominantly

261   exists in a moderately stable homo- or heterooligomeric form, which migration in SDS-PAGE

262   corresponds to an apparent molecular weight of 210 kDa, however, this anomaly awaits further

263   studies.

264   Also, we found that BAPN (even at a rather high concentration of 1 mM) had no significant

265   effect on sensitivity of fresh cells to osmotic stress, on formation of hypotonically-resistant cysts,

266   or on growth rate in both conventional (IAO) and defined (MHTU) media.

267   **DISCUSSION**

268 Amino acid sequence alignments (Fig. 5-6) of LOX proteins demonstrate poor overall

269 conservation (for example, high variability in the number of disulfide bonds) with only a few

270 hyperconserved amino acid residues like Cu-binding His and LTQ formation (*Zhang et al,*

271 *2018).* A fundamental aspect that needs to be emphasized is the fact that relatively little research

272 has been carried out on the influence of HGT with the subsequent adaptation of the catalytic

273 properties of the enzymes to a new host.

274 Refolding efficiency is not significantly affected by NaCl concentration. This surprising fact

275 could reflect the history of prokaryote LOX genes: halophile archaea may have acquired these

276 genes from microorganisms with a rather different requirement for salt. The ancient HGT event

277 may had even originated from a halophobic organism, followed by "domestication" that

278 suppressed the formation of misfolded protein. Besides, LOX may had served as an antibiotic

279 resistance enzyme under aerobic conditions. This, however, is unlikely in extant *H. turkmenica,*

280 since Archaea are usually highly resistant to both polymyxin and common aminoglycosides.

281 Also, HTU-LOX oxidizes some peptide antibiotics and theoretically this feature may be useful

282 for competition with other species of haloarchaea (*Besse et al, 2015*) in the natural habitat of *H.*

283 *turkmenica*. The low expression level of the enzyme suggests that HTU-LOX plays a modest

284 functional role in increasing availability of nitrogen from non-typical amines. Its promiscuous

285 substrate specificity and negligible enzymatic activity in *H. turkmenica* cells (Supplement) make

286 it difficult to demonstrate this fact experimentally.

287 HTU-LOX accepts glycine, β-alanine, and γ-aminobutyric acid as substrates. This observation is

288 unusual, because the presence of any acidic groups in vicinity of the amino group almost

289 completely prevents oxidation by most amine oxidases. Thus, it is safe to conclude that the

290 HTU-LOX has a relaxed substrate specificity in comparison with its mammalian homologue

291 (*Shah et al, 1993*). Perhaps a low selection pressure on the lysyl oxidase gene allowed it to lose

292 substrate specificity. This, however, may be useful for biotechnological purposes as a starting

293 point for molecular evolution in any direction.

294 Another interesting fact is that the amine oxidase activity of the truncated protein HTU-QV is

295 much higher than that of the longer one, HTU-AA. This observation is in line with the general

296 view that LOX catalytic domain is usually (except for animal LOXL2-4 and homologs) preceded

297 by an autoinhibitory sequence, together forming a propeptide. In the case of HTU-LOX, the

298   autoinhibitory sequence corresponds to the stretch from Ala^{39} to Gln^{92}. However, the inhibition

299   is relatively inefficient, and this may also reflect the evolution of HTU-LOX gene after the in-

300   Archaea HGT that resulted in a partial degradation of the autoinhibitory function of the

301   propeptide. LOX genes in Archaea underwent at least two independent HGTs (*Grau-Bove et al,*

302   *2015*) and this is just an example of HGT in Archaea (*Papke et al, 2004, Papke et al, 2015).* The

303   widespread occurrence of these HGT events may also indicate that the transferred genes not

304   necessarily possess indispensable functions in every species.

305   Immunoblotting experiments allow us to hypothesize that HTU-LOX in *H. turkmenica*

306   predominantly exists in a moderately stable homo- or heterooligomeric form, which migration in

307   SDS-PAGE corresponds to an apparent molecular weight of 210 kDa. This anomaly awaits

308   further studies but may have interesting implications, for example, explain lack of detectable

309   amine oxidase activity in *Haloterrigena* cells.

310   What is the origin of the animal lysyl oxidase? Has it emerged in primitive animals at the

311   beginning of their evolution through HGT from Eubacteria? Or, conversely, LOX genes, which

312   have important functions in animals, made their way several times into the world of prokaryotes?

313   The second option seems highly unlikely due to splitting of animal ORFs into exons and

314   molecular phylogeny (*Grau-Bové et al, 2015*) but cannot be excluded completely. In any respect,

315   the most parsimonious explanation of the evolution of the catalytic LOX domain is that inter-

316   kingdom saltations of LOX genes between distant branches of Life occurred more than once.

317   **CONCLUSIONS**

318   *H. turkmenica* LOX (HTU-LOX) was successfully expressed in *E. coli.* Optimal refolding

319   conditions are different from those for the growth of the host cells. HTU-LOX has a relaxed

320   substrate specificity in comparison with mammalian LOX, benzylamine is a poor substrate for

321   both, and sensitivity to 3-aminopropionitrile is conserved in HTU-LOX. N-terminal truncation of

322   HTU-LOX increases its activity. Cultured *H. turkmenica* does not exhibit any detectable amine

323   oxidase activity, and expression level of the HTU-LOX is low. Therefore, native function of *H.*

324   *turkmenica* lysyl oxidase may be cryptic.

