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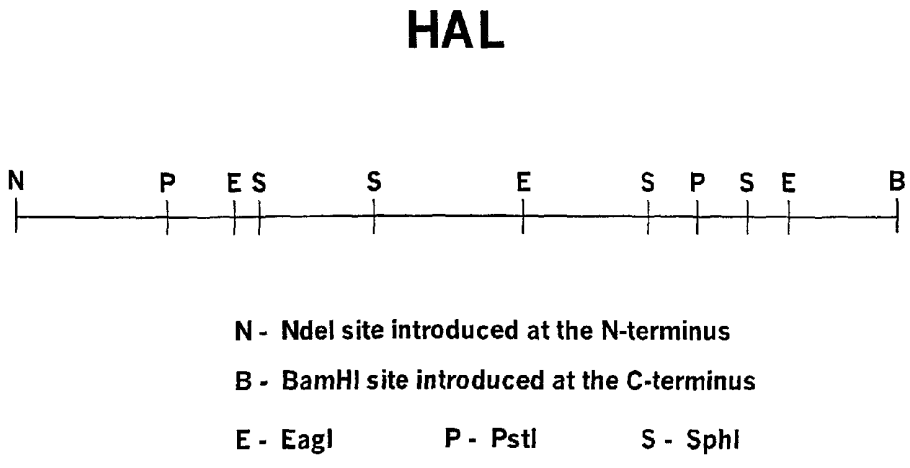
(54) Title: CLONING, OVEREXPRESSION AND THERAPEUTIC USES OF BIOACTIVE HISTIDINE AMMONIA LYASE

Restriction pattern of the HAL coding region cut with selected enzymes.



(57) Abstract: Histidine ammonia lyase (HAL) isolated from *Corynebacteriaceae* can decrease serum histidine levels, induce accumulation of urocanic acid, and is not inhibited by L-histidinol. As a result, histidine ammonia lyases similar to the one isolated from *Corynebacteriaceae* are uniquely suitable for combination therapy with L-histidinol to treat histidine- and/or histamine-dependent pathologies, for example, infectious viruses, such as human Respiratory Syncytial Virus (RSV), Herpes Simplex Virus (HSV), and Human Immunodeficiency Virus (HIV), as well as cancers.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

Cloning, Overexpression and Therapeutic Use of Bioactive Histidine Ammonia Lyase

FIELD OF THE INVENTION

The present invention relates generally to bioactive, amino acid-degrading enzymes, more specifically to a histidine ammonia lyase derived from a bacterium belonging to the family *Corynebacteriaceae,* and to conservative variants thereto. Also described is the use of histidine ammonia lyase, singly or combined with L-histidinol, for treating various viral diseases.

BACKGROUND OF THE INVENTION

Histidine ammonia lyase (EC 4.3.1.3) catalyzes the conversion of L-histidine to urocanic acid and ammonia. This is the first step in the degradation of histidine in both mammals and bacteria. A deficiency in this enzyme results in histidinemia, which is characterized by high serum histidine levels.

An isolated histidine ammonia lyase enzyme is one agent for treating increased histidine levels. Several lines of evidence indicate that *in vivo* depletion of serum histidine concentrations by histidine ammonia lyase could have additional therapeutic value. For example, histidine ammonia lyases have been shown in *in vivo* animal models to have potential therapeutic value against certain tumors. Roberts *et al.*, *Cancer Treat. Rep.* 63:1045 (1979); Jack *et al.*, *Leukemia Res.* 7:421 (1983).

Therapeutically useful (bioactive) enzymes generally display characteristics that are predictors of usefulness *in vivo*. These factors are outlined in Holcenberg and Roberts *et al., Ann. Rev. Pharmacol. Toxicol.* 17: 97 (1977), and include high activity at physiological pH and no requirement for exogenous cofactors. The histidine ammonia lyase isolated from a bacterium of the family *Corynebacteriaceae,* herein denoted as "HAL," has been partially characterized by Roberts *et al., Cancer Treat. Rep.* 63: 1045 (1979). HAL demonstrates a broad useful pH range with approximately 75% of activity

exogenous cofactors. The histidine ammonia lyase isolated from a bacterium of the family *Corynebacteriaceae,* herein denoted as "HAL," has been partially characterized by Roberts *et al., Cancer Treat. Rep.* 63: 1045 (1979). HAL demonstrates a broad useful pH range with approximately 75% of activity being retained at pH 7.2. The plasma half-life of HAL in mice is eight hours. The usefulness of this enzyme for histidine depletion *in vivo* is evident from the observation that single intraperitonial injections of 400 IU/kg effectively depleted plasma histidine in mice for up to 24 hours. However, the *Corynebacteriaceae* HAL which Roberts *et al.* described was not in purified form. As a result, many of the therapeutically beneficial properties associated with this HAL were unknown.

Histidine ammonia lyases have been isolated from several bacterial, animal, mammalian and plant sources. Shibatani *et al., Eur. J. Biochem.* 55: 263-269 (1975). Km values of these enzymes range between 1 and 20 mM. Shibatani (1975), *supra*; Wu *et al., Gene.* 115: 19-25 (1992); Jack *et al., Leukemia Research*, 7: 421-429 (1983); Khanna and Chang, *Int'l J. Artifical Organs* 13: 189-195 (1990). Genes coding for histidine ammonia lyases have been cloned from a number of organisms (Consevage, M. W. and A. T. Phillips. 1990. *Journal of Bacteriology*. 172 (5): 2224-2229; Oda, M. Sugishita, A. and K. Furukawa. 1988. *J. Bacteriology*. 170(7): 3199-3205; Wu, P. C., Kroening, T. A., White, P. J. and Kendrick, K. E. 1992. *J. Bacteriology*. 174(5): 1647-1655; Taylor, R. G., Lambert, M. A., Sexsmith, E., Sadler, S. J., Ray, P. N., Mahuran, D. J. and McInnes, R. R. 1990. *J. Biol. Chem.* 265(30): 18192-18199). Biochemical characterization has shown that most histidine ammonia lyases are inhibited by EDTA and thiol reagents (Shibatani, T., Kakimoto, T. and I. Chibata. 1975. *Eur. J. Biochem.* 55: 263-269; Okamura, H., Nishida, T. and H. Nakajawa. 1974. *J. Biochem.* 75: 139-152). A bioactive histidine ammonia lyase from a bacterium identified as *Kurthia* species was described by Jack, *et al.* in 1983 (Jack, G. W., Wiblin, C. N. and P. C. McMahon. 1983.

Leukemia Research, 7(3): 421-429.) The *Kurthia* species histidine ammonia lyase was reported to have a K_m of 1.25 mM with a half-life of 6-7 hours in mice. Chemical modification of the *Kurthia* histidine ammonia lyase did not increase the biological half-life of this enzyme. However, while HAL isolated from *Corynebacteriaceae* was effective in reducing ascites tumors in mice with high cell challenge (10^7 cells per mouse), the histidine ammonia lyase isolated from *Kurthia* was reported to be effective only at low tumor cell challenge levels (10^3 to 10^5 cells per mouse).

L-histidinol is an analog of histidine that is capable of altering histidine metabolism. Alteration of histidine metabolism by L-histidinol has provided therapeutic benefit. Histidine is required for several cellular processes, including protein synthesis and formation of histamine, both of which are required for tumor growth (Watanabe, *et al*, 1982. *Biochem. and Biophys. Res. Comm.* 109:478-485.; Bartholeyns and Bouclier. 1982. *Cancer Res.* 44:639-645.; Hakii, *et al*, 1986. *J. Cancer Res. and Clin. Oncol.* 111:177-181). Histidine is a direct precursor of histamine and is converted to histamine by the enzyme histidine decarboxylase (HDC). L-histidinol interferes with this conversion by inhibiting HDC. Therefore, L-histidinol can act therapeutically by inhibiting HDC, which is induced by strong tumor promoting phorbol esters (Mitra, *et al*, 1993. *J. Cellular Physiol.*, 156:348-357). L-histidinol possesses some anti-tumor activity, as well as an ability to reverse resistance of certain tumor cell lines to some antineoplastic compounds (Stolfi, R.L. and Martin, D.S. 1990. *Chemotherapy*, 36 (6): 435-440; Warrington, R.C., Fang W. D. and L. U. Zhang, 1996. *Anticancer Research* 16 (6B):3641-3646; Warrington, R. C. and Fang W. D. 1989. *Journal of the National Cancer Institute*. 81 (10): 798-803). L-histidinol is also able to enhance the efficacy of certain anti-cancer drugs, when both are administered to a patient simultaneously. (Warrington, R. C. and W. D. Fang. 1991. *Anticancer Research*, 11 (5): 1869-1874; Warrington, R. C., Cheng, I. And W. D. Fang. 1994. *Anticancer Research*, 14 (2A): 367-372;

Warrington, R. C., Cheng, I., Zhang, L. and W. D. Fang. 1993. *Anticancer Research*, 13 (6A): 2107-2112; Warrington, R. C. 1992. *Biochemistry and Cell Biology*, 70 (5): 365-375; Zaharko, D., Plowman, J., Waud, W., Dykes, D. and L. Malspeis. 1992. *Cancer Research*, 52 (13): 3604-3609). For example, the therapeutic index of chemotherapeutic agents is increased by combining treatment with L-histidinol, since L-histidinol reduces the toxicity of normal chemotherapeutic agents to normal cells but not to cancer cells (Warrington, R. C., Fang, W. D., Zhang, L. Shieh, M. and M. H. Saier, Jr. 1996. *Anticancer Research*, 16 (6B): 3635-3639; Warrington, R. C., Fang W. D., Zhang, L., Shieh, M. and M. H. Saier, Jr. 1996. *Anticancer Research*, 16 (6B): 3629-3633; Badary, O. A., Nagi, M. N., Al-Sawaf, H. A, Al-Harbi, M., and A. M. Al-Bekairia. 1997. *Nephron*, 77 (4): 435-439; Al-Shabanah, O. A., Badary, O. A., Al-Gharably, N. M. and H. A. Al-Sawaf. 1998. *Pharmacological Research*, 38 (3): 225-230; Badary, O. A. 1999. *Experimental Nephrology*, 7 (4): 323-327).

In theory, the use of L-histidinol with a histidine ammonia lyase offers a therapeutic approach to depleting serum histamine and lowering histidine levels. L-histidinol has limited usefulness as a single agent due to its low half-life (Zaharko, D., Plowman, J., Ward, W., Dykes, D., and L. Malspeis, 1992. *Cancer Research.* 52: 3604-3609) and its mode of action as a competitive inhibitor. Accordingly, L-histidinol must be present in very high concentrations in order to competitively inhibit cellular processes involving histidine. Reduced histidine levels would enhance the effectiveness of L-histidinol, by allowing cells to uptake the L-histidinol more readily.

Nevertheless, a histidine ammonia lyase suitable for combination therapy with a histidine analog, such as L-histidinol, must have the additional property of not being inhibited by L-histidinol. One prevalent characteristic of all known isolated histidine ammonia lyases is their inhibition in the presence of a histidine analog, like histidinol. For example, histidine ammonia lyases isolated from bacteria such as *Achromobacter liquidum* and *Streptomyces griseus* are

inhibited by L-histidinol and L-histidinol phosphate, respectively, with a K_i of 4.58 and 0.27 mM, respectively (Shibatani, T. *et al.* 1975. *Eur. J. Biochem.* 55: 263-269; Wu, P. C. *et al.* 1995. *Gene.* 115(1-2): 19-25).

Due to their enzymatic inhibition by histidinol, previously described histidine ammonia lyases have not been suitable candidates for use in combination therapies with these histidine analogs for treating pathologies such as cancer. Accordingly, there is a present and unmet need for a histidine ammonia lyase that possesses the relevant properties associated with previously isolated histidine ammonia lyases, yet maintains the ability to deplete histidine in the presence of L-histidinol.

In addition to cancer, viral diseases such as Human Respiratory Syncytial Virus (RSV), Herpes Simplex Virus (HSV) and Human Immunodeficiency Virus (HIV), infect millions worldwide and cause major health problems. RSV, a common cause of winter outbreaks of acute respiratory disease, in 1998 resulted in 90,000 hospitalizations and 4,500 deaths and is the largest cause of lower respiratory tract disease among infants and young children in the United States (CDC. 1997. *MMWR.* 46(49); 1163-1165). Herpes Simplex Virus infects an even larger portion of the population. The Centers for Disease Control estimated that in 1998, 45 million people ages 12 and older, or one out of five of total adolescent and adult population, was infected with the Herpes Simplex Virus. The Joint United Nation Programme on HIV/AIDS (UNAIDS) estimates that worldwide 33.6 million persons are infected with HIV/AIDS and 2.6 million people died in 1999 from this disease.

Human infectious viruses vary widely in the way they enter cells, replicate inside the cells, and subsequently get released from infected cells. RNA viruses have single- or double-stranded RNA as their genomes, which are naked or enveloped. The RNA strand can be either in a positive or negative form. RNA viruses enter the cell, make copies of their RNA genome, and direct the synthesis of messenger RNA to code for structural and regulatory proteins.

Finally, the genome is assembled with structural proteins and the virus is released. DNA viruses have single- or double-stranded DNA genomes that can be either non-enveloped or enveloped. Retroviruses are also RNA viruses but they involve DNA in their replication process. Thus, each virus is unique in its infection and multiplication process.

One common theme in viral replication is the ability of a virus to utilize the human cellular machinery for its multiplication. This makes drug development against viruses very difficult. In the past, antiviral therapy has focused on development of appropriate vaccines or inhibiting unique processes in viral replication. This often renders such therapy very specific for a type or subtype of viruses. Currently, vaccines are the main line of defense against viruses. Vaccines are developed specifically for each virus type and subtype, and are useful only against that particular virus type/subtype.

Therapies also have been developed that take advantage of unique processes in viral replication. For example, reverse transcriptase is unique to retroviruses. Nucleotide analogs and non-nucleotide reverse transcriptase inhibitors have been developed that inhibit reverse transcriptase without affecting other polymerases. However, such therapy is limited to combating only retroviruses. Yet another approach that targets a unique viral replication process is the use of protease inhibitors against HIV. But since these inhibitors target a specific enzyme, HIV protease, they cannot be effective against a wide range of viruses. Yet another example of a virus-specific therapy is the use of the antiviral compound ganciclovir, which is effective against Herpes Simplex Virus. Ganciclovir is specifically cytotoxic to herpes infected cells. Although ganciclovir therapy may be beneficial to combating the Herpes Simplex Virus, it has limited or no application for treating other viruses.

Accordingly, there is a great need for a therapeutic agent that can be effective against a broad spectrum of viruses. There has been no indication heretofore that a peptide having a histidine ammonia lyase activity could

effectively treat infectious viral agents. Thus, a substantial therapeutic and market potential exists for a histidine ammonia lyase that is effective against infectious viral agents.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the invention to provide a purified polypeptide having a histidine ammonia lyase activity that is not substantially inhibited by a histidine analog, such as histidinol.

It is a further object of the invention to provide a method for treating cancer, using a histidine analog, such as histidinol and a purified polypeptide having a histidine ammonia lyase activity that is not substantially inhibited by such compounds.

It is still a further object of the invention to provide a method for treating a viral infection, using a purified polypeptide having a histidine ammonia lyase activity.

These and other objects of the invention will become apparent to one of ordinary skill in the art upon reading the present application.

In one aspect, the invention provides an isolated polypeptide having histidine ammonia lyase activity, wherein the histidine ammonia lyase activity is not substantially decreased in the presence of a histidine analog such as histidinol. The invention also provides a polypeptide having the preceding characteristics, which comprises a peptide sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6. The invention also provides a polypeptide having the preceding characteristics, which comprises a peptide sequence selected from the group consisting of SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, and SEQ ID NO: 11. The invention further provides a method for PEGylating an isolated polypeptide having the preceding characteristics, comprising reacting a PEG with the polypeptide

In a methodological aspect, the invention provides a method for treating a patient suffering from a viral disorder, comprising administering to a patient suffering from a viral infection a therapeutic amount of a polypeptide having histidine ammonia lyase activity.

The invention further provides a method for treating a patient suffering from a cancer, comprising administering to the patient suffering from the cancer 1) a therapeutic amount of an isolated polypeptide having histidine ammonia lyase activity, wherein said histidine ammonia lyase activity is not substantially decreased in the presence of a histidine analog such as histidinol and 2) a therapeutic amount of a histidine analog.

In a further methodological approach, the invention provides a method for treating disease, comprising administering to a patient 1) a therapeutically effective amount of a polypeptide having histidine ammonia lyase activity and 2) administering to the patient a therapeutically effective amount of a chemotherapeutic agent or a retroviral vector. Consistent with this methodology, the invention provides a method for treating disease according to the previous method, wherein upon the administration of the polypeptide, non-diseased cells of the patient enter a reversible quiescent state.

The invention also provides a method for delivering an immunosuppressant to a patient, comprising: 1) administering to a patient a therapeutically effective amount of a polypeptide having histidine ammonia lyase activity, wherein the polypeptide generates trans-urocanic acid (t-UA) *in vivo*; and 2) subjecting the patient to an irradiating agent, wherein the irradiating agent causes the photoisomerization of t-UA to its cis isomer (c-UA), and wherein said cis isomer comprises an immunosuppressive property.

The present invention also includes an isolated DNA sequence comprising SEQ ID NO: 7, as well as an expression vector comprising SEQ ID NO: 7. In addition, the invention provides a method for treating a patient

comprising constructing an expression vector comprising SEQ ID NO: 7 and introducing the expression vector into the patient.

The present invention further includes an isolated DNA sequence comprising SEQ ID NO: 12, as well as an expression vector comprising SEQ ID NO: 12. In addition, the invention provides a method for treating a patient comprising constructing an expression vector comprising SEQ ID NO: 12 and introducing the expression vector into the patient.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the restriction pattern of the HAL coding region cut with selected enzymes.

Figure 2 lists the experimentally derived peptide sequences of HAL

Figure 3 depicts the SphI digestion pattern of HAL gene showing oligonucleotide and subclones.

Figure 4 depicts a histidine ammonia lyase overexpressing plasmid.

Figure 5 is an SDS-PAGE illustration, showing expression of HAL in *E. coli*. 30 μg samples were loaded onto a 10% SDS-PAGE gel. Lane 1: Sample taken at 1 hour following induction. Lane 2: Sample taken at 2 hours following induction. Lane 3: Sample taken at 3 hours following induction. Lane 4: Sample taken at 4 hour following induction.

Figure 6 is a picture of the SDS-PAGE showing purification of HAL from *E. coli*. Lanes 1 and 4 contain 10 and 20 μg respectively of crude extract. Lanes 2 and 5 contain 5 and 10 μg respectively of phenyl sepharose pooled fractions. Lanes 3 and 6 contain 5 and 10 μg respectively of Q-sepharose pooled fractions.

Figure 7 is a graph depicting the effect of temperature on HAL.

Figure 8 is a graph depicting the effect of pH on HAL.

Figure 9 is a chart illustrating the effect of HAL and Histidinol on HSV. Lane 1: Control. Lane 2: HAL alone (0.003 U/ml) Lane 3: L-histidinol alone (0.5 mM). Lane 4: HAL and L-histidinol (0.003 U/ml and 0.5 mM respectively).

Figure 10 depicts the effectiveness of L-histidinol as a single agent and in combination with HAL. Lane 1: Control. Lane 2: L-histidinol (0.1 mM). Lane 3: L-histidinol (0.5 mM). Lane 4: L-histidinol (1.0 mM). Lane 5: L-histidinol (1.5 mM). Lane 6: L-histidinol 3.0 mM).

Figure 11 depicts the effect of HAL and Histidinol on RSV. Lane 1: Control. Lane 2: HAL alone (0.005 U/ml). Lane 3: L-histidinol alone (3.0 mM). Lane 4: HAL and L-histidinol (0.005 U/ml and 3.0 mM respectively).

Figure 12 depicts the effect of HAL on RMuLV. Lane 1: Control. Lane 2: HAL (0.001 U/ml). Lane 3: HAL (0.002 U/ml). Lane 4: HAL (0.004 U/ml).

Figure 13 illustrates a first peptide sequence pileup of HAL from various bacteria, including *Corynebacteriaceae, B. subtilis, S. griseus, P. putida.*

Figure 14 is a second peptide sequence pileup of HAL from various bacteria, including *Corynebacteriaceae, S. griseus*, and *D. radiodurans.*

Figure 15 is a comparison between the amino acid sequence of *S. griseus* ("STRG") and *Corynebacteriaceae* ("HAL"); positions of an amino acid identity are delineated by "*".

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present inventors have discovered that certain polypeptides, known as histidase or histidine ammonia lyases, can decrease serum histidine levels and induce accumulation of urocanic acid, and yet are not inhibited by analogs of histidine, such as histidinol. By virtue of this discovery, a bioactive histidine ammonia lyase according to the invention can be used to treat allergic

reactions and pathologies characterized by increased levels of or need for histidine and/or histamine, such as cancer and infectious viruses.

The Inventive Polypeptides and Nucleic Acids

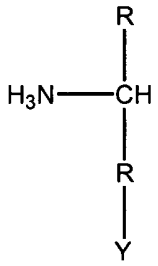
In one of its aspects, the present invention provides a polypeptide, commonly known as "histidase" or "histidine ammonia lyase," that depletes L-histidine serum levels, in turn producing urocanic acid—a beneficial by-product of histidine breakdown. A histidine ammonia lyase (EC 4.3.1.3) catalyzes the nonoxidative elimination of the alpha-amino group of histidine. Although L-histidinol is able to alter histidine metabolism, alteration of histidine metabolism *via* depletion of histidine with a histidine ammonia lyase would provide similar therapeutic benefits, yet would do so in an even more effective and potentially less toxic manner than L-histidinol. An additional advantage for treatment with histidine ammonia lyase is that one of the products of its action, urocanic acid, promises to have protective and beneficial effects on the immune system, as reported by Noonan *et al., Immunol. Today* 13: 250-254 (1992).

In another aspect, the invention contemplates a polypeptide that is able to retain its histidine ammonia lyase activity in the presence of a histidine analog, like histidinol. As defined herein, a "histidine analog" refers to histidine variants, like histidinol, including therapeutic salts thereof. Histidinol, as a representative histidine analog, possesses many beneficial therapeutic uses, including the ability to inhibit the production of histamine from histidine. Histidinol is also able to alter protein synthesis pathways, by causing deacylation of histidyl tRNA. Because the histidine ammonia lyase activity of a polypeptide according to this invention is not substantially decreased in the presence of a histidine analog, like histidinol, it is uniquely suitable among all other known histidine ammonia lyases for combination therapy with such compounds.

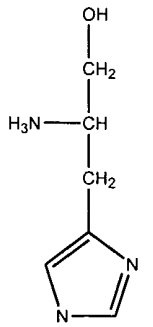
Nucleic acids encoding the inventive peptides also are contemplated, as are conservative variants thereof, in accordance with the

"sequence identity" discussion below. The inventive nucleic acids are, of course, useful in preparing the inventive proteins by recombinant means and in implementing gene therapy treatments analogous to the protein-based treatments, discussed below.

