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Review

Ureases from pathogenic Gram-positive bacteria in the genus *Staphylococcus*

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ABSTRACT

Many species of bacteria in the genus *Staphylococcus* show a positive test for the enzyme urease, which catalyzes the degradation of urea to form two molecules of ammonia and one molecule of bicarbonate. Under physiological conditions, the ammonia becomes protonated to form ammonium ions, which raises the local pH and can contribute to the virulence of the bacteria. These bacteria can cause skin, joint, soft tissue, gastric, and urinary tract infections as well as bacteremia. Among the best characterized urease-positive pathogenic species of *Staphylococcus* are *S. aureus*, *S. cohnii* subsp. *urealyticum*, *S. epidermidis*, *S. leei*, *S. lugdunensis, S. saprophyticus*, *S. warneri*, and *S. xylosus*. This review summarizes the properties of these bacteria and describes the native and subunit structures of their urease proteins. Sequence comparisons are used to show the common features of multiple isolates of *S. aureus*, *S. epidermidis*, and *S. saprophyticus*, and to compare the sequences of the urease subunits of these species to those from jack bean (*Canavalia ensiformis*) and other pathogenic bacteria including *Helicobacter pylori*, *Klebsiella aerogenes*, *Proteus mirabilis*, and *Ureaplasma urealyticum*. The effects of various chemical and natural product inhibitors on the urease enzymes are described, as is the regulation of their synthesis. This summary demonstrates the key role of this enzyme in these bacteria and suggests further lines of research.

KEYWORDS: antimicrobial agents, infection, pathogen, *Staphylococcus*, urease, virulence factor.

INTRODUCTION

The genus *Staphylococcus* includes a very large number of species that share a number of common characteristics [1]. The bacteria are generally described as spherical Gram-positive prokaryotes which are often found in pairs, tetrads, or small clusters. They are not motile and do not form spores or resting stages. The cell walls contain a peptidoglycan in which the diamino acid lysine is often crosslinked to D-alanine through short chains of glycine or other amino acid residues. The walls frequently contain teichoic acids but are not usually encapsulated. The DNA contains 30-40 mole% G+C and is about 2-3 Mbp in size. Most staphylococci are moderately halophilic and can grow in media containing 5% to 10% NaCl. These microorganisms are facultative anaerobes that can grow by fermentation of sugars to form D- or L-lactic acid or by aerobic respiration using electron transport chains containing menaquinones and cytochromes a and b. They are positive for catalase but negative for oxidase activity. The host or niche range may be narrow or broad and many species are opportunistic pathogens of humans.

The List of Prokaryotic names with Standing in Nomenclature (LPSN) now includes 71 species or

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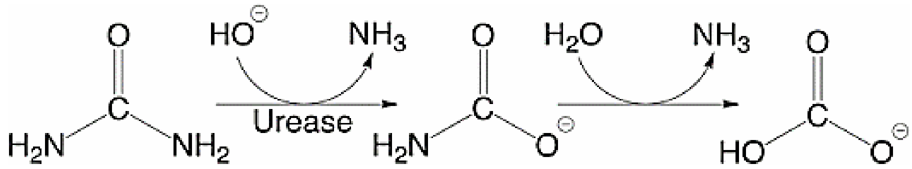
child taxa with published and correct names for the genus *Staphylococcus* [2]. The various species in this genus have been divided into clusters or groups and arranged into phylogenies based on the results of DNA/DNA hybridization experiments [3], 16S rRNA sequences [4], the presence of multiple loci in complete genomic sequences [5], the use of specific virulence factors [6], and other major phenotypic characteristics [7, 8]. This last feature includes the presence or absence of coagulase, sensitivity or resistance to novobiocin, the presence or absence of urease, the formation of hemolysins, the potential for nitrate reduction, the production of acid aerobically from specific sugars, and the presence or absence of phosphatase, ornithine decarboxylase, or pyrrolidinyl arylamidase activities. Of the 37 species listed in Bergey's Manual of Systematics of Archaea and Bacteria, 26 have been reported to be positive for urease activity [1]. This review focuses on those staphylococci which are sometimes pathogenic to humans and whose urease activity has been documented in the scientific literature. However, the urease protein has been characterized biochemically in only a few of these species and its role as an essential virulence factor has only occasionally been demonstrated conclusively.

UREASES FROM PATHOGENIC SPECIES OF *STAPHYLOCOCCUS*

Urease (urea amidohydrolase, EC 3.5.1.5) catalyzes the chemical reaction in which urea is degraded to form two molecules of ammonia and one molecule of bicarbonate [9].



urea bicarbonate

The initial reaction in which one of the amino groups is removed is enzyme-catalyzed while the second reaction in which the other amino group is removed is spontaneous. Under physiological conditions, the two ammonia products become protonated to form two ammonium ions (NH_4^+). The initial carbonic acid product (H_2CO_3) ionizes to form bicarbonate (HCO_3^-) and H^+. This leads to a net decrease in the H^+ concentration and raises the surrounding pH.

The urease enzyme is a nickel-containing protein that is typically composed of three types of subunits: the γ subunit or UreA protein (about 11 kDa) is encoded by *ureA* gene, the β subunit or UreB protein (about 15 kDa) is encoded by the *ureB* gene, and the α subunit or UreC protein (about 62 kDa) is encoded by the *ureC* gene [10, 11]. The complete enzyme is most often a trimer of trimers and has a $(\alpha\beta\gamma)_3$ structure with two Ni^{2+} ions at the active site. Table 1 lists in alphabetical order eight of the major *Staphylococcus* species whose urease proteins have been characterized. In some cases, this has been the result of direct biochemical studies but in others it has come from predicted features of genomic sequences. The table also shows the most common pathogenic effects of these species.

Staphylococcus aureus

Staphylococcus aureus is one of the most commonly encountered bacterial pathogens. *S. aureus* differs from most other species of *Staphylococcus* in that it is coagulase-positive, forms pigmented colonies, and exhibits poor growth in 15% NaCl [1, 7]. Although it is often associated with skin and wound infections, *S. aureus* is also a major cause of respiratory infections, cardiovascular infections, and bacteremia [12]. While these infections can be treated with antibiotics, a growing number of isolates of *S. aureus* are resistant to methicillin (MRSA). Among the

Table 1. Urease-positive pathogenic species of *Staphylococcus*.

Species	Pathogenic effects	Major urease subunits[a]	Native urease mass[b]	Predicted structure[c]
S. aureus	skin/soft tissue, respiratory, cardiovascular, urinary tract infections; bacteremia	α (UreC) - 61.8 kDa β (UreB) - 15.3 kDa γ (UreA) - 11.3 kDa	ND	$(\alpha\beta\gamma)_3$
S. cohnii subsp. *urealyticum*	renal stone; bacteremia	α (UreC) - 61.5 kDa β (UreB) - 15.6 kDa γ (UreA) - 11.2 kDa	ND	$(\alpha\beta\gamma)_3$
S. epidermidis	bacteremia; urinary tract infections	α (UreC) - 62.0 kDa β (UreB) - 14.9 kDa γ (UreA) - 11.1 kDa	ND	$(\alpha\beta\gamma)_3$
S. leei	gastric infection	α (UreC) - 65 kDa β (UreB) - 20 kDa γ (UreA) - 12 kDa	480 kDa	$(\alpha\beta\gamma)_5$
S. lugdunensis	skin/soft tissue infections, endocarditis, bone/joint, urinary tract infections	α (UreC) - 62.0 kDa β (UreB) – 14.9 kDa γ (UreA) - 11.1 kDa	ND	$(\alpha\beta\gamma)_3$
S. saprophyticus	urinary tract infections	α (UreC) - 61.9 kDa β (UreB) - 15.2 kDa γ (UreA) - 11.3 kDa	427 kDa	$(\alpha\beta\gamma)_4$
S. warneri	bacteremia; heart valve endocarditis; urinary tract infections	α (UreC) - 62.0 kDa β (UreB) - 15.0 kDa γ (UreA) - 11.1 kDa	ND	$(\alpha\beta\gamma)_3$
S. xylosus	bacteremia; urinary tract infections	α (UreC) – 61.8 kDa β (UreB) - 15.4 kDa γ (UreA) - 11.2 kDa	300 kDa	$(\alpha\beta\gamma)_3$ or $(\alpha\beta\gamma)_4$

[a]subunit sizes predicted from genomic data.
[b]native mass based on nondenaturing gel electrophoresis or gel filtration chromatography.
[c]predicted structures can be found in reference [11].
ND = not determined.

virulence factors that contribute to the pathogenicity of this bacterium are its ability to form a variety of protein toxins, its capacity to avoid host defenses, its potential to invade host tissues, and its success in forming biofilms. The last factor is particularly important in terms of the role of *S. aureus* as a catheter-associated urinary tract pathogen in post-surgical patients or older adults. The biofilm can block urine flow through the catheter and promote more severe disease. Although the formation of urease is less often discussed as a virulence factor, almost all natural and clinical isolates of *S. aureus* including methicillin-resistant ones contain the *ureABCEFGD* gene cluster needed for urease formation [13]. Urease activity can be detected in many of these samples [14, 15]. The activity of the urease can lead to a local change in pH and incontinence-associated dermatitis [16].

The genes for the urease from *S. aureus* Newman and *S. aureus* U500 were cloned onto a plasmid and expressed in *Staphylococcus carnosus* which is urease negative [17]. While the parental strains of *S. aureus* showed good urease activity in a basal peptone/yeast extract medium, this activity was not induced by urea. However, activity in the strains carrying the recombinant plasmids was noticeably reduced. The expression of the *ure* gene cluster was shown to increase 3- to 4-fold over a period of 48 hours when the bacteria were grown in a biofilm compared to free (planktonic) cells [18]. The formation of urease in *S. aureus* appears to be part of a complex response to an acidic environment. It has been demonstrated that expression of the urease genes is positively regulated by AgrA and CcpA, and negatively regulated by CodY and Rot [19-20]. Strains of *S. aureus* expressing a high level of urease activity contribute more than others to persistent urinary tract infections [21].

The urease enzyme from *S. aureus* seems to be composed of UreC (α), UreB (β), and UreA (γ) subunits with the typical $(\alpha\beta\gamma)_3$ structure but has not been well characterized biochemically. The amino acid sequences of the three subunits based on genomic DNA sequences have been reported in the UniProtKB database. The UreC (α) subunit is expected to contain 571 amino acids for a total molecular mass of 61,780 daltons. The UreB (β) subunit is expected to contain 136 amino acids for a total molecular mass of 15,264 daltons. The UreA (γ) subunit is expected to contain 100 amino acids for a total molecular mass of 11,273 daltons. If the native enzyme does have a $(\alpha\beta\gamma)_3$ structure, its mass would be about 265 kDa. It has been reported that *S. aureus* has a nickel ABC-transporter system which could provide the Ni^{2+} ions necessary for catalysis [22]. The role of the urease in the pathogenicity of *S. aureus* needs to be investigated further, but as the studies noted above suggest, it may be to neutralize the acidic pH of the skin surface or the urinary system.

Staphylococcus cohnii

Staphylococcus cohnii is a coagulase-negative species that is novobiocin-resistant and most often isolated from skin [1]. It has been divided into two subspecies: *S. cohnii* subsp. *cohnii* and *S. cohnii* subsp. *urealyticus* (*S. cohnii* subsp. *urealyticum*) based on colony size, pigmentation, alkaline phosphatase, β-galactosidase, and urease activity [23]. More recent genomic studies confirm these differences and indicate that the isolates in each group should be considered two separate species [24-26]. This has now been reported in the List of Prokaryotic names with Standing in Nomenclature (LPSN). Although *S. cohnii* subsp. *urealyticus* it is not often associated with urinary tract infections, it has been identified in a case of renal staghorn stones [27] and in a case of bacteremia [28].

The urease enzyme from *S. cohnii* subsp. *urealyticus* has not been characterized biochemically, but the genome does contain sequences encoding UreC (α), UreB (β), and UreA (γ) proteins. The amino acid sequences have been reported in the UniProtKB database. The UreC (α) subunit is expected to contain 567 amino acids for a total molecular mass of 61,479 daltons. The UreB (β) subunit is expected to contain 138 amino acids for a total molecular mass of 15,576 daltons. The UreA (γ) subunit is expected to contain 100 amino acids for a total molecular mass of 11,170 daltons. If the native enzyme does have the characteristic $(\alpha\beta\gamma)_3$ structure, its mass would be about 265 kDa. The role of the urease in the pathogenicity *of S. cohnii* subsp. *urealyticus* needs to be investigated further but it may be to neutralize the pH of skin or the urinary system.

Staphylococcus epidermidis

Staphylococcus epidermidis is a coagulase-negative species which can be isolated from most sites on the skin of humans and other mammals [1]. Although it is often considered a commensal microbe with benign effects on the host, it readily forms biofilms and produces other virulence factors that allow it to act as an opportunistic pathogen [29-31]. Infections due to *S. epidermidis* are most often related to the use of catheters that have been inserted into the cardiovascular system. Attachment of the bacteria to the plastic surfaces can lead to bacteremia [32]. Other infections may also occur due to prosthetic joints, shunts, or heart valves. Among the virulence factors that may be released by *S. epidermidis* in a biofilm are various exoenzymes including cysteine-, serine-, or metalloproteases and lipases. The bacteria are also capable of forming a urease activity which may be found in dental plaque [33, 34]. This urease activity has been found to be useful as a marker for bacterial adherence to plastic [35]. The expression of genes encoding urease subunits and urease activity has been measured in biofilms containing both *S. aureus* and *S. epidermis* [36]. The genes for the major urease subunits were downregulated in biofilms containing both species compared to monospecific biofilms. This was associated with a reduction in urease activity itself. This was found to be related to a decrease in metabolic activity and acid production by the bacteria.

Although *S. epidermidis* is rarely a cause of a urinary tract infection, there are a growing number of examples of where this has occurred in both female adults and children [37-40]. The genomes of several of the *S. epidermidis* isolates have been sequenced and analyzed [41-43]. The genes for the urease subunits were cloned into a plasmid and expressed in *Staphylococcus carnosus* [44]. Using an *in vitro* transcription/translation system, it was shown that they encoded proteins similar to those of other staphylococci. The native enzyme protein has not been characterized biochemically, but the predicted amino acid sequences have been reported in the UniProtKB database. The UreC (α) subunit is expected to contain 571 amino acids for a total molecular mass of 62,085 daltons. The UreB (β) subunit is expected to contain 133 amino acids for a total molecular mass of 14,916 daltons. The UreA (γ) subunit is expected to contain 100 amino acids for a total molecular mass of 11,152 daltons. If the native enzyme does have the characteristic $(\alpha\beta\gamma)_3$ structure, its mass would be about 264 kDa. The role of the urease from *S. epidermidis* needs to be studied further but it may be to neutralize the pH of the skin.

Staphylococcus leei

Staphylococcus leei strain SL100 was originally isolated as a coccoid bacterium from stomach biopsy samples of patients with gastric disorders in which the Gram-negative bacterium *Helicobacter pylori* was also present [45]. It was later isolated as a pure culture of spherical cells that formed large clusters, were positive for urease and catalase activities, and were able to bind to gastric mucin under acidic conditions. Based on its phenotypic similarities to *Staphylococcus cohnii* and *Staphylococcus xylosus*, it was named *S. leei*. Further characterization of the bacteria indicated that *S. leei* is closely related to *Staphylococcus saprophyticus* and *S. xylosus* based on its 16S rRNA sequence but it is coagulase positive [46].