325   **ACKNOWLEDGMENTS**

326

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382

383 **FIGURE LEGENDS**

384 **Table**

385 **Substrate efficiencies of recombinant *Haloterrigena* lysyl oxidase (HTU-QV variant) in**
386 **comparison with mammalian enzymes from aorta.** V_{max}/K_m ratios normalized with respect to
387 tyramine. Values for polymers and lysozyme calculated per amine groups. Data for LOX from

388 bovine aorta from (*Shah et al, 1993*) (note that conditions used were significantly different from
389 this study). For HTU-LOX the short variant QV has been used. ND – no data. Z – rate too low for
390 accurate determination. CK – complex kinetics with inhibition by substrate at high concentrations.
391

392 **Figure 1. Electrophoretic analysis of expression and purification of recombinant N-**
393 **terminally truncated *H. turkmenica* LOX (HTU-QV).** 1 – molecular weight marker proteins; 2
394 – *E. coli* proteins before addition of IPTG; 3 – expression induced with IPTG; 4 – purified HTU-
395 QV protein.

396 **Fig. 2. Folding of recombinant *H. turkmenica* lysyl oxidase (HTU-QV).** Folding by dialysis. A
397 – Influence of pH; B – effect of NaCl; C – 1 mM salts of various metals added to the dialysis
398 buffer; D – different concentrations of $CuSO_4$.

399 **Fig. 3. pH dependence of recombinant *H. turkmenica* lysyl oxidase.** Amine oxidase reaction
400 rates for HTU-QV protein in comparison with LOX from sheep aorta were measured in universal
401 borate-phosphate-acetate buffer with histamine as the substrate.

402

403 **Figure 4. Immunoblotting with anti-HTU-LOX antibodies and proteins from *H. turkmenica***
404 **cells.** Chemiluminescence of bound HRP-labeled antibodies; positions of molecular weight
405 markers on the left and in the middle. 1-3 – affinity purified antibodies against HTU-QV, the
406 catalytic domain of LOX from *H. turkmenica*. 4-6 – negative control with rabbit serum against an
407 unrelated antigen (*Dmitriev et al, 2009*). 1,2,4,5 – proteins of *H. turkmenica* grown to log-phase
408 (lanes 1 and 4 were loaded with three times more protein than lanes 2 and 5). 3,6 – proteins of
409 *Halorubrum sp. VKK1262* (loading equal to lanes 2 and 5).

410

411 **Fig. 5. Multiple alignment of archaeal lysyl oxidases.** Obtained using Muscle algorithm.
412 (HaloterrigenalimiCola – *Haloterrigena limicola*, HaloterrigenaturCk – *Haloterrigena*
413 *turkmenica*, NatronoCoCCusjeotgali – *Natronococcus jeotgali,* NitrosopumilussalariaDB31 and
414 Nitrosopimulus – *Nitrosopumilus sequnces.* Yellow – cysteine residues marked in yellow; red –

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415 LTQ-forming lysine and tyrosine; purple – three hyperconserved histidine residues necessary for

416 the binding of Cu^{2+}.

417

418 **Fig. 6. Multiple alignment of the conserved segments of catalytic domains from all lysyl**

419 **oxidases representing different kingdoms.** Obtained using Muscle algorithm from consensus

420 sequences of different taxa. ARCHAE – Archaeal LOX sequences, DELTA –

421 *Deltaproteobacteria*, FUNGI – fungal LOXes, ANIMA – various animal LOXes, LOW –

422 *Mesomycetozoa* and *Orthonectida*, short – LOX from *Truepera radiovitrix, Deinococcus*

423 *pimensis, Nitrospira nitrosa,* and a few samples from Parcubacteria*,* BETA –

424 *Betaproteobacteria*, Actinshort – *Amycolatopsis mediterranei* LOX and closest homologues,

425 STREPTOM – LOX from *Streptomyces*, ACTINvar – other actinomycetal LOXes, BACIES – all

426 other eubacterial LOXes. Yellow – cysteine residues marked in yellow; red – LTQ-forming

427 lysine and tyrosine; purple – three hyperconserved histidine residues necessary for the binding of

428 copper.

429

Table 1(on next page)

Substrate efficiencies of recombinant *Haloterrigena* lysyl oxidase (HTU-QV variant) in comparison with mammalian enzymes from aorta.