Histidine analogs, according to the invention, include compounds of the following structure:



wherein each R is independently a 1-, 2- or 3- carbon alkyl, a 2- to 3-carbon alkene, or a 2- to 3-carbon alkyne, wherein each R independently is optionally substituted one or members of the group consisting of -OH, -SH and =O; and Y is a 5- or 6-membered heterocyclic ring, having one or two hetero atoms selected from the group consisting of N, S and O, including esters and therapeutically effective salts thereof. In some preferred analogs Y is a five-membered ring, having one or two N hetero atoms and in more preferred compounds Y is an imidazole moiety. In certain preferred compounds N is 1. R specifically may be a 1-carbon alkyl. Representative esters include phosphoric acid esters and carboxylic acid esters (especially C1-3). Analogs can include histidinol, histidinal, imidazole glycerol phosphate, imidazole acetol phosphate, and histidinol phosphate. Histidinol has the following strucuture:



The polypeptides of the present invention have a molecular structure that confers the functional characteristics described herein. In a preferred embodiment, the region is conserved that corresponds to the active site, denoted by SEQ ID NO: 1. Accordingly, the peptide sequences delineated by SEQ ID NOS: 2, 3, 4, and 5 are encompassed by the invention because they conserve the active site of the novel polypeptide. Likewise, SEQ ID NOS: 8, 9, and 10 conserve the active site of the novel polypeptide and, accordingly, are contemplated by the invention.

Within the present invention, moreover, are molecules that do not contain the active site, but are variants of the aforementioned peptides by virtue of one or more conservative substitution, such as cysteine for serine--both of which are sulfur-containing amino acids--that maintain histidine ammonia lyase activity in the presence of a histidinol analog. A "conservative substitution" may be made, for instance, on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues involved.

Thus, the overall structure and composition of inventive polypeptides are important only insofar as they confer the appropriate functional characteristics, *i.e.*, histidine-depleting and relative resistance to a histidine analog, such as histidinol. Given the properties of the individual amino acids comprising the disclosed protein products, some rational substitutions will be recognized by the skilled worker. For example: (a) nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan, and methionine; (b) polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine; (c) positively charged (basic) amino acids include arginine, lysine, and histidine; and (d) negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Substitutions typically may be made within groups (a)-(d). In addition, glycine and proline may be substituted for one another based on their ability to disrupt α-helices. Similarly, certain amino

acids, such as alanine, cysteine, leucine, methionine, glutamic acid, glutamine, histidine and lysine are more commonly found in α-helices; while valine, isoleucine, phenylalanine, tyrosine, tryptophan and threonine are more commonly found in β-pleated sheets. Glycine, serine, aspartic acid, asparagine, and proline are commonly found in turns. Some preferred substitutions may be made among the following groups: (i) S and T; (ii) P and G; and (iii) A, V, L and I. Given the known genetic code, and recombinant and synthetic DNA techniques, the skilled scientist can readily construct DNAs encoding the conservative amino acid variants.

In general, both the DNA and protein molecules of the invention can be defined with reference to "sequence identity." Some molecules have at least 60% identity. Preferred molecules are those having at least about 65% sequence identity, more preferably at least 65% or 70% sequence identity. Other preferred molecules have at least 80%, more preferably at least 80% or 85%, sequence identity. Particularly preferred molecules have at least about 90% sequence identity, more preferably at least 90% sequence identity. Most preferred molecules have at least about 95%, more preferably at least 95%, sequence identity. As used herein, two nucleic acid molecules or proteins are said to "share significant sequence identity" if the two contain regions which possess greater than 85% sequence (amino acid or nucleic acid) identity.

"Sequence identity" is defined herein with reference the Blast 2 algorithm, which is available at the NCBI (http://www.ncbi.nlm.nih.gov/BLAST), using default parameters. References pertaining to this algorithm include: those found at http://www.ncbi.nlm.nih.gov/BLAST/blast_references.html; Altschul, S.F., Gish, W., Miller, W., Myers, E.W. & Lipman, D.J. 1990. *J. Mol. Biol.* 215:403-410; Gish, W. & States, D.J. 1993. *Nature Genet.* 3:266-272; Madden, T.L., Tatusov, R.L. & Zhang, J. 1996. *Meth. Enzymol.* 266:131-141; Altschul, S.F., Madden, T.L., Schäffer, A.A., Zhang, J., Zhang, Z., Miller, W. &

Lipman, D.J. 1997. *Nucleic Acids Res.* 25:3389-3402; and Zhang, J. & Madden, T.L. 1997. *Genome Res.* 7:649-656.

To this end, SEQ ID NO: 6, for example, delineates sequence variations that are contemplated by the invention. The amino acid positions not represented by "X" represent 1) highly conserved regions among known histidine ammonia lyases (*see e.g.,* Figure 15), as well as 2) amino acids that are unique to the polypeptide isolated from *Corynebacteriaceae*. Regions corresponding to the absence of an amino acid are denoted by "-", shown in figure 14. Amino acid positions delineated by "X" represent regions where the amino acid can vary without departing from the invention. According to SEQ ID NO: 6, the amino acids represented by "X" can be an amino acid that is present in the corresponding position of any other histidine ammonia lyase. For instance, figure 14 denotes Alanine at position 14 of the HAL isolated from *Corynebacteriaceae*. In histidine ammonia lyases isolated from those species depicted in figure 14, the amino acid at the position corresponding to position 14 in *Corynebacteriaceae* are: threonine, alanine, valine, leucine, asparagine, aspartic acid, and proline, as shown in figure 14. Accordingly, position 14 of the polypeptide contemplated by the invention can be represented by any one of these amino acids. To further illustrate the contemplated variation, the amino acid corresponding to position 241 can be Phenylalanine, leucine, tyrosine, alanine, or cysteine.