The protein with urease activity was isolated after cell disruption by treatment with lysozyme and sonication, and purified by chromatography on DEAE-cellulose, Sephadex G-200, and hydroxyapatite columns [47]. It eluted from the gel filtration column with an apparent molecular mass of 260 kDa and gave a single band with a mass >200 kDa during electrophoresis on nondenaturing polyacrylamide gels. When the preparation was denatured by boiling in 0.4% sodium dodecyl sulfate (SDS) prior to electrophoresis, two subunits with molecular masses of 65 kDa and 21 kDa were observed. This suggested that the native enzyme has a $\alpha_3\beta_3$ structure, which is more like that of *H. pylori* than the other species of *Staphylococcus*. A larger scale high performance liquid chromatography (HPLC) purification scheme was later developed and used to purify the urease after cell disruption in a Dynomill with glass beads [48]. The lysate was clarified by filtration though a 0.2 μm hollow fiber filter and subjected to chromatography on Q-Sepharose, Porus HP2, Sephacryl S-300, and hydroxyapatite columns. SDS-polyacrylamide gel electrophoresis of this sample indicated the presence of three subunits with molecular masses of 65 kDa, 21 kDa, and 12 kDa. Gel filtration chromatography and nondenaturing gel electrophoresis suggested the native mass was 480 kDa, which was consistent with a $(\alpha\beta\gamma)_5$ structure. Although the authors do not explain the differences between the two purification procedures, it is possible that the first one did not effectively separate the β and γ subunits.

The urease genes from *S. leei* were amplified by polymerase chain reaction (PCR) using primers based on the *S. xylosus* sequence and analyzed. Three open reading frames were identified which were designated *ureA*, *ureB*, and *ureC*. These were predicted to code for polypeptides of 100 amino acids (about 12 kDa), 134 amino acids (about 20 kDa), and 571 amino acids (about 65 kDa), respectively. These would correspond to the γ, β, and α subunits seen in other species of *Staphylococcus*. A native enzyme with a $(\alpha\beta\gamma)_5$ structure would have a predicted molecular mass of 485 kDa. When the plasmid containing the *S. leei* urease genes was introduced into an expression strain of *E. coli*, there was only weak production of the α and β subunits and no urease activity. The UniProtKB data base currently only lists two subunits for the urease protein: UreA or α composed of 571 amino acids for a total molecular mass of 61,942, and UreB or β composed of 134 amino acids for a total molecular mass of 14,930. The structure of this enzyme thus needs to be further investigated and the complete sequence of the genome of *S. leei* remains to be determined. The role of this urease in the pathogenicity of *S. leei* also needs to be studied, but it may be to neutralize the acid in the stomach.

Staphylococcus lugdunensis

Staphylococcus lugdunensis is a coagulase-negative novobiocin-sensitive species that grows at temperatures of 30 °C to 45 °C and shows variable pigmentation and urease activity [1]. It was first isolated in 1988 in France [49], and while it has sometimes been confused with *Staphylococcus aureus*, it has markedly different properties [50]. It can be isolated as a commensal microbe from moist areas of the skin but has been increasingly found as an opportunistic pathogen [51-53]. Among the diseases associated with *S. lugdunensis* are skin and soft tissue infections, endocarditis, bacteremia, and bone and joint infections related to prosthetic devices or fractures. The bacteria form a range of virulence factors including adhesins, proteins used to release hemoglobin from erythrocytes and extract heme, and binding factors for fibrinogen and host proteins. They can also form biofilms which are unusual in that they are rich in proteins rather than polysaccharides. Although *S. lugdunensis* is rarely a cause of urinary tract infections, there are a growing number of cases, particularly since matrix assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS) has become available as a tool for identification [54, 55]. Most of these were community-acquired infections rather than catheter-associated infections and were more frequently observed in adult males rather than adult females [55, 56]. Urinary tract infections (UTIs) have also been demonstrated although infrequently in children [57-59].

The genome of *S. lugdunensis* has been sequenced and found to contain 2.6 Mbp. Although most isolates test positive for urease activity, the enzyme has not been characterized biochemically. The predicted amino acid sequences of the urease subunits have been reported in the UniProtKB database. The UreC (α) subunit is expected to contain 571 amino acids for a total molecular mass of 62,184 daltons. The UreB (β) subunit is expected to contain 135 amino acids for a total molecular mass of 15,201 daltons. The UreA (γ) subunit is expected to contain 100 amino acids for a total molecular mass of 11,125 daltons. If the native enzyme has the characteristic $(\alpha\beta\gamma)_3$ structure, its mass would be about 266 kDa. The role of the urease from *S. lugdunensis* needs to be studied further, although it may be to neutralize the pH of the skin.

Staphylococcus saprophyticus

Staphylococcus saprophyticus is a coagulase-negative oxidase-negative novobiocin-resistant species which is most often isolated from skin [1]. It has been divided into two subspecies, *S. saprophyticus* subsp. *saprophyticus* and *S. saprophyticus* subsp. *bovis* on the basis of colony size, the ability to reduce nitrate, the fermentation of D-ribose, and the mammals from which the bacteria were originally isolated. *S. saprophyticus* subsp. *saprophyticus* is the most common urease-positive Gram-positive urinary tract pathogen [60, 61]. It is most often associated with uncomplicated or community acquired UTIs, particularly in adolescent or adult sexually active women. These infections show a pronounced seasonal variation and are most common in the late summer and early fall. However, *S. saprophyticus* can also be a cause of catheter-associated infections in post-surgical patients or older institutionalized males.

S. saprophyticus forms a variety of virulence factors including the adhesive and autolytic protein Aas, uroadherence factor UafA, the fibronectin-binding adhesin UafB, the collagen-binding protein SdrI, and the surface associated lipase Ssp [61-64]. It also can make several surface polysaccharides including a capsular polysaccharide and a polysaccharide intercellular adhesin (PIA) containing *N*-acetylglucosamine that is synthesized by the products of the *icaADBC* genes. The bacteria can form complex biofilms which allow them to attach to the uroepithelium and to plastic catheters [65-67].

The urease enzyme from *S. saprophyticus* has been extensively studied. It was first isolated from a clinical sample and purified from a cell lysate by gel filtration chromatography on a Sephacryl S-300 column [68]. The purified protein showed an apparent molecular mass of 420,000 $\pm$ 16,000 and seemed to be composed of two subunits with masses of 70,000 and 63,000. The enzyme activity was not inducible by urea but showed a K_m for urea of 6.64 mM. A urease-negative mutant was constructed by nitrosoguanidine mutagenesis, and when the urease-positive and urease-negative bacteria were introduced into a rat model system, only the urease-positive bacteria developed a UTI with bladder stones. In later experiments, the urease genes from a type strain of *S. saprophyticus* were cloned onto a plasmid and introduced first into the urease-negative bacterium *S. carnosus* and then into a urease-negative mutant of *S. saprophyticus* [69]. The strain carrying the plasmid then showed signs of a urinary tract infection. When the genes on the plasmid were expressed *in vitro*, multiple polypeptides were produced, including ones with masses of 70 kDa, 47 kDa, 29 kDa, 27 kDa, 20 kDa, and 17 kDa. The structure of the enzyme was later clarified by biochemical experiments in which the urease was purified by chromatography on Q-Sepharose, Phenyl-Sepharose, and Sephacryl-300 columns, and by isoelectric focusing [70]. A purification factor of more than 800-fold was achieved. This preparation gave a native molecular mass as determined by gel filtration chromatography and nondenaturing gel electrophoresis of 420,000 $\pm$ 25,000. SDS-PAGE of the enzyme gave three bands with molecular masses of 72,400 kDa, 20,400 kDa, and 13,900 kDa, respectively. These corresponded to the α, β, and γ subunits found in other staphylococcal ureases. The native mass of 420,000 indicated that the enzyme probably has a $(\alpha\beta\gamma)_4$ structure. There were four atoms of Ni per molecule rather than the two Ni^{2+} ions seen with most other ureases. Unlike most ureases, the protein from *S. saprophyticus* did not contain any cysteine residues including one near the active site [70].

The genome of *S. saprophyticus* was later sequenced which allowed further clarification of the subunits [71]. According to the UniProtKB database, the UreC (α) subunit is expected to contain 571 amino acids for a total molecular mass of 61,942 daltons. The UreB (β) subunit is expected to contain 134 amino acids for a total molecular mass of 14,930 daltons. The UreA (γ) subunit is expected to contain 100 amino acids for a total molecular mass of 11,313 daltons. If the native enzyme has the expected $(\alpha\beta\gamma)_4$ structure, its mass would be about 353 kDa. If the native enzyme has a $(\alpha\beta\gamma)_5$ structure, its mass would be about 441 kDa, more like that of *S. leei*. This discrepancy indicates that the native structure of the *S. saprophyticus* enzyme should be re-evaluated. The activity of urease from *S. saprophyticus* leads to the formation of ammonium ions, which raises the urinary pH and can lead to the formation of urinary stones and catheter encrustations [72]. This can cause the blockage of catheters [73].

Staphylococcus warneri

Staphylococcus warneri is a coagulase-negative oxidase-negative species that forms sticky raised colonies [1]. It is normally found on the skin and is novobiocin-sensitive. Although it is often considered to be nonpathogenic, *S. warneri* has been found to be associated with cases of bacteremia [74, 75], prosthetic heart valve endocarditis [76], and urinary tract infection [77] in adults. It has also been found in neonatal infants [78]. Among the virulence factors formed by this bacterium are polysaccharides associated with biofilm formation and proteins involved in adhesion to epithelial cells [79]. Genes encoding various enterotoxins have been identified in samples from human food sources [80] and the complete genomes of several *S. warneri* samples have been assembled [81]. While the bacteria test positive for urease activity, the enzyme has not been characterized biochemically. The predicted amino acid sequences of the urease subunits have been reported in the UniProtKB database. The UreC (α) subunit is expected to contain 571 amino acids for a total molecular mass of 62,057 daltons. The UreB (β) subunit is expected to contain 135 amino acids for a total molecular mass of 15,046 daltons. The UreA (γ) subunit is expected to contain 100 amino acids for a total molecular mass of 11,109 daltons. If the native enzyme has the characteristic $(\alpha\beta\gamma)_3$ structure, its mass would be about 265 kDa.

Staphylococcus xylosus

Staphylococcus xylosus is a coagulase-negative novobiocin-resistant species that often forms pigmented colonies [1]. It is usually found on the skin of humans and other animals, particularly mice [82]. While it is rarely pathogenic, it has been found in a few cases of pyelonephritis [83] and bacteremia [84, 85]. The bacteria can form biofilms which can be inhibited by the flavonoid rutin [86] and analysis of its genome indicates the potential to form a number of other host-binding protein which can act as virulence factors [85, 87, 88].

The urease from *S. xylosus* C2a was purified from a cell lysate prepared after disruption of the bacteria with the enzyme lysostaphin and centrifugation at 60,009 x g [89]. After dialysis of the extract, the protein was purified by chromatography on Q-Sepharose, phenyl-Sepharose, Sephacryl-300, and Mono Q HR5 columns. The final preparation was tested for purity by isoelectric focusing. The molecular mass of the native enzyme as estimated by gel filtration chromatography was 300 kDa. There were about four Ni^{2+} ions per molecule, similar to that seen in *S. saprophyticus*. The protein was composed of three types of subunits with masses of 64 kDa, 17.8 kDa, and 16.3 kDa as estimated by SDS-polyacrylamide gel electrophoresis.

The gene cluster coding for this urease was later cloned and sequenced [90]. It contained three open reading frames encoding subunits with predicted masses of 11,000 (UreA or γ), 15,400 (UreB or β), and 61,800 (UreC or α). There were no cysteine residues in any of the subunits [90]. This was again similar to the enzyme from *S. saprophyticus* but different from most of other ureases. Based on the previous work indicating the presence of four Ni^{2+} ions per molecule, it was suggested that the native enzyme has a $(\alpha\beta\gamma)_4$ structure with one Ni^{2+} bound to each trimer. According to the sequences in the UniProtKB database, the UreC subunit contains 571 amino acids with a predicted molecular mass of 61,813, the UreB subunit contains 137 amino acids with a predicted molecular mass of 15,432, and the UreA subunits contains 100 amino acids with a predicted molecular mass of 11,256. A native enzyme with a $(\alpha\beta\gamma)_3$ structure would have a mass of about 265 kDa. A native enzyme with a $(\alpha\beta\gamma)_4$ structure would have a mass of 354 kDa. There have been no recent studies on this enzyme and so the nature of the native form remains to be resolved.

VARIATIONS IN UREASE SUBUNITS WITHIN A *STAPHYLOCOCCUS* SPECIES

Several isolates of the most extensively studied *Staphylococcus* species possessing urease activity have been characterized. To determine if there are any significant variations in the reported or expected amino acid sequences of the UreC (α), UreB (β), and UreA (γ) urease subunits, the sequences for *S. aureus*, *S. epidermidis*, and *S. saprophyticus* were retrieved from the UniProtKB database and analyzed. These sequences are listed in Table 2.

Staphylococcus aureus

For *S. aureus*, there are 27 sequences for UreC, almost all of which are 571 amino acids long. However, the sequence for A0A380DRV6 is 575 amino acids in length with four extra amino acids (LGGT) at the carboxyl-terminal end. The sequence for A0AAE8T112 is only 516 amino acids in length and is missing 55 amino acids from the amino-terminal end. There is a high degree of sequence identity (>98%) for the remaining sequences, with only an occasional amino acid substitution. Most of these are functionally conservative (for example, isoleucine for valine or arginine for lysine), except for the replacement of a serine residue with a proline at position 294 in sequences A0A6G4IRA0,

Table 2. Variations in urease subunits in pathogenic *Staphylococcus* species.