V_{max}/K_m ratios normalized with respect to tyramine. Values for polymers and lysozyme calculated per amine groups. Data for LOX from bovine aorta from (*Shah et al, 1993*) (note that conditions used were significantly different from this study). For HTU-LOX the short variant QV has been used. ND – no data. Z – rate too low for accurate determination. CK – complex kinetics with inhibition by substrate at high concentrations.
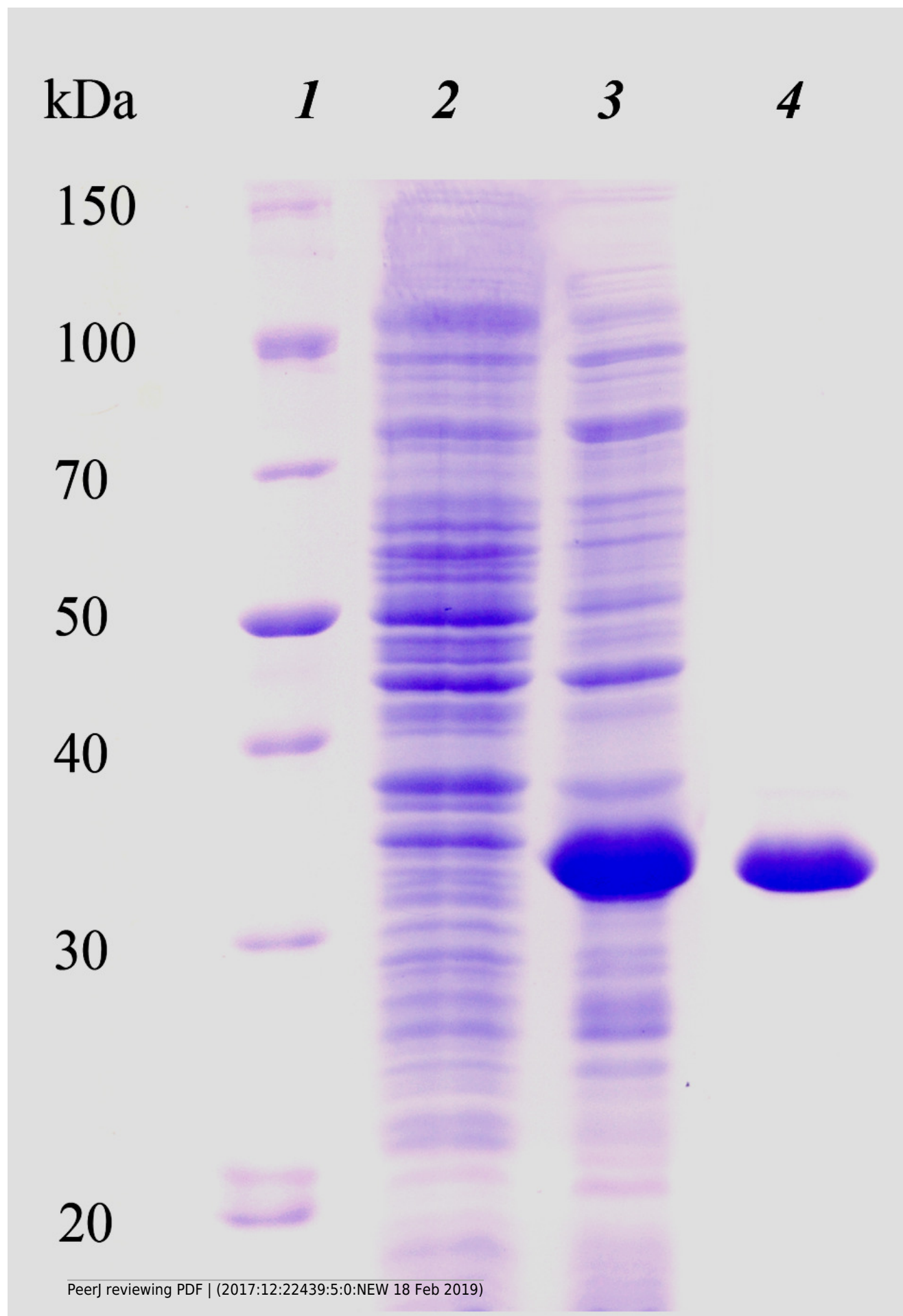
1

substrate	HTU-LOX	Sheep	Bovine
L-lysine	0.058	0.028	ND
cadaverine	0.370	1.070	1.09
histamine	0.550	0.920	ND
taurine	1.120	0.120	ND
glycine	0.020	z	ND
β-alanine	0.005	z	ND
GABA	0.015	z	ND
methylamine	0.020	ND	ND
substance P	0.068	ND	ND
lysozyme	CK	0.080	ND
polyallylamine	CK	0.080	ND
amikacin	0.260	ND	ND
capreomycin	0.120	0.190	ND
polymyxin	0.780	ND	ND
benzylamine	z	0.170	0.52
hexylamine	0.28	1.140	0.14

2

3

4   **Substrate efficiencies of recombinant *Haloterrigena* lysyl oxidase (HTU-QV variant) in**

5   **comparison with mammalian enzymes from aorta.** V_{max}/K_m ratios normalized with respect to

6   tyramine. Values for polymers and lysozyme calculated as for molar amine groups. Data for LOX

7   from bovine aorta from (*Shah et al, 1993*) (note that conditions used were significantly different

8   from this study). For HTU-LOX the short variant QV has been used. ND – no data. Z – rate too

9   low for accurate determination. CK – complex kinetics with inhibition by substrate at high

10 concentrations.