The Following Legend is used to describe the species associated with the peptides disclosed in figure 14.

```
983831      : HAL
      CAC21618    : Streptomyces coelicolor
HUTH_STRGR : Streptomyces griseus
HUTH_DEIRA : Deinococcus radiodurans
4      BAB16159    : Agrobacterium rhizogenes
5      Q9KWE4      : Agrobacterium rhizogenes
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HUTH_BACSU : Bacillus subtilis
7      Q9KSQ4      : Vibrio cholerae
8      Q9HU85      : Pseudomonas aeruginosa
9      Q9KBE6      : Bacillus halodurans
HUTH_PSEPU : Pseudomonas putida
HUTH_RHIME : Rhizobium meliloti
12     Q9HU90      : Pseudomonas aeruginosa
HUTH_HUMAN : Human
HUTH_CAEEL : Caenorhabditis elegans
15     Q9HLI6      : Thermoplasma acidophilum
HUTH_MOUSE : Mouse
17     BAB29407    : Mus musculus (Mouse)
18     HUTH_RAT    : Rat
18     AAG53586    : uncultured bacterium pCosAS1
20     Q9KKE0      : Rhizobium meliloti
21     Q9HQD5      : Halobacterium sp
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A further example, as shown by SEQ ID NO: 11, delineates other contemplated peptides, which can be formulated by referencing the histidine ammonia lyases set forth in Figure 13. As in SEQ ID NO: 6, the amino acid positions not represented by "X" represent 1) highly conserved regions among known histidine ammonia lyases, as well as 2) amino acids that are unique to the polypeptide isolated from *Corynebacteriaceae*. Regions that may correspond to the absence of an amino acid are denoted by "-", shown in figure 13. Amino acid positions delineated by "X" represent regions where the amino acid can vary without departing from the invention. The amino acids represented by "X" can be an amino acid that is present in the corresponding position of any other histidine ammonia lyase. For instance, figure 13 denotes Threonine at position 8 of the HAL isolated from *Corynebacteriaceae*. In histidine ammonia lyases isolated from other species, the amino acid at the position corresponding to

position 8 in *Corynebacteriaceae* are threonine, isoleucine, alanine, glutamate, and valine, also shown in figure 13. Accordingly, position 8 of the polypeptide contemplated by the invention can be represented by any one of these amino acids. To further illustrate the contemplated variation, the amino acid corresponding to position 307 can be alanine, aspartate, glycine, glutamate, or arginine.

In addition to having varying peptide sequences, the polypeptides contemplated by the invention can possess varying molecular weights, without departing from the invention, so long as one or more of the novel properties, as disclosed herein, are maintained. Accordingly, a polypeptide can have a monomeric molecular weight between about 30,000 to 67,000 daltons. More preferably, the monomeric molecular weight is between about 45,000 and 60,000 daltons. It is most preferred that the monomeric molecular weight is about 56,000 daltons.

Therapeutic Compositions

The proteins of the present invention can be formulated according to known methods to prepare pharmaceutically useful compositions, whereby the inventive molecules, or their functional derivatives, are combined in a mixture with a pharmaceutically acceptable carrier vehicle. Suitable vehicles and their formulation, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in *Remington's Pharmaceutical Sciences* (16th ed., Osol, A., Ed., Mack, Easton PA (1980)). In order to form a pharmaceutically acceptable composition suitable for effective administration, such compositions will contain an effective amount of one or more of the proteins of the present invention, together with a suitable amount of carrier vehicle.

Pharmaceutical compositions for use in accordance with the present invention may be formulated in conventional manner using one or more physiologically acceptable carriers or excipients. Thus, the inventive polypeptides

and their physiologically acceptable salts and solvate may be formulated for administration by inhalation or insufflation (either through the mouth or the nose) or oral, buccal, parenteral or rectal administration.

For oral administration, the pharmaceutical compositions may take the form of, for example, tablets or capsules prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (*e.g.*, pregelatinised maize starch, polyvinylpyrrolidone or hydroxypropyl methylcellulose); fillers (*e.g.*, lactose, microcrystalline cellulose or calcium hydrogen phosphate); lubricants (*e.g.*, magnesium stearate, talc or silica); disintegrants (*e.g.*, potato starch or sodium starch glycolate); or wetting agents (*e.g.*, sodium lauryl sulfate). The tablets may be coated by methods well known in the art. Liquid preparations for oral administration may take the form of, for example, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (*e.g.*, sorbitol syrup, cellulose derivatives or hydrogenated edible fats); emulsifying agents (*e.g.*, lecithin or acacia); non-aqueous vehicles (*e.g.*, almond oil, oily esters, ethyl alcohol or fractionated vegetable oils); and preservatives (*e.g.*, methyl or propyl-p-hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring and sweetening agents as appropriate.

Preparations for oral administration may be suitably formulated to give controlled release of the active compound. For buccal administration the composition may take the form of tablets or lozenges formulated in conventional manner.

For administration by inhalation, the novel polypeptide for use according to the present invention is conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. In the case of a

pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, *e.g.* gelatin for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

The novel polypeptide may be formulated for parenteral administration by injection, *e.g.*, by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, *e.g.*, in ampules or in multi-dose containers, with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

The compounds may also be formulated in rectal compositions such as suppositories or retention enemas, *e.g.*, containing conventional suppository bases such as cocoa butter or other glycerides.

In addition to the formulations described previously, the novel polypeptide may also be formulated as a depot preparation. Such long acting formulations may be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compounds may be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

The compositions may, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the active ingredient. The pack may for example comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration.

Methods of the Invention:

Therapeutic Rationale

Viral fighting properties

In one embodiment, the inventive polypeptides possess hitherto unknown uses for treating human infectious viruses, including DNA and RNA viruses. It has been discovered by the present inventors the novel polypeptides are surprisingly potent inhibitors of RNA, DNA and retroviruses viruses. Histidine ammonia lyase therapy by itself and in combination with histidinol is effective against these three main groups of viruses. The unique broad-spectrum antiviral activity of HAL is a highly desirable characteristic for an antiviral agent.

Specific viruses that can be treated according to the invention include, but are not limited to, human Respiratory Syncytial Virus (RSV), Herpes Simplex Virus (HSV) and Human Immunodeficiency Virus (HIV). The latter virus can be treated in accordance with the present invention, based on the observation that inventive polypeptide was able to inhibit viral replication in the Rauscher Murine Leukemia Virus, a model virus for HIV. Other treatable viruses include the following closely related viruses.

Respiratory syncytial virus belongs to the family Paramyxoviridae. The other human infectious viruses belonging to the family Paramyxoviridae include: Parainfluenza 1, 2, 3, 4 viruses which cause upper respiratory disease, bronchitis/bronchiolitis, pneumonia; mumps virus, and measles virus. The family Paramyxoviridae is very closely related to Rhabdoviridae and Filoviridae because the viruses belonging to these families contain a single-stranded RNA (negative sense) genome which is non-segmented and enveloped. Human infectious viruses belonging to Rhabdoviridae are vesicular stomatitis-Indiana, New Jersey, cocal viruses, chandipura virus, Piry virus, Isfahan virus, rabies

virus, Mokola virus, and Duvenhage virus. Human infectious viruses belonging to the family Filoviridae include Marburg and Ebola viruses. More broadly, Respiratory Syncytial Virus is an RNA virus.

Other RNA viruses that cause human infections include the following: polioviruses 1, 2, and 3; coxsackieviruses B1-B6; human echoviruses 1-9, 11-27, and 29-34; human enteroviruses 1-113; Norwalk virus and similar viruses that belong to the family Caliciviruses that cause gastroenteritis in humans; eastern equine encephalitis virus; western equine encephalitis virus; Venezualan equine encephalitis virus; chikungunya virus; O'nyong-nyong virus; Ross River virus; Mayarovirus; rubella virus; yellow fever virus; dengue viruses; Western Nile virus; St. Louis encephalitis virus; Japanese encephalitis virus; Murray Valley encephalitis virus; Rocio virus; tick-borne encephalitis viruses; human coronaviruses 229-E and OC43; upper respiratory tract infection, probably pneumonia and possibly gastroenteritis; influenza A, B, and C viruses; Bunyamwera virus; Bwamba virus; Oriboca virus; Oropouche virus; Gwama virus; California encephalitis virus; LaCrosse virus; Tahyna virus; Sandfly fever-Naples virus; Crimean-Congo hemorrhagic fever virus; Hantaan virus (Korean hemorrhagic fever, hemorrhagic fever with renal syndrome, nephropalthia epidemica); lymphocytic choriomeningitis (LCM) virus; Lassa virus; Machupa virus (Bolivian hemorrhagic fever); Junin virus (Argentine hemorrhagic fever); reovirus 1, 2, and 3; Orungo virus (febrile illness in Nigeria and Uganda); Kemerovo virus (febrile illness in Russia and Egypt); human rotaviruses, Colorado tick fever virus.

Rauscher Murine Leukemia virus belongs to the family Retroviridae. Viruses that belong to this family have a single-stranded (positive sense), non-segmented enveloped genome, but they involve a DNA step in replication. Human infectious viruses belonging to this family include type C oncoviruses such as human T-lymphotropic virus 1 (HTLV-I, adult T-cell leukemia) and human T-lymphotropic virus 2 (HTLV-II, possibly associated with

hairy-cell leukemia), human immunodeficiency viruses 1 and 2 (HIV 1 and HIV 2) that cause acquired immunodeficiency syndrome (AIDS) and other viruses, related to HIV 1 and HIV 2, which cause AIDS-like disease.

Herpes Simplex Virus belongs to the family Herpesviridae. Viruses belonging to the family Herpesviridae have a double-stranded enveloped genome, a property that they share with viruses belonging to the families Poxviridae and Iridoviridae. Human infectious viruses belonging to the family Herpesviridae include Herpes Simplex Viruses 1 and 2, cercopilthecine, herpesvirus 1 (B-virus), varicella-Zoster virus, human cytomegalovirus, EB virus, and human herpesvirus 6. Human infectious viruses belonging to Poxviridae include variola virus (smallpox, alastrim), vaccinia virus, monkeypox virus, cowpox virus, orf virus (contagious pustular dermatitis), pseudo-cowpox (milker's nodule) virus, yabapox virus, tanapox virus, and molluscum contagiosum virus. More broadly, Herpes Simplex Virus is a DNA virus and other human infectious viruses in this category are hepatitis B virus; human parvovirus B-19, parvovirus RA-1, and other parvoviruses that cause gastroenteritis; human papillomaviruses (HPV) 1-48); polyomaviruses such as JC, SV40 and BK; and Adenoviruses such as Mastadenovirus h1-h49.

Polypeptides that have a histidase activity are able to combat viruses by inhibiting viral replication, for example, in the absence of a histidine analog. However, a greater therapeutic benefit is achieved, when treating viruses, if the polypeptides of the invention are employed in conjunction with a histidine analog, like histidinol, as shown in Example 11. In fact, a synergistic effect is observed when HAL- and histidine analog-directed therapies are combined, also shown in Example 11. Accordingly, the invention contemplates polypeptides that can be used to treat infectious viruses by virtue of the polypeptide's histidine depleting activity, either alone or in combination with a histidine analog.

Cancer fighting properties

In another aspect, the inventive polypeptide is able to function as an anticancer agent. *In vitro*, the polypeptides of the invention are effective in controlling the growth of a variety of human tumors. For example, the growth of different prostate and ovarian cancer cell lines has been inhibited by the inventive polypeptides, as shown in Example 12.

By virtue of their anti-carcinogenic activity *in vitro*, the polypeptides of the invention can also be used to inhibit malignant tumor proliferation *in vivo*. In addition, any of the novel polypeptides are a suitable candidate for an anticancer agent that can be used in combination therapy with other anticancer agents, as described below. In particular, the polypeptides of the present invention can be administered to a patient in the presence of a histidine analog, like histidinol, due to the novel polypeptides' ability to retain histidine ammonia lyase activity in the presence of such compounds.

There are numerous types of cancers that can be treated according to the invention, including prostate and ovarian cancer, and glioblastomas. Other types of cancers that may be treated include: chronic and acute leukemia, cancer of the bone, brain, breast cartilage, cervix, esophagus, kidney, larynx, liver, lung, pancreas, and uterus. In addition, the polypeptides of the invention may be used to combat Hodgkin's Disease, lymphoma, melanoma, multiple myeloma, colo-rectal, and testicular cancer.

Quiescent-inducing properties

It has been discovered that incubation in histidine-deficient medium has been able to cause non-transformed mammalian cells to enter a reversible inactive, or "quiescent," state at a specific point during the cell cycle, called the "restriction point." Newman *et al.* 1983. *Anticancer Research.* 43:4703. This quiescent state is characterized by an absence of DNA synthesis and reduced rates of ribosomal RNA and protein synthesis. These and a series of

other metabolic events associated with growth quiescence are reversible, and have been termed the "negative pleiotypic response." In contrast to the reversible arrest of normal cells by nutritional manipulations, transformed cell lines seem to have lost their ability to stop proliferation at the restriction point, as reported by Pardee *et al. Annual Rev. Biochem.* 47:715-750 (1974); and Pardee, *Proc. Natl. Acad. Sci. U.S.A.* 71: 1286-1290 (1974) and Newman *et al.,* (1983), *supra.*

This fundamental difference between normal and malignant cells can be exploited to selectively kill transformed cells under conditions that leave normal cells intact. For example, chemotherapeutic drugs function preferentially against proliferating cells, with no significant capacity to discriminate between cycling normal and cycling tumor tissues. Previously, Newman *et al., Anticancer Res.* 43: 4703 (1983) were able to drive a cell line (BALB/3T3) into a quiescent state by incubating the cell line in a histidine-deficient medium. This methodology protected the cells from the lethal effects of Methotrexate. Warrington, *Anticancer Res.* 6: 451 (1986), and *Biochem. Cell Biol.* 70: 365 (1992), reported similar findings, but instead used a histidine analog in place of a histidine-deficient medium. These findings led to the conclusion by Warrington (1986), *supra,* that cancer chemotherapeutic agents are selective when the tumor cell population has a higher growth fraction than normal cells. Thus, arresting the growth of normal cells without impacting the growth of tumor cells would confer selectivity to the anti-proliferative drugs commonly used in cancer chemotherapy.

In this context, a histidine ammonia lyase is a suitable candidate to selectively deplete the circulating histidine, since the histidine depleting activity of histidine ammonia lyase will cause growth arrest in normal cells, without affecting the growth of tumor cells. A histidine ammonia lyase can also be used in combination with a histidine analog such as L-histidinol, where the histidine ammonia lyase activity is not substantially decreased in the presence of the

histidine analog. Accordingly, chemotherapeutic drugs would be less inclined to react with quiescent cells and confer less toxicity to a patient, thereby increasing the therapeutic index of cancer chemotherapy.

In one embodiment, patients who would undergo cancer chemotherapy first can be given an injection of an effective dose of a histidine ammonia lyase (*e.g.* between 1 μg and 1 gram per kg body weight, administered intravenously). About twenty-four hours after histidine ammonia lyase injection, a conventional chemotherapeutic agent, such as one of those described herein, can then be administered to the patient. However, the invention also contemplates a method of administering several doses of a conventional chemotherapeutic agent to a patient after about 24 hours following the injection of a histidine ammonia lyase. The type of chemotherapy will vary with the type of cancer and also will be based on the suitability of the chemotherapeutic agent to a particular patient.

In yet another aspect of the invention, a histidine ammonia lyase can be used to enhance the specificity of cancer gene therapy. Retroviral vectors are one of the commonly used vehicles to deliver therapeutic genes for selectively killing tumor cells. However, retroviruses deliver DNA into growing cells without significant capacity to discriminate between cycling normal and cycling tumor tissues. Accordingly, retroviral therapy also suffers from the problem of killing high concentrations of non-targeted, healthy (i.e. non-tumor) cells that are proliferating at a given time in the human body. To obviate these problems, a histidine ammonia lyase can be first administered to a patient, thereby causing normal (i.e. non-tumor) cells to enter a reversible quiescent state. For example, an intravenous injection of 1 μg to 1 g of HAL reacted with polyethylence glycol ("PEGylated HAL") per kg body weight can be given to a patient 24 hours prior to the injection of retroviral vector. This treatment would arrest the growth of normal cells without affecting the growth of cancer cells. As a result, retroviral vectors would selectively target proliferating tumor cells.

Immunosuppressant Properties

In another embodiment, the invention contemplates methodologies for delivering an immunosuppressant to a patient. The products of the enzymatic action of histidine ammonia lyase are trans-urocanic acid (t-UA) and ammonia. Irradiation at approximately 310 nm causes the photoisomerization of t-UA to its cis isomer (c-UA), as noted by Hanson *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* 95: 10576-10578 (1998). Cis-urocanic acid is believed to play the role of one of the UVB-induced immunosuppressive mediators (Kripke, *Cancer Res.* 54: 6102-6105 (1994); and Norval *et al.*, *Photochem. Photobiol.* 62: 209-217 (1995)). This immunosuppressive property of urocanic acid can be used, for example, to treat immune system disorders and to prevent rejection of transplanted organs.

Although in theory, such an approach promises to provide a therapeutic benefit, small molecules like urocanic acid are rapidly cleared from circulation, thereby limiting their use as effective immunosuppressors over prolonged periods of time. However, it has been discovered that PEGylated HAL has a long circulatory half-life in mice (over 48 hours). Thus, an effective dose (1 μg to 1 g per kg body weight) of a histidine ammonia lyase can be used to generate circulating urocanic acid for prolonged periods of time. In turn, a cis-isomerizing agent, such as UVB irradiation, can be used to cause local immunosuppression (for conditions such as psoriasis), or systemic immunosuppression, the process of which subjects the patient to whole body irradiation. In one example, whole body irradiation can be employed according to the invention, to combat organ rejection following transplantation.

In another embodiment, selective immunosuppression can be achieved by targeting the UVB irradiation. For example, psoriasis could be treated by an injection of a histidine ammonia lyase followed by selective irradiation of the affected areas. Selective UVB irradiation, following the

injection of a histidine ammonia lyase into a patient, can also be used to treat conditions like arthritis.

In a further embodiment, localization and/or specificity of immunosuppression could also be achieved by targeting a histidine ammonia lyase to specific organs. To this end, the invention contemplates a fusion protein comprising one or more targeting peptide sequences in addition to the coding regions of a selected histidine ammonia lyase. Pasqualini *et al. Nature* 380: 364-366 (1996), have reported the success of targeting various proteins to specific organs *via* this methodology.

Treatment Methods

Therapeutic methods involve administering to a subject in need of treatment a therapeutically effective amount of a polypeptide contemplated by the invention. "Therapeutically effective" is employed here to denote the amount of a peptide that is of sufficient quantity to inhibit or reverse cancer growth (*e.g.*, induce apoptosis). Some methods contemplate combination therapy with known cancer medicaments or therapies, for example, chemotherapy (preferably using compounds of the sort listed above) or radiation. The patient may be a human or non-human animal. A patient typically will be in need of treatment when suffering from a pathology such as a cancer or virus described above.

As previously demonstrated, the histidine ammonia lyase activity of the novel polypeptide is not substantially decreased in the presence of a histidine analog, like histidinol. A typical method, accordingly, involves administering to a patient both the novel polypeptide and the selected histidinol according to the methods described herein. In one embodiment, the novel polypeptide can be administered simultaneously with a chosen histidinol. In another embodiment, the novel polypeptide is first administered to a patient, followed by a selected histidinol. In yet another embodiment, a histidine analog, such as histidinol, is first administered to a patient followed by the novel

polypeptide. The invention also contemplates administering multiple dosages of the novel polypeptide or chosen histidinol in conjunction with the methods described herein (i.e. administering two or more dosages of the novel polypeptide, followed by at least one dosage of a histidine analog, like histidinol).

Administration during *in vivo* treatment may be by any number of routes, including parenteral and oral, but preferably parenteral. Intracapsular, intravenous, intrathecal, and intraperitoneal routes of administration may be employed, and generally intravenous is preferred. The skilled artisan will recognize that the route of administration will vary depending on the disorder to be treated.

Determining a therapeutically effective amount of the novel polypeptide, according to this invention, largely will depend on particular patient characteristics, route of administration, and the nature of the disorder being treated. General guidance can be found, for example, in the publications of the International Conference on Harmonisation and in REMINGTON'S PHARMACEUTICAL SCIENCES, chapters 27 and 28, pp. 484-528 (Mack Publishing Company 1990).

Determining a therapeutically effective amount specifically will depend on such factors as toxicity and efficacy of the medicament. Toxicity may be determined using methods well known in the art and found in the foregoing references. Efficacy may be determined utilizing the same guidance in conjunction with the methods described below in the Examples. A pharmaceutically effective amount, therefore, is an amount that is deemed by the clinician to be toxicologically tolerable, yet efficacious. Efficacy, for example, can be measured by the induction or substantial induction of T lymphocyte cytotoxicity at the targeted tissue or a decrease in mass of the targeted tissue.

Suitable dosages can be preferably from about one microgram per kg body weight to one gram per kg body weight, and more preferably from 2 milligrams to 10 mg per kg body weight.

The compositions, since they are useful in cancer treatment, may be formulated in conjunction with other conventional methods of treatment. Conventional methods include administering a histidine analog, like histidinol. Such forms of treatment also include conventional chemotherapeutic agents. Conventional chemotherapeutic agents include alkylating agents, antimetabolites, various natural products (*e.g.*, vinca alkaloids, epipodophyllotoxins, antibiotics, and amino acid-depleting enzymes), hormones and hormone antagonists. Specific classes of agents include nitrogen mustards, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogues, pyrimidine analogues, purine analogs, platinum complexes, adrenocortical suppressants, adrenocorticosteroids, progestins, estrogens, antiestrogens and androgens. Some exemplary compounds include cyclophosphamide, chlorambucil, methotrexate, fluorouracil, cytarabine, thioguanine, vinblastine, vincristine, doxorubincin, daunorubicin, mitomycin, cisplatin, hydroxyurea, prednisone, hydroxyprogesterone caproate, medroxyprogesterone, megestrol acetate, diethyl stilbestrol, ethinyl estradiol, tomoxifen, testosterone propionate and fluoxymesterone. In treating breast cancer, for example, tamoxifen is particularly preferred.

The invention further contemplates the administering to a patient a peptide of the invention in conjunction with alkylating agents, antimetabolites, various natural products (*e.g.*, vinca alkaloids, epipodophyllotoxins, antibiotics, or amino acid-depleting enzymes) hormones and hormone antagonists. Specific classes of agents include nitrogen mustards, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogues, pyrimidine analogues, purine analogs, platinum complexes, adrenocortical suppressants, adrenocorticosteroids, progestins, estrogens, antiestrogens and androgens. Some exemplary compounds include cyclophosphamide, chlorambucil, methotrexate, fluorouracil, cytarabine,

thioguanine, vinblastine, vincristine, doxorubincin, daunorubicin, mitomycin, cisplatin, hydroxyurea, prednisone, hydroxyprogesterone caproate, medroxyprogesterone, megestrol acetate, diethyl stilbestrol, ethinyl estradiol, tomoxifen, testosterone propionate and fluoxymesterone.

Construction of the inventive polypeptide

A polypeptide according to this invention can be isolated by conventional means and the present invention is not limited to any particular method of producing the desired polypeptide contemplated herein. According to the contemplated recombinant methods of production, however, the invention provides recombinant DNA constructs comprising one or more of the nucleotide sequences of the domains described in the present invention. The recombinant constructs of the present invention comprise a vector, such as a plasmid or viral vector, into which a DNA or DNA fragment, typically bearing an open reading frame, is inserted, in either orientation. The invention further contemplates cells containing these vectors.

To this end, the DNA that encodes a novel polypeptide is first isolated using well known techniques. For instance, Example 1 provides one non-limiting method for isolating such targeted genomic DNA. This methodology includes culturing selected cells before extracting the genomic DNA from the culture, followed by subjecting the DNA to a series of restriction enzymes, whereby generated genomic DNA fragments can be studied and isolated by conventional techniques, for example, agarose gel electrophoresis.

Next, a vector can be selected and, likewise, cut with a restriction enzyme to generate a vector fragment by a methodology that is consistent with the procedure used to isolate the genomic DNA. Suitable vectors include bacterial and mammalian expression systems, as described below. After a suitable vector is selected, varying concentrations of the DNA fragment ("insert") can be placed into contact with the vector, as shown in Example 1, to

determine the best insert:vector ratio for transformation of the DNA fragments. The transformants can then be cultured to generate copies of the DNA fragments.

To isolate the domains of a novel polypeptide from the generated DNA fragment, DNA probes can be designed by virtue of selecting sequences that are highly conserved in known histidine ammonia lyases. As shown in Example 1, the Wisconsin Graphics GCG package pileup program provides one method of determining highly conserved regions. A selected probe can be used to screen the targeted genomic DNA library, for example, using technology as disclosed by Ausubel *et al.*, CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, (John Wiley & Sons, 1994). Example 1 provides a non-limiting embodiment for utilizing suitable probes to screen the genomic library, followed by a series of steps to purify the genomic clones.

Bacterial Expression

Useful expression vectors for bacterial use are constructed by inserting a structural DNA sequence encoding a desired protein together with suitable translation initiation and termination signals in operable reading phase with a functional promoter. The vector will comprise one or more phenotypic selectable markers and an origin of replication to ensure maintenance of the vector and, if desirable, to provide amplification within the host. Suitable prokaryotic hosts for transformation include *E. coli, Bacillus subtilis, Salmonella typhimurium* and various species within the genera Pseudomonas, Streptomyces, and Staphylococcus, although others may, also be employed as a matter of choice. In a preferred embodiment, the prokaryotic host is *E. coli*, as shown in Example 3.

Bacterial vectors may be, for example, bacteriophage-, plasmid- or cosmid-based. These vectors can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids typically containing elements of the well known cloning vector pBR322 (ATCC 37017). Such commercial vectors include, for example, GEM 1 (Promega Biotec, Madison, WI,

USA), pBS, phagescript, PsiX174, pBluescript SK, pBs KS, pNH8a, pNH16a, pNH18a, pNH46a (Stratagene); pTrc99A, pKK223-3, pKK233-3, pKK232-8, pDR540, and pRIT5 (Pharmacia). A preferred vector according to the invention is Bluescript vector (pBSSK) - (Stratagene).

These "backbone" sections are combined with an appropriate promoter and the structural sequence to be expressed. Bacterial promoters include lac, T3, T7, lambda P_R or P_L, trp, and ara. The T7 promoter is preferred.

Following transformation of a suitable host strain and growth of the host strain to an appropriate cell density, the selected promoter is derepressed/induced by appropriate means (e.g., temperature shift or chemical induction) and cells are cultured for an additional period. Cells are typically harvested by centrifugation, disrupted by physical or chemical means, and the resulting crude extract retained for further purification.

Eukaryotic Expression

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include selected mouse L cells, such as thymidine kinase-negative (TK) and adenine phosphoribosul transferase-negative (APRT) cells. Other examples include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, *Cell 23*:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences. DNA sequences derived from the SV40 viral genome, for example, SV40 origin, early promoter, enhancer, splice, and polyadenylation sites may be used to provide the required non-transcribed genetic elements.

Mammalian promoters include CMV immediate early, HSV thymidine kinase, early and late SV40, LTRs from retrovirus, and mouse metallothionein-I. Exemplary mammalian vectors include pWLneo, pSV2cat, pOG44, pXT1, pSG (Stratagene) pSVK3, pBPV, pMSG, and pSVL (Pharmacia).

In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, the coding sequence of interest may be ligated to an adenovirus transcription/translation control complex, *e.g.*, the late promoter and tripartite leader sequence. This chimeric gene may then be inserted in the adenovirus genome by *in vitro* or *in vivo* recombination. Insertion in a non-essential region of the viral genome (*e.g.*, region E1 or E3) will result in a recombinant virus that is viable and capable of expressing a target protein in infected hosts. (*E.g.*, See Logan *et al.*, 1984, *Proc. Natl. Acad. Sci.* USA 81:3655-3659).

Extension of half-life and prevention of antibody formation to the inventive peptide (PEGylating)

The invention also contemplates a polypeptide that can be induced to have an increased half-life. To this end, the polypeptide is manipulated by conventional techniques, such as modification with polyethylene glycol (PEGylation). According to this methodology, a suitable amount of a PEGylating agent is reacted with a polypeptide of the invention before introducing the polypeptide to a targeted cell culture or tissue. In one embodiment, the PEGylating agent is BTC-PEG 5000 (Shearwater Polymers, Inc.); however, the invention contemplates other PEGylating agents. Example 10 provides a non-limiting method to construct a PEGylated peptide in accordance with the invention.

A PEGylated polypeptide has practical applications both *in vitro* and *in vivo*. For example, a polypeptide's ability to sustain its enzymatic properties for an increased amount of time would permit a decrease in the dosage

necessary to ameliorate one or more symptoms associated with a targeted pathology. In addition, a PEGylated polypeptide can possess an increased resistance to antibody-mediated depletion in the host. According to this embodiment, the PEGylating agent is believed to inhibit a host's antibody-mediated response against the polypeptide.

Gene Therapy Applications:

By virtue of discovering the DNA sequences that encode the novel polpypeptides disclosed herein, the invention contemplates the use of these sequences in gene therapy approaches. To this end, a promoter and the DNA that encodes a polypeptide according to the invention is inserted into a vector, which is then introduced into a subject suffering from a pathology, such as a cancer or infectious virus.

The construction of a suitable vector can be achieved by any of the methods well-known in the art for the insertion of exogenous DNA into a vector. *See, e.g.*, Sambrook *et al.*, *Molecular Cloning* (Cold Spring Harbor Press 2d ed. 1989), which is incorporated herein by reference. In addition, the prior art teaches various methods of introducing exogenous genes into cells *in vivo*. *See* Rosenberg *et al.*, *Science 242*:1575-1578 (1988), and Wolff *et al.*, *PNAS* 86:9011-9014 (1989), which are incorporated herein by reference. The routes of delivery include systemic administration and administration *in situ*. Well-known techniques include systemic administration with cationic liposomes, and administration *in situ* with viral vectors. Any one of the gene delivery methodologies described in the prior art is suitable for the introduction of a recombinant vector containing an inventive gene according to the invention into a MTX-resistant, transport-deficient cancer cell. A listing of present-day vectors suitable for the purpose of this invention is set forth in Hodgson, *Bio/Technology 13*: 222 (1995), which is incorporated by reference.

For example, liposome-mediated gene transfer is a suitable method for the introduction of a recombinant vector containing an inventive gene according to the invention into a MTX-resistant, transport-deficient cancer cell. The use of a cationic liposome, such as DC-Chol/DOPE liposome, has been widely documented as an appropriate vehicle to deliver DNA to a wide range of tissues through intravenous injection of DNA/cationic liposome complexes. *See* Caplen *et al.*, *Nature Med.* 1:39-46 (1995) and Zhu *et al.*, *Science 261*:209-211 (1993), which are herein incorporated by reference. Liposomes transfer genes to the target cells by fusing with the plasma membrane. The entry process is relatively efficient, but once inside the cell, the liposome-DNA complex has no inherent mechanism to deliver the DNA to the nucleus. As such, the most of the lipid and DNA gets shunted to cytoplasmic waste systems and destroyed. The obvious advantage of liposomes as a gene therapy vector is that liposomes contain no proteins, which thus minimizes the potential of host immune responses.

As another example, viral vector-mediated gene transfer is also a suitable method for the introduction of the vector into a target cell. Appropriate viral vectors include adenovirus vectors and adeno-associated virus vectors, retrovirus vectors and herpesvirus vectors.

Adenoviruses are linear, double stranded DNA viruses complexed with core proteins and surrounded by capsid proteins. The common serotypes 2 and 5, which are not associated with any human malignancies, are typically the base vectors. By deleting parts of the virus genome and inserting the desired gene under the control of a constitutive viral promoter, the virus becomes a replication deficient vector capable of transferring the exogenous DNA to differentiated, non-proliferating cells. To enter cells, the adenovirus fibre interacts with specific receptors on the cell surface, and the adenovirus surface proteins interact with the cell surface integrins. The virus penton-cell integrin interaction provides the signal that brings the exogenous gene-containing virus into a cytoplasmic endosome. The adenovirus breaks out of the endosome and moves to the nucleus, the viral capsid

falls apart, and the exogenous DNA enters the cell nucleus where it functions, in an epichromosomal fashion, to express the exogenous gene. Detailed discussions of the use of adenoviral vectors for gene therapy can be found in Berkner, *Biotechniques* 6:616-629 (1988) and Trapnell, *Advanced Drug Delivery Rev.* 12:185-199 (1993), which are herein incorporated by reference. Adenovirus-derived vectors, particularly non-replicative adenovirus vectors, are characterized by their ability to accommodate exogenous DNA of 7.5 kB, relative stability, wide host range, low pathogenicity in man, and high titers (10^4 to 10^5 plaque forming units per cell). *See* Stratford-Perricaudet *et al.*, *PNAS* 89:2581 (1992).

Adeno-associated virus (AAV) vectors also can be used for the present invention. AAV is a linear single-stranded DNA parvovirus that is endogenous to many mammalian species. AAV has a broad host range despite the limitation that AAV is a defective parvovirus which is dependent totally on either adenovirus or herpesvirus for its reproduction *in vivo*. The use of AAV as a vector for the introduction into target cells of exogenous DNA is well-known in the art. *See, e.g.*, Lebkowski *et al.*, *Mole. & Cell. Biol.* 8:3988 (1988), which is incorporated herein by reference. In these vectors, the capsid gene of AAV is replaced by a desired DNA fragment, and transcomplementation of the deleted capsid function is used to create a recombinant virus stock. Upon infection the recombinant virus uncoats in the nucleus and integrates into the host genome.