Species	Major subunit	Predicted sequence and strain[a]	Residues
Staphylococcus aureus	UreC (α)	P67405: *S. aureus* (strain MW2), P67404: *S. aureus* (strain N315), Q6GEE4: *S. aureus* (strain MRSA252), Q5HDR8: *S. aureus* (strain COL), Q6G732: *S. aureus* (strain MSSA476), A6U414: *S. aureus* (strain JH1), Q2FEK3: *S. aureus* (strain USA300), A5IV71: *S. aureus* (strain JH9), A6QJD0: *S. aureus* (strain Newman), A8Z387: *S. aureus* (strain USA300/TCH1516), P67403: *S. aureus* (strain Mu50/ATCC 700699), A7X5M3: *S. aureus* (strain Mu3/ ATCC700698), Q2YYQ6: *S. aureus* (strain bovine RF122/ET3-1), Q2G2K5: *S. aureus* (strain NCTC 8325/PS 47), A0A380DRV6: *S. aureus* (575 AA), A0A6A9GVJ7: *S. aureus*, A0A658BLK7: *S. aureus*, A0A2Y8TGG8: *S. aureus*, A0A641A967: *S. aureus*, A0A6A8FCS3: *S. aureus*, A0A6G4IRA0: *S. aureus*, A0AAE8TII2: *S. aureus* (516 AA), A0A224B4Q6: *S. aureus*, A0AAE3D4Z7: *S aureus*, A0A7U7EZU1: *S. aureus* subsp. *aureus* ST228, A0A0E1XE69: *S, aureus* subsp *aureus* MN8, W8U8P5: *S. aureus*	571
	UreB (β)	P67407: *S. aureus* (strain N315), Q6GEE5: *S. aureus* (strain MRSA252), Q5HDR9: *S. aureus* (strain COL), Q6G733: *S. aureus* (strain MSSA476), Q8NV90: *S. aureus* (strain MW2), A5IV70: *S. aureus* (strain JH9), A6U413: *S. aureus* (strain JH1), Q2FEK4: *S. aureus* (strain USA300), A6QJC9: *S. aureus* (strain Newman), A8Z386: *S. aureus* (strain USA300/TCH1516), P67406: *S. aureus* (strain Mu50/ATCC 700699), A7X5M0: *S. aureus* (strain Mu3/ATCC 700698), Q2YYQ7: *S. aureus* (strain bovine RF122/ET3-1), Q2G2K6: *S. aureus* (strain NCTC 8325/PS 47), A0A380ENP3: *S. aureus*, A0A8G2I073: *S. aureus*, A0A5S9C4Y2: *S. aureus*, A0A6A8FEJ0: *S. aureus*, A0A380DTE5: *S. aureus*, A0AA40JP37: *S. aureus*, A0A133PYH6: *S. aureus* (143 AA), A0A2S6D070: *S. aureus*, A0A2S6DCE7: *S. aureus*,A0AAE8TI47: *S. aureus*, A0A3A2IZI6: *S. aureus*, X5DXN5: *S. aureus*	136
	UreA (γ)	P67720: *S. aureus* (strain MW2), Q5HD50: *S. aureus* (strain COL), Q6G734: *S, aureus* (strain MSSA476), P67719: *S. aureus* (strain N315), Q6GEE6: *S. aureus* (strain MRSA252), A51V69: *S. aureus* (strain JH9), A6QJC8: *S. aureus* (strain Newman), A6U412: *S. aureus* (strain JH1), Q2FEK5: *S. aureus* (strain USA300), A8Z385: *S. aureus* (strain USA300/TCH1516), P67718: *S. aureus* (strain Mu50/ATCC 700699), A7X5L9: *S. aureus* (strain Mu3/ ATCC700698), Q2YYQ8: *S. aureus* (strain bovine RF122/ET3-1), Q2FVW5: *S. aureus* (strain NCTC 8325 /PS 47), A0A0E1XC19: *S. aureus* subsp. *aureus* MN8, A0A135PYG4: *S.* aureus (89 AA), A0A0D1G4V9: *S. aureus*	100

Table 2 continued..

Species	Major subunit	Predicted sequence and strain[a]	Residues
Staphylococcus epidermidis	UreC (α)	Q8CNC9: *S. epidermidis* (strain ATCC 12228/FDA PCI 1200), Q5HLW1: *S. epidermidis* (strain ATCC 35984/RP62A), A0A0E1VB93: *S. epidermidis* W23144, A0A4Y7VQ95: *S. epidermidis,* A0A2G7HW93: *S. epidermidis*	571
	UreB (β)	Q8CND0: *S. epidermidis* (strain ATCC 12228/FDA PCI 1200), Q5HLW2: *S. epidermidis* (strain ATCC 35984 / RP62A), A0A0E1VHP6: *S. epidermidis* W23144, A0A0N1EU57: *S. epidermidis*	133
	UreA (γ)	Q8CND1: *S. epidermidis* (strain ATCC 12228/FDA PCI 1200), Q5HLW3: *S. epidermidis* (strain ATCC 35984/RP62A), A0A0N1EL21: *S. epidermidis*	100
Staphylococcus saprophyticus	UreC (α)	Q4A0J5: *S. saprophyticus subsp. saprophyticus* (strain ATCC 15305/DSM 20229/NCIMB 8711 NCTC 7292/S-41), A0A380HJV3: *S. saprophyticus*	571
	UreB (β)	Q4A0J4: *S. saprophyticus* subsp. *saprophyticus* (strain ATCC 15305/DSM 20229/NCIMB 8711/NCTC 7292/S-41), A0A380HHW9: *S. saprophyticus*	134
	UreA (γ)	Q4A0J3: *S. saprophyticus* subsp. *saprophyticus* (strain ATCC 15305/DSM 20229/NCIMB 8711/NCTC 7292/S-41). A0A380HIH3: *S. saprophyticus*	100

[a]sequences from the UniProtKB database.

QEGEE4, A0A2Y8TGG8, and A0A0E1XE69, a glycine with a cysteine at position 372 in sequence A0A6A8FCS3, a glutamic acid with a valine at position 397 in sequence Q6GEE4, and an arginine with a cysteine at position 401 in sequence A0A6G4IRA0. Sequence A0A380DRV6 also differs from the others in that it has the residues LNSKILLI before the four extra amino acids at the carboxyl-terminal end.

For *S. aureus*, there are 26 UreB amino acid sequences, almost all of which are 136 residues long. Sequence A0A133PYH6 has 143 amino acids with the added sequence MFKGGSQ at the amino-terminal end. Again, there is a high degree of sequence identity (>98%) with only occasional amino acid substitutions. Most of these are conservative except for the replacement of a proline residue by an alanine at position 33 in sequence A0A6AA8FEJ, a glutamic acid residue by an alanine at position 115 in sequences P67407, A5IV70, A6U413, P67406, A7X5M0, and A0A342IZ16), an alanine by an aspartic acid at position 123 in sequences A0AA40JP37, A0A380DTE5, Q2YYQ7, A0A256D070, A0A256DCE7, A0A8G2I073, and A0A5S9C4Y2, and a glycine by an arginine at position 133 in sequences A6QJC9 and A0A380ENP3.

For *S. aureus*, there are 17 UreA sequences with a length of about 100 amino acids. These sequences show a perfect 100% match, except for sequence A0A135PYG4 which is missing 11 amino acids from the amino terminal end and is only 89 residues long.

Staphylococcus epidermidis

For *S. epidermidis,* there are 5 sequences for UreC, all of which are 571 amino acids long. There is >99% sequence identity except for three conservative substitutions at positions 245 (A0A4Y7VQ95), 400 (A0A4Y7VQ95), and 519 (A0A0E1VB93). For *S. epidermidis*, there are 4 UreB amino acid sequences, all of which are 133 residues long. There is a perfect match among these sequences except for one conservative substitution at position 15 in sequence A0A0E1VHP6. For *S. epidermidis* there are 3 UreA sequences with a length of 100 amino acids. These sequences show a perfect 100% match.

Staphylococcus saprophyticus

For *Staphylococcus saprophyticus*, there are only 2 reported sequences for each of the subunits. The UreC sequences are 571 amino acids in length and a perfect match. The UreB sequences are 134 residues long and show 97% identity. Sequence Q4A0J4 has a leucine at position 109 while sequence A0A380HHW9 has a proline at this position. Sequence Q4A0J4 has a serine at position 113 while sequence A0A380HHW9 has a glycine at this position. Sequence Q4A0J4 has a glutamic acid at position 127 while sequence A0A380HHW9 has a lysine at this position. The UreA sequences are noticeably different in that while sequence Q4A0J3 is 100 amino acids long like that of the other *Staphylococcus* species, sequence A0A380HIH3 is 133 residues long with the additional 33 amino acids at the amino-terminal end. The rest of the sequence is identical to that of Q4A0J3 except that the first methionine residue is replaced by a valine. The 33-residue extension begins with another methionine residue.

COMPARISON OF UREASE SUBUNITS AMONG *STAPHYLOCOCCUS* SPECIES

To determine if there are any significant variations in the reported or expected amino acid sequences of the UreC (α), UreB (β), and UreA (γ) urease subunits among the *Staphylococcus* species with significant urease activity, the sequences of the subunits from *S. aureus*, *S. cohnii*, *S. epidermidis*, *S. leei*, *S. lugdunensis*, *S. saprophyticus*, *S. warneri*, and *S. xylosus* were retrieved from the UniProtKB database and analyzed. The sequences from the type strain were used when several are available and they are all listed in Table 3. There is currently no entry for a UreA sequence from *S. leei*.

The multiple sequence alignments for the three subunits are shown in Figure 1. The abbreviations for the species in the sequences shown are *S. aureus* - STAAE, *S. lugdunensis* - STALU, *S. epidermidis* - STAES, *S. warneri* - STAWA, *S. cohnii* - STACC, *S. leei* - STAP, *S. saprophyticus* - STAS1, and *S. xylosus* – STAXY.

UreC subunits

The UreC subunit is the largest at 571 amino acid residues and shows some sequence variation.

Table 3. Comparative analysis of urease subunits from *Staphylococcus* species.

***Staphylococcus* species**	**UreC (α) sequence[a]**	**UreB (β) sequence[a]**	**UreA (γ) sequence[a]**
S. aureus (strain Newman)	A6QJD0	A6QJC9	A6QJC8
S. cohnii subsp. *cohnii*	A0A0M2NZJ5	A0A0M2P659	A0A0M2P3P3
S. epidermidis (strain ATCC 12228)	Q8CNC9	Q8CND0	Q8CND1
S. leei	A3RJV8	A3RJV7	not available
S. lugdunensis	A0A133QAH3	A0A133QAT9	A0A4Q9WG78
S. saprophyticus (ATCC 15305)	Q4A0J5	Q4A0J4	Q4A0J3
S. warneri	A0A2T4Q2L4	A0A2T4Q2L0	A0A2t4PIT2
S. xylosus	P42873	P42874	P42875

[a]sequences from UniProKB database.

The overall sequence identity for the eight sequences is 74.1%. Individual pairwise identities range from 96.8% (Q4A0J5 – *S. saprophyticus* vs. P42873 - *S. xylosus*) to 84.5% (A6QJD0 – *S. aureus* vs. A0A0M2NZJ5 – *S. cohnii*). Most of the amino acid replacements are conservative substitutions (for example, isoleucine for valine or arginine for lysine), but others involve replacing one charged amino acid with one of the opposite charge (for example, glutamic acid for arginine). Some amino acid replacements are more likely to have an effect on the secondary structure of the protein (for example, substituting proline for serine) since they would disrupt or alter an α-helix.

UreB subunits

The UreB subunit shows the most variation in sequence length, with the reported sequences ranging from 133 amino acids to 138 amino acids. The overall sequence identity for the eight sequences is 48.9%. Some of the amino acid replacements are again conservative, but others are more substantial and involve changes from nonpolar to polar residues, or changes from polar residues to charged residues. The greatest variation occurs between residue 108 and the carboxyl-terminal end.

UreA subunits

All of the sequences are 100 amino acid residues long, but as noted above, there is no entry for *S. leei*. The overall sequence identity is 76.0%. Most of the amino acid replacements are conservative, except for replacement of an alanine residue in *S. aureus* at position 26 by a glutamine in four of the sequences, a lysine residue in *S. aureus* at position 66 by a glutamic acid or alanine, an aspartic acid residue in *S. aureus* at position 74 by an alanine in six of the sequences, and a histidine residue in *S. aureus* at position 75 by a serine, asparagine, or aspartic acid in five of the sequences.

PHYLOGENETIC TREES BASED ON UREASE SUBUNIT SEQUENCES

Traditionally, phylogenetic trees for microorganisms have been based on similarities in 16S rRNA sequences [4, 91, 92]. To determine if the sequences of the urease subunits from these selected *Staphylococcus* species are consistent with these relationships, new trees were generated based on the amino acid sequences of the UreA, UreB, and UreC subunits. These are shown in Figure 2 and compared to a similar tree for the same *Staphylococcus* species based on 16S rRNA nucleotide sequences. There is no reported complete genomic or 16S rRNA sequence for *S. leei*.