Figure 1

Electrophoretic analysis of recombinant N-terminally truncated *H. turkmenica* LOX (HTU-QV) expression and purification.
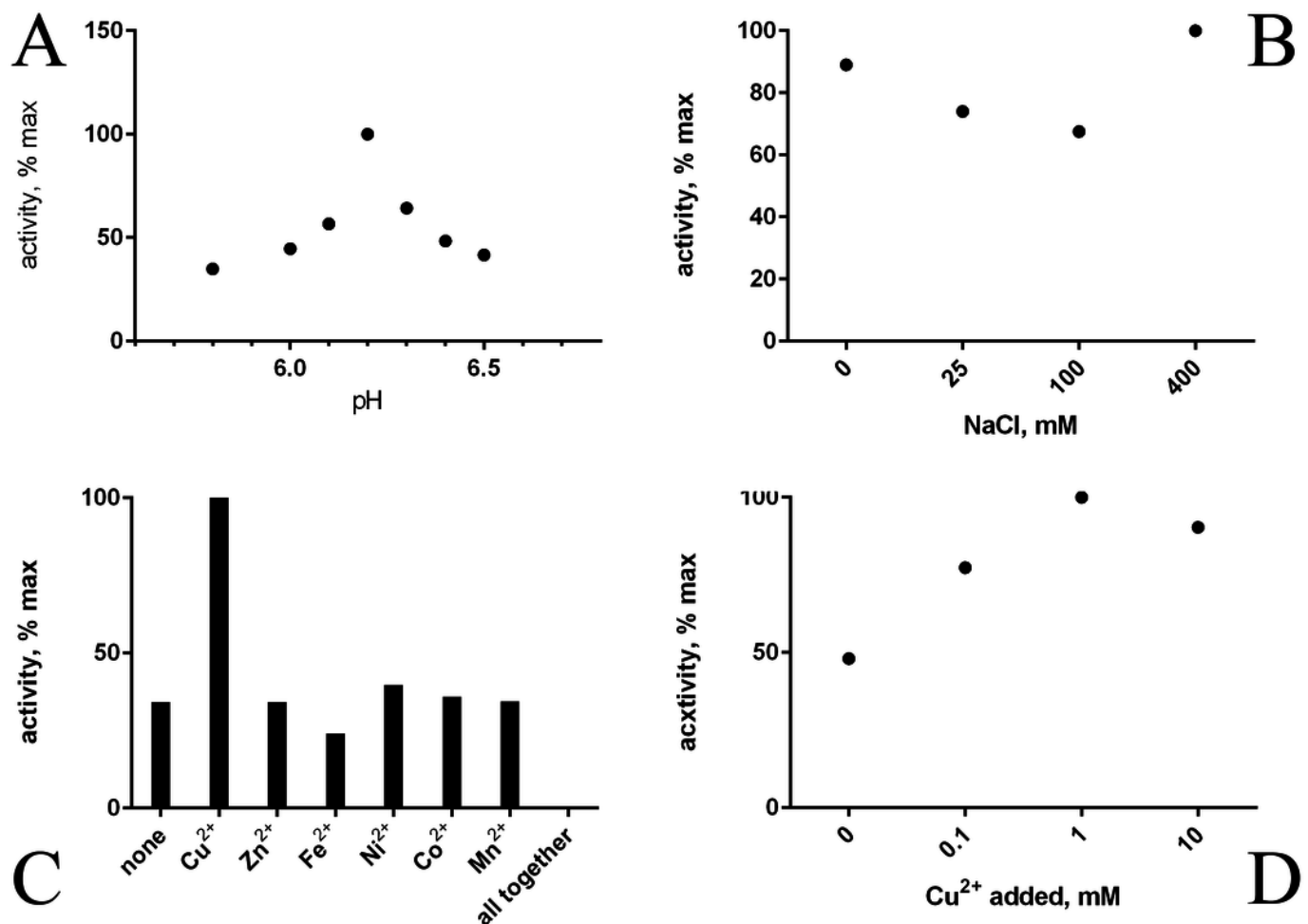
1 - molecular weight marker proteins; 2 - *E. coli* proteins before addition of IPTG; 3 – Expression induced with IPTG; 4 – purified HTU-QV protein.