Another suitable virus-based gene delivery mechanism is retroviral vector-mediated gene transfer. In general, retroviral vectors are well-known in the art. *See* Breakfield *et al.*, *Mole. Neuro. Biol.* 1:339 (1987) and Shih *et al.*, in *Vaccines* 85: 177 (Cold Spring Harbor Press 1985). A variety of retroviral vectors and retroviral vector-producing cell lines can be used for the present invention. Appropriate retroviral vectors include Moloney Murine Leukemia Virus, spleen necrosis virus, and vectors derived from retroviruses such as Rous Sarcoma Virus, Harvey Sarcoma Virus, avian leukosis virus, human immunodeficiency virus, myeloproliferative sarcoma virus, and mammary tumor virus. These vectors

include replication-competent and replication-defective retroviral vectors. In addition, amphotropic and xenotropic retroviral vectors can be used. In carrying out the invention, retroviral vectors can be introduced to a tumor directly or in the form of free retroviral vector producing-cell lines. Suitable producer cells include fibroblasts, neurons, glial cells, keratinocytes, hepatocytes, connective tissue cells, ependymal cells, chromaffin cells. *See* Wolff *et al.*, *PNAS 84*:3344 (1989).

Retroviral vectors generally are constructed such that the majority of its structural genes are deleted or replaced by exogenous DNA of interest, and such that the likelihood is reduced that viral proteins will be expressed. *See* Bender *et al.*, *J. Virol.* 61:1639 (1987) and Armento *et al.*, *J. Virol. 61*:1647 (1987), which are herein incorporated by reference. To facilitate expression of the novel protein, a retroviral vector employed in the present invention must integrate into the genome of the host cell genome, an event which occurs only in mitotically active cells. The necessity for host cell replication effectively limits retroviral gene expression to tumor cells, which are highly replicative, and to a few normal tissues. The normal tissue cells theoretically most likely to be transduced by a retroviral vector, therefore, are the endothelial cells that line the blood vessels that supply blood to the tumor. In addition, it is also possible that a retroviral vector would integrate into white blood cells both in the tumor or in the blood circulating through the tumor.

The spread of retroviral vector to normal tissues, however, is limited. The local administration to a tumor of a retroviral vector or retroviral vector producing cells will restrict vector propagation to the local region of the tumor, minimizing transduction, integration, expression and subsequent cytotoxic effect on surrounding cells that are mitotically active.

Both replicatively deficient and replicatively competent retroviral vectors can be used in the invention, subject to their respective advantages and disadvantages. For instance, for tumors that have spread regionally, such as lung cancers, the direct injection of cell lines that produce replication-deficient vectors

may not deliver the vector to a large enough area to completely eradicate the tumor, since the vector will be released only from the original producer cells and their progeny, and diffusion is limited. Similar constraints apply to the application of replication deficient vectors to tumors that grow slowly, such as human breast cancers which typically have doubling times of 30 days versus the 24 hours common among human gliomas. The much shortened survival-time of the producer cells, probably no more than 7-14 days in the absence of immunosuppression, limits to only a portion of their replicative cycle the exposure of the tumor cells to the retroviral vector.

The use of replication-defective retroviruses for treating tumors requires producer cells and is limited because each replication-defective retrovirus particle can enter only a single cell and cannot productively infect others thereafter. Because these replication-defective retroviruses cannot spread to other tumor cells, they would be unable to completely penetrate a deep, multilayered tumor *in vivo*. *See* Markert *et al., Neurosurg. 77*: 590 (1992). The injection of replication-competent retroviral vector particles or a cell line that produces a replication-competent retroviral vector virus may prove to be a more effective therapeutic because a replication competent retroviral vector will establish a productive infection that will transduce cells as long as it persists. Moreover, replicatively competent retroviral vectors may follow the tumor as it metastasizes, carried along and propagated by transduced tumor cells. The risks for complications are greater, with replicatively competent vectors, however. Such vectors may pose a greater risk then replicatively deficient vectors of transducing normal tissues, for instance. The risks of undesired vector propagation for each type of cancer and affected body area can be weighed against the advantages in the situation of replicatively competent verses replicatively deficient retroviral vector to determine an optimum treatment.

Both amphotropic and xenotropic retroviral vectors may be used in the invention. Amphotropic viruses have a very broad host range that includes

most or all mammalian cells, as is well known to the art. Xenotropic viruses can infect all mammalian cells except mouse cells. Thus, amphotropic and xenotropic retroviruses from many species, including cows, sheep, pigs, dogs, cats, rats, and mice, *inter alia* can be used to provide retroviral vectors in accordance with the invention, provided the vectors can transfer genes into proliferating human cells *in vivo*.

Clinical trials employing retroviral vector therapy treatment of cancer have been approved in the United States. *See* Culver, *Clin. Chem. 40*: 510 (1994). Retroviral vector-containing cells have been implanted into brain tumors growing in human patients. *See* Oldfield *et al., Hum. Gene Ther. 4*: 39 (1993). These retroviral vectors carried the HSV-1 thymidine kinase (HSV-tk) gene into the surrounding brain tumor cells, which conferred sensitivity of the tumor cells to the antiviral drug ganciclovir. Some of the limitations of current retroviral based cancer therapy, as described by Oldfield are: (1) the low titer of virus produced, (2) virus spread is limited to the region surrounding the producer cell implant, (3) possible immune response to the producer cell line, (4) possible insertional mutagenesis and transformation of retroviral infected cells, (5) only a single treatment regimen of pro-drug, ganciclovir, is possible because the "suicide" product kills retrovirally infected cells and producer cells and (6) the bystander effect is limited to cells in direct contact with retrovirally transformed cells. *See* Bi *et al.*, *Human Gene Therapy 4*: 725 (1993).

Yet another suitable virus-based gene delivery mechanism is herpesvirus vector-mediated gene transfer. While much less is known about the use of herpesvirus vectors, replication-competent HSV-1 viral vectors have been described in the context of antitumor therapy. *See* Martuza *et al., Science 252*: 854 (1991), which is incorporated herein by reference.

The following examples are intended to be illustrative and not limiting.

WORKING EXAMPLES

EXAMPLE 1: *Isolation of the DNA encoding HAL*

The bacterium from the family *Corynebacteriaceae* that produces bioactive histidine ammonia lyase (HAL) was grown in 100 ml of Luria broth overnight at 30°C. The cells were harvested and resuspended in 10 ml of 50 mM Tris (pH 7.5) with 10 mM EDTA. Solid lysozyme was added to 0.2 mg/ml and the suspension was incubated at 4°C for 30 minutes. Following this incubation, the suspension was frozen for several hours at -70°C. Upon thawing, SDS was added to 0.1% and proteinase K was added to 0.2 mg/ml and was incubated at 37°C overnight. Next, RNAse was added to 0.1 mg/ml and the mixture incubated at 55°C for 30 minutes. The resulting DNA was extracted five times with an equal volume of phenol/chloroform (1:1) and precipitated with 2 volumes of absolute ethanol. The DNA was spooled out on a glass Pasteur pipette, washed with ice cold 70% ethanol, and resuspended in a minimal amount of TE buffer.

Genomic DNA was restricted with Sau3AI over a time course of 1 hour. Every ten minutes, an aliquot was removed, taken to 10 mM EDTA, and analyzed by agarose gel electrophoresis. The time point that showed an average fragment size of 1 - 5 kb was loaded in its entirety and resolved on an agarose gel. The 1- 5 kb fragments were isolated with DEAE filter paper, phenol extracted and ethanol precipitated.

The Bluescript vector pBSSK- (Stratagene) was restricted with BamHI and treated with arctic shrimp alkaline phosphatase (USB). The treated, linearized vector was subjected to agarose gel electrophoresis and the linear species were isolated as above with DEAE filter paper.

The resulting vector and genomic DNA fragment concentrations were measured and ligations conducted. These were done using 150 ng of vector

in a 10 μl reaction volume. Vector concentration was kept constant and insert was varied at stoichiometries of 0X, 0.5X, 1X, 2X, and 5X that of the vector. Ligations were performed at 4°C overnight. After ligation, reactions were diluted to 30 μl with water and heated to 65°C for ten minutes.

The diluted ligation reactions were used to transform freshly prepared electrocompetent XL-1Blue MRF' (Stratagene) cells. Transformed ligations were test plated on MacConkey agar to judge the best insert:vector ratio. Once the optimum ratio had been determined, this was used exclusively for transformation.

Transformants were plated at a cell density of 3 000 - 10 000 cfu per nylon filter on 150 mm plates containing LB agar with 50 μg/ml ampicillin. Duplicate replica filters were produced and processed for colony hybridization.

DNA probes were designed using regions of known histidine ammonia lyases that had a strong probability of being conserved in HAL. Using the Wisconsin Graphics GCG package pileup program, the peptide sequences of the known histidine ammonia lyases from *B. subtilis*, *S. griseus*, *P. putida*, and rat were aligned and examined for highly conserved regions. Several of these were chosen as candidates for probe design. Using the DNA sequences of cloned genes from *Corynebacterium* , a codon preference table was derived. From this a backtranslation was performed resulting in the most likely DNA sequence for the protein region of interest.

Two of the resulting probes (TM63 and TM74), shown in Table 1, below, were labeled, mixed, and used to screen the above genomic library. Oligos were labeled with γ^{32}PATP using T4 polynucleotide kinase as described (Ausubel, *et al*, eds, 1994. "Current Protocols in Molecular Biology," John Wiley and Sons, Inc.,) and cleaned up using Elutips (Schleicher & Schuell). Hybridization of duplicate filters was carried out in a Bellco hybridization oven at 37°C using the SSPE protocol as described (Ausubel, *et al.*, eds, "Current

Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994). Filters were washed in 6X SSC with 0.5%SDS (Ausubel, *et al*, eds, "Current Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994) at 37°C. Filters were then washed at successively higher temperatures in 3 M TMAC (Ausubel, *et al*, eds, "Current Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994) until very little radioactivity could be detected with a survey meter (generally 45 - 55°C). Upon exposure to X-Ray film (Kodak X-Omat), colonies which were evident on both replicate filters were picked with a wooden toothpick and transferred to a fresh nylon filter overlaid onto an LB/ampicillin plate. This procedure was repeated until a homogeneous population was achieved.

Table 1: oligonucleotides with DNA sequence and approximate coordinates relative to the ATG start codon.

Name	Length	Sequence (5' to 3')	Coordinates
TM63	30	CGCGTTCAGGACGCATACTCCGTTCGCTGC	838-867
TM74	24	GCCCATGGAAACGTGGTCTTCCTG	1370 - 1393
TM85	21	ATCATCATGCCCGAGTCCACA	1156 - 1176
TM87	21	GCCATCAGGAAGACCACGTTT	990 - 971
TM89	20	ATGCAGGAAGACCACGTTTC	1246 - 1265
TM91	21	ATCGAGGTCCGCCAATGCCAT	648 - 628
TM92	18	ACCGGAGCAGCCCAGTGA	441 - 424
TM93	20	TGCTTGAAGTATTGCGCCAG	1403 - 1422
TM94	18	GATCCTCGGGTGCGATGT	226 - 209
TM95	18	ATGCTGATCGGGCTTCGT	92 - 74

TM96	27	ATTTGATTCATATGGCTTCCGCTCCTC	-11 - +16
TM97	28	ATCTTGGATCCGAACATGGTGCGTTGCA	Beyond C-Terminus
TM98	18	AGCACCAGAT CGATGCAC	128 - 145
TM99	18	TGGCATGGGTGAACCGGT	267 - 284
TM101	18	ATCAGCGTTGAAGCCCAG	682 – 699
TM103	18	ACGTGCTGGACTTCCTTG	1019 - 1036
TM105	18	GTGCATAAGGCCCTCGAA	1501 - 1518
TM106	18	GAGCTTCGAGGGCCTTAT	1522 - 1505
TM109	18	CGAGCAACGCAGCGAGTA	870 - 853

Purified clones were confirmed by DNA sequencing and comparison to known peptide sequence and to known histidine ammonia lyases from the literature as well as peptide sequence from authentic histidine ammonia lyase from a bacterium belonging to the family *Corynebacteriaceae*. Using this protocol a primary clone, pHUT23, was isolated and identified as containing HAL coding sequence.

An oligo, TM85, was synthesized to the N-terminal-most region of pHUT23 and used to further screen the genomic library. This resulted in two clones, pHUT26 and pHUT28 which contain sequences more toward the N-terminus of the gene. These clones represent the C-terminal 2/3 of the gene. Another oligo, TM91, was synthesized based on the N-terminal-most sequence. This oligo was used to re-screen existing library plates. This resulted in the isolation of pHUT30, containing the N-terminal 1/3 of the gene. The authenticity of this gene was confirmed by comparison of peptide sequences obtained from the original enzyme isolated from a bacterium from the family *Corynebacteriaceae*.

Using both the full-length gene and the genomic sub-clones, the histidine ammonia lyase gene was sequenced in both directions by Sanger's chain-termination DNA sequencing method (USB). The purified double-stranded templates, shown with the primers used in Table 1, were denatured by the standard alkaline-denaturation method.

The sequence data revealed that the intact gene encompasses 1533 base pairs (see SEQ ID NO: 12), encoding a protein of 511 amino acids (see SEQ ID NO: 10). Expression of this open reading frame in *E. coli* results in a single, approximately 55 kDa polypeptide, as detected by denaturing polyacrylamide gel electrophoresis (see figure 5). Appearance of this 55 kDa peptide corresponds with induction of histidine ammonia lyase activity (conversion of L-histidine to urocanic acid), an activity detected by us under these conditions exclusively in *E. coli* harboring a plasmid containing the full-length HAL gene.

EXAMPLE 2: *Peptide sequencing of HAL*

HAL from a bacterium from the family *Corynebacteriaceae* that had been partially purified using ammonium sulfate and DEAE - Sephadex was resolved by SDS-PAGE. The separated material was electrophoretically transferred to Immobilon-P and stained with Coomassie Brilliant Blue. The major band of 55 000 daltons was excised and subjected to N-terminal sequencing. This fraction was sent to Commonwealth Biotechnologies, Inc. (Richmond, VA), cleaved with BrCN, HPLC purified, and fractions sequenced. The data are shown in Figure 2.

EXAMPLE 3: *Expression of Histidine Ammonia Lyase from a bacterium from the family* Corynebacteriaceae *Using a High-Efficiency Prokaryotic Expression System*

The *E. coli* expression plasmid pHUT102, depicted in Figure 4, is designed to express HAL DNA sequences from the strong phage T7 gene 10 promoter using T7 RNA Polymerase. This vector, pSN75, is a derivative of pET11b (Novagen) that has an additional transcriptional terminator inserted upstream of the T7 promoter. This provides the target cassette in as transcriptionally silent a context as possible.

Two mutagenic oligonucleotides, TM96 and TM97 (see Table 1), were synthesized based on histidine ammonia lyase sequence. The N-terminal oligo adds an NdeI site at the ATG start codon and the C-terminal oligo adds a BamHI site just beyond the C-terminus. These were used to thermal cycle amplify the HAL gene out of the genome of a bacterium from the family *Corynebacteriaceae* . The resulting fragment was restricted with NdeI and BamHI and cloned into pSN75, resulting in pHUT102. This provides histidine ammonia lyase under the control of a T7 promoter with flanking transcriptional terminators to prevent readthrough transcription.

A T7 expression system containing kanamycin as a selectable marker was constructed by cloning the 1 kb kanamycin resistance Pst I cassette excised from pUC4K into the unique Pst I site of pSN75. The new vector, pSN75K is ampicillin sensitive and kanamycin resistant. The Nde I- BamHI fragment containing the HAL coding region was excised from pHUT102 and cloned into NdeI + BamHI cut pSN75K. This expression construct, pHUT200, could readily be used for clinical production of HAL since there is no need to use penicillin antibiotics during the production.

For the purpose of expression, pHUT200 was transformed into BL21(λDE3) harboring pLysS and grown in Terrific broth at 28°C to OD600 of

0.6. The culture was induced for 4 hours with 0.4mM IPTG and harvested. Cells were lysed and analyzed by SDS-PAGE and enzyme assay. As measured by these assays, we estimate that the HAL is produced to approximately 30% of the total cell protein (see Figure 5) and 8% of the soluble protein, representing about 0.2g per liter of culture.

EXAMPLE 4: *Expression of HAL in a Vector That Directs Periplasmic Localization.*

The NdeI/BamHI fragment from pHUT102 was excised and purified. The vector, pET12c (Novagen), was likewise cut with NdeI/BamHI and purified. These fragments were ligated and transformed into XL-1 Blue MRF'. The clone, pHUT114, containing the HAL gene as a fusion with a T7 phage periplasmic localization signal under control of a T7 promoter.

For the purpose of expression, pHUT114 was transformed into BL21(λDE3) and grown in Terrific broth supplemented with 75mM NaCl at 28°C to OD600 of 0.6 under antibiotic selection. The culture was induced for 4 hours with 0.4mM IPTG and harvested. Spheroplasts were prepared (Ausubel, *et al*, eds, "Current Protocols in Molecular Biology," John Wiley and Sons, Inc., 1994) and the supernatant was assayed for enzyme.

This approach yielded active, periplasmic HAL. However, microscopic examination showed the formation of inclusion bodies. This resulted in relatively low per volume yield and a specific activity comparable to the cellularly localized material. At this point, this approach offers no clear advantage. However, if inclusion body formation could be minimized by altering growth and induction conditions, it is possible that the enzyme could be purified directly from the culture medium.

EXAMPLE 5: *Fed Batch Fermentation for the Production of HAL from a bacterium from the family* Corynebacteriaceae.

Fermentation of pHUT200 in BL21λ21(DE3)pLysS is performed at 30°C in the following media: Base media- 20 g/L yeast extract and 1.67 g/L $(NH_4)_2SO_4$, supplemented with 17.2 ml of 1 M KH_2PO_4, 36.7 ml of 1 M K_2HPO_4, 1 ml/L of 2% $CaCl_2.H_2O$, 1 ml/L of 10% thiamine-HCl, 10 ml/L of Trace Metal Solution (6 g/liter Fe(III)Citrate, 1.5 g/L $MnCl_2.H_2O$, 0.8 g/L $Zn(CH_3COO)_2.2H_2O$, 0.3 g/L H_3BO_3, 0.25 g/L $Na_2MoO_4.2H_2O$, 0.25 g/L $CoCl_2.6H_2O$, 0.15 g/L $CuCl_2.2H_2O$, 0.84 g/L EDTA), 10 ml/L of 20% $MgSO_4.7H_2O$, and 10 ml/L of 50% glucose. Bring to final volume of 1 Liter with ddH_2O. When pH increases by 0.01 the feed media is initiated. The feed media is as follows: 1.5 g/L $(NH_4)_2SO_4$, 274 g/L yeast extract, 7.5 ml/L $MgSO_4.7H_2O$, and 400 ml 50% glucose. The feed was stopped when the pH decreased by 0.01. Thus, pH was maintained by the feed control loop. The fermentation run is induced at OD_{600} = 5.0 with 1 mM IPTG. The dissolved oxygen is maintained at 20%, and induction is continued for 4 hours. The final OD_{600} = 32, and the yield is approximately 1 gram of HAL per liter. The amount of protein following different periods of induction is shown in the SDS-PAGE gel in figure 5.

EXAMPLE 6: *Purification of HAL from* E. coli.

A simple purification method involving two acetone precipitation steps and one Q-sepharose column. Following resuspension of the cell paste in one-tenth volume of 50 mM TRIS pH 8.0 the pellet is sonicated four times and centrifuged. An equal volume of acetone is added to the supernatant. The solution is then centrifuged at 14,000 rpm for 15 minutes. The supernatant is retained and an equal volume of acetone is again added and again centrifuged. Following the second acetone precipitation the pellet is resuspended in 50 mM

TRIS pH 8.0. The resuspension is then loaded onto a Q-sepharose column (5 mg protein/ ml Q-sepharose) in 20 mM TRIS pH 8.0. The column is then washed with 20 mM TRIS pH 8.0 with 0.1 M KCl. Elution is performed with a 200 ml gradient from 0.1 M to 0.6 M KCl at a flow rate of 1 ml/min. Phenyl sepharose can then be used to further purify the enzyme. An example of purification via this scheme is depicted in figure 6.

Several potential alternate methods of purification have also been used successfully. HAL is resistant to heating at 70°C. Thus, heating and centrifugation can be used to remove precipitated contaminant proteins. Additionally, HAL does not precipitate with the addition of ammonium sulfate to 30% saturation. Therefore, addition of 30% ammonium sulfate and centrifugation can also be used to remove contaminant proteins. This precipitation can then be followed by purification via a phenyl sepharose column.

EXAMPLE 7: *Recovery of HAL from inclusion bodies.*

The pellet obtained from the sonicate of EXAMPLE 6 was washed in 100mM sodium phosphate, pH6 containing 0.5% Triton X-100 by trituration. The washed inclusion bodies were collected by centrifugation at 4°C in an SS34 rotor at 10 000 rpm for 10 minutes. This was repeated twice more, resulting in purified inclusion bodies.

A small amount of this material was solubilized in 50 mM Tris, pH8 with 8 M urea. Two hundred micrograms of this material was bound to 0.5 ml of DEAE-sephadex equilibrated in the same buffer. The resin was collected by centrifugation and eluted with 1 ml of 50 mMTris, pH8 with 0.5 M NaCl. This material was directly assayed for histidine ammonia lyase activity. A typical recovery yielded approximately 1 - 5 % of the total histidine ammonia lyase in the active conformation.

EXAMPLE 8: *Characterization of HAL.*

Purified HAL has been determined to have approximately 40 I.U./mg of activity at 37°C. The temperature optimum was found to be 45°C (figure 7). The graph shows that the enzyme maintains a significant level of activity at physiological temperature conditions. Also below is a graph depicting the effect of pH on HAL activity. The activity profile of HAL at various pH is depicted in figure 8. The enzyme is active over a wide range of pH, with highest activity around pH 8.2 and high activity in physiological conditions.

Both reduced glutathione and DTT caused inhibition of HAL, but the inhibition was not complete. Both compounds were required at 15 mM concentrations to reduce the activity by half. EDTA was found to completely inhibit the reaction in concentrations as low as 1 mM. This inhibition was reversible with the presence of Mn^{2+} at 1 x 10^{-5} M concentration.

Histidine ammonia lyase produced in *E. coli* was purified to near homogeneity as detailed above. Female mice weighing 18 - 22 grams were injected intraperitoneally with 1500 IU/kg body weight. HAL activity was monitored by assaying plasma obtained via retro-orbital bleeding two and ten hours following injection. These experiments using the recombinant enzyme showed an *in vivo* half-life of approximately 3 hours.

EXAMPLE 9: *Comparison of HAL to Other Histidine Ammonia Lyases.*

HAL has a significant advantage over other histidine ammonia lyases in that it is relatively resistant to inhibition by L-histidinol. L-histidinol is a histidine analog. Because L-histidinol acts as a competitive inhibitor it must be present in high concentrations to have an effect on histidine dependent reactions. Using HAL to reduce the overall histidine pool would therefore greatly increase the effectiveness of L-histidinol. However, other histidine ammonia lyase enzymes have been shown to be strongly inhibited by L-histidinol. Because the

Corynebacteriaceae histidine ammonia lyase (HAL) is not inhibited by L-histidinol at therapeutic L-histidinol levels it has a great advantage over other histidine ammonia lyases. Histidine ammonia lyase isolated from *Achromobacter liquidum* and *Streptomyces griseus* have been shown to be inhibited by L-histidinol and L-histidinol phosphate respectively with a Ki of 4.58 and 0.27 mM (Shibatani, T. *et al.* 1975.; Wu, P. C. *et al.* 1995). Enzyme kinetic studies in our laboratory using *Streptomyces griseus* histidine ammonia lyase, showed that L-histidinol was able to completely inhibit the enzyme even at equal molar concentration to histidine. However, with HAL 20% of activity still is retained when L-histidinol is present at 10 times the concentration of L-histidine. We demonstrated the Ki of L-histidinol for HAL to be 24.3 to 33.4 mM.

EXAMPLE 10: *Extension of Half-life and Prevention of Antibody Formation to HAL Using PEGylation.*

A PEGylation strategy has been developed using BTC-PEG 5000 (Shearwater Polymers, Inc.). HAL is reconstituted in 50 mM sodium phosphate buffer pH 8.0 and dialyzed against that same buffer. Dialysis is continued for three hours. Following dialysis the protein concentration is adjusted to 5 mg/ml. BTC-PEG is added in a ratio of 1:10 and incubated for one hour at RT after dissolving the BTC-PEG. The solution is then dialyzed against 50 mM sodium phosphate pH 7.5 to remove unbound PEG. The extent of PEGylation is then determined by using a fluorescamine assay. Different ratios of PEG to enzyme were tested to determine the % PEGylation each ratio would yield. Repeated fluorescamine assays showed that 1:10 PEGylation with BTC-PEG provided about 40 - 45 % PEGylation protection of the enzyme.

When HAL was injected into mice, the bioactive half-life was determined to be less than four hours. To determine the half-life of the enzyme a known amount of units of enzyme is injected intraperitonially into several mice. Blood is then drawn retro-orbitally from different mice at 4-hour intervals. The

blood is then centrifuged and serum is used to perform the histidine ammonia lyase assay as previously described. The half-life is then determined by comparing the time at which units/ml in serum is half of its value following the first four hour time-point.

A 30 Unit HAL intraperitonial injection yielded only 3 Units of active HAL in the blood following four hours and the half-life was found to be less than one hour. HAL was then PEGylated using BTC-PEG. This increased the half-life of the enzyme in the blood to more than 48 hours. The PEGylation of the enzyme also affords it protection against antibody-mediated depletion in the host. UnPEGylated protein will elicit an antibody response that will clear the enzyme from the blood following one week of treatment. The antibody response is greatly delayed in mice receiving PEGylated HAL. In three out of five mice active enzyme was still being recovered following 79 days of treatment and following 119 days in 2 of the five mice.

We have also used a higher molecular weight PEG, BTC 20,000, as well as other PEGs to successfully modify HAL.

EXAMPLE 11: *Anti-viral Activity of HAL.*

HAL was tested for its anti-viral activity against a variety of infectious viruses *in vitro*. Effectiveness against Herpes Simplex Virus (HSV) was assayed using the following method. Confluent VERO cells from a T-175 flask are tyrpsinized and split into as many T-25 flasks as the experiment requires. The cells are grown in RPMI-1640 with 10% newborn calf serum-heat inactivated, and L-glutamine. After cells have grown to confluence remove the media and add 0.5 ml of diluted virus (make a series of 1:5 dilution). Dilution of virus is prepared in RPMI-1640 with 2% NCS. The cells are then incubated for 1 hour at 37°C and then 5 ml of RPMI-1640 with 2 % NCS with or without the test compounds is added and incubation is allowed to continue for 1 day. After 24 hours the flasks are sealed with parafilm and frozen at -70°C. The cells are

then allowed to thaw at RT to lyse dead intact cells, releasing virus. The viral suspension is then centrifuged to remove cell debris. The viral dilution that caused complete lysis will be used in the plaque assay. The plaque assay is set up by trypsinizing cells from a T-75 and resuspending the cells in RPMI-1640 with 10% NCS and poured into 6-well plate at 2 ml/well. The cells are incubated overnight at 37°C. The media is then removed by suction and 0.2 ml of the viral dilution is added and allowed to incubate for one hour. During this hour the agar is prepared and stored at 41°C to prevent hardening. The agar concentrations are as follows: ½ vol. 2X BME (Gibco), 2 % Pen/Strep, 2 % NCS, and 1 % agar. After the hour incubation 2 ml of the BME/agar is carefully added to the wells, so as not to disturb the cell monolayer. The plate is left at RT for twenty to thirty minutes to allow the agar to harden, and then the cells are incubated for 48 hours at 37°C. The cells are then stained used BME/agar containing neutral red. The BME/agar is prepared as before with the addition of neutral red stain (Gibco) to a final concentration of 5%. 2 ml of this agar is added and allowed to harden before 24 hour incubation at 37°C. Plaques are then counted and the cells fixed for a permanent record.

One positive experimental result has been seen against HSV. Herpes Simplex Viruses are double-stranded DNA viruses belonging to the family *Herpesviridae*. Several viruses in this group, including Herpes Simplex Virus Type 1, Herpes Simplex Virus Type 2, Varicella-Zoster Virus, Epstein-Barr Virus, and Cytomegalo Virus cause serious and often fatal infections in human beings. Results of the experiments using HSV have indicated that HAL does inhibit HSV replication and that when given in conjunction with L-histidinol even significantly greater inhibition is observed. Use of HAL alone in a concentration of 0.005 U/ml resulted in a reduction in plaque forming units of approximately 200 fold versus control. Use of 0.01 U/ml HAL resulted in over 1,000-fold decrease in plaque forming units/ml. However, when given in conjunction with L-histidinol the effect is greatly enhanced and the concentration

of both compounds can be significantly lowered while inhibition is increased. When L-histidinol is given at 0.1 and 0.5 mM concentrations no inhibition is observed, and only 5-fold inhibition is observed at 1 mM. However, when 0.5 mM L-histidinol is given in combination with 0.003 U/ml HAL the inhibition is near 100% (less than 500 PFU versus 1.25 x 10^8 in control). These results are shown in figure 9.

Another successful study has been with Respiratory Syncytial Virus (RSV). RSV is another highly infectious disease causing agent. It causes lower respiratory-tract infections such as bronchitis and pneumonia in infancy and early childhood, with nearly 50% of infants suffering from an RSV infection during their first winter. Experiments were performed in tissue culture using the RSV Plaque Assay. The RSV plaque assay is performed similarly to the HSV assay. When performing the RSV assay Hep2 cells are used to propagate the RSV and the cells are grown in EMEM media with 2 % FBS, L-glutamine and Antibiotic-Antimycotic. The virus stock is diluted in the medium and added to each well with or without the desired testing compound. The plates are incubated for 2 hours at 37°C and the virus is removed. 0.5% agarose is added to the media and incubated for 5 days at 37°C. The plates are then fixed with 10 % formalin and stained with crystal violet. When HAL and L-histidinol were present in 0.005 U/ml and 3 mM respectively, no inhibition of RSV plaque numbers is seen, as is shown in figure 11. However, when these same concentrations were used in combination the resulting plaque assay showed no higher than background levels of plaques. These results indicate the strong synergy that exists with these two drugs and provides promise for HAL as an effective antiviral therapy.

Use of HAL in combination with L-histidinol is thus shown to greatly reduce the therapeutic index of both drugs. This makes effective treatment of these highly infectious diseases with low toxicity a good possibility.

Rauscher Murine Leukemia Virus (RMuLV) belongs to the family *retroviradae*, the group of viruses that also includes the Human Immunodeficiency Virus (HIV). We have used RMuLV as a model for drug development against HIV. In these studies we use mouse SC-1 cells persistently infected with RMuLV to test the effect of HAL against virus replication. Briefly, the cells are plated on a 96-well plate in RPMI medium containing 10% Fetal Calf Serum and glutamine. After 24 hours of growth, the cells are treated with various test compounds for 24 hours. The supernatant is then tested for reverse transcriptase activity as described in Roberts, J. and W. G. McGregor (Roberts, J. and W. G. McGregor. 1991. *J. General Virology*. 72: 299-305). Results depicted in Figure 12 show that HAL given at 0.004 U/ml inhibits reverse transcription by over 70%.

Example 12: *Effectiveness of HAL as an Anticancer Agent.*

We have used a sensitive *in vitro* cancer drug screening assay to study the effect of HAL on various tumor cell lines. Briefly, each cell line is inoculated into microtiter plates, and pre-incubated for 24 hours at 37°C. Subsequently, the test agents are added and the culture is incubated for an additional 48 hours at 37°C. End-point determination of cell growth is performed by *in situ* fixation of cells, followed by staining with a protein-binding dye, sulforchodamine B (SRB) (Monks, A., Scudiero, D. Skehan, P., Shoemaker, R., Paull, L., Vistica, D., Hose, C., Langley, J., Cronise, P., Vaigro-Wolff, A. *et al.* 1991. *Journal of National Cancer Institute.* 83(11): 757-766). Two human prostate cancer cell lines, LNCaP and PC-3, were tested using this assay. We found the growth of human prostate cancer cell line LNCaP was inhibited by 69% by 0.005 U/ml HAL, and PC-3 is inhibited by 81% by 0.01 U/ml HAL In addition to prostate cancer, three cell lines of human ovarian cancer were tested. The growth of ovarian cancer cell lines SKOV-3 and MA148 were inhibited by 78% and 95% respectively by 0.01 U/ml HAL, and OVCA3

is inhibited by 53% with 0.005 U/ml HAL. In addition to the prostate and ovarian cancer cell lines C6 glioblastoma cells were tested and HAL was found to inhibit growth by 95% at a concentration of 0.01 U/ml.

The results of this widely accepted assay are very encouraging. Clearly, HAL is very effective in controlling the growth of a wide variety of human tumors *in vitro* and is potentially an effectively anticancer therapeutic.

WE CLAIM:

1. An isolated polypeptide having histidine ammonia lyase activity, wherein said histidine ammonia lyase activity is not substantially decreased in the presence of a histidine analog.

2. A polypeptide according to claim 1, wherein the histidine analog is histidinol.

3. An isolated polypeptide according to claim 1, comprising a sequence selected from the group consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 6.

4. An isolated polypeptide according to claim 3, wherein the polypeptide has a monomeric molecular weight between about 30,000 to 70,000 daltons.

5. An isolated polypeptide according to claim 4, wherein the polypeptide has a monomeric molecular weight of about 56,000 daltons.

6. A method for PEGylating a polypeptide, comprising reacting a PEG with the polypeptide according to claim 1.

7. A method of treatment, comprising administering to a patient suffering from a viral infection a therapeutic amount of a polypeptide having histidine ammonia lyase activity.

8. A method according to claim 7, wherein the histidine ammonia lyase activity is not substantially decreased in the presence of a histidine analog.

9. A method according to claim 8, wherein the histidine analog is histidinol.

10. A method according to claim 8, further comprising administering to a patient in need of treatment a therapeutic amount of a histidine analog.

11. A method according to claim 8, wherein the virus is selected from the group consisting of Herpes Virus Type 1, Herpes Simplex Virus Type 2, Varicella-Zoster Virus, Epstein-Barr virus, Cytomegalovirus, Respiratory Syncytial Virus, and Human Immunodeficiency Virus.

12. A method for treating a patient suffering from a cancer, comprising administering to the patient suffering from said cancer a therapeutic amount of the polypeptide in claim 1 and a therapeutic amount of a histidine analog.

13. A method for treating disease, comprising administering to a patient a therapeutically effective amount of a polypeptide having histidine ammonia lyase activity, and administering to said patient a therapeutically effective amount of a chemotherapeutic agent or a retroviral vector.

14. A method according to claim 13, wherein upon the administration of said polypeptide, non-diseased cells of said patient enter a reversible quiescent state.

15. A method according to claim 13, wherein the polypeptide is a PEGylated polypeptide.

16. A method for delivering an immunosuppressant to a patient, comprising: administering to a patient a therapeutically effective amount of a polypeptide having histidine ammonia lyase activity, wherein said polypeptide generates trans-urocanic acid (t-UA) *in vivo*; and subjecting the patient to an irradiating agent, wherein said irradiating agent causes the photoisomerization of

t-UA to its cis isomer (c-UA), and wherein said cis isomer comprises an immunosuppressive property.

17. A method according to claim 16, wherein the irradiating agent is UVB irradiation, and wherein the polypeptide is a PEGylated polypeptide.

18. A method according to claim 17, wherein the patient has an immune system disorder.

19. A method according to claim 18, wherein the UVB radiation is localized.

20. A method according to claim 16, further comprising administering to the patient a transplanted organ.

21. An isolated DNA sequence comprising SEQ ID NO: 7.

22. An expression vector comprising the DNA sequence of claim 21.

23. A method for treating a patient comprising constructing an expression vector according to claim 22 and introducing said expression vector into the patient.

Figure 1: Restriction pattern of the HAL coding region cut with selected enzymes.

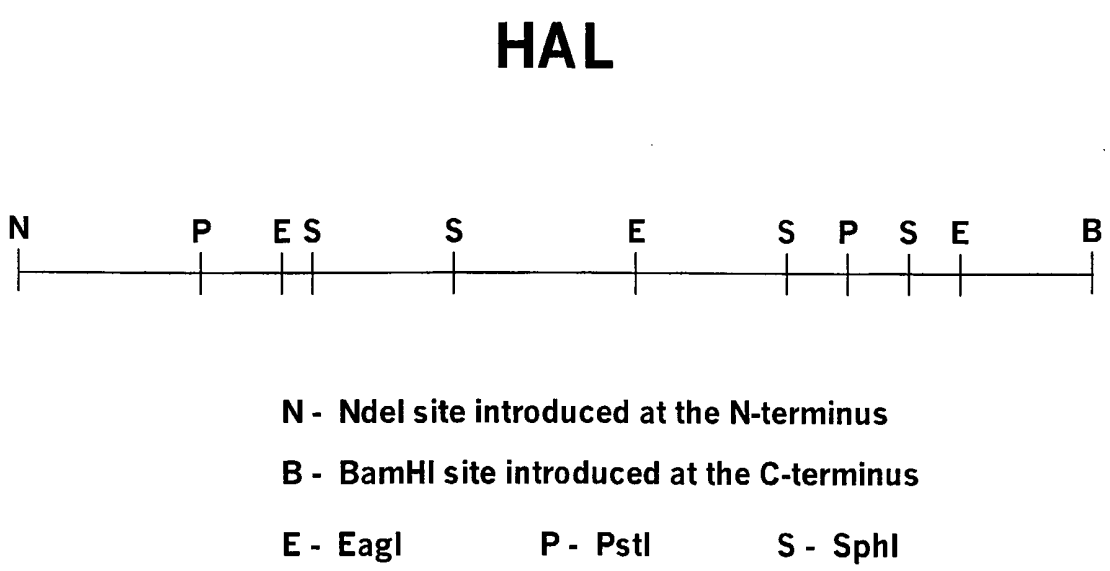


Figure 2: Experimentally derived peptide sequences of HAL

N-terminal

(M)ASAPQITLGLSGATAD

Internal

(M)ALADLDELLDEA

(M)GEPVEREVLRA

Figure 3: SphI digestion pattern of HAL gene showing oligonucleotide and subclones.

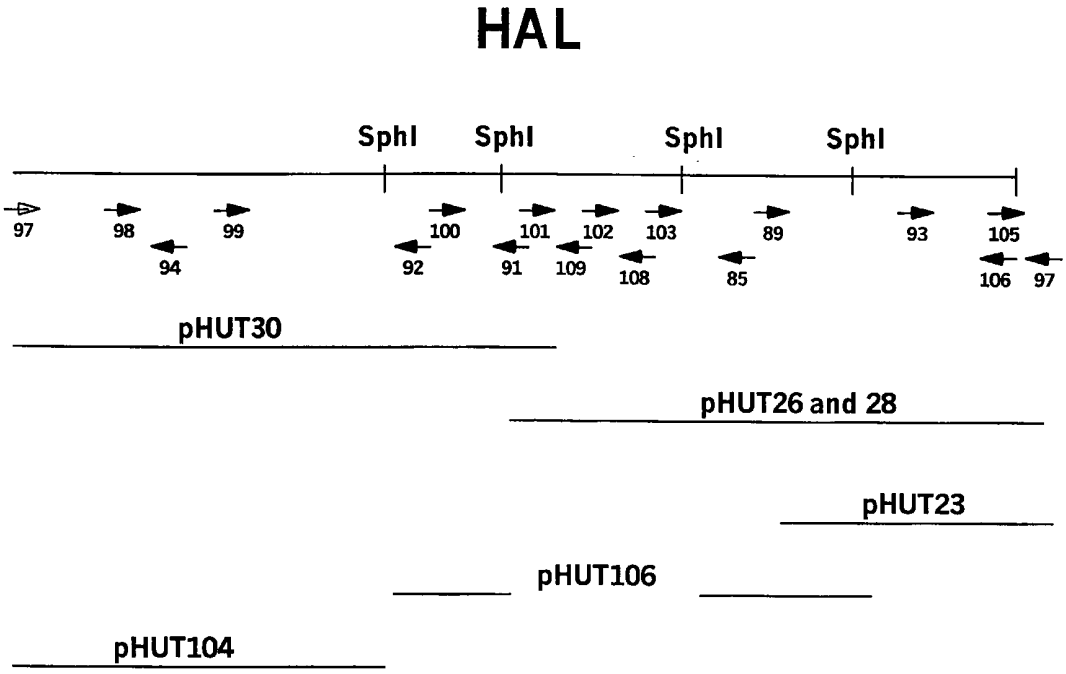


Figure 4: Histidine ammonia lyase overexpressing plasmid.

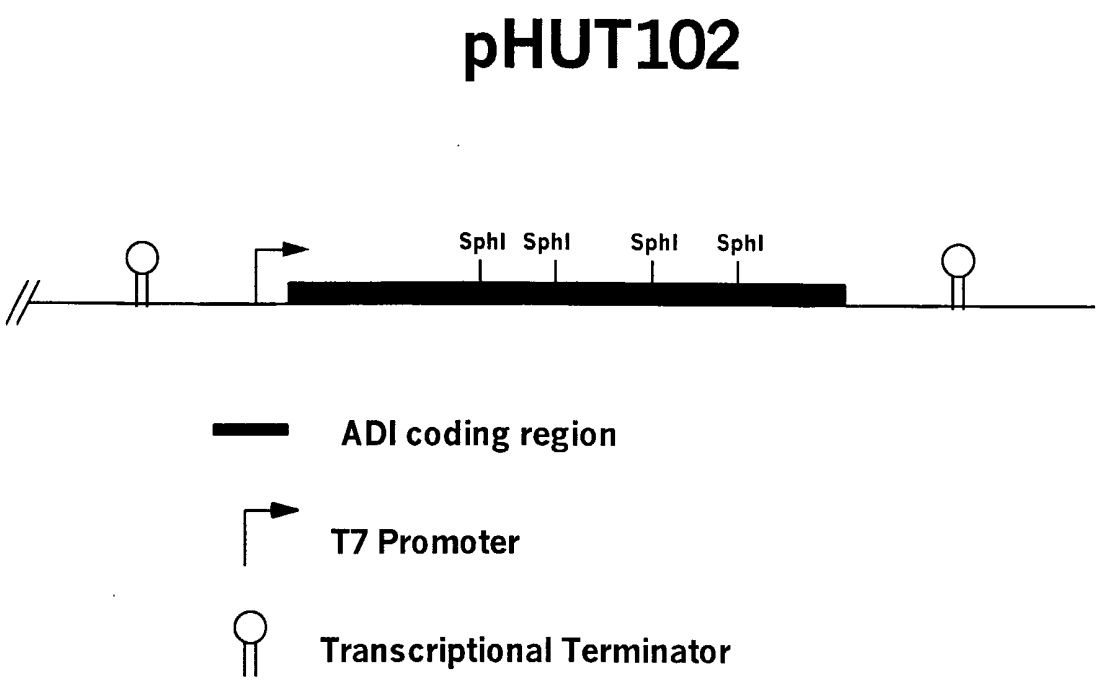


Figure 5: SDS-PAGE showing expression of HAL in *E. coli*.

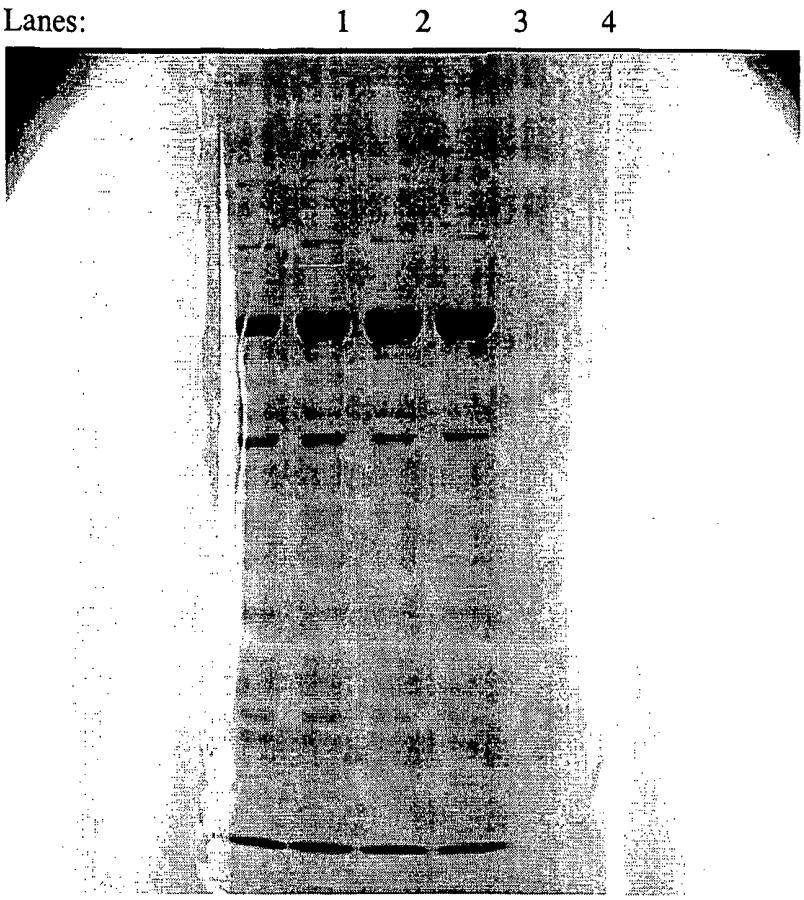


Figure 6: SDS-PAGE showing purification of HAL from *E. coli*

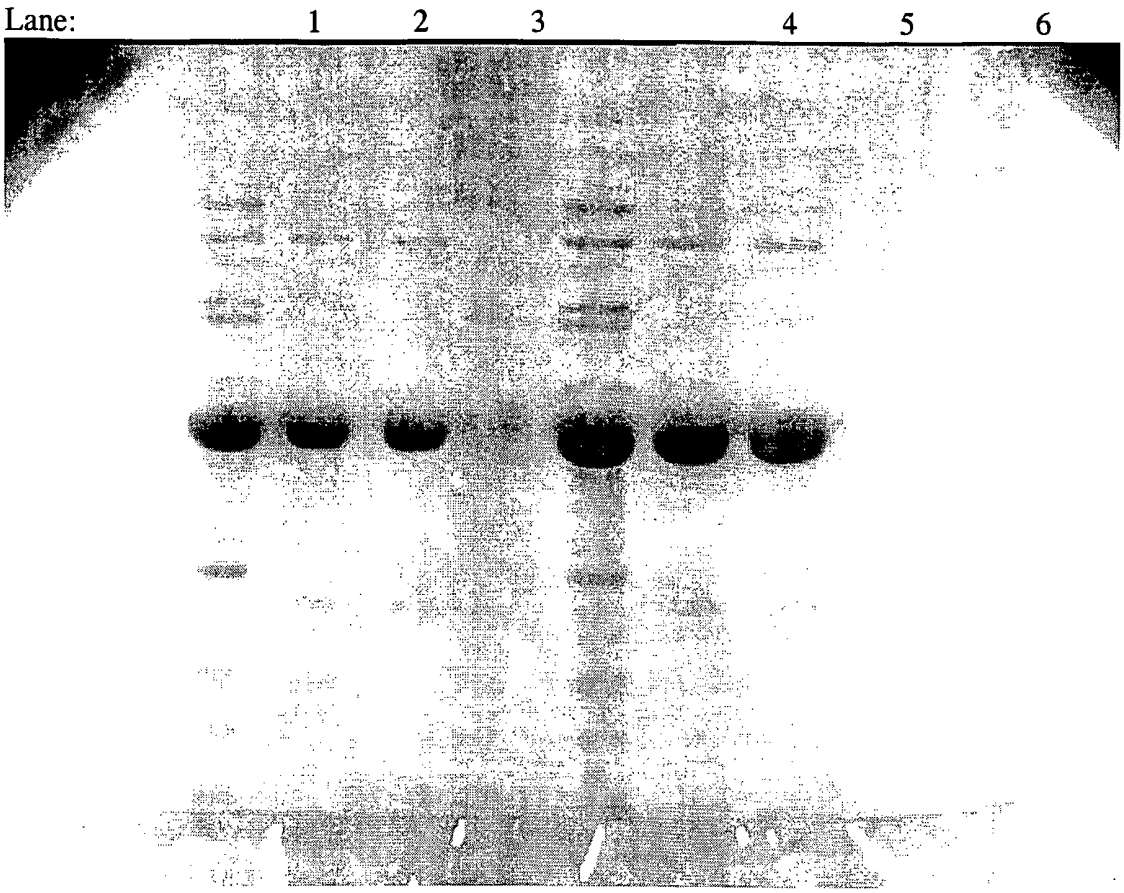


Figure 7: Effect of Temperature on HAL

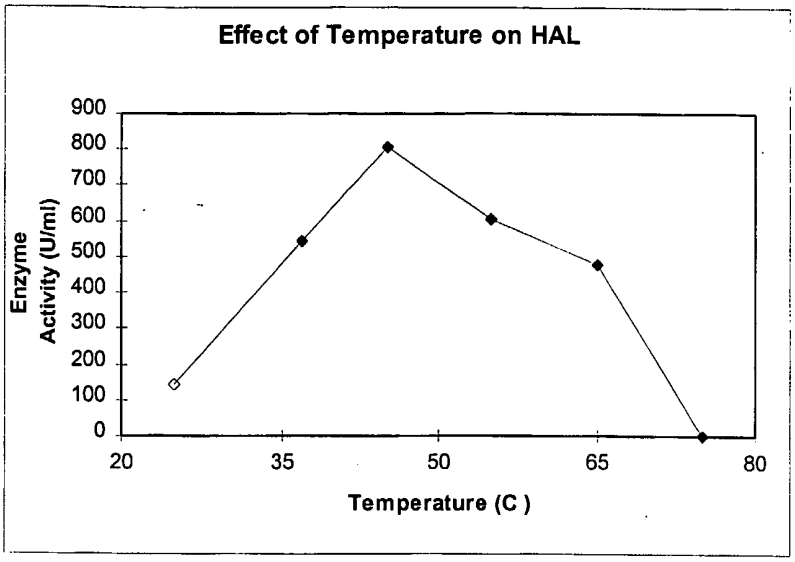


Figure 8: Effect of pH on HAL.

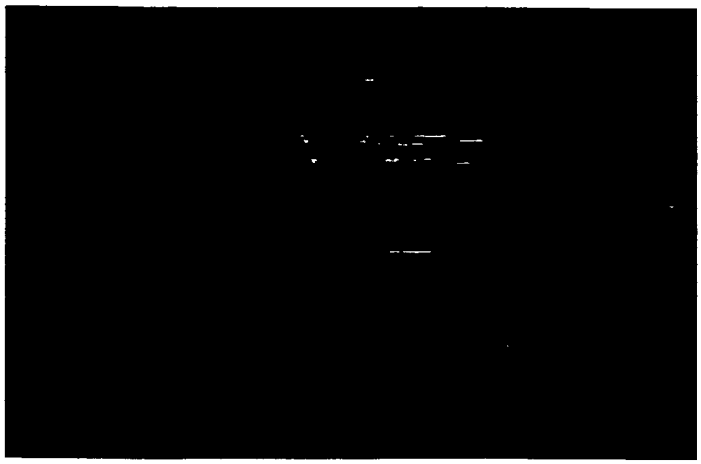


Figure 9: Effect of HAL and Histidinol on HSV.

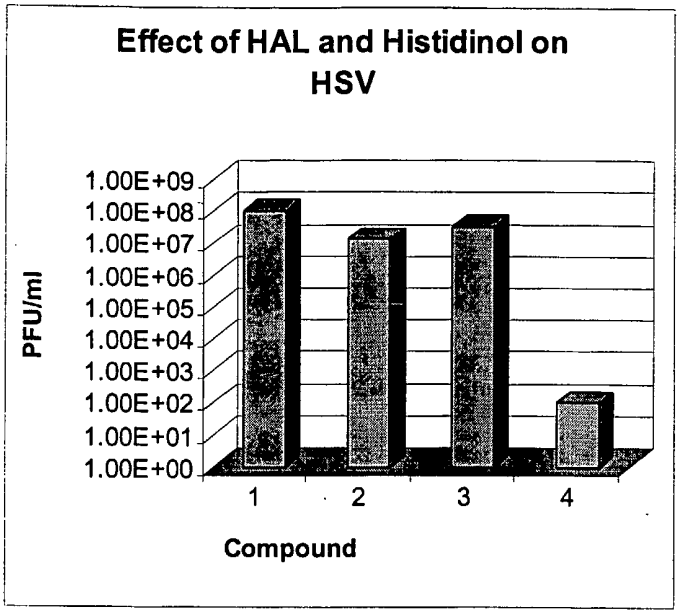


Figure 10: Effectiveness of L-histidinol as a Single Agent

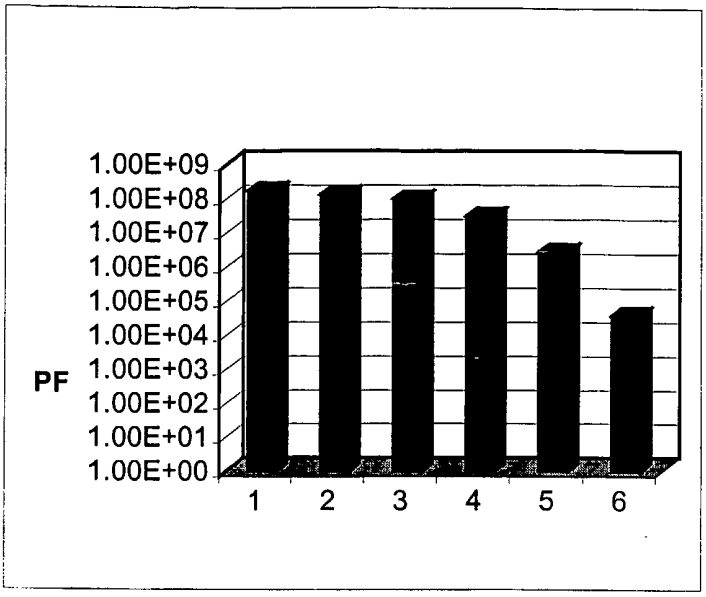


Figure 11: Effect of HAL and Histidinol on RSV.

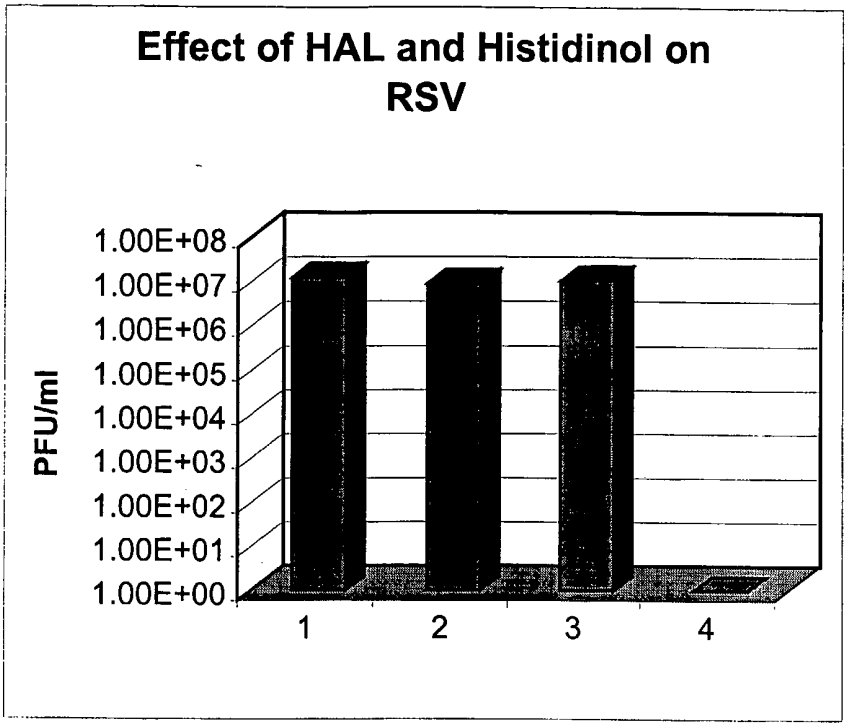


Figure 12: Effect of HAL on RMuLV.

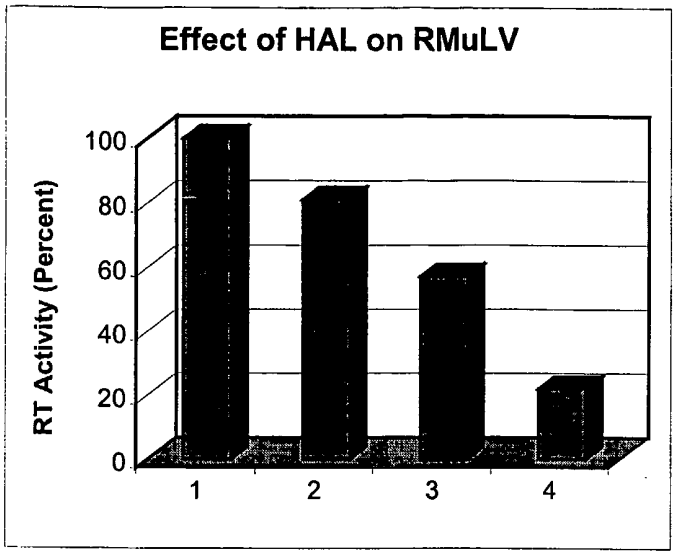


Figure 13: Histidine ammonia lyase peptide sequence pileup