A. UreC sequences

```
sp|A6QJD0|URE1_STAAE                MSFKMTQNQYTSLYGPTVGDSIRLGDTNLFAQIEKDYAVYGEEATFGGGKSIRDGMAQNP    60
tr|A0A133QAH3|A0A133QAH3_STALU      MSFKMTQSQYTSLYGPTVGDSVRLGDTNLFAQVEKDYANYGDEATFGGGKSIRDGMAQNP    60
sp|Q8CNC9|URE1_STAES                MSFKMTQSQYTSLYGPTVGDSVRLGDTNLFAQVEKDYANYGDEATFGGGKSIRDGMAQNP    60
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA      MSFKMTQSQYTSLYGPTVGDAVRLGDTNLFAEVEKDYANYGDEATFGGGKSIRDGMAQNP    60
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC          MTQSQYTSLYGPTIGDSVRLGDTNLFAKVEKDFATYGDEATFGGGKSIRDGMAQNP    56
tr|A3RJV8|A3RJV8_9STAP              MSFKMTQSQYTSLYGPTVGDSVRLGDTNLFARVERDYATYGDEAAFGGGKSIRDGMAQNP    60
sp|Q4A0J5|URE1_STAS1                MSFKMTQSQYTSLYGPTVGDSVRLGDTNLFARVERDYATYGDEAAFGGGKSIRDGMAQNP    60
sp|P42873|URE1_STAXY                MSFKMTQSQYTSLYGPTVGDSVRLGDTNLFARVEKDYATYGDEAAFGGGKSIRDGMAQNP    60
                                        ***.*********:**::*********.:*:*:* **:**:***************

sp|A6QJD0|URE1_STAAE                RVTRDDVNVADLVISNAVIIDYDKVVKADIGIKNGYIFAIGNAGNPDIMDNVDIIIGSTT    120
tr|A0A133QAH3|A0A133QAH3_STALU      NVTRDDRKVADLVLTNALIIDYDKVVKADIGVKNGYIMGIGKAGNPDIMDNVDIIIGATT    120
sp|Q8CNC9|URE1_STAES                NVTRDDKNVADLVLTNALIIDYDKIVKADIGIKNGYIFKIGKAGNPDIMDNVDIIIGATT    120
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA      NVTRDDKNVADLVLTNAMIIDYDKIVKADIGIKNGYIFAIGKAGNPDIMDNVNIIIGSTT    120
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC      NVTRDDKKVADLVITNALILDYDKVIKADIVVKNGYIMKIGKAGNPDIMDNVDIVIGATT    116
tr|A3RJV8|A3RJV8_9STAP              NVTRDDKQVADLVITNAMIIDYDKIVKADIGVKNGYIMKIGKAGNPDIMDNVDIIIGATT    120
sp|Q4A0J5|URE1_STAS1                NVTRDDKQVADLVITNAMIIDYDKIVKADIGVKNGYIMKIGKAGNPDIMDNVDIIIGATT    120
sp|P42873|URE1_STAXY                NVTRDDKQVADLVITNALILDYDKIVKADIGVKNGYIMKIGKAGNPDIMDNVDIIIGATT    120
                                    .***** :*****::**:*:****::**** :*****: **:**********:*:**:**

sp|A6QJD0|URE1_STAAE                DIIAAEGKIVTAGGIDTHVHFINPEQAEVALESGITTHIGGGTGASEGSKATTVTPGPWH    180
tr|A0A133QAH3|A0A133QAH3_STALU      DIIAAEGKIVTAGGIDTHVHFINPEQAEVALESGITTHIGGGTGASEGTKATTVTPGPWH    180
sp|Q8CNC9|URE1_STAES                DIIAAEGKIVTAGGIDTHVHFINPEQAEVALESGITTHIGGGTGASEGAKATTVTPGPWH    180
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA      DIISAEGKIVTAGGIDTHVHFINPEQAEVALESGITTHIGGGTGASEGAKATTVTPGPWH    180
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC      DVIAAEGKIITAGAIDTHVHFINPEQSLVALESGTTTLIGGGTGASEGSKATTVTPGPWY    176
tr|A3RJV8|A3RJV8_9STAP              DIISAEGKIVTAGGIDTHVHFINPEQSQVALESGITTHIGGGTGASEGTKATTVTPGPWH    180
sp|Q4A0J5|URE1_STAS1                DIISAEGKIVTAGGIDTHVHFINPEQSQVALESGITTHIGGGTGASEGTKATTVTPGPWH    180
sp|P42873|URE1_STAXY                DIISAEGKIVTAGGIDTHVHFVNPEQSQVALESGITTHIGGGTGASEGAKATTVTPGPWH    180
                                    *:*:*****:***.*******:****: ****** ** **********:**********:

sp|A6QJD0|URE1_STAAE                IHRMLEAAEGLPINVGFTGKGQATNPTALIEQINAGAIGLKVHEDWGATPSALSHALDVA    240
tr|A0A133QAH3|A0A133QAH3_STALU      IHRMLEVAEALPINVGFTGKGQAVNHTALIEQIHAGAIGLKVHEDWGATPSALRHALDVA    240
sp|Q8CNC9|URE1_STAES                IHRMLEAAEEMPINVGFTGKGQAVNHTALIEQIHAGAIGLKVHEDWGATPSALSHALDVA    240
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA      IHRMLEAAEALPINVGFTGKGQAVNHTALIEQIHAGVIGLKVHEDWGATPSALSHALDVA    240
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC      IHRMLEAAEQIPLNIGFTGKGQAVNHTALVEQIHAGVIGLKVHEDWGATPSALDHALQVA    236
tr|A3RJV8|A3RJV8_9STAP              LHRMLLAAESLPLNIGFTGKGQAVNHTALVEQIHAGAIGLKVHEDWGATPSALDHALQVA    240
sp|Q4A0J5|URE1_STAS1                LHRMLLAAESLPLNIGFTGKGQAVNHTALVEQIHAGAIGLKVHEDWGATPSALDHALQVA    240
sp|P42873|URE1_STAXY                LHRMLLAAESLPLNIGFTGKGQAVNHTALVEQIHAGAIGLKVHEDWGATPSALDHALQVA    240
                                    :**** .** :*:*:********.* ***:***:**.**************** ***:**
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Figure 1

Figure 1 continued..

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sp|A6QJD0|URE1_STAAE             DEFDVQIALHADTLNEAGFMEDTMAAVKDRVLHMYHTEGAGGGHAPDLIKSAAFSNILPS   300
tr|A0A133QAH3|A0A133QAH3_STALU   DEFDVQIALHADTLNEAGFMEDTMAAVKDRVLHMYHTEGAGGGHAPDLIKSASYPNILPS   300
sp|Q8CNC9|URE1_STAES             DEFDVQVALHADTLNEAGFMEDTMAAVKDRVLHMYHTEGAGGGHAPDLIKSAAYSNILPS   300
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA   DEFDVQIALHADTLNEAGFMEDTMKAVNNRVLHMYHTEGAGGGHAPDLIKSASYSNILPS   300
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC   DDYDIQVALHADTLNEAGFMEETMAAVKDRVLHMYHTEGAGGGHAPDLIKSASYSNILPS   296
tr|A3RJV8|A3RJV8_9STAP           DDYDVQIALHADTLNEAGFMEETMAAVKDRVLHMYHTEGAGGGHAPDLIKSAAYANILPS   300
sp|Q4A0J5|URE1_STAS1             DDYDVQIALHADTLNEAGFMEETMAAVKDRVLHMYHTEGAGGGHAPDLIKSAAYANILPS   300
sp|P42873|URE1_STAXY             DDYDVQIALHADTLNEAGFMEETMAAVKDRVLHMYHTEGAGGGHAPDLIKSAAYSNILPS   300
                                 *::*:*:**************:** **::***********************:: *****

sp|A6QJD0|URE1_STAAE             STNPTLPYTHNTVDEHLDMVMITHHLNAAIPEDIAFADSRIRKETIAAEDVLQDMGVFSM   360
tr|A0A133QAH3|A0A133QAH3_STALU   STNPTLPYTQNTVDEHLDMVMITHHLNASIPEDIAFADSRIRKETIAAEDVLQDMGVFSM   360
sp|Q8CNC9|URE1_STAES             STNPTLPYTHNTVDEHLDMVMITHHLNASIPEDIAFADSRIRKETIAAEDVLQDMGVFSM   360
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA   STNPTLPYTHNTVDEHLDMVMITHHLNASIPEDIAFADSRIRKETIAAEDVLQDMGVFSM   360
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC   STNPTLPYTHNTIDEHLDMVMITHHLNASIPEDVAFADSRIRKETIAAEDVLQDLGVFSM   356
tr|A3RJV8|A3RJV8_9STAP           STNPTLPYTVNTIDEHLDMVMITHHLNASIPEDIAFADSRIRKETIAAEDVLQDMGVFSM   360
sp|Q4A0J5|URE1_STAS1             STNPTLPYTVNTIDEHLDMVMITHHLNASIPEDIAFADSRIRKETIAAEDVLQDMGVFSM   360
sp|P42873|URE1_STAXY             STNPTLPYTVNTIDEHLDMVMITHHLNASIPEDIAFADSRIRKETIAAEDVLQDIGVFSM   360
                                 ********* **:***************:****:********************:*****

sp|A6QJD0|URE1_STAAE             ISSDSQAMGRVGEVITRTWQVAHRMKEQRGPLDGDFEHNDNNRIKRYIAKYTINPAITHG   420
tr|A0A133QAH3|A0A133QAH3_STALU   ISSDSQAMGRVGEVIIRTWQVAHRMKEQRGVLEGDTEYNDNHRIKRYIAKYTINPAITHG   420
sp|Q8CNC9|URE1_STAES             VSSDSQAMGRVGEVVTRTWQVAHRMKEQRGPLDGDFEYHDNNRIKRYIAKYTINPAITHG   420
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA   ISSDSQAMGRVGEVITRAWQTAHRMKEQRGPLEGDSEYNDNNRIKRYIAKYTINPAITHG   420
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC   VSSDSQAMGRVGEVITRTWQVAHRMKEQRGALEGDGEYNDNNRIKRYIAKYTINPAITHG   416
tr|A3RJV8|A3RJV8_9STAP           VSSDSQAMGRVGEVITRTWQVAHRMKEQRGLLDGDSEYNDNNRIKRYIAKYTINPAITHG   420
sp|Q4A0J5|URE1_STAS1             VSSDSQAMGRVGEVITRTWQVAHRMKEQRGLLDGDSEYNDNNRIKRYIAKYTINPAITHG   420
sp|P42873|URE1_STAXY             VSSDSQAMGRVGEVITRTWQVAHRMKEQRGSLDGDSEYNDNNRIKRYIAKYTINPAITHG   420
                                 :*************: *:**.********* *:** *::**:******************

sp|A6QJD0|URE1_STAAE             ISEYVGSIEPGKLADIVLWDPIFFGVKPELVVKGGLINSAVNGDANGSIPTSEPMKYRKM   480
tr|A0A133QAH3|A0A133QAH3_STALU   ISDYVGSIESGKLADLVMWDPIFFGVKPELVIKGGLINSAVNGDANGSIPTSEPQKYRKM   480
sp|Q8CNC9|URE1_STAES             ISDYVGSVEAGKLADLVMWEPEFFGAKPDLVVKGGMINSAVNGDANGSIPTSEPLKYRKM   480
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA   ISDYVGSIESGKLADLVLWDPAFFGVKPELVVKGGLINVAVNGDANGSIPTSEPMKYRRM   480
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC   ISEYVGSIDEGKLADIIMWDPAFFGVKPELVLKGGMINTAINGDANGSIPISEPLKYRNM   476
tr|A3RJV8|A3RJV8_9STAP           ISDYVGSIDEGKLADIILWEPAFFGVKPDVIVKGGLINAAINGDANGSIPTSEPLKYRKM   480
sp|Q4A0J5|URE1_STAS1             ISDYVGSIDEGKLADIILWEPAFFGVKPDVIVKGGLINAAINGDANGSIPTSEPLKYRKM   480
sp|P42873|URE1_STAXY             ISDYVGSIDEGKLADIIMWEPAFFAVKPDVIVKGGLINPAINGDANGSIPTSEPLKYRKM   480
                                 **:****:: *****:::*:* **..**::::***:** *:********* *** ***.*
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Figure 1

Figure 1 continued..

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sp|A6QJD0|URE1_STAAE              YGQYGGNLTSTSMTFVSKTAYENGINRALNLKRMVRPVKNIRQLSKADMKNNSATPKLDV    540
tr|A0A133QAH3|A0A133QAH3_STALU    YGQYGTNIQHTAITFVSTTAYENGIYNLLNLKRMVRPVHGIRNLSKKDMKHNHATPKLDV    540
sp|Q8CNC9|URE1_STAES              YGQFGGNITHTAMTFVSNTAYENGIYRQLNLKRMVRPVRNIRNLTKADMKNNNATPKIDV    540
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA    YGQYGGNITHTAMTFVSNTAYENGIYRQLNLQRMVRPVKNIRNLTKADMKNNSATPKLDV    540
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC    YGQLGGNMQSTAITFVSSTAYENDIGRSLGLKRKLRPVKNIRKLTKADMKNNNATPKLDV    536
tr|A3RJV8|A3RJV8_9STAP            YGQLGGNLQSTSMTFVSTTAYENDIGKLLGLKRKLRPVHNIRKLSKKDMKNNNATPDLDV    540
sp|Q4A0J5|URE1_STAS1              YGQLGGNLQSTSMTFVSTTAYENDIGKLLGLKRKLRPVHNIRKLSKKDMKNNNATPDLDV    540
sp|P42873|URE1_STAXY              YGQLGGNMQGTSMTFVSTTAYENDIGKLLGLKRKLRPVHNIRKLTKADMKNNSATPKIDV    540
                                  *** * *:  *::****.*****.* . *.*:* :***:.**:*:* ***:* ***.:**

sp|A6QJD0|URE1_STAAE              DPQTYEVYVDGEKITSNAATELPLTQRYFLF      571
tr|A0A133QAH3|A0A133QAH3_STALU    DPQTYEVFVDGEKITSEAATELPLTQRYFLF      571
sp|Q8CNC9|URE1_STAES              DPQTYEVFVDGNKITSEAATELPLTQRYFLF      571
tr|A0A2T4Q2L4|A0A2T4Q2L4_STAWA    DPQTYEVYVDGEKITSEPATELPLTQRYFLF      571
tr|A0A0M2NZJ5|A0A0M2NZJ5_STACC    DPQTYEVFVDGKKITSEPATELPLTQRYFLF      567
tr|A3RJV8|A3RJV8_9STAP            DPQTYEVFVDGEKITSEPATELPLTQRYFLF      571
sp|Q4A0J5|URE1_STAS1              DPQTYEVFVDGEKITSEPATELPLTQRYFLF      571
sp|P42873|URE1_STAXY              DPQTYEVFVDGEKITSEPATELPLTQRYFLF      571
                                  *******:***:****: *************
```

B. UreB sequences

```
sp|Q8CND0|URE2_STAES              -MIPGEIIVKNTEIEVNKHHPETVIEVKNTGDRPIQVGSHFHFFEANKALEFDREKAYGK    59
sp|A6QJC9|URE2_STAAE              -MIPGEIITKSTEVEINNHHPETVIEVENTGDRPIQVGSHFHFYEANAALDFEREMAYGK    59
tr|A0A133QAT9|A0A133QAT9_STALU    -MIPGEIKVKNTDIEINQGHPETVLTVKNTGDRPIQVGSHFHFYEANKALKFEREKTYGK    59
tr|A0A2T4Q2L0|A0A2T4Q2L0_STAWA    -MIPGEIRIKNTDIEINPNSPETVLEVKNTGDRPIQIGSHFHFFEANLALDFERNKAYGK    59
tr|A0A0M2P659|A0A0M2P659_STACC    MMKPGEIIVKRTEIDVNKGLHATVITVKNTGDRPIQVGSHYHFFEVNPALDFNREHAYGK    60
tr|A3RJV7|A3RJV7_9STAP            -MKPGEIIVKRTEIEVNQGHNATILNVKNTGDRPIQVGSHYHFFEANPALQFDHEKAYGK    59
sp|Q4A0J4|URE2_STAS1              -MKPGEIIVKRTEIEVNQGHNATILNVKNTGDRPIQVGSHYHFFEANPALQFDHEKAYGK    59
sp|P42874|URE2_STAXY              -MKPGEIIVKRTEIEVNRGHNATILDVKNTGDRPIQVGSHYHFFEANPALQFEREKAYGK    59
                                   * ****  * *::::*     *:: *:********:***:**:*.* **.*::: :***

sp|Q8CND0|URE2_STAES              HLDIPAGAAVRFEPGDEKKVQLVEYSGRRKIYGFRGLVDGDIDEERVFRPNDSNQ-NAAV    118
sp|A6QJC9|URE2_STAAE              HLDIPAGAAVRFEPGDKKEVQLVEYAGKRKIFGFRGMVNGPIDESRVYRPTDENDEYAGV    119
tr|A0A133QAT9|A0A133QAT9_STALU    HLDIPAGAAVRFEPGDEKKVQLVEYAGHRNIYGFRGMVNGKIDETRVYRPTGEHDSYAGV    119
tr|A0A2T4Q2L0|A0A2T4Q2L0_STAWA    HLDIPAGAAVRFEPGDVKQVQLVEYAGNRRIYGFRGFVNGPIDGSRVYRPTGEDDKYAGV    119
tr|A0A0M2P659|A0A0M2P659_STACC    RLDIPAGAAVRFEPGDEKEIQLVEYKGSRNIFGFHGKVNGPIDDTRVFKPDDYTKESDII    120
tr|A3RJV7|A3RJV7_9STAP            RLDIPAGAAVRFEPGDEKEVQLVEYSGKRKIYGFHGDVNGSIDESRVYKLEDDSTATEVI    119
sp|Q4A0J4|URE2_STAS1              RLDIPAGAAVRFEPGDEKEVQLVEYSGKRKIYGFHGDVNGSIDESRVYKLEDDSTATEVI    119
sp|P42874|URE2_STAXY              RLDIPAGAAVRFEPGDEKEVQLVEYSGKRRIFGFHGEVNGPIDEARVYKAEDDDSATEII    119
                                  :************** *::***** * *.*:**:* *:* **  **::  .       :
```