Figure 2

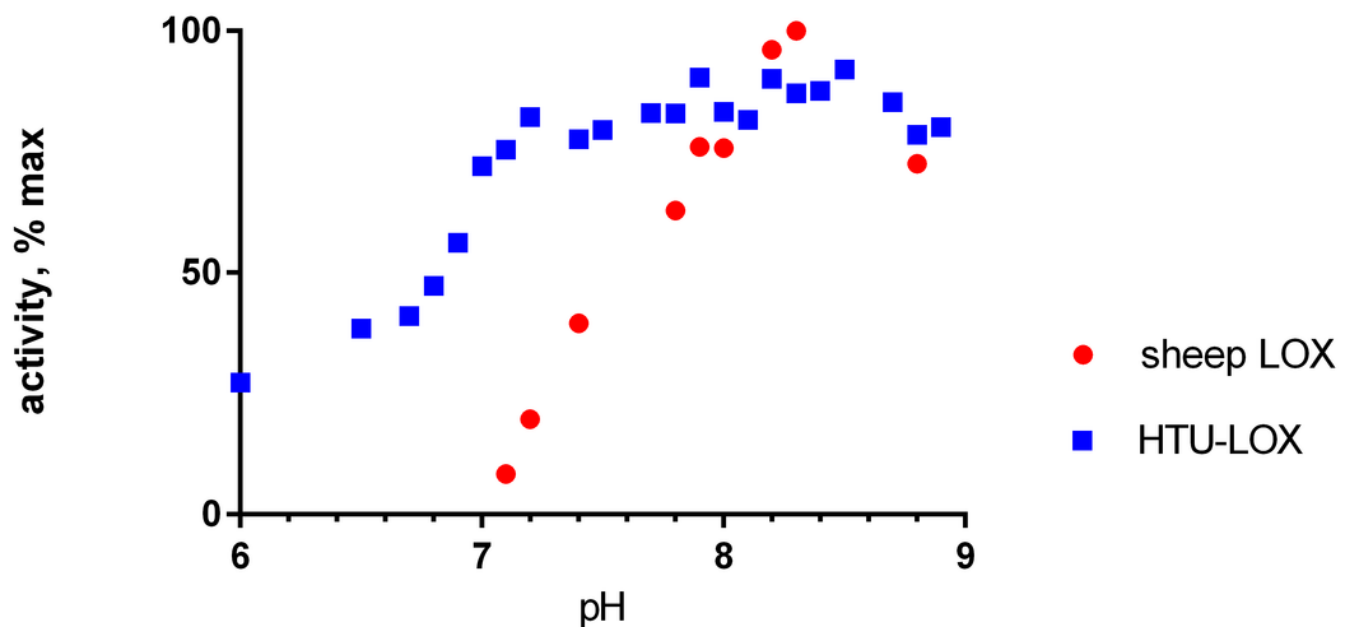Folding of recombinant *H. turkmenica* lysyl oxidase (HTU-QV).

Folding by dialysis. A – Influence of pH; B – effect of NaCl; C – 1 mM salts of various metals added to the dialysis buffer; D – different concentrations of $CuSO_4$.

Figure 3

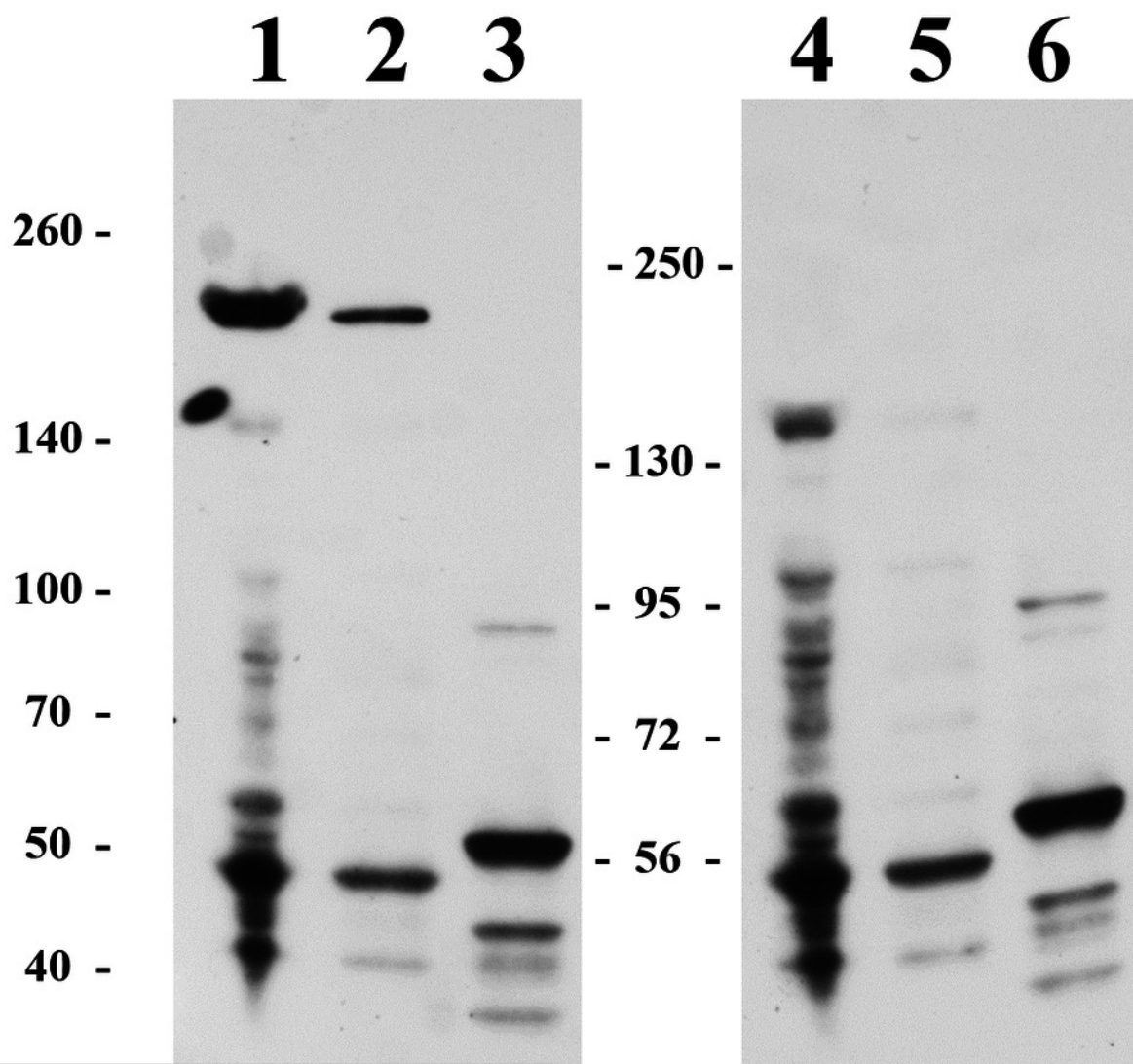pH dependence of recombinant *H. turkmenica* lysyl oxidase.