```
HUTH_PSEPU      ------------------------------------------------------------
-
HUTH_RHIME      ------------------------------------------------------------
-
HUTH_MOUSE
MPRYTVHVRGEWLAVPCQDGKLTVGWLGREAVRRYMKNKPDNGGFTSVDEVQFLVHRCKG
HUTH_RAT
MPRYTVHVRGEWLAVPCQDGKLSVGWLGREAVRRYMKNKPDNGGFTSVDEVRFLVRRCKG
HUTH_HUMAN
MPRYTVHVRGEWLAVPCQDAQLTVGWLGREAVRRYIKNKPDNGGFTSVDDAHFLVRRCKG
HUTH_CAEEL      -MRLQVQIGTECVVVPCKP-DDTIHAVAKKSVEKLRRLRPK----
LPLADDYFEVRRTVG
HUTH_BACS       ------------------------------------------------------------
-
HUTH_STRGR      ------------------------------------------------------------
-
HUTH_CORY       ------------------------------------------------------------
-


HUTH_PSEPU      ------------------------------------------------------------
-
HUTH_RHIME      ------------------------------------------------------------
-
HUTH_MOUSE      LGLLDNEDELEVALEDNEFVEVVIEGDVMS--------PDFIPSQPEGVFLYSKYR---
-
HUTH_RAT        LGLLDNEDLLEVALEDNEFVEVVIEGDVMS--------PDFIPSQPEGVFLYSKYR---
-
HUTH_HUMAN      LGLLDNEDRLEVALENNEFVEVVIEGDAMS--------PDFIPSQPEGVYLYSKYR---
-
HUTH_CAEEL
NSLLDPEDLVSDVLKDSDFIIVAASVEETEDAKEAKKQEEIDNARAEIEKIDNRRRKVSF
HUTH_BACS       ------------------------------------------------------------
-
HUTH_STRGR      ------------------------------------------------------------
-
HUTH_CORY       ------------------------------------------------------------
-