Figure 1

Figure 1 continued..

```
sp|Q8CND0|URE2_STAES                 K-NDAGEDNANKKGGK--      133
sp|A6QJC9|URE2_STAAE                 F-GDNGAENVNKKGRKRS      136
tr|A0A133QAT9|A0A133QAT9_STALU       F-NDQGEENLNKKGGQD-      135
tr|A0A2T4Q2L0|A0A2T4Q2L0_STAWA       F-NDEGHDNVNKKGGTK-      135
tr|A0A0M2P659|A0A0M2P659_STACC       DKATLSEDNANKESGYKR      138
tr|A3RJV7|A3RJV7_9STAP               AEQDKTSENANKGRG---      134
sp|Q4A0J4|URE2_STAS1                 AEQDKTSENANKGRG---      134
sp|P42874|URE2_STAXY                 AEENKVSENANKESGYNR      137
                                            :* **
```

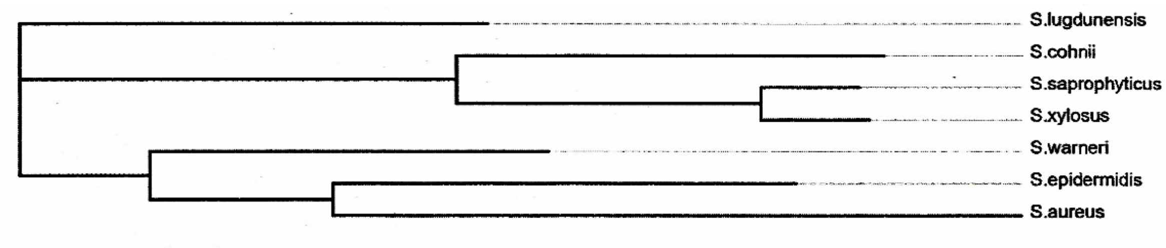
C. UreA sequences

```
sp|A6QJC8|URE3_STAAE                 MHFTQREQDKLMIVVAAEVARRRKARGLKLNHPEALALISDELLEGARDGKTVAELMSYG    60
tr|A0A2T4PIT2|A0A2T4PIT2_STAWA       MHFTQREQDKLMIVVAAEVARRRKARGLKLNHPEALALISDELLEGARDGKTVAELMSFG    60
sp|Q8CND1|URE3_STAES                 MHFTQREQDKLMIVVAAEVARRRKARGLKLNHPEALALISDELLEGARDGKTVAELMSYG    60
tr|A0A4Q9WG78|A0A4Q9WG78_STALU       MHFTQREQDKLMIVVAAEVARRRKARGLKLNHPEALALISDELLEGARDGKTVAELMSYG    60
tr|A0A0M2P3P3|A0A0M2P3P3_STACC       MHFTQREQDKLMLVIASDLARRRQQRGLKLNYPEAAAIISFELLEGARDGKSVAELMSYG    60
sp|Q4A0J3|URE3_STAS1                 MHFTQREQDKLMLVIAADLARRRQQRGLKLNYPEAVAIISFELLEGARDGKTVAELMSYG    60
sp|P42875|URE3_STAXY                 MHFTQREQDKLMLVIAADLARRRQQRGLKLNYPEAVAIISFELLEGARDGKTVAELMSYG    60
                                     ************:*:*:::****: ******:*** *:** **********:******:*

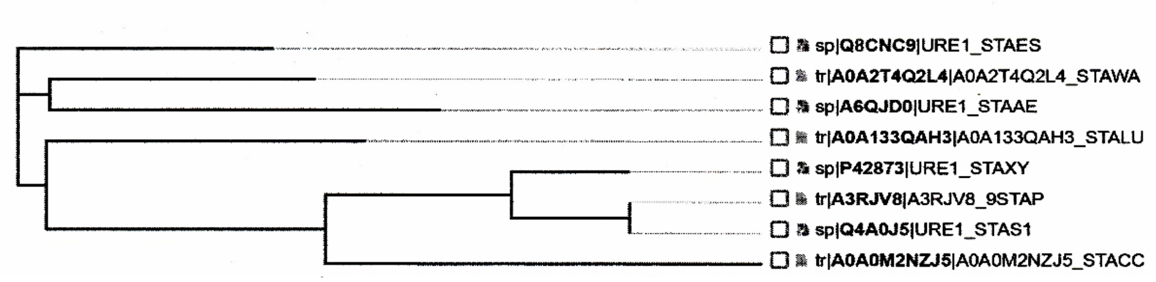
sp|A6QJC8|URE3_STAAE                 RQILNKEDVMDGVEHMITDIEIEATFPDGTKLITVHHPIV    100
tr|A0A2T4PIT2|A0A2T4PIT2_STAWA       KTILNEEDVMDGVASMITEIEIEATFPDGTKLITVHHPIV    100
sp|Q8CND1|URE3_STAES                 KTILNEEDVMDGVANMITELEIEATFPDGTKLITVHHPIV    100
tr|A0A4Q9WG78|A0A4Q9WG78_STALU       KTILNEEDVMDGVASMITELEIEATFPDGTKLITVHHPIV    100
tr|A0A0M2P3P3|A0A0M2P3P3_STACC       KQILSADDVMEGVADMLTDLEIEATFPDGTKLITVHHPIV    100
sp|Q4A0J3|URE3_STAS1                 KQILNEDDVMEGVADMLTEMEIEATFPDGTKLITVHHPIV    100
sp|P42875|URE3_STAXY                 KQILGEDDVMEGVADMLTEMEIEATFPDGTKLITVHHPIV    100
                                     : **. :***:**  *:*::********************
```

Figure 1. Multiple sequence alignments of urease subunits from *Staphylococcus* species.

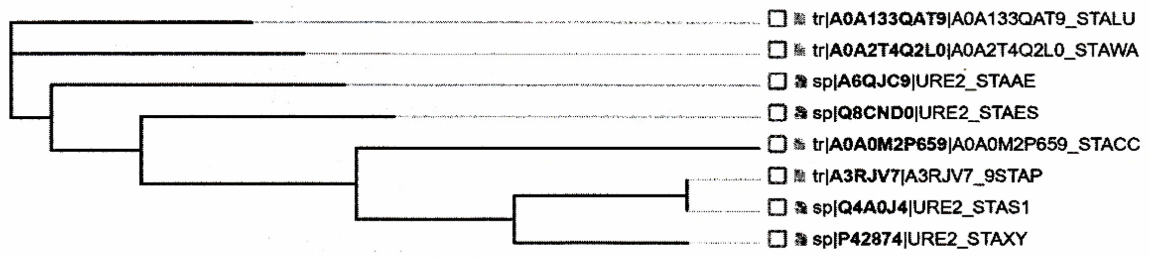
A. 16S rRNA nucleotide sequences



B. UreC amino acid sequences



C. UreB amino acid sequences



D. UreA amino acid sequences

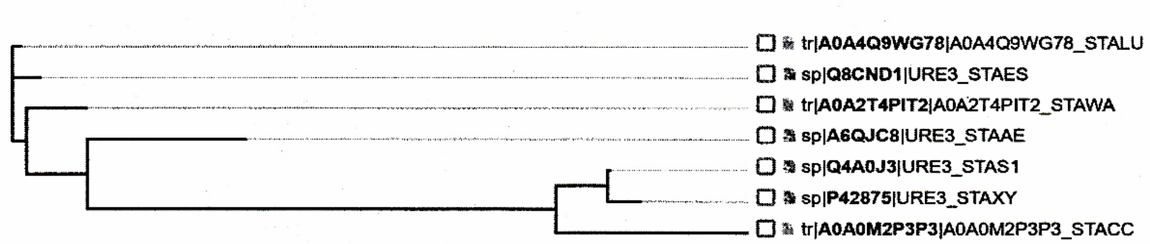


Figure 2. Phylogenetic trees of *Staphylococcus* sequences.

In the 16S rRNA tree, *S. aureus* and *S. epidermidis* are related but separated from a branch that leads to *S. warneri*. The 16S rRNA sequences of *S. saprophyticus* and *S. xylosus* are very closely related. These two species are in a different lineage which includes a branch that leads to *S. cohnii*. The 16S rRNA sequences in these three species are more distantly related to that of *S. lugdunensis*.

For the UreC sequences, the amino acid sequence for *S. leei* (A3RJV8) is most similar to that of *S. saprophyticus* (Q4A0J5). Together with the UreC sequence from *S. xylosus* (P42873), these species form a branch that is distantly related to the sequence for *S. cohnii* (A0A0M2NZJ5). This branch is separated from the one leading to the sequence from *S. lugdunensis* (A0A133QAH3), and well separated from the branches for *S. aureus* (A6QJD0), *S. warneri* (A0A2T4Q2L4), and *S. epidermidis* (Q8CNC9).

For the UreB amino acid sequences, the sequence of *S. leei* (A3RJV7) is again most similar to that of *S. saprophyticus* (Q4A0J5). Together with the UreB sequence from *S. xylosus* (P42874), these species form a branch that is distantly related to the sequence for *S. cohnii* (A0A0M2P659). This branch is aligned with the one leading to the sequence from *S. epidermidis* (Q8CND0), and well separated from the branches for *S. aureus* (A6QJC9), *S. warneri* (A0A2T4Q2L0), and *S. lugdunensis* (A0A133QAT9).

For the UreA subunits, the sequences for *S. saprophyticus* (Q4A0J3) and *S. xylosus* (P42875) are most closely related. They are found in a branch related to the sequence for *S. cohnii* (A0A0M2P3P3). This lineage is distantly related to the sequence from *S. aureus* (A6QJC8). These UreA sequences are well separated from the branch leading to *S. warneri* (A0A2T4PIT2). The sequences for *S. epidermidis* (A8CND1) and *S. lugdunensis* (A0A4Q9WG78) are only distantly related to the rest of the *Staphylococcus* species. These UreA relationships are similar to but in some ways different from those for UreC and UreB. The three urease protein trees thus are not the same as the 16S rRNA tree.

COMPARISON OF *STAPHYLOCOCCAL* UREASES TO OTHER SPECIES

There is an extensive literature on the ureases from other organisms. These include jack bean (*Canavalia ensiformis*), which has often been studied as a model system, as well as the Gram-negative bacteria *Helicobacter pylori*, *Klebsiella aerogenes*, *Morganella morganii, Proteus mirabilis*, and *Providencia stuartii* and the cell-wall deficient Gram-positive microbe *Ureaplasma urealyticum* [9-11, 93]. To compare the sequences of the *S. saprophyticus* and *S. aureus* enzymes with those of *Canavalia ensiformis, H. pylori*, *K. aerogenes*, *P. mirabilis*, and *U. urealyticum,* the other related sequences were retrieved from the UniProKB database and aligned with them. The alignments are shown in Figure 3. The abbreviations for the species in the sequences shown are *Ureaplasma urealyticum* - UREUR, *Canavalia ensiformis* – CANEN, *Helicobacter pylori* – HELPJ, *Staphylococcus saprophyticus* - STAS1, *Staphylococcus aureus* – STAAE, *Proteus mirabilis* – PROMH, and *Klebsiella aerogenes* – KLEAE.

The enzyme from jack bean (*Canavalia ensiformis*) is unusually long compared to the others (840 amino acids) and the native enzyme occurs as a dimer of two identical subunits. The carboxyl-terminal end of the jack bean enzyme shows significant sequence similarity to the UreC sequences from the bacteria. The *H. pylori* enzyme is composed of two subunits, one that is similar to UreC and one that is a combination of both UreB and UreA. The UreB or β subunit of *H. pylori* contains 569 amino acids and corresponds to the UreC sequence of the other bacteria. The UreA or α subunit of *H. pylori* contains 238 amino acids and has a sequence corresponding to staphylococcal UreA at its amino-terminal end and a sequence corresponding to staphylococcal UreB at its carboxyl-terminal end.

Of the 571 amino acid residues in the *S. saprophyticus* and *S. aureus* UreC sequences, 209 (36.6%) are identical to those in other five sequences. Of the 134 or 136 residues in the *S. saprophyticus* and *S. aureus* UreB sequences, 31 (23.0%) are identical to those in the other four bacterial sequences. Of the 100 residues in the *S. saprophyticus* and *S. aureus* UreA sequences, 38 (38.0%) are identical to those in the other four bacterial sequences. Many of the amino acid substitutions in all three sequences are conservative. Phylogenetic trees like those shown in Figure 2 indicate that the UreC sequences of *S. aureus* and *S. saprophyticus* are most closely related to that of *Ureaplasma urealyticum*. The UreB sequences, on the other hand, are most closely related to those of *Klebsiella aerogenes* and *Proteus mirabilis*, and the UreA sequences are most closely related to that of *Helicobacter pylori*.

The active site of almost all ureases is characterized by the presence of two Ni^{2+} ions, which are complexed with a set of histidine,

A. UreC sequences

```
sp|P0CB00|URE1_UREUR      ------------------------------------------------------------     0
sp|P07374|UREA_CANEN      MKLSPREVEKLGLHNAGYLAQKRLARGVRLNYTEAVALIASQIMEYARDGEKTVAQLMCL     60
sp|P69997|URE1_HELPJ      ------------------------------------------------------------     0
sp|Q4A0J5|URE1_STAS1      ------------------------------------------------------------     0
sp|A6QJD0|URE1_STAAE      ------------------------------------------------------------     0
sp|P17086|URE1_PROMH      ------------------------------------------------------------     0
sp|P18314|URE1_KLEAE      ------------------------------------------------------------     0

sp|P0CB00|URE1_UREUR      ------------------------------------------------------------     0
sp|P07374|UREA_CANEN      GQHLLGRRQVLPAVPHLLNAVQVEATFPDGTKLVTVHDPISRENGELQEALFGSLLPVPS     120
sp|P69997|URE1_HELPJ      ------------------------------------------------------------     0
sp|Q4A0J5|URE1_STAS1      ------------------------------------------------------------     0
sp|A6QJD0|URE1_STAAE      ------------------------------------------------------------     0
sp|P17086|URE1_PROMH      ------------------------------------------------------------     0
sp|P18314|URE1_KLEAE      ------------------------------------------------------------     0

sp|P0CB00|URE1_UREUR      ------------------------------------------------------------     0
sp|P07374|UREA_CANEN      LDKFAETKEDNRIPGEILCEDECLTLNIGRKAVILKVTSKGDRPIQVGSHYHFIEVNPYL     180
sp|P69997|URE1_HELPJ      ------------------------------------------------------------     0
sp|Q4A0J5|URE1_STAS1      ------------------------------------------------------------     0
sp|A6QJD0|URE1_STAAE      ------------------------------------------------------------     0
sp|P17086|URE1_PROMH      ------------------------------------------------------------     0
sp|P18314|URE1_KLEAE      ------------------------------------------------------------     0

sp|P0CB00|URE1_UREUR      ------------------------------------------------------------     0
sp|P07374|UREA_CANEN      TFDRRKAYGMRLNIAAGTAVRFEPGDCKSVTLVSIEGNKVIRGGNAIADGPVNETNLEAA     240
sp|P69997|URE1_HELPJ      ------------------------------------------------------------     0
sp|Q4A0J5|URE1_STAS1      ------------------------------------------------------------     0
sp|A6QJD0|URE1_STAAE      ------------------------------------------------------------     0
sp|P17086|URE1_PROMH      ------------------------------------------------------------     0
sp|P18314|URE1_KLEAE      ------------------------------------------------------------     0
```