In comparison with LOX from sheep aorta amine oxidase reaction rates were measured for HTU-QV protein if universal borate-phosphate-acetate buffer with histamine as the substrate.

Figure 4

Immunoblotting detection of lysyl oxidase in *H. turkmenica* cells with anti-HTU-LOX antibodies.

Chemiluminescence of bound HRP-labeled antibodies; positions of molecular weight markers on the left and in the middle. 1-3 – affinity purified antibodies against HTU-QV, the catalytic domain of LOX from *H. turkmenica*. 4-6 – negative control with rabbit serum against an unrelated antigen (*Dmitriev et al, 2009*). 1,2,4,5 – proteins of *H. turkmenica* grown to log-phase (lanes 1 and 4 were loaded with three times more protein than lanes 2 and 5). 3,6 – proteins of *Halorubrum sp. VKK1262* (loading equal to lanes 2 and 5).

Figure 5

Multiple alignment of archaeal lysyl oxidases.

Obtained using Muscle algorithm. (HaloterrigenalimiCola – *Haloterrigena limicola*, HaloterrigenaturCk – *Haloterrigena turkmenica*, NatronoCoCCusjeotgali – *Natronococcus jeotgali,* NitrosopumilussalariaDB31 and Nitrosopimulus – *Nitrosopumilus sequnces.* Yellow – cysteine residues marked in yellow, red – LTQ-forming lysine and tyrosine, purple – three hyperconserved histidine residues necessary for the binding of Cu^{2+}.

```
HaloterrigenalimiCola      MKIKRIKGRKRAAALIGVFLIVVAGIGIITLGGVAVDNPFTVSDRSTDTPTTSE-SEGTT
HaloterrigenaturCk         MILNWIKERKRATALVGVLLIVVAGVGMITLGGVAADNPFTVSDSSTDTSTTSG-SEDTA
NatronoCoCCusjeotgali      -----MKDRKRATVLVGIILIAVVGAGIITLGDVTVNNPFTVNDSNTDTSTTSE-SEGTA
NitrosopumilussalariaBD31  -----------------------------MFAAPMIMDAAAAKGGNGNGNNNGGN
Nitrosopumilus             --MTYTKKIFRKTTLIPVLLAI----------GFMFTTPMLLDVAAAPGGNGNG-NGGST
                                             *: :.     :         .    .  . .

HaloterrigenalimiCola      DEGATPADEENAATPPPTTVESDPKPS-----DDHVEDRTGVNFVPGVENFNVSTEVFDE
HaloterrigenaturCk         NEEATPVDEENSTT-PST-TESDSEPSDDQVEDDQVEDQPEVNFVPGVRNFDVSIEEFDE
NatronoCoCCusjeotgali      NEEATPVDKENPAT-SSTPAESNSEPS-----DAQVEDKPEVNFVPGVRDFSISTEEFDE
NitrosopumilussalariaBD31  DETTIPTNALLPDVSPGVPKHLNIHNQ---------QQKEFLRFTNVWANLGPGTLEFEP
Nitrosopumilus             ---SIPSDALLPDISPGVPKHLNIHNQ---------QQNEFLRFTNTWNNVGVGALEFEP
                            : *: .  . . :  .               ::. :.*.    :... *:

HaloterrigenalimiCola      -SSPDVEDGFVTPGEHRLLRFDMIIYNMGDADAELGRPENR-----PDLFEYSES HGHAH
HaloterrigenaturCk         -SSADVEDGFVTPGEHRLLRFDMIIYNVGDADAELGHPENR-----SDLFEYSDS HNHAH
NatronoCoCCusjeotgali      -SSTDVEDGFVTPGEHRLLRFDTIIYNLGDADAELGHPENR-----SDQFEYSDS HNHAH
NitrosopumilussalariaBD31  LFPDPDADEGTTQDA----------FQNLYDDEGNFGLTDQNVWHENVSQFIFHEA HNHWH
Nitrosopumilus             VFPDSDAVEGTTQDA----------FQNLYDDAGNFAIPSQKIWSTTVSEFIFHET HNHWH
                            ...*. :*  .    . : *:  * .:: ..:.   . * : ::*.**

HaloterrigenalimiCola      LKGFNNYILL-DESGE----------RTGAVR KQT F CLRDLYQTRSTASSSPQ---FD C
HaloterrigenaturCk         LKGFNKYKIL-DEAGN----------EMNAGK KQT F CLRDNFQTRSNASSSAK---FD C
NatronoCoCCusjeotgali      LKGFNKYALF-DESGN----------EMDMGK KQT F CLRDDFQTRSNASSSAK---FN C
NitrosopumilussalariaBD31  IDNVGEFAVRAYDPNNPDVPGDIV--DDAASI K VGFC ITNVFKYNGEESPTSQRIYWD C
Nitrosopumilus             ISDIGEFSIRSDDNGVPGEIAKNVNGDDVAAVK VGFC IADVYKYNGDNSPTSQRVYWD C
                            :....:: :   . .              *   **: : :: ..  *.:.:   ::*

HaloterrigenalimiCola      E--YQGISAGWADE Y DASLPGQYIVIDDLPDGEYTLQATTNAAGTI--NET C DGDNTVRV
HaloterrigenaturCk         D--YQGISAGWADV Y PASLPGQYLVIDDLPDGEYTLQATTNAEGTI--DEK C DDDNTVRV
NatronoCoCCusjeotgali      D--YQGISAGWADV Y PASLPGQYLVIDGLPDGEYTLHATTNAEGTI--DEK C DDDNTVRV
NitrosopumilussalariaBD31  EVGLQGIQPGWVDQ Y HQSVEGNEININTKVPNGTYFLTHTWNPANAFVDADNSNNVSWMKF
Nitrosopumilus             EVGLQGIAPGWADQ Y HQSVEGNEINITDLPNGTYFLVHKWNPANAFVDADVSNDESWMKF
                            :   *** .**.* *  *: *: : *  :*:* * *  . *. .:: : .: . :...

HaloterrigenalimiCola      DL--------SINNDTVTVHTPQSHYVRPSA C -------
HaloterrigenaturCk         DL--------RINNDTVTVHSSQDDYVKPPS C -------
NatronoCoCCusjeotgali      DL--------RINNDTVTVLSSQEDHVKPSA C -------
NitrosopumilussalariaBD31  ELTDDGNGNRKINEIEGFAPE C QDDDSTPGI C GDINKNS
Nitrosopumilus             DLTDDGNGNRKIVEIEGFAPE C QGDGSTPGI C GEINKNN
                            :*            *: .     *     * *
```