HUTH_PSEPU      ------------
TELTLKPGTLTLAQLRAIHAAPVRLQLDASAAPAIDASVACVEQIIA
HUTH_RHIME      ------------
MTVILRPGSVPLSDLETIYWTGAPARLDAAFDAGIAKAAARIAEIVA
HUTH_MOUSE      ----------
EPEKYIALDGDSLSTEDLVNLGKGRYKIKLTSIAEKKVQQSREVIDSIIK
HUTH_RAT        ----------
EPEKYIALDGDSLSTEDLVNLGKGHYKIKLTSIAEKKVQQSREVIDSIIK
HUTH_HUMAN      ----------
EPEKYIELDGDRLTTEDLVNLGKGRYKIKLTPTAEKRVQKSREVIDSIIK
HUTH_CAEEL
ADSLAPMVLAPPTKLLILDGNSLLPEDLVRCEKGECAIQLSMESEDRIRKARTFLEKIAS
HUTH_BACS       --------------
MVTLDGSSLTTADVARVLFDFEEAAASEESMERVKKSRAAVERIVR
HUTH_STRGR      ----------
MDMHTVVVGTSGTTAEDVVAVARHGARVELSAAAVEALAAARLIVDALAA
HUTH_CORY       ---------
MASAPQITLGLSGATADDVIAVARHEARISISPQVLEELASVRAHIDALAS
```

Figure 13 cont'd.

```
HUTH_PSEPU
EDRTAYGINTGFGLLASTRIASHDLENLQRSLVLSHAAGIGAPLDDDLVRLIMVLKINSL
HUTH_RHIME
GNAPVYGINTGFGKLASIKIDSSDVATLQRNLILSHCCGVGQPLTEDIVRLIMALKLISL
HUTH_MOUSE
ERTVVYGITTGFGKFARTVIPANKLQELQVNLVRSHSSGVGKPLSPERCRMLLALRINVL
HUTH_RAT
ERTVVYGITTGFGKFARTVIPANKLQELQVNLVRSHSSGVGKPLSPERCRMLLALRINVL
HUTH_HUMAN
EKTVVYGITTGFGKFARTVIPINKLQELQVNLVRSHSSGVGKPLSPERCRMLLALRINVL
HUTH_CAEEL
EHRAVYGVTTGFGTFSNVTIPPEKLKKLQLNLIRSHATGYGEPLAPNRARMLLALRINIL
HUTH_BACS
DEKTIYGINTGFGKFSDVLIQKEDSAALQLNLILSHACGVGDPFPECVSRAMLLLRANAL
HUTH_STRGR
KPEPVYGVSTGFGALASRHIGTELRAQLQRNIVRSHAAGMGPRVEREVVRALMFLRLKTV
HUTH_CORY
ADTPVYGISTGFGALATRHIAPEDRAKLQRSLIRSHAAGMGEPVEREVVRALMFLRAKTL


HUTH_PSEPU
SRGFSGIRRKVIDALIALVNAEVYPHIPLKGSVGASGDLAPLATMSLVLLGEGKARYKGQ
HUTH_RHIME
GRGASGVRLELVRLIEAMLDKGVIPLIPEKGSVGASGDLAPLAHMAAVMMGHGEAFFAGE
HUTH_MOUSE
AKGYSGISLETLKQVIEAFNASCLSYVPEKGTVGASGDLAPLSHLALGLIGEGKMWSPKS
HUTH_RAT
AKGYSGISLETLKQVIEVFNASCLSYVPEKGTVGASGDLAPLSHLALGLIGEGKMWSPKS
HUTH_HUMAN
AKGYSGISLETLKQVIEMFNASCLPYVPEKGTVGASGDLAPLSHLALGLVGEGKMWSPKS
HUTH_CAEEL
AKGHSGISVENIKKMIAAFNAFCVSYVPQQGTVGCSGDLCPLAHLALGLLGEGKMWSPTT
HUTH_BACS
LKGFSGVRAELIEQLLAFLNKRVHPVIPQQGSLGASGDLAPLSHLALALIGQGEVFFEGE
HUTH_STRGR
ASGHTGVRPEVAQTMADVLNAGITPVVHEYGSLGCSGDLAPLSHCALTLMGEGEAEGPDG
HUTH_CORY       ASGRS-
VRPVVLETMVGMLNAGITPVVREYGSLGCSGDLAPLSHCALVLMGEGEATDAHG

HUTH_PSEPU      -
WLSATEALAVAGLEPLTLAAKEGLALLNGTQASTAYALRGLFYAEDLYAAAIACGGLSV
HUTH_RHIME      -
RMKGDAALKAAGLSPVTLAAKEGLALINGTQVSTALALAGLFRAHRAGQAALITGALST
HUTH_MOUSE
GWADAKYVLEAHGLKPIVLKPKEGLALINGTQMITSLGCEALERASAIARQADIVAALTL
HUTH_RAT
GWADAKYVLEAHGLKPIVLKPKEGLALINGTQMITSLGCEAVERASAIARQADIVAALTL
HUTH_HUMAN
GWADAKYVLEAHGLKPVILKPKEGLALINGTQMITSLGCEAVERASAIARQADIVAALTL
HUTH_CAEEL
GWQPADVVLKKNNLEPLELGPKEGLALINGTQMVTALGAYTLERAHNIARQADVIAALSL
HUTH_BACS       -
RMPAMTGLKKAGIQPVTLTSKEGLALINGTQAMTAMGVVAYIEAEKLAYQTERIASLTI
HUTH_STRGR
TVRPAGELLAAHGIAPVELREKEGLALLNGTDGMLGMLVMALADLRNLYTSADITAALSL
HUTH_CORY
DIRPVPELFAEAGLTPVELAEKEGLALVNGTDGMLGQLIMALADLDELLDIADATAAMSV
```

Figure 13 cont'd.

```
HUTH_PSEPU      EAVLGSRSPFDARIHE-ARGQRGQIDTAACFRDLLGDSSEVSLSHKNCD----
KVQDPYS
HUTH_RHIME      DAAMGSSAPFHPDIQH-CAAIRARSTRAAALRQLLTG-SPIRQSHIEGDE---
RVQDPYC
HUTH_MOUSE      EVLKGTTKAFDTDIHA-VRPHRGQIEVAFRFRSLLDS-
DHHPSEIAESHRFCDRVQDAYT
HUTH_RAT        EVLKGTTKAFDTDIHA-VRPHRGQIEVAFRFRSLLDS-
DHHPSEIAESHRFCDRVQDAYT
HUTH_HUMAN      EVLKGTTKAFDTDIHA-LRPHRGQIEVAFRFRSLLDS-
DHHPSEIAESHRFCDRVQDAYT
HUTH_CAEEL      DVLKGTTRAYDPDIHR-IRPHRGQNLSALRLRALLHS-
EANPSQIAESHRNCTKVQDAYT
HUTH_BACS       EGLQGIIDAFDEDIHL-ARGYQEQIDVAERIRFYLSD-SGLTTSQGE-----
LRVQDAYS
HUTH_STRGR      EALLGTDKVLAPELHA-IRPHPGQGVSADNMSRVLAG-SGLTGHHQDDAP---
RVQDAYS
HUTH_CORY       EAQLGTDQVFRAELHEPLRPHPGQGRSAQNMFAFLAD-SPIVASHREGDG---
RVQDAYS


HUTH_PSEPU
LRCQPQVMGACLTQLRQAAEVLGIEANAVSDNPLVFAAEGDVISGGNFHAEPVAMAADNL
HUTH_RHIME      IRCQPQVDGACLDLLRSVAATLTIEANAVTDNPLVLSDN-
SVVSGGNFHAEPVAFAADQI
HUTH_MOUSE
LRCCPQVHGVVNDTIAFVKDIITTELNSATDNPMVFASRGETISGGNFHGEYPAKALDYL
HUTH_RAT
LRCCPQVHGVVNDTIAFVKDIITTELNSATDNPMVFASRGETISGGNFHGEYPAKALDYL
HUTH_HUMAN
LRCCPQVHGVVNDTIAFVKNIITTELNSATDNPMVFANRGETVSGGNFHGEYPAKALDYL
HUTH_CAEEL
LRCVPQVHGVVHDTIEFVREIITTEMNSATDNPLVFADREEIISGGNFHGEYPAKALDFL
HUTH_BACS
LRCIPQVHGATWQTLGYVKEKLEIEMNAATDNPLIFNDGDKVISGGNFHGQPIAFAMDFL
HUTH_STRGR      VRCAPQVNGAGRDTLDHAALVAGRELASSVDNPVVLPDG-
RVESNGNFHGAPVAYVLDFL
HUTH_CORY       LRCSPQVTGAARDTIAHARLVATRELAAAIDNPVVLPSG-
EVTSNGNFHGAPVAYVLDFL


HUTH_PSEPU      ALAIAEIGSLSERRISLMMDKHMS-
QLPPFLVENGGVNSGFMIAQVTAAALASENKALSH
HUTH_RHIME
ALAVCEIGAISQRRIALLVDPALSLRLPAFLAKKPGLNSGLMIAEVTSAALMSENKQLSH
HUTH_MOUSE      AIGVHELAAISERRIERLCNPSLS-
ELPAFLVAEGGLNSGFMIAHCTAAALVSESKALCH
HUTH_RAT        AIGVHELAAISERRIERLCNPSLS-
ELPAFLVAEGGLNSGFMIAHCTAAALVSESKALCH
HUTH_HUMAN      AIGIHELAAISERRIERLCNPSLS-
ELPAFLVAEGGLNSGFMIAHCTAAALVSENKALCH
HUTH_CAEEL      AIAVAELAQMSERRLERLVNKELS-
GLPTFLTPDGGLNSGFMTVQLCAASLVSENKVLCH
HUTH_BACS       KIAISELANIAERRIERLVNPQLN-
DLPPFLSPHPGLQSGAMIMQYAAASLVSENKTLAH
HUTH_STRGR
AIVAADLGSICERRTDRLLDKNRSHGLPPFLADDAGVDSGLMIAQYTQAALVSEMKRLAV
HUTH_CORY
AIAVADLGSIAERRTDRMLDPARSRDLPAFLADDPGVDSGMMIAQYTQAGLVAENKRLAV
```

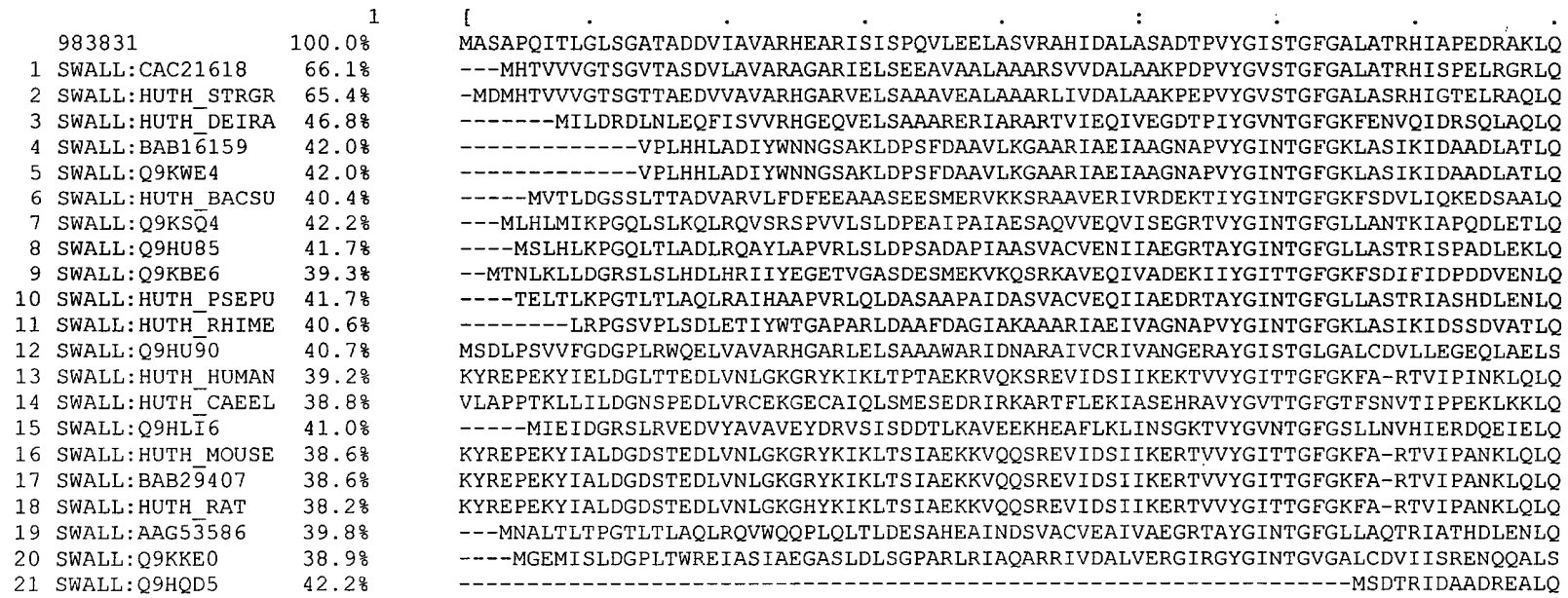
Figure 13 cont'd.

```
HUTH_PSEPU      PHSVDSLPTSANQEDHVSMAPAAGKRLWEMAENTRGVPAIEWLGACQGLDLRKG-LKTS
HUTH_RHIME      PASVDSTPTSANQEDHVSMACHGARRLLQMTENLFSIIGIEALAAVQGIEFRAP-LTTS
HUTH_MOUSE      PSSVDSLSTSAATEDHVSMGGWAARKALRVVEHVEQVLAIELLAACQGIEFLRP-LKTT
HUTH_RAT        PSSVDSLSTSAATEDHVSMGGWAARKALRVIEHVEQVLAIELLAACQGIEFLRP-LKTT
HUTH_HUMAN      PSSVDSLSTSAATEDHVSMGGWAARKALRVIEHVEQVLAIELLAACQGIEFLRP-LKTT
HUTH_CAEEL      PSSVDSIPTSCNQEDHVSMGGFAARKALTVVEHVEAVLAMELLAACQGIEFLKP-LIST
HUTH_BACS       PASVDSIPSSANQEDHVSMGTIAARHAYQVIANTRRVIAIEAICALQAVEYRGI-EHAA
HUTH_STRGR
PASADSIPSSAMQEDHVSMGWSAARKLRTAVDNLARIVAVELYAATRAIELRAAEGLTPA
HUTH_CORY       PA-VDSIPSSAMQEDHVSLGWHAARKLPTSVANLRRILAVEMLIAGRALDLRAP-LKPG

HUTH_PSEPU      AKLEKARQALRSEVA-HYDRDRFFAPDIEKAVELLAKG---S-LTGLLPAGVLPSL---
-
HUTH_RHIME      PELQKAAAAVRGVSS-SIEEDRYMADDLKAAGDLVASG---R-LAAAVSAGILPKLEN-
HUTH_MOUSE      TPLEKVYDLVRSVVR-
PWIKDRFMAPDIEAAHRLLLDQKVWEVAAPYIEKYRMEHIPESR
HUTH_RAT        TPLEKVYDLVRSVVR-
PWIKDRFMAPDIEAAHRLLLDQKVWEVAAPYIEKYRMEHIPESR
HUTH_HUMAN      TPLEKVYDLVRSVVR-
PWIKDRFMAPDIEAAHRLLLEQKVWEVAAPYIEKYRMEHIPESR
HUTH_CAEEL      APLHKIYQLVRSVAP-
PLNEDRYMKPEIDAVLEMIRENRIWEAVLPHLETLEAMEELDPD
HUTH_BACS       SYTKQLFQEMRKVVP-SIQQDRVFSYDIERLTDWLKK----ESLIPDHQNKELRGMNI-
HUTH_STRGR      PASEAVVAALRAAGAEGPGPDRFLAPDLAAADTFVREG---R-LVAAVEPVTGPLA---
-
HUTH_CORY       PATGAVLEVLRSKVA-GPGQDRFLSAELEAAYDLLANG---S-VHKALEAHLPE-----
-


HUTH_PSEPU      ----------------------------
HUTH_RHIME      ----------------------------
HUTH_MOUSE      PLSPTAFSLESLRKNSATIPESDDL----
HUTH_RAT        PLSPTAFSLESLRKNSATIPESDDL----
HUTH_HUMAN      PLSPTAFSLQFLHKKSTKIPESEDL----
HUTH_CAEEL      ALRQFTKTPTGIVQDRSMIPISDDEESIE
HUTH_BACS       ----------------------------
HUTH_STRGR      ----------------------------
HUTH_CORY       ----------------------------
```

Figure 14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983831 100.0% MASAPQITLGLSGATADDVIAVARHEARISISPQVLEELASVRAHIDALASADTPVYGISTGFGALATRHIAPEDRAKLQ
1 SWALL:CAC21618 66.1% ---MHTVVVGTSGVTASDVLAVARAGARIELSEEAVAALAAARSVVDALAAKPDPVYGVSTGFGALATRHISPELRGRLQ
2 SWALL:HUTH_STRGR 65.4% -MDMHTVVVGTSGTTAEDVVAVARHGARVELSAAAVEALAAARLIVDALAAKPEPVYGVSTGFGALASRHIGTELRAQLQ
3 SWALL:HUTH_DEIRA 46.8% -------MILDRDLNLEQFISVVRHGEQVELSAAARERIARARTVIEQIVEGDTPIYGVNTGFGKFENVQIDRSQLAQLQ
4 SWALL:BAB16159 42.0% -------------VPLHHLADIYWNNGSAKLDPSFDAAVLKGAARIAEIAAGNAPVYGINTGFGKLASIKIDAADLATLQ
5 SWALL:Q9KWE4 42.0% -------------VPLHHLADIYWNNGSAKLDPSFDAAVLKGAARIAEIAAGNAPVYGINTGFGKLASIKIDAADLATLQ
6 SWALL:HUTH_BACSU 40.4% -----MVTLDGSSLTTADVARVLFDFEEAAASEESMERVKKSRAAVERIVRDEKTIYGINTGFGKFSDVLIQKEDSAALQ
7 SWALL:Q9KSQ4 42.2% ---MLHLMIKPGQLSLKQLRQVSRSPVVLSLDPEAIPAIAESAQVVEQVISEGRTVYGINTGFGLLANTKIAPQDLETLQ
8 SWALL:Q9HU85 41.7% ----MSLHLKPGQLTLADLRQAYLAPVRLSLDPSADAPIAASVACVENIIAEGRTAYGINTGFGLLASTRISPADLEKLQ
9 SWALL:Q9KBE6 39.3% --MTNLKLLDGRSLSLHDLHRIIYEGETVGASDESMEKVKQSRKAVEQIVADEKIIYGITTGFGKFSDIFIDPDDVENLQ
10 SWALL:HUTH_PSEPU 41.7% ----TELTLKPGTLTLAQLRAIHAAPVRLQLDASAAPAIDASVACVEQIIAEDRTAYGINTGFGLLASTRIASHDLENLQ
11 SWALL:HUTH_RHIME 40.6% --------LRPGSVPLSDLETIYWTGAPARLDAAFDAGIAKAAARIAEIVAGNAPVYGINTGFGKLASIKIDSSDVATLQ
12 SWALL:Q9HU90 40.7% MSDLPSVVFGDGPLRWQELVAVARHGARLELSAAAWARIDNARAIVCRIVANGERAYGISTGLGALCDVLLEGEQLAELS
13 SWALL:HUTH_HUMAN 39.2% KYREPEKYIELDGLTTEDLVNLGKGRYKIKLTPTAEKRVQKSREVIDSIIKEKTVVYGITTGFGKFA-RTVIPINKLQLQ
14 SWALL:HUTH_CAEEL 38.8% VLAPPTKLLILDGNSPEDLVRCEKGECAIQLSMESEDRIRKARTFLEKIASEHRAVYGVTTGFGTFSNVTIPPEKLKKLQ
15 SWALL:Q9HLI6 41.0% -----MIEIDGRSLRVEDVYAVAVEYDRVSISDDTLKAVEEKHEAFLKLINSGKTVYGVNTGFGSLLNVHIERDQEIELQ
16 SWALL:HUTH_MOUSE 38.6% KYREPEKYIALDGDSTEDLVNLGKGRYKIKLTSIAEKKVQQSREVIDSIIKERTVVYGITTGFGKFA-RTVIPANKLQLQ
17 SWALL:BAB29407 38.6% KYREPEKYIALDGDSTEDLVNLGKGRYKIKLTSIAEKKVQQSREVIDSIIKERTVVYGITTGFGKFA-RTVIPANKLQLQ
18 SWALL:HUTH_RAT 38.2% KYREPEKYIALDGDSTEDLVNLGKGHYKIKLTSIAEKKVQQSREVIDSIIKERTVVYGITTGFGKFA-RTVIPANKLQLQ
19 SWALL:AAG53586 39.8% ---MNALTLTPGTLTLAQLRQVWQQPLQLTLDESAHEAINDSVACVEAIVAEGRTAYGINTGFGLLAQTRIATHDLENLQ
20 SWALL:Q9KKE0 38.9% ----MGEMISLDGPLTWREIASIAEGASLDLSGPARLRIAQARRIVDALVERGIRGYGINTGVGALCDVIISRENQQALS
21 SWALL:Q9HQD5 42.2% ----------MSDTRIDAADREALQ

Figure 14, cont'd.