Figure 3

Figure 3 continued..

```
sp|P0CB00|URE1_UREUR    ------------------------------MFKISRKNYSDLYGITTGDSVRLGDTNLWV    30
sp|P07374|UREA_CANEN    MHAVRSKGFGHEEEKDASEGFTKEDPNCPFNTFIHRKEYANKYGPTTGDKIRLGDTNLLA    300
sp|P69997|URE1_HELPJ    ------------------------------MKKISRKEYVSMYGPTTGDKVRLGDTDLIA    30
sp|Q4A0J5|URE1_STAS1    -----------------------------MSFKMTQSQYTSLYGPTVGDSVRLGDTNLFA    31
sp|A6QJD0|URE1_STAAE    -----------------------------MSFKMTQNQYTSLYGPTVGDSIRLGDTNLFA    31
sp|P17086|URE1_PROMH    ------------------------------MKTISRQAYADMFGPTTGDRLRLADTELFL    30
sp|P18314|URE1_KLEAE    ------------------------------MSNISRQAYADMFGPTVGDKVRLADTELWI    30
                                                         : :. * . :* *.** :**.**:*

sp|P0CB00|URE1_UREUR    KVEKDLTTYGEESVFGGGKTLREGMGMNSTMKLDDKLGNAEVMDLVITNALILDYTGIYK    90
sp|P07374|UREA_CANEN    EIEKDYALYGDECVFGGGKVIRDGMGQSCGHP--P----AISLDTVITNAVIIDYTGIIK    354
sp|P69997|URE1_HELPJ    EVEHDYTIYGEELKFGGGKTLREGMSQSNNPSKE-------ELDLIITNALIVDYTGIYK    83
sp|Q4A0J5|URE1_STAS1    RVERDYATYGDEAAFGGGKSIRDGMAQNPNVTRDD----KQVADLVITNAMIIDYDKIVK    87
sp|A6QJD0|URE1_STAAE    QIEKDYAVYGEEATFGGGKSIRDGMAQNPRVTRDD----VNVADLVISNAVIIDYDKVVK    87
sp|P17086|URE1_PROMH    EIEKDFTTYGEEVKFGGGKVIRDGMGQSQVVSAE-------CVDVLITNAIILDYWGIVK    83
sp|P18314|URE1_KLEAE    EVEDDLTTYGEEVKFGGGKVIRDGMGQGQMLAAD-------CVDLVLTNALIVDHWGIVK    83
                        .:* * : **:*  ***** :*:**. .               * :::**:*:*:  : *

sp|P0CB00|URE1_UREUR    ADIGIKNGKIASIGKSGNPHLTDGVD--MVVGISTEVSAGEGKIYTAGGLDTHVHWLEPE    148
sp|P07374|UREA_CANEN    ADIGIKDGLIASIGKAGNPDIMNGVFSNMIIGANTEVIAGEGLIVTAGAIDCHVHYICPQ    414
sp|P69997|URE1_HELPJ    ADIGIKDGKIAGIGKGGNKDMQDGVKNNLSVGPATEALAGEGLIVTAGGIDTHIHFISPQ    143
sp|Q4A0J5|URE1_STAS1    ADIGVKNGYIMKIGKAGNPDIMDNVD--IIIGATTDIISAEGKIVTAGGIDTHVHFINPE    145
sp|A6QJD0|URE1_STAAE    ADIGIKNGYIFAIGNAGNPDIMDNVD--IIIGSTTDIIAAEGKIVTAGGIDTHVHFINPE    145
sp|P17086|URE1_PROMH    ADIGIKDGRIVGIGKAGNPDVQPNVD--IVIGPGTEVVAGEGKIVTAGGIDTHIHFICPQ    141
sp|P18314|URE1_KLEAE    ADIGVKDGRIFAIGKAGNPDIQPNVT--IPIGAATEVIAAEGKIVTAGGIDTHIHWICPQ    141
                        ****:*:* *  **:.** .:  .*   : :*  *:  :.** * ***.:* *:*:: *:

sp|P0CB00|URE1_UREUR    IVPVALDGGITTVIAGGTGMNDGTKATTVSPGKFWVKSALQAADGLPINAGFLAKGQGME    208
sp|P07374|UREA_CANEN    LVYEAISSGITTLVGGGTGPAAGTRATTCTPSPTQMRLMLQSTDDLPLNFGFTGKGSSSK    474
sp|P69997|URE1_HELPJ    QIPTAFASGVTTMIGGGTGPADGTNATTITPGRRNLKWMLRAAEEYSMNLGFLAKGNASN    203
sp|Q4A0J5|URE1_STAS1    QSQVALESGITTHIGGGTGASEGTKATTVTPGPWHLHRMLLAAESLPLNIGFTGKGQAVN    205
sp|A6QJD0|URE1_STAAE    QAEVALESGITTHIGGGTGASEGSKATTVTPGPWHIHRMLEAAEGLPINVGFTGKGQATN    205
sp|P17086|URE1_PROMH    QAQEGLVSGVTTFIGGGTGPVAGTNATTVTPGIWNMYRMLEAVDELPINVGLFGKGCVSQ    201
sp|P18314|URE1_KLEAE    QAEEALVSGVTTMVGGGTGPAAGTHATTCTPGPWYISRMLQAADSLPVNIGLLGKGNVSQ    201
                            .: .*:** :.****   *:.*** :*.   :   * :.:   :* *: .**   :
```

Figure 3

Figure 3 continued..

```
sp|P0CB00|URE1_UREUR      -DPIFEQIVAGACGLKIHEDWGATGNAIDLALTVAEKTDVAVAIHTDTLNEAGFVEHTIA    267
sp|P07374|UREA_CANEN      PDELHEIIKAGAMGLKLHEDWGSTPAAIDNCLTIAEHHDIQINIHTDTLNEAGFVEHSIA    534
sp|P69997|URE1_HELPJ      DASLADQIEAGAIGFKIHEDWGTTPSAINHALDVADKYDVQVAIHTDTLNEAGCVEDTMA    263
sp|Q4A0J5|URE1_STAS1      HTALVEQIHAGAIGLKVHEDWGATPSALDHALQVADDYDVQIALHADTLNEAGFMEETMA    265
sp|A6QJD0|URE1_STAAE      PTALIEQINAGAIGLKVHEDWGATPSALSHALDVADEFDVQIALHADTLNEAGFMEDTMA    265
sp|P17086|URE1_PROMH      PEAIREQITAGAIGLKIHEDWGATPMAIHNCLNVADEMDVQVAIHSDTLNEGGFYEETVK    261
sp|P18314|URE1_KLEAE      PDALREQVAAGVIGLKIHEDWGATPAAIDCALTVADEMDIQVALHSDTLNESGFVEDTLA    261
                             : : : **. *:*:*****:*  *:  .* :*:. *: : :*:*****.*  *.::

sp|P0CB00|URE1_UREUR      AMKGRTIHAYHTEGAGGGHAPDILESVKYAHILPASTNPTIPYTVNTIAEHLDMLMVCHH    327
sp|P07374|UREA_CANEN      AFKGRTIHTYHSEGAGGGHAPDIIKVCGIKNVLPSSTNPTRPLTSNTIDEHLDMLMVCHH    594
sp|P69997|URE1_HELPJ      AIAGRTMHTFHTEGAGGGHAPDIIKVAGEHNILPASTNPTIPFTVNTEAEHMDMLMVCHH    323
sp|Q4A0J5|URE1_STAS1      AVKDRVLHMYHTEGAGGGHAPDLIKSAAYANILPSSTNPTLPYTVNTIDEHLDMVMITHH    325
sp|A6QJD0|URE1_STAAE      AVKDRVLHMYHTEGAGGGHAPDLIKSAAFSNILPSSTNPTLPYTHNTVDEHLDMVMITHH    325
sp|P17086|URE1_PROMH      AIAGRVIHVFHTEGAGGGHAPDVIKSVGEPNILPASTNPTMPYTINTVDEHLDMLMVCHH    321
sp|P18314|URE1_KLEAE      AIGGRTIHTFHTEGAGGGHAPDIITACAHPNILPSSTNPTLPYTLNTIDEHLDMLMVCHH    321
                          *. .*.:* :*:**********::      ::**:***** * * **  **:**:*: **

sp|P0CB00|URE1_UREUR      LNPKVPEDVAFADSRIRSQTIAAEDLLHDMGAISIMSSDTLAMGRIGEVVTRSWQMAHKM    387
sp|P07374|UREA_CANEN      LDREIPEDLAFAHSRIRKKTIAAEDVLNDIGAISIISSDSQAMGRVGEVISRTWQTADKM    654
sp|P69997|URE1_HELPJ      LDKSIKEDVQFADSRIRPQTIAAEDTLHDMGIFSITSSDSQAMGRVGEVITRTWQTADKN    383
sp|Q4A0J5|URE1_STAS1      LNASIPEDIAFADSRIRKETIAAEDVLQDMGVFSMVSSDSQAMGRVGEVITRTWQVAHRM    385
sp|A6QJD0|URE1_STAAE      LNAAIPEDIAFADSRIRKETIAAEDVLQDMGVFSMISSDSQAMGRVGEVITRTWQVAHRM    385
sp|P17086|URE1_PROMH      LDPSIPEDVAFAESRIRRETIAAEDILHDMGAISVMSSDSQAMGRVGEVILRTWQCAHKM    381
sp|P18314|URE1_KLEAE      LDPDIAEDVAFAESRIRRETIAAEDVLHDLGAFSLTSSDSQAMGRVGEVILRTWQVAHRM    381
                          *:  : **: **.**** :****** *:*:* :*: ***: ****:***: *:** *.:

sp|P0CB00|URE1_UREUR      KAQFGALKGDSEFNDNNRVKRYVAKYTINPAIAHGIDSYVGSIEVGKLADIVAWEPKFFG    447
sp|P07374|UREA_CANEN      KAQTGPLKCDSSDNDNFRIRRYIAKYTINPAIANGFSQYVGSVEVGKLADLVMWKPSFFG    714
sp|P69997|URE1_HELPJ      KKEFGRLKEEKGDNDNFRIKRYLSKYTINPAIAHGISEYVGSVEVGKVADLVLWSPAFFG    443
sp|Q4A0J5|URE1_STAS1      KEQRGLLDGDSEYNDNNRIKRYIAKYTINPAITHGISDYVGSIDEGKLADIILWEPAFFG    445
sp|A6QJD0|URE1_STAAE      KEQRGPLDGDFEHNDNNRIKRYIAKYTINPAITHGISEYVGSIEPGKLADIVLWDPIFFG    445
sp|P17086|URE1_PROMH      KLQRGTLAGDSADNDNNRIKRYIAKYTINPALAHGIAHTVGSIEKGKLADIVLWDPAFFG    441
sp|P18314|URE1_KLEAE      KVQRGALAEETGDNDNFRVKRYIAKYTINPALTHGIAHEVGSIEVGKLADLVVWSPAFFG    441
                          * : * *  :   *** *::**::*******:::*:   ***:: **:**:: *.* ***
```

Figure 3

Figure 3 continued..

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sp|P0CB00|URE1_UREUR      AKPYYVVKMGVIARCVAGDPNASIPTCEPVIMRDQFGTYGRSLTSTSVSFVSKIGLENGI    507
sp|P07374|UREA_CANEN      TKPEMVIKGGMVAWADIGDPNASIPTPEPVKMRPMYGTLGKAGGALSIAFVSKAALDQRV    774
sp|P69997|URE1_HELPJ      VKPNMIIKGGFIALSQMGDANASIPTPQPVYYREMFAHHGKAKYDANITFVSQAAYDKGI    503
sp|Q4A0J5|URE1_STAS1      VKPDVIVKGGLINAAINGDANGSIPTSEPLKYRKMYGQLGGNLQSTSMTFVSTTAYENDI    505
sp|A6QJD0|URE1_STAAE      VKPELVVKGGLINSAVNGDANGSIPTSEPMKYRKMYGQYGGNLTSTSMTFVSKTAYENGI    505
sp|P17086|URE1_PROMH      VKPALIIKGGMVAYAPMGDINAAIPTPQPVHYRPMYACLGKAKYQTSMIFMSKAGIEAGV    501
sp|P18314|URE1_KLEAE      VKPATVIKGGMIAIAPMGDINASIPTPQPVHYRPMFGALGSARHHCRLTFLSQAAAANGV    501
                          .**  ::* *.:  .  ** *.:*** :*:  *  :.  *       : *:*  .    :

sp|P0CB00|URE1_UREUR      KEEYKLEKELLPVKNCRSINKKSMKWNSATPNLEVDPQTFDAAVDYNDLENWLEQPAAEL    567
sp|P07374|UREA_CANEN      NVLYGLNKRVEAVSNVRKLTKLDMKLNDALPEITVDPESYTVKADGK-------------    821
sp|P69997|URE1_HELPJ      KEELGLERQVLPVKNCRNITKKDMQFNDTTAHIEVNPETYHVFVDGK-------------    550
sp|Q4A0J5|URE1_STAS1      GKLLGLKRKLRPVHNIRKLSKKDMKNNNATPDLDVDPQTYEVFVDGE-------------    552
sp|A6QJD0|URE1_STAAE      NRALNLKRMVRPVKNIRQLSKADMKNNSATPKLDVDPQTYEVYVDGE-------------    552
sp|P17086|URE1_PROMH      PEKLGLKSLIGRVEGCRHITKASMIHNNYVPHIELDPQTYIVKADGV-------------    548
sp|P18314|URE1_KLEAE      AERLNLRSAIAVVKGCRTVQKADMVHNSLQPNITVDAQTYEVRVDGE-------------    548
                               *.  :  * . * : * .*  *.   .: :: ::: . .*

sp|P0CB00|URE1_UREUR      AKKLKKTANGKYVLDAEPLTEAPLAQRYFLF   598
sp|P07374|UREA_CANEN      ------------LLCVSEATTVPLSRNYFLF   840
sp|P69997|URE1_HELPJ      ------------EVTSKPANKVSLAQLFSIF   569
sp|Q4A0J5|URE1_STAS1      ------------KITSEPATELPLTQRYFLF   571
sp|A6QJD0|URE1_STAAE      ------------KITSNAATELPLTQRYFLF   571
sp|P17086|URE1_PROMH      ------------PLVCEPATELPMAQRYFLF   567
sp|P18314|URE1_KLEAE      ------------LITSEPADVLPMAQRYFLF   567
                                       :  .   :::: :
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B. UreB sequences

```
sp|P0CB01|URE2_UREUR       ------------------------------------------------------------    0
sp|P14916|URE23_HELPY      MKLTPKELDKLMLHYAGELAKKRKEKGIKLNYVEAVALISAHIMEEARAGKKTAAELMQE    60
sp|Q4A0J4|URE2_STAS1       ------------------------------------------------------------    0
sp|A6QJC9|URE2_STAAE       ------------------------------------------------------------    0
sp|P17087|URE2_PROMH       ------------------------------------------------------------    0
sp|P18315|URE2_KLEAE       ------------------------------------------------------------    0
```

Figure 3

Figure 3 continued..