Figure 6

Multiple alignment of the conserved segments of catalytic domains from all lysyl oxidases representing different kingdoms.

Obtained using Muscle algorithm from consensus sequences of different taxa. ARCHAE – Archaeal LOX sequences, DELTA – *Deltaproteobacteria*, FUNGI – fungal LOXes, ANIMA – various animal LOXes, LOW – *Mesomycetozoa* and *Orthonectida*, short – LOX from *Truepera radiovitrix, Deinococcus pimensis, Nitrospira nitrosa,* and a few samples from Parcubacteria, BETA – *Betaproteobacteria*, Actinshort – *Amycolatopsis mediterranei* LOX and closest homologues, STREPTOM – LOX from *Streptomyces*, ACTINvar – other actinomycetal LOXes, BACIES – all other eubacterial LOXes. Yellow – cysteine residues marked in yellow; red – LTQ-forming lysine and tyrosine; purple – three hyperconserved histidine residues necessary for the binding of copper.

```
ARCHEA      -------KESNSEPSDDQVEDKPEVNFVPGVRNFGVSTEEFDESSPDVEDGFVTPGEHRL
DELTA       -------------VDADVISRTVYIERRTFAADACEVYEGCVGAPGR---------RRL
FUNGI       -------------DADWLQKHLYIDYVDAAEDPCLINEGCLTGPPA-----------
ANIMA       ----------MDALLVQQTAHLEDRPLYLLGCAMEENCLASSAYQVEPGWPYGTRRL
LOW         ----------MNSNNAQSTLVLSAGHLYNTQCAMEEGCLASGAW---------RKL
BACIES      ---------------QCPPGTNCELLPDLVILPRFTRSQIKEYSNDDPY---------YGGQ
short       ---------------PNRLLPDLVIYPPSELSIVGSEKTG-----------RRE
BETA        --------------ATTNRLPNLKPLPASNLSLVADSAGGST--------------
ACTINshort  ----------------LPDLRQAPIGDLQVQTG-----PS---------GQVR
ACTINvar    ---------------------------------AK-----------AVR
STREPTOM    QAPAPALKANAKRPTKATVPNVPKPDLRSLPAYGITVSDGYEDVPG----------KDY
```

```
ARCHEA      LRFDMIIYNLG-DADAELGRPE-------------------------------N
DELTA       LRFSVSIPNLG-SAAVIPPPE-------------------------------E
FUNGI       ----------DRDDP---------------------------------N
ANIMA       LRFTARIWNRG-TADFLPK--------------------------------R
LOW         LRFSASFWNFG-TADFLP---------------------------------N
BACIES      LRFAATIANIG-DGPMETRGYCGTLGVVSNSICPDGSYPRQVLFQRIYSLKDKNLSSVDR
short       IKFATTVWNIGKSGPLELIGTV-----------DPATNKTRVYQRIKNRGGESAS---R
BETA        LRFNTTSWNKG-SGPLVLGAGA------------VDTGSGKQQVFQRVFLSNGGYFV---P
ACTINshort  LRFTTSIVNV--DGPLLLVAHR-----------DSTDVPFMAVQAI-QSDGSIADV--E
ACTINvar    LRFTAAEWNAG-DGPLLLYGRR-----------DSATDTMDVRQYFFDAKHGQVQR--Q
STREPTOM    LAFSANVWNAG-PAKLVVDGFR-----------SPGKELMDAYQYFYDAKGKQVGY--T
```

```
ARCHEA      RPDQFEYSES--HGHAH-LKGFNKYAI--LDESGN---EMNAGKRQTFCLRDVFQTRS--