```
                          81     [        .         1         .         .         .         .         :         .  160
   983831              100.0%      RSLIRSHAAGMGEPVEREVVRALMFLRAKTLASGRTGVRPVVLETMVGMLNAGITPVVREYGSLGCSGDLAPLSHCALVL
 1 SWALL:CAC21618       66.1%      RNIVRSHAAGMGPRVEREVVRALMFLRLKTVCSGRTGVRPEVAQTMADVLNAGITPVVHEYGSLGCSGDLAPLSHCALTL
 2 SWALL:HUTH_STRGR     65.4%      RNIVRSHAAGMGPRVEREVVRALMFLRLKTVASGHTGVRPEVAQTMADVLNAGITPVVHEYGSLGCSGDLAPLSHCALTL
 3 SWALL:HUTH_DEIRA     46.8%      HNLIVSHAIGMGEPLPAEVVRGMLLLRAQSLSLGHSGVRVEVVELLLALLNADALPVVPSQGSVGASGDLAPLAHLALGL
 4 SWALL:BAB16159       42.0%      RNLILSHCCGVGAPLPENVVRLIMALKLISLGRGASGVRIELIRLIEGMLEKGVIPVIPEKGSVGASGDLAPLAHMSATM
 5 SWALL:Q9KWE4         42.0%      RNLILSHCCGVGAPLPENVVRLIMALKLISLGRGASGVRIELIRLIEGMLEKGVIPVIPEKGSVGASGDLAPLAHMSATM
 6 SWALL:HUTH_BACSU     40.4%      LNLILSHACGVGDPFPECVSRAMLLLRANALLKGFSGVRAELIEQLLAFLNKRVHPVIPQQGSLGASGDLAPLSHLALAL
 7 SWALL:Q9KSQ4         42.2%      KSIVLSHAAGIGELMSDETVRLMMLLKINSLARGYSGIRLEVIQALIELVNNQIYPCVPKKGSVGASGDLAPLAHMSTVL
 8 SWALL:Q9HU85         41.7%      RSIVLSHAAGVGEALDDAMVRLVMLLKVNSLARGFSGIRRKVIDALIALINAEVYPHIPLKGSVGASGDLAPLAHMSLVL
 9 SWALL:Q9KBE6         39.3%      HNLIYSHACGVGSPFPETVSRTMLVLRANALLKGFSGVRPLVIERLLALVNANIHPVIPQQGSLGASGDLAPLSHLALVL
10 SWALL:HUTH_PSEPU     41.7%      RSLVLSHAAGIGAPLDDDLVRLIMVLKINSLSRGFSGIRRKVIDALIALVNAEVYPHIPLKGSVGASGDLAPLAHMSLVL
11 SWALL:HUTH_RHIME     40.6%      RNLILSHCCGVGQPLTEDIVRLIMALKLISLGRGASGVRLELVRLIEAMLDKGVIPLIPEKGSVGASGDLAPLAHMAAVM
12 SWALL:Q9HU90         40.7%      RNTLLSHACGVGEPLRDEQTRAIICAAVANYSQGKSGLDRSLVEGLLALLNHGITPQVPAQGSVGY---LTHMAHVGIAL
13 SWALL:HUTH_HUMAN     39.2%      VNLVRSHSSGVGKPLSPERCRMLLALRINVLAKGYSGISLETLKQVIEMFNASCLPYVPEKGTVGASGDLAPLSHLALGL
14 SWALL:HUTH_CAEEL     38.8%      LNLIRSHATGYGEPLAPNRARMLLALRINILAKGHSGISVENIKKMIAAFNAFCVSYVPQQGTVGCSGDLCPLAHLALGL
15 SWALL:Q9HLI6         41.0%      KNLIRSHSSGVGDYLENRYVRAIMAVRLNSLAAGYSAVSADLLNMMVEMLNRDVIPAVPKYGSVGASGDLAPLAHIGLAM
16 SWALL:HUTH_MOUSE     38.6%      VNLVRSHSSGVGKPLSPERCRMLLALRINVLAKGYSGISLETLKQVIEAFNASCLSYVPEKGTVGASGDLAPLSHLALGL
17 SWALL:BAB29407       38.6%      VNLVRSHSSGVGKPLSPERCRMLLALRINVLAKGYSGISLETLKQVIEAFNASCLSYVPEKGTVGASGDLAPLSHLALGL
18 SWALL:HUTH_RAT       38.2%      VNLVRSHSSGVGKPLSPERCRMLLALRINVLAKGYSGISLETLKQVIEVFNASCLSYVPEKGTVGASGDLAPLSHLALGL
19 SWALL:AAG53586       39.8%      RSLVLSHAAGVGEPLDDDIVRLMMVLKINSLARGFSGIRLSVIQALIALVNAGVYSVDPAKGSVGASGDLAPLAHMSLTL
20 SWALL:Q9KKE0         38.9%      RNIILSHACGVGDPLGRVEARAVMAAQIANLTHGYSGVRVETAEMLLALLNADIIPLIPSRGSVGY------LTHAALVL
21 SWALL:Q9HQD5         42.2%      ANLVRSHAAGAGSELDTAAVRALLVTRLNALAKGYSGIRERVLDVLVGLLNEGVHPVVPSRGSLGASGDLAPLAHMSRVL
```

Figure 14, cont'd.

```
                                161    [       .       .       .       2       .       .       .       . 240
   983831                100.0%       MGEGEATDAHGDIRPVPELFAEAGLTPVELAEKEGLALVNGTDGMLGQLIMALADLDELLDIADATAAMSVEAQLGTDQV
 1 SWALL:CAC21618         66.1%       MGEGDAEGPDGTVRPAGELLAAHGIAPVELREKEGLALLNGTDGMLGMLVMALADLDTLYKSADITAALTMEALLGTDRV
 2 SWALL:HUTH_STRGR       65.4%       MGEGEAEGPDGTVRPAGELLAAHGIAPVELREKEGLALLNGTDGMLGMLVMALADLRNLYTSADITAALSLEALLGTDKV
 3 SWALL:HUTH_DEIRA       46.8%       IGLGDI-EYQGQVRPAADVLAELGLSPVQLQAKEGLALINGTQLMGSLLALALHDAQVLLGTANLAAAMTVEARYGSHRP
 4 SWALL:BAB16159         42.0%       MGEGEAF-YQGVQMPSKDALAKAGLSPVVLAAKEGLALINGTQTSTALALAGLFRAHRAAQSALVTGALSTDAAMGSSAP
 5 SWALL:Q9KWE4           42.0%       MGEGEAF-YQGVQMPSKDALAKAGLSPVVLAAKEGLALINGTQTSTALALAGLFRAHRAAQSALVTGALSTDAAMGSSAP
 6 SWALL:HUTH_BACSU       40.4%       IGQGEVF-FEGERMPAMTGLKKAGIQPVTLTSKEGLALINGTQAMTAMGVVAYIEAEKLAYQTERIASLTIEGLQGIIDA
 7 SWALL:Q9KSQ4           42.2%       LGEGQAR-YNGKIISGLEAMKIAGLEPITLAPKEGLALLNGTQASTAFALEGLFVAEDLFASATVCGAMSVEAALGSRRP
 8 SWALL:Q9HU85           41.7%       IGESRARH-RGEWLPAAEALAVAGLEPLTLAAKEGLALLNGTQVSTAYALRGLFEAEDLFAAATVCGGLSVEAMLGSRAP
 9 SWALL:Q9KBE6           39.3%       LGEGEVF-YKGTKTKASFALKEEEIEPITLTAKEGLALINGTQAMTAMGVIAYLEAEKLAFQSEIIASLTMEGLRGIIDA
10 SWALL:HUTH_PSEPU       41.7%       LGEGKAR-YKGQWLSATEALAVAGLEPLTLAAKEGLALLNGTQASTAYALRGLFYAEDLYAAAIACGGLSVEAVLGSRSP
11 SWALL:HUTH_RHIME       40.6%       MGHGEAFFAGERMKGDAALKA-AGLSPVTLAAKEGLALINGTQVSTALALAGLFRAHRAGQAALITGALSTDAAMGSSAP
12 SWALL:Q9HU90           40.7%       LGIGEVS-YRGSVVPAAAALAAEGLATVRLGAKDGLCLVNGTPCMTGLACLALDDAQRLAQWADVIGAMSFEALRGQLAA
13 SWALL:HUTH_HUMAN       39.2%       VGEGKMWSPKSGWADAKYVLEAHGLKPVILKPKEGLALINGTQMITSLGCEAVERASAIARQADIVAALTLEVLKGTTKA
14 SWALL:HUTH_CAEEL       38.8%       LGEGKMWSPTTGWQPADVVLKKNNLEPLELGPKEGLALINGTQMVTALGAYTLERAHNIARQADVIAALSLDVLKGTTRA
15 SWALL:Q9HLI6           41.0%       MGEGKAF-FEGRLMDSARALEKAGLKPYQFKEKEGVALINGTSFMSGILSIAVMDAHDILENAIRSALLSFEALGGTSKA
16 SWALL:HUTH_MOUSE       38.6%       IGEGKMWSPKSGWADAKYVLEAHGLKPIVLKPKEGLALINGTQMITSLGCEALERASAIARQADIVAALTLEVLKGTTKA
17 SWALL:BAB29407         38.6%       IGEGKMWSPKSGWADAKYVLEAHGLKPIVLKPKEGLALINGTQMITSLGCEALERASAIARQADIVAALTLEVLKGTTKA
18 SWALL:HUTH_RAT         38.2%       IGEGKMWSPKSGWADAKYVLEAHGLKPIVLKPKEGLALINGTQMITSLGCEAVERASAIARQADIVAALTLEVLKGTTKA
19 SWALL:AAG53586         39.8%       LGEGKAR-YRGEWLPAATALQKAGLAPVTLAAKEGLALLNGTQASTAFALRGLFEAEDLFASAVVCGALTTEAVLGSRRP
20 SWALL:Q9KKE0           38.9%       IGHGSAMQGTERLSGADAL-ARLGLAPLRLEAKEGLSLVNGTPCATGLAALALARTERLFAWADAAAAMTYE-NLGSQAN
21 SWALL:Q9HQD5           42.2%       IGEGQA-DVAGERMPAAEALAAADLEPVTLQAKEGLALINGTQLTTGVAALALVDAERVLRSADTAGALTTEVTMSTTAS
```

Figure 14, cont'd.

```
                          241   [       :       .       .       .       3       .       . 320
   983831              100.0%      FRAELHEPLRPHPGQGRSAQNMFAFLADSPIVASHREGDGRVQDAYSLRCSPQVTGAARDTIAHARLVATRELAAAIDNP
 1 SWALL:CAC21618       66.1%      LAPELHA-IRPHPGQAASAANMAAVLKGSGLTGHHQDDAPRVQDAYSVRCAPQVAGAGRDTMAHAGLVAERELAAAVDNP
 2 SWALL:HUTH_STRGR     65.4%      LAPELHA-IRPHPGQGVSADNMSRVLAGSGLTGHHQDDAPRVQDAYSVRCAPQVNGAGRDTLDHAALVAGRELASSVDNP
 3 SWALL:HUTH_DEIRA     46.8%      FQPDV-VGLRPHPGALAVAAELREFLAGSEIAPSHLTGDGKVQDAYSLRAVPQVHGATWDALAQAERVLAVEFASVTDNP
 4 SWALL:BAB16159       42.0%      FHPDIHT-LRGHKGQIDAGSALRNLLQGSEIRESHIEGDERVQDPYCIRCQPQVDGACLDLLASVARTLEIEANAVTDNP
 5 SWALL:Q9KWE4         42.0%      FHPDIHT-LRGHKGQIDAGSALRNLLQGSEIRESHIEGDERVQDPYCIRCQPQVDGACLDLLASVARTLEIEANAVTDNP
 6 SWALL:HUTH_BACSU     40.4%      FDEDIHLA-RGYQEQIDVAERIRFYLSDSGLTTS--QGELRVQDAYSLRCIPQVHGATWQTLGYVKEKLEIEMNAATDNP
 7 SWALL:Q9KSQ4         42.2%      FDPRIHR-VRGHRTQMDAATAYRHLLVSSEIGQSHSNCE-KVQDPYSLRCQPQVMGACLQQIRSAAEVLEVEANSVSDNP
 8 SWALL:Q9HU85         41.7%      FDARIHAA-RGQRGQIDVAAAYRDLLASSEVARSHEKCD-KVQDPYSLRCQPQVMGACLTQMRQAAEVLEIEANAVSDNP
 9 SWALL:Q9KBE6         39.3%      FDEQIHFA-RGYVEQVDVARRMESYLQDSQLTT--RQGELRVQDAYSLRCIPQVHGATWQTLRYVKEKLEIEMNAATDNP
10 SWALL:HUTH_PSEPU     41.7%      FDARIHEA-RGQRGQIDTAACFRDLLGDSSEVSSHKNCD-KVQDPYSLRCQPQVMGACLTQLRQAAEVLGIEANAVSDNP
11 SWALL:HUTH_RHIME     40.6%      FHPDIQHCAAIRARSTRAAA-LRQLLTGSPIRQSHIEGDERVQDPYCIRCQPQVDGACLDLLRSVAATLTIEANAVTDNP
12 SWALL:Q9HU90         40.7%      FDAEI-VALKPHPGMQRVAANLRALLAGSQVLENAR--GIRTQDALSIRSIPQIHGACRDQLAHARQIET-ELNSATDNP
13 SWALL:HUTH_HUMAN     39.2%      FDTDIHA-LRPHRGQIEVAFRFRSLLSDSEIAESHRFCD-RVQDAYTLRCCPQVHGVVNDTIAFVKNIITTELNSATDNP
14 SWALL:HUTH_CAEEL     38.8%      YDPDIHR-IRPHRGQNLSALRLRALLNPSQIAESHRNCT-KVQDAYTLRCVPQVHGVVHDTIEFVREIITTEMNSATDNP
15 SWALL:Q9HLI6         41.0%      FTPWILGA-RPHLGQVAIGNRFREYLTGSDIV--KRADSVKVQDAYTLRCIPQVYGSVADVIDYVENVLSVEINSATDNP
16 SWALL:HUTH_MOUSE     38.6%      FDTDIHA-VRPHRGQIEVAFRFRSLLSDSEIAESHRFCD-RVQDAYTLRCCPQVHGVVNDTIAFVKDIITTELNSATDNP
17 SWALL:BAB29407       38.6%      FDTDIHA-VRPHRGQIEVAFRFRSLLSDSEIAESHRFCD-RVQDAYTLRCCPQVHGVVNDTIAFVKDIITTELNSATDNP
18 SWALL:HUTH_RAT       38.2%      FDTDIHA-VRPHRGQIEVAFRFRSLLSDSEIAESHRFCD-RVQDAYTLRCCPQVHGVVNDTIAFVKDIITTELNSATDNP
19 SWALL:AAG53586       39.8%      FDARIHE-VRGQRGQIDAAALFRHVLTDTSAIASHHNCD-KVQDPYSLRCQPQVMGACLTQMRQVAEVLLVESNAVSDNP
20 SWALL:Q9KKE0         38.9%      AFAELPLALRQSPGLSAVGEGLRDWLADSPMLAG--TAGTRTQDPLSLRAVPQVHGAARDAFGQVAEIVDRELASVTDNP
21 SWALL:Q9HQD5         42.2%      CAPAIHE-VRPHDGQAVSARHIRNLTAGSEVLDHHRDCD-RVQDAYSIRCLPQVHGAVRDALDHLRAAVATELNSATDNP
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Figure 14, cont'd.

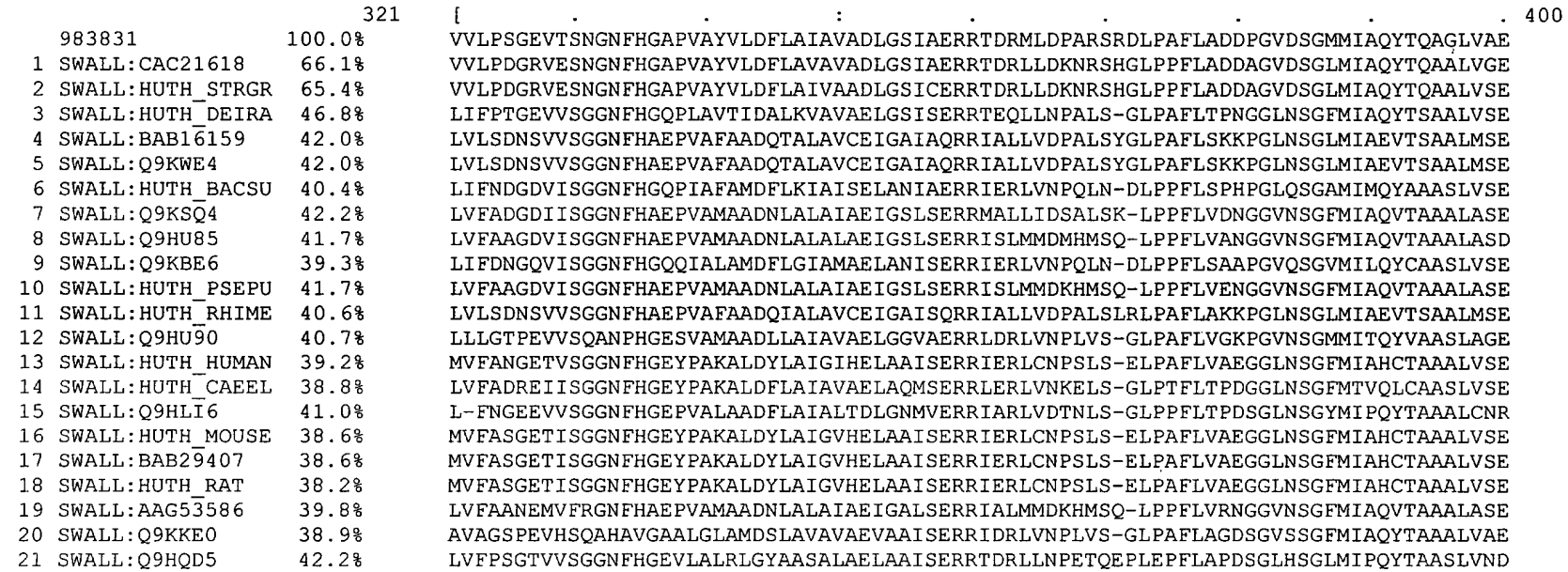

Figure 14, cont'd.

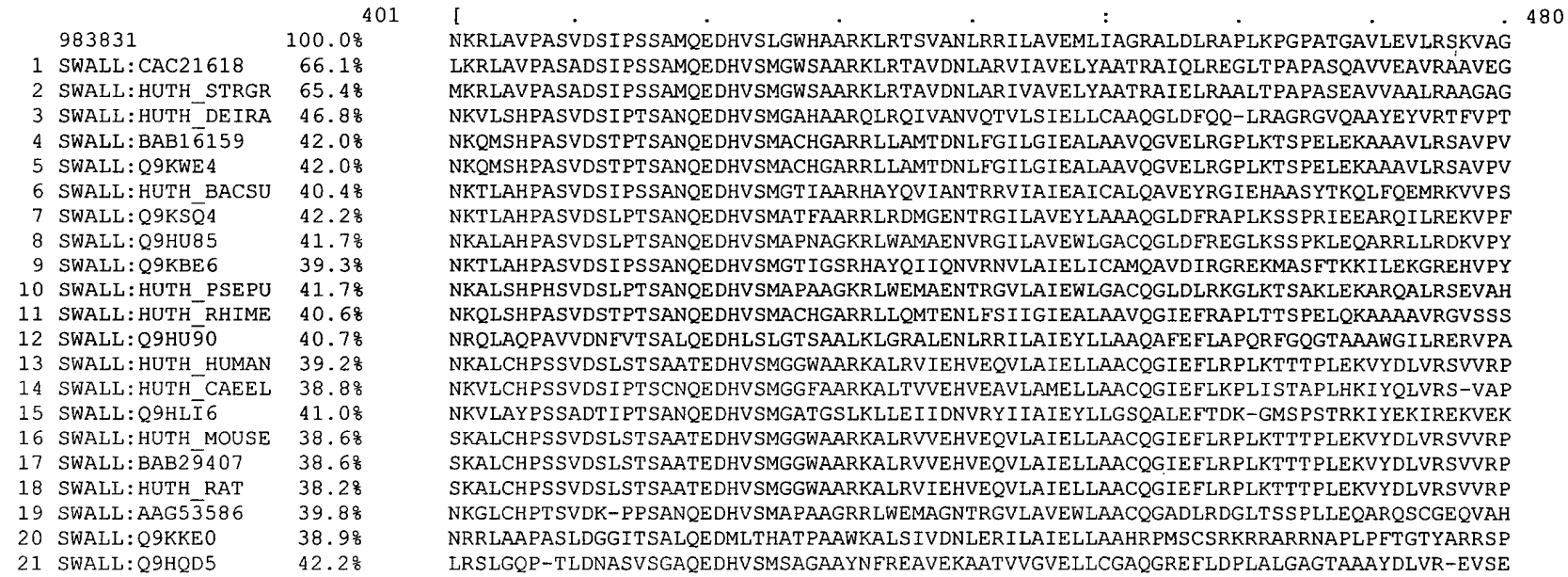

Figure 14, cont'd.

```
                          481    [        .         5         .  ] 513
   983831              100.0%      PGQDRFLSAELEAAYDLLANGSVHKALEAHLPA
 1 SWALL:CAC21618       66.1%      PGPDRHLAPDLAAADAFVRAGHLVAAAESVTGP
 2 SWALL:HUTH_STRGR     65.4%      PGPDRFLAPDLAAADTFVREGRLVAAVEPVTGP
 3 SWALL:HUTH_DEIRA     46.8%      LTEDRYFRPDLLRLRGELVSGRVAQAADTQAPA
 4 SWALL:BAB16159       42.0%      LEDDRYMATDLKAAIEVVASGALVSAISSGLPV
 5 SWALL:Q9KWE4         42.0%      LEDDRYMATDLKAAIEVVASGALVSAISSGLPV
 6 SWALL:HUTH_BACSU     40.4%      IQQDRVFSYDIERLTDWLKKESLIPDHQNKELR
 7 SWALL:Q9KSQ4         42.2%      YDKDRYFAPDIEKANALL-QLAVHNRLMPDQLL
 8 SWALL:Q9HU85         41.7%      YQEDRFFAPDIEAASQLLASGCLNALLPARLLP
 9 SWALL:Q9KBE6         39.3%      IDQDRMFAKDIERAAKWLKDGSWDFTKMREKER
10 SWALL:HUTH_PSEPU     41.7%      YDRDRFFAPDIEKAVELLAKGSLTGLLPAGLPS
11 SWALL:HUTH_RHIME     40.6%      IEEDRYMADDLKAAGDLVASGRLAAAVSAGLPK
12 SWALL:Q9HU90         40.7%      YDTDRWLAPDIASAAAILGERKSLARLAASIGD
13 SWALL:HUTH_HUMAN     39.2%      WIKDRFMAPDIEAAHRLLLEQKVWEVAAPYIEK
14 SWALL:HUTH_CAEEL     38.8%      PNEDRYMKPEIDAVLEMIRENRIWEAVLPHLET
15 SWALL:Q9HLI6         41.0%      LDHDRPPSFDIETIRKMMDKKEFISALP-----
16 SWALL:HUTH_MOUSE     38.6%      WIKDRFMAPDIEAAHRLLLDQKVWEVAAPYIEK
17 SWALL:BAB29407       38.6%      WIKDRFMAPDIEAAHRLLLDQKVWEVAAPYIEK
18 SWALL:HUTH_RAT       38.2%      WIKDRFMAPDIEAAHRLLLDQKVWEVAAPYIEK
19 SWALL:AAG53586       39.8%      YDDDRFFAPDIEAAISLLNKGSLVGLLPAFL--
20 SWALL:Q9KKE0         38.9%      PIATIVR----------------------------
21 SWALL:Q9HQD5         42.2%      PAGDRALADDMAAVGDLVRAGLVEDAVARALDA
```

Figure 14, cont'd.