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sp|P0CB01|URE2_UREUR       ---------------------------------MSGSSNQFTPGKLVPGAINFA--EGEI     25
sp|P14916|URE23_HELPY      GRTLLKPDDVMDGVASMIHEVGIEAMFPDGTKLVTVHTPIEANGKLVPGELFLK--NEDI     118
sp|Q4A0J4|URE2_STAS1       ---------------------------------------------MKPGEIIVK--RTEI     13
sp|A6QJC9|URE2_STAAE       ---------------------------------------------MIPGEIITK--STEV     13
sp|P17087|URE2_PROMH       ---------------------------------------------MIPGEIRVNAALGDI     15
sp|P18315|URE2_KLEAE       ---------------------------------------------MIPGEYHVK--PGQI     13
                                                                        : **         ::


sp|P0CB01|URE2_UREUR       VMNEGREAKVISIKNTGDRPIQVGSHFHLFETNSALVFFDEKGNEDKERKVAYGRRFDIP     85
sp|P14916|URE23_HELPY      TINEGKKAVSVKVKNVGDRPVQIGSHFHFFEVNRCLDF---------DREKTFGKRLDIA     169
sp|Q4A0J4|URE2_STAS1       EVNQGHNATILNVKNTGDRPIQVGSHYHFFEANPALQF---------DHEKAYGKRLDIP     64
sp|A6QJC9|URE2_STAAE       EINNHHPETVIEVENTGDRPIQVGSHFHFYEANAALDF---------EREMAYGKHLDIP     64
sp|P17087|URE2_PROMH       ELNAGRETKTIQVANHGDRPVQVGSHYHFYEVNEALRF---------ARKETLGFRLNIP     66
sp|P18315|URE2_KLEAE       ALNTGRATCRVVVENHGDRPIQVGSHYHFAEVNPALKF---------DRQQAAGYRLNIP     64
                            :*   :    : : * ****:*:***:*: *.* .* *          :: : * :::*


sp|P0CB01|URE2_UREUR       SGTAIRFEPGDKKEVSVIDLVGTREVWGVNGLVNGKLKK---------------------     124
sp|P14916|URE23_HELPY      SGTAVRFEPGEEKSVELIDIGGNRRIFGFNALVDRQADNESKKIALHRAK-ERGFHGAKS     228
sp|Q4A0J4|URE2_STAS1       AGAAVRFEPGDEKEVQLVEYSGKRKIYGFHGDVNGSIDESRVYKLEDDSTATEVIAEQDK     124
sp|A6QJC9|URE2_STAAE       AGAAVRFEPGDKKEVQLVEYAGKRKIFGFRGMVNGPIDESRVYRPTDENDEYAGVFGDNG     124
sp|P17087|URE2_PROMH       AGMAVRFEPGQSRTVELVAFAGKREIYGFHGKVMGKLESEKK------------------     108
sp|P18315|URE2_KLEAE       AGTAVRFEPGQKREVELVAFAGHRAVFGFRGEVMGPLEVNDE------------------     106
                           :* *:*****:.: *.::   * * ::*... *    .


sp|P0CB01|URE2_UREUR       ------------       124
sp|P14916|URE23_HELPY      DDNY-VKTIKE-       238
sp|Q4A0J4|URE2_STAS1       TSENANKGRG--       134
sp|A6QJC9|URE2_STAAE       AENVNKKGRKRS       136
sp|P17087|URE2_PROMH       ------------       108
sp|P18315|URE2_KLEAE       ------------       106
```

Figure 3

Figure 3 continued..

C. UreA sequences

```
sp|P14916|URE23_HELPY     MKLTPKELDKLMLHYAGELAKKRKEKGIKLNYVEAVALISAHIMEEARAGKKTAAELMQE     60
sp|Q4A0J3|URE3_STAS1      MHFTQREQDKLMLVIAADLARRRQQRGLKLNYPEAVAIISFELLEGARDGK-TVAELMSY     59
sp|A6QJC8|URE3_STAAE      MHFTQREQDKLMIVVAAEVARRRKARGLKLNHPEALALISDELLEGARDGK-TVAELMSY     59
sp|P0CB02|URE3_UREUR      MNLSLREIQKLLVTVAADVARRRLARGLKLNYSEAVALITDHVVEGARDGK-LVADLMQS     59
sp|P17088|URE3_PROMH      MELTPREKDKLLLFTAGLVAERRLAKGLKLNYPEAVALISCAIMEGAREGK-TVAQLMSE     59
sp|P18316|URE3_KLEAE      MELTPREKDKLLLFTAALVAERRLARGLKLNYPESVALISAFIMEGARDGK-SVASLMEE     59
                          *.:: :* :**::  *. :*.:*  :*:***: *::*:*:  ::* ** **  .*.**.

sp|P14916|URE23_HELPY     GRTLLKPDDVMDGVASMIHEVGIEAMFPDGTKLVTVHTPIEANGKLVPGELFLKNEDITI     120
sp|Q4A0J3|URE3_STAS1      GKQILNEDDVMEGVADMLTEMEIEATFPDGTKLITVHHPIV-------------------     100
sp|A6QJC8|URE3_STAAE      GRQILNKEDVMDGVEHMITDIEIEATFPDGTKLITVHHPIV-------------------     100
sp|P0CB02|URE3_UREUR      AREVLRVDQVMEGVDTMVGIIQVEVTFPDGTKLVSVHSPIYK------------------     101
sp|P17088|URE3_PROMH      GRTVLTAEQVMEGVPEMIKDVQVECTFPDGTKLVSIHSPIV-------------------     100
sp|P18316|URE3_KLEAE      GRHVLTREQVMEGVPEMIPDIQVEATFPDGSKLVTVHNPII-------------------     100
                          .: :*  ::**:**  *:  : :*  ****:**:::* **

sp|P14916|URE23_HELPY     NEGKKAVSVKVKNVGDRPVQIGSHFHFFEVNRCLDFDREKTFGKRLDIASGTAVRFEPGE     180
sp|Q4A0J3|URE3_STAS1      ------------------------------------------------------------     100
sp|A6QJC8|URE3_STAAE      ------------------------------------------------------------     100
sp|P0CB02|URE3_UREUR      ------------------------------------------------------------     101
sp|P17088|URE3_PROMH      ------------------------------------------------------------     100
sp|P18316|URE3_KLEAE      ------------------------------------------------------------     100