DELTA       NPDLYVVYDEC--HQHEH-LVNFASYEL--RDADNN---VVAVGRRQGFYLVD-MEPYC--
FUNGI       NPPYWHWDTC--HEHWH-FTAYANYRL--LSANGS---EVVAQGHRNGFCLED-SLCDE--
ANIMA       PRHSWEWHAC--HQHYHSMEVFAHYDL--LDLNGT---KVAEGHRASFCLED-TECDG--
LOW         PDDGPEWHEC--HNHYH-ISNFANYTI--TGSAGN---QLTQGHRQSFCLED-VKCLP--
BACIES      PAGTNYYDNQPGHNHYH-VDDWVEFRLVKIEP-GKRASIIAKGRRVSYCLFDSGICNNAD
short       TAGYFEYHPD--HEHWHLFNDFATYELWTLNADGSLETLVATSGRVTFCLMDTTAVDP--
BETA        VAGGFEWHPA--HNHFH-FDDFALYTLQPVNAPGG---VVRTGSRTTFCLMDSTRIDS--
ACTINshort  TPASLYYEPADGHDHWH-LLDFEYYQL--RRPDGG---VVVTDRRNGFCIGDRYRVRD--
ACTINvar    TAGTMYYEPAPMHQHWH-LLDFARYQL--RTPDGE---TVVRDRRNGFCLADRYADVD--
STREPTOM    PTGTMEWDPRPGHEHWH-FTDFASYRL--LKADKK---ETVRSGREAFCLANTDAVD---
                      :     ** * *  . :  : :             . *  : : : *
```

```
ARCHEA      ------NASSS-----------AKFDCE-------YQGISAGWADVYP-ASLPGQYLVI-D
DELTA       ------DAAPR-----------AYTCG-------GQGISPGWSDTYA-ADTPCQWLDV-T
FUNGI       ------GVAP-----------FYNCT-------NQGITMGCHDLYD-AGLGCQWIDI-T
ANIMA       ------GVQRR--------    -YCANYG----DQGISVNCWDTYR-HDIDCQWIDI-T
LOW         ------SLLPK-----------YICN-------NQGISVGCADSYSVSNIDCQWIDI-T
BACIES      SLCTINGTVVYGERNLSNYGLGNYASCN-----AMKQGISVGGGYDTYG-VMYEGQFLQLPK
short       ------YPLPN---APGGP--TYSSCG-----NNVQGISVGWGDTYG-AKLAGQEIDL-T
BETA        ------SLPG---APGQA--VYSTCG-----RTIGGISVGWGDTYG-AHLPGQEIDF-T
ACTINshort  ------DLPG---RPADPYVLGHMCG-PAALTVKMGISVGWGDDYK-HTLPFQWLDI-T
ACTINvar    ------YTLPNAVWRPENT-DLATSCGDPSSLDVREGISVGS-DDYR-YTVDFQWLDI-T
STREPTOM    ------YTVKNANWHPDNT-DLSTACGQENSISVREVLDVGSGDTYT-QDLPGQSFDI-T
                            :  .  * *        * : .
```

```
ARCHEA      DL-------PDGEYTLQATTNAEGTIDEKCDDDNTVRVDLRI------------------
DELTA       DV-------PDGTYTLRVGVDTRDIVDEGDVHPNTVDVPVR------------------
FUNGI       DLHLQPGYSPNTEYTLSVILNPEKAIPETDYSNNAAV----------------------
ANIMA       DV-------PPGNYILKVVVNPEFAVAESDFTNNAVRCNIRY-----------------
LOW         PL-------KSGWYVLNVYVNPDKRVTESDYTNNVFHVLFRF-----------------
BACIES      GL-------ASGTYILEIE-DPTGSFYEKNRSNNLFRMPIVIEKQ--------------
short       DV-------PDGRYLLRVEVDPEDRIEELDYDNNFSVTFVEI-----------------
BETA        GN-------ADGTYQLRIVIDPNKVIIESDESNNASCVLISIRKPNTVTVLDSSGSCSTA
ACTINshort  GL-------PAGRYDLVNVADPDGALLEKNYDNNASWVDISVTSP--------------
ACTINvar    HV-------PSGTYDLVNTVNPDRTL-ETSYDNNSSSIAIVLGGT--------------
STREPTOM    GL-------PNGTYYIQVLANPENRLKETNHKNNSALRKVVLGGK--------------
                    *  . :      :.   .  *        *
```