KEY:

983831 : HAL
1 CAC21618 : Streptomyces coelicolor
2 HUTH_STRGR : Streptomyces griseus
3 HUTH_DEIRA : Deinococcus radiodurans
4 BAB16159 : Agrobacterium rhizogenes
5 Q9KWE4 : Agrobacterium rhizogenes
6 HUTH_BACSU : Bacillus subtilis
7 Q9KSQ4 : Vibrio cholerae
8 Q9HU85 : Pseudomonas aeruginosa
9 Q9KBE6 : Bacillus halodurans
10 HUTH_PSEPU : Pseudomonas putida
11 HUTH_RHIME : Rhizobium meliloti
12 Q9HU90 : Pseudomonas aeruginosa
13 HUTH_HUMAN : Human
14 HUTH_CAEEL : Caenorhabditis elegans
15 Q9HLI6 : Thermoplasma acidophilum
16 HUTH_MOUSE : Mouse
17 BAB29407 : Mus musculus (Mouse)
18 HUTH_RAT : Rat
18 AAG53586 : uncultured bacterium pCosAS1
20 Q9KKE0 : Rhizobium meliloti
21 Q9HQD5 : Halobacterium sp

Figure 15

```
STRG      6   VVVGTSGTTAEDVVAVARHGARVELSAAAVEALAAARLIVDALAAKPEPVYGVSTGFGAL
"HAL"     7   ITLGLSGATADDVIAVARHEARISISPQVLEELASVRAHIDALASADTPVYGISTGFGAL
                 * ** ** ** ***** **   *    * **  *   ****    **** *******

STRG,     66  ASRHIGTELRAQLQRNIVRSHAAGMGPRVEREVVRALMFLRLKTVASGHTGVRPEVAQTM
HAL       67  ATRHIAPEDRAKLQRSLIRSHAAGMGEPVEREVVRALMFLRAKTLASGRTGVRPVVLETM
              * ***  * ** ***   ********  ************ ** *** ***** *  **

STRG      126 ADVLNAGITPVVHEYGSLGCSGDLAPLSHCALTLMGEGEAEGPDGTVRPAGELLAAHGIA
HAL       127 VGMLNAGITPVVREYGSLGCSGDLAPLSHCALVLMGEGEATDAHGDIRPVPELFAEAGLT
                 ********* ******************* *******    *  **  ** *  *

STRG      186 PVELREKEGLALLNGTDGMLGMLVMALADLRNLYTSADITAALSLEALLGTDKVLAPELH
HAL       187 PVELAEKEGLALVNGTDGMLGQLIMALADLDELLDIADATAAMSVEAQLGTDQVFRAELH
              **** ******* ******** * ******  *   ** *** * ** **** *   ***

STRG      246 A-IRPHPGQGVSADNMSRVLAGSGLTGHHQDDAPRVQDAYSVRCAPQVNGAGRDTLDHAA
HAL       247 EPLRPHPGQGRSAQNMFAFLADSPIVASHREGDGRVQDAYSLRCSPQVTGAARDTIAHAR
                 ******* ** **   ** *     *     ******* ** *** ** ***  **

STRG      305 LVAGRELASSVDNPVVLPDGRVESNGNFHGAPVAYVLDFLAIVAADLGSICERRTDRLLD
HAL       307 LVATRELAAAIDNPVVLPSGEVTSNGNFHGAPVAYVLDFLAIAVADLGSIAERRTDRMLD
              *** ****   ******* * * ******************  ****** ****** **
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Figure 15, Cont'd.

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STRG     365  KNRSHGLPPFLADDAGVDSGLMIAQYTQAALVSEMKRLAVPASADSIPSSAMQEDHVSMG
HAL      367  PARSRDLPAFLADDPGVDSGMMIAQYTQAGLVAENKRLAVPASVDSIPSSAMQEDHVSLG
                **  ** ***** ***** ******** ** * ******** ************** *

STRG     425  WSAARKLRTAVDNLARIVAVELYAATRAIELRAAEGLTPAPASEAVVAALRAAGAEGPGP
HAL      427  WHAARKLRTSVANLRRILAVEMLIAGRALDLRAP--LKPGPATGAVLEVLRSKVA-GPGQ
              * ******* * ** ** ***   * **  ***   * * **  **   **   * ***

STRG     485  DRFLAPDLAAADTFVREGRLVAAVE
HAL      484  DRFLSAELEAAYDLLANGSVHKALE
              ****   * **      *    * *
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SEQ ID NO: 1

1 LNAGITPVVR EYGSLGCSGD LAPLSHCALV LMGEGEA

SEQ ID NO: 2

1 GMLNAGITPV VREYGSLGCS GDLAPLSHCA LVLMGEGEAT

SEQ ID NO: 3

1 MASAPQITLG LSGATADDVI AVARHEARIS ISPQVLEELA SVRAHIDALA

51 SADTPVYGIS TGFGALATRH IAPEDRAKLQ RSLIRSHAAG MGEPVEREVV

101 RALMFLRAKT LASGRTGVRP VVLETMVGML NAGITPVVRE YGSLGCSGDL

151 APLSHCALVL MGEGEATDAH GDIRPVPELF AEAGLTPVEL AEKEGLALVN

201 GTDGMLGQLI MALADLDELL DIADATAAMS VEAQLGTDQV FRAELHEPLR

251 PHPGQGRSAQ NMFAFLADSP IVASHREGDG RVQDAYS

SEQ ID NO: 4

1 MASAPQITLG LSGATADDVI AVARHEARIS ISPQVLEELA SVRAHIDALA

51 SADTPVYGIS TGFGALATRH IAPEDRAKLQ RSLIRSHAAG MGEPVEREVV

101 RALMFLRAKT LASGRTGVRP VVLETMVGML NAGITPVVRE YGSLGCSGDL

151 APLSHCALVL MGEGEATDAH GDIRPVPELF AEAGLTPVEL AEKEGLALVN

201 GTDGMLGQLI MALADLDELL DIADATAAMS VEAQLGTDQV FRAELHEPLR

251 PHPGQGRSAQ NMFAFLADSP IVASHREGDG RVQDAYSLRC SPQVTGAARD

301 TIAHARLVAT RELAAAIDNP VVLPSGEVTS NGNFHGAPVA YVLDFLAIAV

351 ADLGSIAERR TDRMLDPARS RDLPAFLADD PGVDSGMMIA QYTQAGLVAE

401 NKRLA

SEQ ID NO: 5

1 MASAPQITLG LSGATADDVI AVARHEARIS ISPQVLEELA SVRAHIDALA

51 SADTPVYGIS TGFGALATRH IAPEDRAKLQ RSLIRSHAAG MGEPVEREVV

101 RALMFLRAKT LASGRTGVRP VVLETMVGML NAGITPVVRE YGSLGCSGDL

151 APLSHCALVL MGEGEATDAH GDIRPVPELF AEAGLTPVEL AEKEGLALVN

201 GTDGMLGQLI MALADLDELL DIADATAAMS VEAQLGTDQV FRAELHEPLR

251 PHPGQGRSAQ NMFAFLADSP IVASHREGDG RVQDAYSLRC SPQVTGAARD

301 TIAHARLVAT RELAAAIDNP VVLPSGEVTS NGNFHGAPVA YVLDFLAIAV

351 ADLGSIAERR TDRMLDPARS RDLPAFLADD PGVDSGMMIA QYTQAGLVAE

401 NKRLAVPASV DSIPSSAMQE DHVSLGWHAA RKLRTSVANL RRILAVEMLI

451 AGRALDLRAP LKPGPATGAV LEVLRSKVAG PGQDRFLSAE LEAAYDLLAN

501 GSVHKALEAH LPA

SEQ ID NO: 6

1 XXXXXXXXXG XSGXTAXDVX AVARHXARXX XSXXXXEXLA XXRXXXDALA

51 XXXXPVYGXS TGFGALAXRH IXXEXRAXLQ RXXXRSHAAG MGXXVEREVV

101 RALMFLRXKT XASGXTGVRP XVXXTMXGXL NAGITPVVXE YGSLGCSGDL

151 APLSHCALXL MGEGEATXXX GXXRPXXELX AXXGXXPVEL XEKEGLALXN

201 GTDGMLGXLX MALADLXXLX XXADXTAAXS XEAXLGTDXV XXXELHXXXR

251 PHPGQGXSAX NMXXXLAXSX XXXXHXXXXX RVQDAYSXRC XPQVXGAXRD

301 TXXHAXLVAX RELAXXXDNP VVLPXGXVXS NGNFHGAPVA YVLDFLAIXX

351 ADLGSIXERR TDRXLDXXRS XXLPXFLADD XGVDSGXMIA QYTQAXLVXE

401 XKRLAVPASX DSIPSSAMQE DHVSXGWXAA RKLRTXVXNL XRIXAVEXXX

451 AXRAXXLRAX XXLXPXPAXX AVXXXLRXXX AXGPGXDRFL XXXLXAAXXX

501 XXXGXXXXAX E

SEQ ID NO: 7

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1     ATGGCTTCCG CTCCTCAAAT AACACTTGGC CTAAGTGGCG CAACCGCAGA

51    CGACGTTATC GCCGTTGCCC GCCACGAAGC CCGCATCAGC ATTTCTCCGC

101   AAGTACTTGA GGAACTGGCT TCCGTCCGAG CACATATCGA TGCACTAGCA

151   TCCGCTGATA CCCCGGTTTA TGGCATTTCA ACCGGCTTTG GCGCGTTGGC

201   AACCCGCCAC ATCGCACCCG AGGATCGCGC CAAGCTGCAG CGCTCCCTCA

251   TCCGTTCCCA CGCTGCTGGC ATGGGTGAAC CGGTGGAGCG CGAAGTGGTC

301   CGCGCATTGA TGTTCTTGCG TGCAAAGACC CTGGCTTCCG GCCGCACGGG

351   CGTTCGCCCG GTTGTCCTTG AGACCATGGT CGGCATGCTC AATGCAGGCA

401   TCACTCCGGT AGTCCGCGAA TACGGTTCAC TGGGCTGCTC CGGTGACTTG

451   GCTCCGCTGT CGCACTGCGC ATTAGTGCTG ATGGGCGAGG GCGAAGCCAC

501   CGATGCCCAC GGCGACATCC GCCCGGTACC GGAACTGTTC GCCGAGGCCG

551   GATTGACCCC TGTCGAACTG GCAGAAAAGG AAGGCCTGGC TCTGGTCAAC

601   GGCACCGACG GCATGCTCGG CCAGCTGATC ATGGCATTGG CGGACCTCGA

651   TGAGCTGCTG GACATCGCCG ATGCCACCGC CGCCATGAGC GTTGAAGCCC

701   AGCTGGGCAC CGATCAGGTA TTCCGCGCAG AACTGCACGA ACCACTGCGC

751   CCGCACCCAG GCCAGGGCCG CAGCGCCCAG AACATGTTCG CCTTCCTGGC

801   CGACTCGCCA ATTGTTGCCT CGCATCGCGA GGGAGACGGC CGAGTGCAGG

851   ATGCCTACTC GCTGCGTTGC TCGCCGCAGG TCACCGGCGC CGCCCGCGAC

901   ACCATTGCTC ATGCCCGCCT GGTCGCCACC CGCGAACTGG CTGCGGCCAT

951   TGACAACCCT GTGGTGCTGC CCAGCGGCGA AGTGACTTCC AACGGCAACT
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1001 TCCACGGCGC ACCGGTAGCC TACGTGCTGG ACTTCCTTGC CATCGCCGTG

1051 GCCGACCTCG GCTCTATCGC CGAGCGCCGC ACCGACCGCA TGCTCGACCC

1101 AGCCCGCTCC CGCGACCTGC CGGCATTCCT GGCCGACGAT CCGGGTGTGG

1151 ACTCGGGCAT GATGATCGCC CAGTACACCC AGGCCGGCTT GGTGGCAGAA

1201 AACAAGCGGC TGGCAGTTCC TGCCAGCGTT GACTCCATCC CATCCTCGGC

1251 CATGCAGGAA GACCACGTTT CCCTGGGCTG GCATGCGGCG CGCAAGCTGC

1301 GCACCTCGGT AGCGAACCTC CGCCGCATTC TCGCAGTGGA AATGCTGATT

1351 GCCGGCCGCG CCCTGGACCT GCGGGCCCCA TTGAAGCCTG GTCCAGCGAC

1401 CGGTGCGGTG CTTGAAGTAT TGCGCAGCAA GGTTGCAGGC CCCGGCCAGG

1451 ACCGCTTCCT TTCCGCAGAA CTGGAAGCAG CCTATGACCT GCTGGCCAAT

1501 GGCTCGGTGC ATAAGGCCCT CGAAGCTCAC CTGCCTGCAT AA

SEQ ID NO: 8

1 MASAPQITLG LSGATADDVI AVARHEARIS ISPQVLEELA SVRAHIDALA

51 SADTPVYGIS TGFGALATRH IAPEDRAKLQ RSLIRSHAAG MGEPVEREVV

101 RALMFLRAKT LASGRSVRPV VLETMVGMLN AGITPVVREY GSLGCSGDLA

151 PLSHCALVLM GEGEATDAHG DIRPVPELFA EAGLTPVELA EKEGLALVNG

201 TDGMLGQLIM ALADLDELLD IADATAAMSV EAQLGTDQVF RAELHEPLRP

251 HPGQGRSAQN MFAFLADSPI VASHREGDGR VQDAYS

SEQ ID NO:9

1 MASAPQITLG LSGATADDVI AVARHEARIS ISPQVLEELA SVRAHIDALA

51 SADTPVYGIS TGFGALATRH IAPEDRAKLQ RSLIRSHAAG MGEPVEREVV

101 RALMFLRAKT LASGRSVRPV VLETMVGMLN AGITPVVREY GSLGCSGDLA

151 PLSHCALVLM GEGEATDAHG DIRPVPELFA EAGLTPVELA EKEGLALVNG

201 TDGMLGQLIM ALADLDELLD IADATAAMSV EAQLGTDQVF RAELHEPLRP

251 HPGQGRSAQN MFAFLADSPI VASHREGDGR VQDAYSLRCS PQVTGAARDT

301 IAHARLVATR ELAAAIDNPV VLPSGEVTSN GNFHGAPVAY VLDFLAIAVA

351 DLGSIAERRT DRMLDPARSR DLPAFLADDP GVDSGMMIAQ YTQAGLVAEN

401 KRLA

SEQ ID NO:10

1 MASAPQITLG LSGATADDVI AVARHEARIS ISPQVLEELA SVRAHIDALA

51 SADTPVYGIS TGFGALATRH IAPEDRAKLQ RSLIRSHAAG MGEPVEREVV

101 RALMFLRAKT LASGRSVRPV VLETMVGMLN AGITPVVREY GSLGCSGDLA

151 PLSHCALVLM GEGEATDAHG DIRPVPELFA EAGLTPVELA EKEGLALVNG

201 TDGMLGQLIM ALADLDELLD IADATAAMSV EAQLGTDQVF RAELHEPLRP

251 HPGQGRSAQN MFAFLADSPI VASHREGDGR VQDAYSLRCS PQVTGAARDT

301 IAHARLVATR ELAAAIDNPV VLPSGEVTSN GNFHGAPVAY VLDFLAIAVA

351 DLGSIAERRT DRMLDPARSR DLPAFLADDP GVDSGMMIAQ YTQAGLVAEN

401 KRLAVPAVDS IPSSAMQEDH VSLGWHAARK LPTSVANLRR ILAVEMLIAG

451 RALDLRAPLK PGPATGAVLE VLRSKVAGPG QDRFLSAELE AAYDLLANGS

501 VHKALEAHLP E

SEQ ID NO: 11

1 XXXXXXXXXG XSGXTAXDVX AVARHXARXX XSXXXXEXLA XXRXXXDALA

51 XXXXPVYGXS TGFGALAXRH IXXEXRAXLQ RXXXRSHAAG MGXXVEREVV

101 RALMFLRXKT XASGXX-VRP XVXXTMX**G**XL NAGITPVVXE YGSLGCSGDL

151 APLSHCALVL MGEGEA**T**XXX GXXRPXXELX AXXGXXPVEL XEKEGLALXN

201 GTDGMLGXLX MALADLXXLX XXADXTAAXS XEAXLGTDXV XXXELHXXXR

251 PHPGQGXSAX NMXXXLAX-S XXXXXHXXXX X---RVQDAY SXRCXPQVXG

301 AXRDTXXHAX LVAXRELAXX XDNPVVLPXG -XVXSNGNFH GAPVAYVLDF

351 LAIXXADLGS IXERRTDRXL DXXRSXXLPX FLADDXGVDS GXMIAQYTQA

401 XLVXEXKRLA VPA-XDSIPS SAMQEDHVSX GWXAARKLXT XVXNLXRIXA

451 VEXXXAXRAX XLRAX-XXXX PAXXAVXXXL RXXXA-GPGQ DRFLXXXLXA

501 AXXXXXXX-- -X-XXXXXEX XXXX

SEQ ID NO: 12

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   1  ATGGCTTCCG CTCCTCAAAT AACACTTGGC CTAAGTGGCG CAACCGCAGA

  51  CGACGTTATC GCCGTTGCCC GCCACGAAGC CCGCATCAGC ATTTCTCCGC

 101  AAGTACTTGA GGAACTGGCT TCCGTCCGAG CACATATCGA TGCACTAGCA

 151  TCCGCTGATA CCCCGGTTTA TGGCATTTCA ACCGGCTTTG GCGCGTTGGC

 201  AACCCGCCAC ATCGCACCCG AGGATCGCGC CAAGCTGCAG CGCTCCCTCA

 251  TCCGTTCCCA CGCTGCTGGC ATGGGTGAAC CGGTGGAGCG CGAAGTGGTC

 301  CGCGCATTGA TGTTCTTGCG TGCAAAGACC CTGGCTTCCG GCCGCAGCGT

 351  TCGCCCGGTT GTCCTTGAGA CCATGGTCGG CATGCTCAAT GCAGGCATCA

 401  CTCCGGTAGT CCGCGAATAC GGTTCACTGG GCTGCTCCGG TGACTTGGCT

 451  CCGCTGTCGC ACTGCGCATT AGTGCTGATG GGCGAGGGCG AAGCCACCGA

 501  TGCCCACGGC GACATCCGCC CGGTACCGGA ACTGTTCGCC GAGGCCGGAT

 551  TGACCCCTGT CGAACTGGCA GAAAAGGAAG GCCTGGCTCT GGTCAACGGC

 601  ACCGACGGCA TGCTCGGCCA GCTGATCATG GCATTGGCGG ACCTCGATGA
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651 GCTGCTGGAC ATCGCCGATG CCACCGCCGC CATGAGCGTT GAAGCCCAGC

701 TGGGCACCGA TCAGGTATTC CGCGCAGAAC TGCACGAACC ACTGCGCCCG

751 CACCCAGGCC AGGGCCGCAG CGCCCAGAAC ATGTTCGCCT TCCTGGCCGA

801 CTCGCCAATT GTTGCCTCGC ATCGCGAGGG AGACGGCCGA GTGCAGGATG

851 CCTACTCGCT GCGTTGCTCG CCGCAGGTCA CCGGCGCCGC CCGCGACACC

901 ATTGCTCATG CCCGCCTGGT CGCCACCCGC GAACTGGCTG CGGCCATTGA

951 CAACCCTGTG GTGCTGCCCA GCGGCGAAGT GACTTCCAAC GGCAACTTCC

1001 ACGGCGCACC GGTAGCCTAC GTGCTGGACT TCCTTGCCAT CGCCGTGGCC

1051 GACCTCGGCT CTATCGCCGA GCGCCGCACC GACCGCATGC TCGACCCAGC

1101 CCGCTCCCGC GACCTGCCGG CATTCCTGGC CGACGATCCG GGTGTGGACT

1151 CGGGCATGAT GATCGCCCAG TACACTCAGG CCGGCTTGGT GGCAGAAAAC

1201 AAGCGGCTGG CAGTTCCTGC AGTTGACTCC ATCCCATCCT CGGCCATGCA

1251 GGAAGACCAC GTTTCCCTGG GCTGGCATGC GGCGCGCAAG CTGCCGACCT

1301 CGGTAGCGAA CCTCCGCCGC ATTCTCGCAG TGGAAATGCT GATTGCCGGC

1351 CGCGCCCTGG ACCTGCGGGC CCCATTGAAG CCTGGTCCAG CGACCGGTGC

1401 GGTGCTTGAA GTATTGCGCA GCAAGGTTGC AGGCCCCGGC CAGGACCGCT

1451 TCCTTTCCGC AGAACTGGAA GCAGCCTATG ACCTGCTGGC CAATGGCTCG

1501 GTGCATAAGG CCCTCGAAGC TCACCTGCCT GAATAA