sp|P14916|URE23_HELPY     EKSVELIDIGGNRRIFGFNALVDRQADNESKKIALHRAKERGFHGAKSDDNYVKTIKE       238
sp|Q4A0J3|URE3_STAS1      ----------------------------------------------------------       100
sp|A6QJC8|URE3_STAAE      ----------------------------------------------------------       100
sp|P0CB02|URE3_UREUR      ----------------------------------------------------------       101
sp|P17088|URE3_PROMH      ----------------------------------------------------------       100
sp|P18316|URE3_KLEAE      ----------------------------------------------------------       100
```

Figure 3. Alignment of urease subunit sequences from multiple organisms.

lysine, and aspartate residues [94]. In the *Klebsiella aerogenes* and *Helicobacter pylori* proteins, these are identified as His249, His275, Asp363, His137, His139, and Lys220. Analysis of the *S. aureus* and *S. saprophyticus* sequences indicates that the same amino acids occur at these positions in those proteins as well.

INHIBITION OF UREASE ACTIVITY

One approach to the treatment of diseases caused by those *Staphylococcus* species with urease activity involves inhibition of the enzyme's function. There is an extensive scientific literature on urease inhibitors which have been isolated from natural sources or synthesized in the laboratory [95-103]. Historically, most experimental studies have used either the enzyme from jack bean (*Canavalia ensiformis*) as a model system or the enzymes from *Helicobacter pylori* and *Proteus mirabilis* because these bacteria are major gastrointestinal or urinary tract pathogens, respectively. Most of the inhibition experiments with *Staphylococcus* species have focused on *S. saprophyticus* because it too causes a significant number of urinary tract infections. Among the general classes of chemical compounds that have been investigated are substrate analogues, hydroxamates, phosphoramides, polyphenolic compounds, and imidazoles. More recently, there has been increased interest in the inhibition of urease activity in *S. saprophyticus* by over-the-counter natural products and plant extracts which often contain complex mixtures of compounds.

Inhibiton of urease activity by specific chemicals

Table 4 summarizes the specific individual chemicals that have been found to inhibit the urease activity in *S. saprophyticus*. While all of the compounds were initially tested for inhibitory activity *in vitro* using cell-free extracts, many have also been tested *in vivo* using washed whole cells or during the growth of bacterial cultures in an artificial urine medium. When grown in this medium, *S. saprophyticus* cultures show a marked increase in pH from 6.5 to more than 8.5 as hydrolysis of urea leads to the formation of ammonium ions. When the urease activity in the bacteria is inhibited, the timing of this increase may be delayed and the extent of the pH change may be reduced.

Among the substrate analogues that inhibit *S. saprophyticus* urease activity are hydroxyurea, boric acid, and dimethylsulfoxide (DMSO). Hydroxyurea has been found to be both a substrate and an inhibitor of several ureases, including those from *Proteus mirabilis*, *Morganella morganii*, *Providencia stuartii*, and *Ureaplasma urealyticum*. It also inhibits the activity from *S. saprophyticus* [70]. Hydroxyurea was used in

Table 4. Inhibition of urease activity in *Staphylococcus saprophyticus* by single chemicals.

Chemical class	Inhibitor	Inhibition in cell extracts	Inhibition in whole cells[a]	Reference
Substrate analogue	hydroxyurea	+	NT	70
	boric acid	+	+	104
	dimethylsulfoxide	+	+	105
Hydroxamic acid	acetohydroxamic acid	+	+	68, 70, 106
	DL-phenyalanine lhydroxamic acid	+	-	106
Phosphoramide	fluorofamide	+	+	106
Polyphenolic compound	(+) catechin hydrate	+	+	106
	(-) epigallocatechin gallate	+	+	106
	resveratrol	+	+	107

[a]NT indicates not tested.

several clinical studies in the 1970s and 1980s with small numbers of patients who were treated concurrently with antibiotics. It was found to maintain urine at an acidic pH and to reduce the reoccurrence of renal stone formation. However, these studies were not continued and most clinical work has focused on other compounds. Boric acid is a rapid reversible competitive inhibitor of urease in cell-free extracts from *Proteus mirabilis*, but it has not been used clinically. A recent series of experiments indicated that when boric acid was combined with dimethylsulfoxide and sodium fluoride, it inhibited the growth of *S. saprophyticus* in artificial urine medium and normal human urine [104]. Dimethylsulfoxide (DMSO) resembles urea and is commonly used as solvent for various nonpolar organic compounds and plant extracts. DMSO was found to inhibit the ureases from *P. mirabilis* and *S. saprophyticus* [105]. Kinetic analysis indicated inhibition was of the noncompetitive type in which the V_{max} decreased and the K_m remained the same. DMSO also inhibited urease activity in whole cells. 10% DMSO slowed the growth rate and reduced the yield in a rich medium (LB) or in LB medium supplemented with 100 mmol L^{-1} urea. When the bacteria were grown in an artificial urine medium containing 10% DMSO, the growth rate was again reduced and the increase in pH associated with urease activity was delayed. A combination of 25 mmol L^{-1} sodium fluoride, 25 mmol L^{-1} boric acid, and 10% DMSO almost completely inhibited growth and the pH increase in cultures grown in human urine [104].

Hydroxamates are a large family of compounds that resemble urea and that can act as urease inhibitors. Acetohydroxamic acid (AHA) is the most extensively studied chemical in this group. In an early study, it was found to inhibit the urease activity from sword bean powder. AHA was later shown to inhibit the activities of the enzyme in cell-free extracts from *Proteus mirabilis*, *Providencia stuartii*, *Morganella morganii*, *Klebsiella pneumoniae*, *Ureaplasma urealyticum*, and *Staphylococcus saprophyticus* [68, 70]. In the case of *S. saprophyticus*, AHA was found to be a competitive inhibitor of urease activity with a K_i of 8.2 μg mL^{-1} in a cell-free extract. It also inhibited urease activity in whole cells and at a concentration of 10 μg mL^{-1} slowed the increase in pH that normally occurs in an artificial urine medium [106]. DL-phenylalanine hydroxamic acid also inhibited the urease activity of *S. saprophyticus* in cell-free extracts [106]. While AHA has been extensively investigated for its clinical potential, it is no longer recommended for use in patients because of its toxicity.

Phosphoramides (or *N*-acylphosphoric triamides or diamides) are a large class of compounds that are potent urease inhibitors. *N*-benzoyl- and *N*-isopentenoyl- phosphoric acid (BPA and IPA) were shown in the 1980s to inhibit ureases in crude extracts from a variety of sources including *Proteus mirabilis*. Since then, the most commonly used inhibitor is fluorofamide [*N*-(diaminophosphinyl)-4-fluorobenzamide]. It has been found to inhibit the urease activities from *Proteus mirabilis* and *Ureaplasma urealyticum*. Fluorofamide inhibited urease activity in cell extracts and whole cells of *S. saprophyticus* [106]. It also blocked the increase in pH that occurred in an artificial urine medium. However, fluorofamide has never been tested in carefully controlled human clinical trials.

Polyphenolic compounds include a large number of chemicals that have two or more hydroxyl groups attached to aromatic or nonaromatic carbon skeletons. The major groups include terpenoids, phenol and phenolic acids, stilbenes, catechins, flavones, coumarin, alkaloids, and quinones. Some are modified by the addition of sugar moieties. The simple compound catechol was shown to inhibit the ureases from jack bean (*Canavalia ensiformis*) and *Sporosarcina pasteurii* by binding irreversibly to cysteine residues at the active site. (+) Catechin hydrate and (-) epigallocatechin gallate are plant polyphenolics that have been reported to have antibacterial and antioxidant activities. Both compounds inhibited the urease activity in cell-free extracts and whole cells of *Staphylococcus saprophyticus* [106]. However, (+) catechin hydrate at a concentration of 1 mg mL^{-1} had no effect on the pH of cultures in an artificial urine medium. As noted above, the urease from *S. saprophyticus* does not contain any cysteine residues but rather has threonine at the active site. This may make it less susceptible to these potential inhibitors. Resveratrol (3,4',5-trihydroxystilbene) is a naturally occurring polyphenolic compound found in over 70 plant species. Increasing concentrations of purified resveratrol up to 5 mmol L^{-1} gradually reduced the urease activity in cell-free extracts of *S. saprophyticus* and *Proteus mirabilis* [107]. In the case of *S. saprophyticus*, it showed simple competitive inhibition kinetics in which V_{max} stayed the same and the apparent K_m increased. Purified resveratrol also showed partial inhibitory activity towards the urease in whole cells (about 50% inhibition at 5 mmol L^{-1}); a commercial product from Herbasway Laboratories had a similar effect. When pure resveratrol was added to cultures of *S. saprophyticus* in artificial culture medium at a concentration of 1 mmol L^{-1} (close to the minimum inhibitory concentration), there was an increase in the lag phase and a somewhat lower yield.

Omeprazole and the related compound lansoprazole are examples of imidazoles, which can also act as inhibitors for some ureases. They were originally described as inhibitors of the urease activity from the gastrointestinal pathogen *Helicobacter pylori* due to covalent modification of cysteine residues at the active site. However, they were found not to inhibit the urease activities from *Proteus mirabilis*, *Proteus vulgaris*, and *Providencia rettgeri*. Omeprazole also did not inhibit the urease activity from *S. saprophyticus* [106]. This is probably because this protein has threonine rather than cysteine at the active site [70].

Inhibition of urease activity by plant extracts

Because prescription drugs can be expensive and require approval of a healthcare provider, many people with urinary tract infections rely on less expensive over-the-counter products that can be obtained in grocery or drug stores or from online sources. Because these are complex mixtures, the concentrations of the specific chemicals in them are usually unknown. While some of these products have the ability to inhibit the urease activity from *S. saprophyticus*, many do not [108]. Table 5 lists the various plant extracts that have been found to inhibit the urease activity in *S. saprophyticus in vitro* and *in vivo*.

Green tea extract is derived from the leaves of *Camellia sinensis* and is widely promoted for the treatment of cancer, kidney disease, diabetes, and hypertension. It is said to have anti-inflammatory, antioxidant, and antibacterial activities. The composition of green tea extract is very complex, but among the phenolic chemicals present are (-) epicatechin, (-) epicatechin-3-gallate, (-) epigallocatechin and (-) epigallocatechin-3-gallate. Analysis of the total concentration of phenolic compounds in three different commercial samples of green tea extract indicated that they ranged from 21.8 mg mL^{-1} to 126.6 mg mL^{-1} [107]. As noted above, (+) catechin and (-) epigallocatechin gallate were found to reduce the urease activity in extracts and whole cells of *S. saprophyticus* [106]. Sprouts™ green tea extract was able to inhibit this activity in cell-free extracts and whole cells of *S. saprophyticus* and to

Table 5. Inhibition of urease activity in *Staphylococcus saprophyticus* by plant extracts.

Extract/plant source	Inhibition in cell extracts	Inhibition in whole cells	Reference
Green Tea/*Camellia sinensis*	+	+	[106, 108]
Uva Ursi/*Arctostaphylos uva-ursi*	+	+	[108, 110]
Cranberry/*Vaccinium macrocarpon*	±	NT	[108]
Garlic/*Allium sativum* L	-	NT	[108]
Curcumin/Turmeric (*Curcuma longa*)	-	+	[111]
Berberine/Goldenseal (*Hydrastis canadensis*)	NT	+	[111]

block the increase in pH that normally occurs during growth in artificial culture medium [106].

Uva Ursi (*Arctostaphylos uva-ursi*) or bearberry is a low-lying shrub that is widely distributed across North America, Europe, and Asia. Commercial preparations of Uva Ursi derived from its leaves or berries are widely promoted for the treatment of both urinary tract infections and prostate disorders and have been shown to have antimicrobial activity, antioxidant activity, and anti-inflammatory activity. Although these extracts contain a wide range of organic compounds, the clinical effects of Uva Ursi extracts have been attributed to arbutin (4-hydroxyphenyl-β-D-glucopyranoside). Arbutin undergoes hydrolysis to yield free D-glucose and hydroquinone, which may then undergo oxidation to form 1,4-benzoquinone (*p*-benzoquinone). Commercial samples of Uva Ursi were found to vary in phenolic compound concentration from 6.8 mg mL^{-1} to 93.6 mg mL^{-1} [109]. Urease activity in cell-free extracts of both *S. saprophyticus* and *Proteus mirabilis* was found to be inhibited by four different commercial samples of Uva Ursi extract [110]. Neither enzyme was sensitive to inhibition by arbutin. The urease from *S. saprophyticus* was inhibited by hydroquinone while the enzyme from *P. mirabilis* was inhibited by both hydroquinone and 1,4-benzoquinone. Both enzymes showed mixed inhibition kinetics. The urease in whole cells of *S. saprophyticus* was sensitive to inhibition by the Uva Ursi extract and hydroquinone while the enzyme in whole cells of *P. mirabilis* was sensitive to the extract, hydroquinone, and 1,4-benzoquinone. When *S. saprophyticus* was grown in an artificial urine medium, the presence of 1% (v/v) Uva Ursi extract slowed the growth rate and delayed the increase in pH.

Extracts of cranberry (*Vaccinium macrocarpon*) have been widely used for the prevention and treatment of urinary tract infections. The extract may be consumed as a juice or concentrated tablets and contains water, sugars, organic acids, and a wide range of compounds including flavonoids and polyphenolics. Cranberry extract has antioxidant and antimicrobial activity against uropathogenic *Escherichia coli* (UPEC) as well as *Proteus mirabilis* and *Staphylococcus saprophyticus*. The evidence for the inhibition of urease activity by cranberry extract or its constituents is very limited. When a cell-free extract from *S. saprophyticus* was treated with a commercial cranberry extract, there was good inhibition of urease activity [108]. However, this was found to be due to the highly acidic nature of the extract and a diluted or neutralized sample of the extract had little effect.

Garlic (*Allium sativum* L) has been used to treat a variety of human illnesses because it has been reported to have antioxidant, anti-inflammatory, and antimicrobial activities. Garlic extracts contain a mixture of sulfur-containing compounds including allicin (diallyl thiosulfinate), diallyl disulfide, and S-allyl cysteine. Crude extracts of garlic as well as extracts of onion, leeks, and cabbage were found to inhibit the urease activity from jack bean *in vitro*. Pure allicin inhibited this urease in a biphasic fashion but inhibition was prevented by boric acid, a reversible competitive inhibitor. Allicin was also found to inhibit the urease activity from *Proteus mirabilis* in both cell extracts and whole cells. On the other hand, a commercial over-the-counter garlic preparation (Krolik) had only a limited effect on the urease activity from *Staphylococcus saprophyticus* [108].

Curcumin (1,7-bis-(4-hydroxy-3-methoxyphenyl)-hepta-1,6-diene-3,5-dione) and its derivatives are the active components of turmeric, an herbal preparation derived from the rhizomes of *Curcuma longa*. Curcumin has been reported to have a wide range of pharmacological uses as an antibiotic, antioxidant, and anti-inflammatory agent, with positive effects for the treatment of cancer, ageing, diabetes, neurological, and cardiovascular diseases. It was found to inhibit the growth of *Proteus mirabilis* and to delay the formation of crystals in an artificial urine medium. Over-the-counter preparations of curcumin as turmeric, which vary in phenolic compound concentration [109], had little effect on the activity of the urease from *Staphylococcus saprophyticus* [106]. This was largely due to the poor solubility of the compound in aqueous solutions. However, it was found to be more effective when combined with the alkaloid berberine [111]. An extract from Golden Seal (*Hydrastis canadensis*) containing this compound was found to inhibit the growth of *S. saprophyticus* in agar plate assays using either a rich peptone medium or artificial urine medium at pH 6.5. A similar effect was observed with pure berberine HCl. The size of the zone of inhibition increased when 20 µg mL^{-1}, 50 µg mL^{-1}, or 100 µg mL^{-1} curcumin was added to the medium. When *S. saprophyticus* was grown in an artificial urine medium at pH 6.5 containing 20 µg mL^{-1} curcumin and increasing amounts of berberine up to 100 µg mL^{-1}, there was a reduction in both the growth rate and the yield of cells. There was complete inhibition of growth when the bacteria were cultured in filter-sterilized human urine containing 20 µg mL^{-1} curcumin and 100 µg mL^{-1} berberine.

REGULATION OF UREASE FORMATION

A potentially novel approach to the treatment of diseases caused by *Staphylococcus* species with urease activity might involve the inhibition of urease subunit formation and assembly of the native enzyme, or inhibition of nickel incorporation into the apoenzyme. Previous studies with genetic mutants of *Staphylococcus saprophyticus* have shown that the loss of urease activity is associated with a loss of virulence [68, 69]. The increase in pH that typically results from urease activity does not occur and urinary stone formation or catheter encrustation is prevented. If urease formation could be specifically inhibited, the pathogenic consequences of a UTI or other infection might be avoided.

Inhibition of urease synthesis

Unlike the situation in *Proteus mirabilis* where transcription of the genes for the urease subunits is regulated by a specific protein called UreR and induced by urea, urease synthesis in *S. saprophyticus* and *S. aureus* is constitutive [93]. The level of expression is, however, influenced by the pH of the growth medium [33]. For *S. saprophyticus*, the specific activity at pH 5.9 was 1.8 times that found at pH 7.2. For *S. aureus*, the specific activity at pH 5.9 was 11.2 times that observed at pH 7.2. Recent studies have indicated that in *S. aureus* this is related to a complex regulatory system called the acid response network [112]. This system mediates the transcription of a large number of genes when the bacteria are exposed to either strong acids like HCl or weak acids such as acetic acid and lactic acid. Exposure to both conditions results in up-regulation of the genes for the urease subunits. This response is dependent on the general transcriptional regulators AgrA, CcpA, and CodY [19]. AgrA and CcpA activate transcription while CodY inhibits it. Genes homologous to the *agr* locus were cloned from a clinical isolate of *S. saprophyticus* and shown to be present in other clinical isolates [113]. Proteins homologous to AgrA, CodY, and CcpA from other species of *Staphylococcus* including *S. epidermidis*, *S. lugdunensis*, *S. saprophyticus*, and *S. xylosus* are listed in the UniProt KB database. The primary effect of increased urease formation is to raise the normally acidic pH at local sites, and strains with high levels of urease activity are better able to cause persistent urinary tract infections [21].

Formation of urease in *K. pneumoniae* is dependent on the presence of particular nitrogen sources [114]. The specific activity of urease is very low when the bacteria are grown with ammonium as the nitrogen source but higher with amino acids such as arginine or histidine as the nitrogen source. The formation of urease in *S. aureus, S. saprophyticus,* and *S. xylosus* is also affected by ammonium sulfate and other nitrogen sources [17, 115]. However, these studies have not been pursued.

Inhibition of nickel incorporation

The ureases from all of the pathogenic species contain Ni^{2+} ions at the active site. One possible strategy to reduce urease activity and prevent or limit infections would be to sequester Ni^{2+} or to prevent its incorporation into the urease apoprotein. The human host protein Calprotectin has been shown to bind two equivalents of Ni^{2+} through coordination complexes with histidine residues [116]. When a urease-positive strain of *Staphylococcus aureus* was grown in a nickel-limited medium or a medium containing Calprotectin and Ni^{2+}, the amount of nickel in the cells was reduced. When these cells were tested for urease activity in the absence of nickel or in the presence of CP+Ni^{2+}, the formation of ammonia was also reduced.

The assembly of the metallocenter in urease depends on the products of the *ureD*, *ureE*, *ureF*, and *ureG* genes [117, 118]. A model for the sequential use of the proteins encoded by these genes in the insertion of Ni^{2+} into the apoenzyme has been proposed, in which UreE acts as a Ni^{2+} chaperone and UreG catalyzes the last key step. UreG is a GTP-binding protein with GTPase activity. Colloidal bismuth sulfate (CBS) can bind to UreG and was found to reduce urease activity in *P. mirabilis*, *K. pneumoniae*, *S. saprophyticus*, *S. aureus*, and *H. pylori*. Inhibition was much greater for intact cells than for cell extracts.

Ni^{2+} is a divalent cation and so cannot freely cross cellular membranes. The uptake of nickel ions prior to their incorporation into urease might be another drug target. Transport across the plasma membrane in *Proteus mirabilis* is facilitated by two ATP-binding cassette (ABC) nickel transport systems: Nik and Ynt. Construction of mutants affecting the ion-binding protein in each system indicated that the Ynt system is the primary mechanism for generating active urease enzyme. The Nik system can contribute to urease activity in a deleted Ynt strain at high nickel concentrations. A similar ABC transport system called Opp2 is found in *Staphylococcus aureus* [22]. This is one of four Opp transporters and includes two membrane proteins and two ATPases encoded by the genes *nikBCDF*. The protein Opp5A is a substrate-binding protein that together with the Opp2 proteins allows the uptake of Ni^{2+}. A second single protein nickel transporter called NixA contributes to Ni^{2+} uptake and a double *nikBCDF nixA* deletion mutant showed no nickel accumulation. The double mutant exhibited very low urease activity and showed no increase in pH in an acidified rich medium. A similar gene cluster occurs in *S. saprophyticus*. No specific inhibitors of these transport systems have yet been identified.

DISCUSSION

As this review demonstrates, the enzyme urease appears to play a key role in the pathogenesis of some species of *Staphylococcus*. Biochemical and genetic analyses of this protein indicate that the urease in these bacteria is structurally and functionally similar to those from other pathogenic bacteria such as *Helicobacter pylori* and *Proteus mirabilis*. However, there are some key differences such as the absence of cysteine residues in the UreC subunit of *S. saprophyticus* and *S. xylosus* and the more extensive regulation of subunit synthesis in *S. aureus* under acidic conditions. Most experimental studies of staphylococcal ureases have focused on *S. aureus* and *S. saprophyticus*, and much less information is currently available about the enzymes from *S. cohnii*, *S. epidermidis*, *S. lugdunensis*, *S. warneri*, and *S. xylosus*. There are many other urease-positive species in this genus which have not been extensively studied, and the work described here can serve as a valuable background for additional experiments.

One of the topics that requires further investigation is the native structure of the urease enzyme. While the genomic data now available has indicated the amino acid sequences of the major UreC, UreB, and UreA subunits, the stoichiometry of the nondenatured protein is still unknown in many cases. This is particularly true for *S. aureus*. Since most of the biochemical work on *S. saprophyticus* and *S. xylosus* was done in the 1980s and 1990s, the urease from all of the species described here should be studied further.

A second key topic is the role of the urease activity in the pathogenesis of these staphylococci. While studies with genetic mutants of *S. saprophyticus* were used to demonstrate that it is an essential virulence factor in urinary tract infections, similar work has not yet been done on the other pathogenic species of *Staphylococcus*. Methods for the genetic manipulation of microorganisms are now much better, and with the availability of the gene sequences for the urease subunits, it should be relatively easy to produce “knock-out” mutants of the other organisms.

A third important topic is the function of individual amino acids in the structure and activity of the urease protein. The availability of a large number of variants in the UreC, UreB, and UreA sequences, particularly for *Staphylococcus aureus*, can provide a good resource for further studies. By using strains in which specific amino acid replacements have been defined, one could look at their effects on the three-dimensional structure or urease activity of the enzyme.

A fourth important topic is the role of accessory urease genes in controlling urease activity. The genes for the urease subunits UreC, UreB, and UreA are often part of large clusters encoding other proteins which are believed to play a role in nickel acquisition and the assembly of the holoenzyme. While the functions of some of these proteins has been extensively studied in Gram-negative bacteria such as *Helicobacter pylori* and *Klebsiella aerogenes,* similar work has not been done with the Gram-positive pathogens.

Finally, the inhibition of urease formation and activity is still a key issue in the control of the diseases associated with these bacteria [103]. While many bacterial infections can be treated with antibiotics, it is often much better to target specific virulence factors such as urease. While many chemical inhibitors or natural product extracts have been described, they have been most often studied with either the jack bean enzyme or enzymes from *H. pylori* or *Proteus mirabilis*. Specific testing of these agents against the enzyme and functions of *S. aureus* and the other pathogenic *Staphylococcus* species is warranted. There are thus many possible avenues for further research.

CONFLICT OF INTEREST STATEMENT

The author declares there are no conflicts of interest.

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